Investigation on electron transfer from spinach thylakoids to modified electrodes in a photosynthetic electrochemical cell

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**INVESTIGATION ON ELECTRON TRANSFER
FROM SPINACH THYLAKOIDS
TO MODIFIED ELECTRODES IN A
PHOTOSYNTHETIC ELECTROCHEMICAL CELL**

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SCHOOL OF CHEMICAL AND BIOMEDICAL ENGINEERING

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SUMMARY

The depleting fossil fuel supply results in attempts to shift the energy prospects toward renewable energy sources. Solar energy has been considered as an attractive alternative energy source as it is abundant, free, and clean. Specifically, electrons generated from the water splitting reaction driven by solar energy have gained interest. Several efforts to utilize solar energy and the water splitting reaction have led to the development of inorganic and organic catalysts that mimic the natural plant water splitting catalyst. Although synthetic catalysts are known to be relatively more stable, recent studies show that the yield is still considerably lower than the natural manganese center. The potential of natural water splitting photosynthetic catalysts and their application in electricity generation are yet to be fully investigated and exploited.

Photosynthesis is a process in which water and carbon dioxide are converted into carbohydrate by using light as energy source. Water is split into electrons, hydrogen, and oxygen catalyzed by the manganese center embedded inside photosystem II (PSII). Some studies have shown that the generated electrons can be transferred directly or indirectly to an electrode resulting in photocurrent generation in photosynthetic electrochemical cell (PEC). In an attempt to obtain a high efficiency PEC design, understanding the electron transfer pathway becomes paramount in enhancing the PEC performance. In our study, we use isolated thylakoids from spinach leaves as the biocatalyst.

Investigation of suitable electrodes to harness the generated electrons based on the photosynthesis reaction is the main focus in this work. Preliminary

investigation shows that the distance between electrode and isolated thylakoids is an important factor to obtain efficient electron transfer, thus a biocompatible electrode is needed. Several well-known electrodes used in inorganic photovoltaic devices, such as indium tin oxide (ITO), multiwalled carbon nanotubes (MWCNT), reduced graphene oxide (rGO), carbon cloth, and graphene are screened in this study.

Thylakoid stability is retained upon interaction with ITO, as indicated by bound protein complexes and photocurrent generation. The bi-directional photocurrent is studied at different conditions, such as the presence of mediator and applied potential, and is confirmed by the presence of generated O_2 and H_2O_2 as the photosynthesis by-products. In the absence of mediator, the generated photocurrent is relatively low, thus an attempt to increase direct electron transfer to ITO and active surface area are needed to enhance PEC performance.

Dispersed MWCNT prepared by non-covalent interaction between MWCNT and Triton X-100 (TX100) surfactant, is electrodeposited on the ITO. A preliminary study shows the proof of charge transfer and biocompatible interaction between dispersed MWCNT and thylakoids based on results generated using three spectroscopic techniques (Raman, absorbance, and fluorescence). Upon MWCNT deposition, higher photocurrent is detected compared to unmodified ITO.

Further investigation is done with various carbon electrodes, such as carbon cloth, graphene, and rGO. Our observations show higher photocurrent using carbon cloth compared to graphene electrode. Carbon cloth modification using deposited rGO results in a negligible photocurrent increase, as hypothesized due to thermodynamic constraints on the transfer of electrons generated by the photosynthesis reaction.

In summary, we show proof of biocompatible interaction and electron transfer between isolated thylakoids and various conducting electrodes. The generated bi-directional photocurrent indicates that the direction of electron transfer can be tuned depending on different treatments, such as applied potential and the presence of redox mediators. The overall results of this study suggest the photosynthesis reaction is capable of generating electricity. The fundamental understanding of electron transfer from an isolated photosynthetic compartment can be applied to living photosynthetic organisms in future studies.

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LIST OF ACRONYM AND ABBREVIATION

BSA	Bovine serum albumin
CIB	Chloroplast isolation buffer
CV	Cyclic voltammetry
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DCPIP	2,6-dichlorophenol-indophenol
ETC	Electron transport chain
FESEM	Field emission scanning electron microscopy
ITO	Indium tin oxide
MWCNT	Multiwalled carbon nanotubes
NHE	Normal hydrogen electrode
OCV	Open circuit voltage
PC	Plastocyanin
PEC	Photosynthetic electrochemical cell
PEM	Proton exchange membrane
PQ	Plastoquinone
PS	Photosystem
rGO	Reduced graphene oxide
SCE	Standard calomel electrode
SM	Silicomolybdic acid
SWCNT	Single-walled carbon nanotubes
TX-100	Triton X-100

CHAPTER 1

Introduction

1.1 Research Background

The world consumes approximately 14TW energy yearly more than 80% of which is derived from fossil fuel. Fossil fuel has limited availability resulting in its annual price increase. Alternative ways to generate renewable and low-cost energy source, therefore, should be explored and investigated. One of the interesting renewable source candidates is the generated electron and hydrogen from water splitting process using photon energy from sunlight as catalyst. Unfortunately, the high standard reduction potential of water at $\sim +0.82$ V vs NHE limits this approach [1]. Although several inorganic catalysts have been reported to successfully oxidize water into oxygen and electron, their production yield is relatively low (~ 0.5 -10%) in addition to their high material price and fabrication process [2].

The photosynthesis protein complexes are the natural biocatalysts for water splitting reaction and are the main focus of this study. Manganese complex embedded in photosystem II (PSII) is the natural water oxidizing catalyst. In the presence of photon energy from light, water is split according to the following reaction: $2\text{H}_2\text{O} \xrightarrow{4h\nu} \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$ [3]. Interestingly, higher yield is observed by using natural catalyst (manganese complex) as compared to inorganic catalysts obtained from chemical synthesis (such as ZrO_2/TaON , NiO/RuO_2 , Ag_3VO_4 , and Ni-ZnS), which has approximately 30% quantum efficiency (QE) [4]. QE indicates the ratio of the generated electrons to the number of photons energy adsorbed.

Electrons generated during water splitting reaction can be used as the main electron source in the PEC development. From PSII, the electrons are channeled to PSI followed by further transfer to the respective electrodes. To investigate the electron transfer between biocatalyst and electrodes, two-chamber or one-chamber PEC configuration are commonly used. Factors such as electrolyte, electrode, photosynthetic biocatalyst, and interaction between biocatalyst and electrode affect the PEC performance [5, 6].

Previous investigations on photosynthetic materials as PEC biocatalyst have been done using isolated photosynthetic compartments such as chloroplast or thylakoids and living unicellular photosynthetic organisms such as green algae and cyanobacteria [7-9]. Despite its excellent water oxidizing performance, isolated PSII bio-catalyst has a relatively short lifetime due to photo damage caused by the formation of reactive oxygen species, such as hydrogen peroxide (H_2O_2) and oxygen radical, as the photosynthesis by-products [10, 11]. Although isolated photosynthetic compartment has a short lifetime activity, its utilization enables detailed investigation on photosynthesis electron transfer without any influences from other metabolic reactions.

The present study focuses on the fundamental of photosynthetic photocurrent generation using isolated thylakoids as biocatalyst. We exploit the potential of several carbon materials (multiwalled carbon nanotubes (MWCNT), reduced graphene oxide (rGO), carbon cloth, and free standing graphene) and transparent conducting indium tin oxide (ITO) as the prospective electrodes to harness the generated electrons from the photosynthesis process due to their

remarkable conductivity properties and biocompatibility. Those materials have been commonly used as electrodes in inorganic photovoltaic (PV) device [12-14].

Direct interaction between biomaterials, especially isolated proteins, and electrode surface may compromise the protein function and stability [15]. For example, pristine nanocarbon material may be toxic to living cells and proteins due to its hydrophobic properties[16]. Hence, an electrode pre-treatment is needed to provide biocompatible interaction between thylakoids and electrode surface.

To further investigate the site of electron transfer, combination between redox mediators and herbicide are added. Electrons can be intercepted by addition of redox mediators, while herbicide is known to inhibit the electron transfer from a specific site of photosynthetic electron transport chain (ETC)[17]. The direction of electron transfer can be deduced from the photocurrent generation. Detailed understanding of electron transfer phenomena is needed to design high efficiency PEC.

1.2 Research Objectives

The present work investigates the feasibility of photosynthetic materials as biocatalyst by using isolated thylakoids from spinach leaves. Electron transfer between thylakoids and electrode is monitored using two-chamber or one-chamber PEC configuration. The overall objectives of this research work are to improve the PEC design through electrode modification and understanding the principle of electron transfer phenomenon. In an attempt to achieve the overall objectives, we break down the specific aims of this project into:

1. To investigate the electron donation site from thylakoids to the electrode by either varying or inhibiting the electron shuttling position using redox mediator or herbicide.
2. To understand the fundamental electron transfer direction by observing the bi-directional photocurrent generation.
3. To obtain high-efficiency PEC design by screening the performance of various working electrodes such as ITO, MWCNT, ITO modified MWCNT (MWCNT/ITO), rGO, carbon cloth, carbon cloth modified rGO (rGO/carbon cloth), and free standing graphene electrode.

The major constraints in utilizing photosynthesis reaction are the stability and the amount of current generated upon illumination. Although high photocurrent can be generated using isolated thylakoids, protein degradation is unavoidable due to the presence of reactive oxygen species (ROS) as the photosynthesis by-products and the lack of repair mechanisms to regenerate the damaged proteins [18]. In contrast, utilization of living unicellular organisms exhibits higher stability based on their ability to repair the degraded protein, while the low electron transfer rate is observed due to the cell membrane resistance and electrons utilization in other living processes.

We believe that upon detailed understanding on the isolated thylakoids electron transfer, similar concept could be applied to living photosynthetic organisms. Thus, our recent work will focus on the fundamental study of isolated thylakoids as the representative of photosynthetic materials.

1.3 Organization of the Thesis

The flow of this thesis is organized as follows:

Chapter 1 Introduction describes the research background and overall objectives of this work.

Chapter 2 Literature Review summarizes the literature studies of the past works and current developments related to our research. This includes the general information of the world's energy consumption, photosynthesis process (including light reaction and water splitting reaction), brief information about the PEC, and the description of electrodes properties used in the experiments.

Chapter 3 Investigation on Electron Transfer from Isolated Spinach Thylakoids to Indium Tin Oxide (ITO). This chapter provides detailed information of the electron transfer between isolated thylakoids and ITO conducting glass based on current generation upon illumination. The site of electron transfer is investigated by addition of redox mediators or herbicide. Further information on the correlation between bi-directional photocurrent and the direction of electron transfer is described in this chapter.

Chapter 4 Interaction and Charge Transfer of Isolated Thylakoids and Multiwalled Carbon Nanotubes. This part describes the interaction and possibility of charge transfer between isolated thylakoids and dispersed MWCNT inside the solution by using three spectroscopy (Raman, UV-Vis, and fluorescence) techniques. The proof of biocompatible interaction and charge transfer are shown.

Chapter 5 Carbon-nanotube Modified Electrode in the Photosynthetic Electrochemical Cell. This chapter highlights the capability of higher photocurrent generation using MWCNT/ITO compared to bare ITO in the absence of mediator. The higher surface area provided by rough electrode surface and higher conductivity upon MWCNT deposition are important factors to enhance the photocurrent generation. The effect of MWCNT thickness toward electrode transparency and photocurrent generation is described.

Chapter 6 Free Standing Graphene and Modified Carbon Cloth Electrodes based on the Photosynthetic Electrochemical Cell (PEC). The feasibility of carbon materials, such as free standing graphene, rGO, and carbon cloth are tested to study the effect of different carbon surface toward electron transfer rate. Modified carbon cloth using deposited rGO reveals no significant photocurrent increase as compared to the untreated carbon cloth due to limited electrons generation during photosynthesis process.

Chapter 7 Conclusion and Future Works describes the overall results of the investigation and some suggestions for the future work.

CHAPTER 2

Literature Review

2.1 World's energy consumption

Energy sources are commonly classified as renewable and non-renewable. Our predominant energy source originates from the non-renewable fossil fuels (natural gas, coal, and petroleum) which are approximately 80% of the total energy used (Figure 2.1). As population grows and energy demand increases proportionally, the limited fossil fuel supply will not be able to suffice the energy need in the future [19]. The depleted fossil fuel results in several attempts to shift the energy prospective from fossil fuels to the renewable energy. Solar, wind, water, and biomass are some examples of common renewable energy sources being utilized.

Figure 2.1 World's energy consumption chart in 2010[20].

2.2 Solar energy and water splitting reaction

Recently solar energy has become an attractive renewable energy source. The sun provides approximately 100,000TW solar energy annually, while the world average yearly energy consumption is calculated at ~14TW [4]. The fact that utilization of 1 hour solar energy can suffice the annual world's energy consumption makes solar energy attractive as the prospective renewable energy to substitute fossil fuels in the future. In addition, solar energy has an advantage as a clean energy source with no greenhouse gas emission during the operation process; unlike the fossil fuel combustion that generates CO₂ as the byproduct. Despite its advantages, only small effective energy concentration (~1kW/m²per hour) is received by the earth's surface [21, 22]. This low energy concentration becomes the main concern in the solar energy utilization and results in the need of efficient light harvesting material. The solar radiance is classified into three spectrums, which are ultraviolet (UV) (5%) at ($\lambda < 400\text{nm}$), visible light (VIS) (40%) at ($400\text{nm} < \lambda < 700\text{nm}$), and infrared (IR) (55%) at ($\lambda > 700\text{nm}$) [22].

Several attempts to utilize the solar energy as alternative energy source have drawn many efforts in the development of photovoltaic device. Electron generation based on water splitting reaction using photon energy from sunlight is an attractive alternative to utilize solar energy as both water and sunlight are renewable sources and cheap. Thus, investigation of suitable water splitting catalyst material becomes an interesting field to be explored. Some approaches of PV development, including inorganic attempts based on the excitation of semiconductor band-gaps and biological attempts through photosynthesis process have been done. Although the inorganic catalysts obtained from chemical reaction are relatively stable, it turns out

that most of the catalysts used are made of metal oxides and only work effectively in the UV spectrum. The low percentage (~5%) of UV light in the solar spectrum inhibits its optimum application. To overcome the limited UV spectrum problem, several investigations to develop inorganic catalysts that work in the visible light have been done, but unfortunately, the catalytic yield is relatively low. The highest value is reported at 6.3% quantum yield using modified ZrO_2/TaON catalyst [23]. Observation on some other well-studied inorganic catalysts (NiO/RuO_2 : ~2.3eV, Ag_3VO_4 ~2.0eV, and Ni-ZnS ~2.3eV) shows that these catalysts are unable to perform direct water splitting reaction without additional external redox agents [2, 4, 24, 25].

In contrast, photosynthesis process uses the visible spectrum, thus speculated to have higher capability to harness the solar energy as compared to the inorganic catalyst. The biological water splitting reaction has 50% QE when red photon is used and 30% QE based on the whole solar spectrum [4]. The high water oxidizing yield provided by nature together with excited electron generation through charge separation in the reaction center are the main driving forces to further explore the feasibility of utilizing photosynthesis reaction to harvest solar energy. Previous works have shown capability of isolated photosynthetic compartment, such as chloroplast or thylakoid [7] and unicellular living unicellular photosynthesis organisms (cyanobacteria and green algae) [8, 9] as biocatalyst.

2.3 Photosynthesis

2.3.1 General information on photosynthesis

Photosynthesis is a biological process of converting light into chemical energy that is necessary for living activities. It is commonly classified into oxygenic and anoxygenic photosynthesis based on the presence or absence of oxygen by-product. In oxygenic photosynthesis, carbon dioxide and water are converted into carbohydrate by using light energy. Oxygenic photosynthesis occurs at cyanobacteria, green bacteria, green algae, and high-class plants [4, 26], with the overall reaction is described as follows:

In high-class plants, oxygenic photosynthesis occurs inside specific photosynthetic compartment (chloroplast) (Figure 2.2A). Chloroplast is a lipid bilayer vesicle containing thylakoids stacks, in which several photosynthetic protein complexes including photosystem I (PSI), photosystem II (PSII), and manganese center are embedded. In intact chloroplast, three main reactions – light reaction, dark reaction, and photophosphorylation – are combined together to sustain the overall photosynthesis process [27]. Dark reaction occurs in the stroma while the light reaction and photophosphorylation occur at the thylakoid. In the light reaction, solar energy is captured to oxidize water into electron, hydrogen, and oxygen. The generated electrons during water splitting reaction is used to reduce NADP^+ from dark reaction, and the formed NADPH is subsequently used to convert the carbon dioxide from atmosphere into carbohydrate (Figure 2.2B).

Figure 2.2 (A) Chloroplast, (B) Three photosynthesis reactions.

2.3.2 Photosynthesis light reaction

Photon absorption, charge separation, excited electron generation, and water splitting reaction are the important steps that build up the overall photosynthesis light reaction. Photosystem is an arrangement of reaction center which is surrounded by light harvesting complexes and light harvesting pigment antennas. The adsorbed photon energy from the antennas is further transferred to reaction center and results in excited electron due to the reaction center charge separation as described in Figure 2.3A.

Two photosystems (PSI and PSII) are embedded in thylakoid membrane. The PSI reaction center has maximum light harvesting capability at 700nm, while PSII has optimum wavelength utilization at 680nm, therefore PSI and PSII are commonly addressed as P700 and P680 [28]. Upon illumination, excited electrons generated from both photosystems are transported to each of the primary electron acceptor to be subsequently used to reduce the NADP^+ . The presence of electron donor is needed to recombine with the reaction center hole in order to sustain the continuity of the photosynthesis process. P700 electron loss is substituted by electron from PSII, while the electron generated during the water oxidizing reaction

replaces the PSII electron loss. The higher standard reduction of P680 ($\sim 1.2\text{V}$ vs NHE) as compared to water ($\sim 0.8\text{V}$ vs NHE) indicates its capability to extract the electron from water [4]. Figure 2.3B shows the electron transport pathway and the protein complexes involved arranged according to their standard reduction potential in the Z-scheme.

Figure 2.3 (A) Schematic of photon harvesting and excited electron generation at the photosystem and (B) Z-scheme of electron transport chain [28].

2.3.3 Light harvesting antenna

Photosynthetic organisms are equipped with light harvesting antennas (LHAs) to capture photon energy. In high-class plants and green algae, LHAs consist of chlorophyll (chlorophyll a and chlorophyll b) and carotenoid. Chlorophyll a has absorbance peaks at ~420nm and 670nm, while chlorophyll b optimum absorbance peaks are detected at ~450nm and 650nm [29]. Carotenoid main function is to protect the reaction center from excess light exposure by dissipating the excess light as heat. Despite its main function, carotenoid is also able to utilize light energy due to its orange pigment color and has two absorbance peaks at 420 and 450nm [30, 31]. According to the specific absorbance spectrum of the LHAs, only visible light range can be utilized by green algae and high-class plant photosynthesis process.

In order to increase the light utilization at the green spectrum, cyanobacteria contain additional antenna accessories (phycobilisome) such as phycoerythrin and phycocyanin. Additional light harvesting antenna (bacteriochlorophyll) is present inside anoxygenic photosynthetic bacteria with the optimum light harvesting capability found in the infrared region [32]. The absorbance spectra of the LHAs from photosynthetic organism at visible light spectrum are presented in Figure 2.4.

Figure 2.4 Light harvesting antennas absorbance spectrum [28].

2.3.4 Oxygen evolving center (OEC) manganese complex

Oxygen Evolving Centre (OEC) is a biological water oxidizing catalyst. It consists of four manganese ions, one calcium ion, and one chloride ion (manganese cluster) that work together to perform water oxidizing reaction through five intermediate states commonly known as the S-states (S₀, S₁, S₂, S₃, and S₄, Figure 2.5A). Three manganese ions form a cubane structure with calcium ion, and another one is located outside the cubane. The metals are linked by oxo bridges [4].

The mechanism of water oxidation is described as follows: two water molecules are bound to OEC. One water molecule is bound to calcium (Ca²⁺) ion and another one is bound to one of the manganese ion that is not contained within the cubane. In the S₀ state, manganese ion binds the water molecule (Mn(IV)) and is oxidized to Mn(V). The water is subsequently deprotonated to an oxo form. Due to the highly oxidized state of Mn(V), electron is donated to other Mn(IV) ions (S₁, S₂, and S₃) and causes a nucleophilic attack by the oxygen of the second water substrate in the Ca²⁺ to form O=O bond of molecular oxygen (S₄) [4, 33].

The deprotonation of substrate water molecule and stabilization of intermediate will be performed by chloride ion (Cl^-) and hydrogen bonding network of the protein side chains, such as Tyr, Glu, Arg, and Gln [33]. Details of the five intermediate states during the water oxidizing reaction are described in Figure 2.5B.

(A)

(B)

Figure 2.5(A) Manganese cluster structure (B) Schematic of water oxidizing reaction in five intermediate states [4].

Utilization of the electron generation during charge separation and water splitting reaction in the photosystem reaction center is the main focus of this study.

Suitable electrode materials are needed to obtain high electron transfer efficiency from the photosynthesis materials, thus we will focus on the screening of different conducting materials, such as ITO and carbon materials (MWCNT, rGO, carbon cloth, and free standing graphene) to obtain high electron transfer efficiency in the PEC design.

2.4 Indium tin oxide (ITO)

Indium tin oxide is a mixture between indium oxide (In_2O_3) and tin oxide (SnO_2) at typical ratio of 90% (w/w) In_2O_3 to 10% SnO_2 (w/w). ITO is commonly fabricated as thin layer conducting film on the substrate. Common methods to fabricate deposited ITO are electron beam evaporation [34, 35], chemical vapor deposition (CVD) [36], pulse laser deposition [13], and magnetron sputtering techniques on the substrate [37].

ITO is a n-type semiconductor with a band-gap between 4.4 and 4.9eV in vacuum [38-40]. It has some advantages, such as transparency in the visible spectrum, good electrical conductivity, low electrical resistivity, and chemical inertness [13]. Modification on ITO conductivity and transparency properties can be done by modifying charge carrier, layer thickness, and deposition condition (temperature and oxygen pressure) [13, 41]. A control of ITO conductivity and transparency is needed during the fabrication process. Increase of ITO thickness and charge carrier result in conductivity increase, while formation of yellowish ITO layer reduces its transparency.

ITO has been widely used for many applications, such as flat panel display, oven windows, organic light-emitting diodes, improving the durability of glass,

electromagnetic shielding, and solar cell [13, 42]. Bare ITO is unable to harness solar energy, thus integration with charge transfer sensitizer is needed. For example, ITO integration with quantum dots (QDs) or synthetic dye (ruthenium complex) in the photovoltaic device [43-45].

Several studies have highlighted the interactions between ITO surface and biomaterials, such as PC12 cells [46, 47], cytochrome c [48], and rabbit plasma protein [49]. No biomaterial morphology and function can be observed upon interaction, justifying the biocompatible interaction. To the best of our knowledge, study of interaction between isolated PSI [50] on ITO surface has only been recently reported, while detail information using whole photosynthetic compartment has not been explored yet.

2.5 Carbon Nanotubes (CNT)

2.5.1 General information of CNT

Carbon nanotubes (CNT) are cylindrical graphene sheet tubes, first synthesized by Iijima [51]. CNT is considered as an interesting electrode due to high tensile strength, thermal conductivity, heat resistance, and conductivity properties [52, 53]. In general, there are two types of carbon nanotubes, which are known as single-walled carbon nanotubes (SWCNT) and multiwalled carbon nanotubes (MWCNT). SWCNT consists of single graphene sheet, while MWCNT consists of several tube arrays, thus providing larger diameter compared to SWCNT (Figure 2.6).

Figure 2.6(A) Single walled carbon nanotubes, (B) Multiwalled carbon nanotubes, showing typical length, width, and distance separation between each of MWCNT graphene layers [54].

Different chirality affects the CNTs electrical conductivity and results in semiconducting and metallic CNTs. Chirality can be determined from a pair of integers (n,m) called chiral vectors, as described by schematic in the Figure 2.7 Metallic or semiconducting CNTs based on different chirality [55]. All nanotubes which the $n-m = 3i$ (i are zero or positive integer) are metallic. Otherwise they are considered to be semiconducting [55, 56].

Figure 2.7 Metallic or semiconducting CNTs based on different chirality [55].

Some of the well-known methods to grow CNT are done through electric arc discharge [57], laser vaporization [58], and chemical vapor deposition (CVD) of hydrocarbon [59, 60]. The CVD method is commonly used for possible control of CNT alignment during the synthesis process [60]. Hydrocarbon gas, such as methane, ethylene, and acetylene, are used as carbon source with the addition of Fe or Co as catalyst. The high temperature decomposes the hydrocarbons resulting in the formation of nanotubes on the substrate according to the catalyst alignment. After CNT growth, purification method using thermal oxidation is commonly employed to remove the amorphous carbon and carbon defect.

2.5.2 Raman spectroscopy to study CNT conductivity

Raman spectroscopy has been employed to investigate the conductivity of carbon material [61, 62]. Upon laser excitation, carbon materials generate several strong peaks according to specific carbon bond vibration at either sp^2 or sp^3 (G, D, G' or 2D, and radial breathing mode (RBM)). G peak represents a graphitic layer and conductivity properties of individual carbon bond stretching and compressing (sp^2 carbon bond) which occurs at $\sim 1580\text{cm}^{-1}$, while the D peak indicates the carbon defect (sp^3 carbon bond) which is normally observed at $\sim 1350\text{cm}^{-1}$ [62]. The G' peak is observed at $\sim 2700\text{cm}^{-1}$, while RBM indicates the diameter of single-walled carbon nanotubes. Screening of electronic properties of different types of carbon materials, including the graphite, graphene, nanotubes (SWCNT or MWCNT), and fullerene can be screened from Raman signal. It has been noted that carbon conductivity is determined by the integrity of sp^2 carbon bond which is observed at

G peak [63, 64], thus high conductivity carbon materials can be indicated by a high ratio of sp^2 and sp^3 carbon bond or the low ratio of I_D/I_G [65].

2.5.3 Bioapplication of CNT

Due to its unique properties and advantages, attempts to integrate CNT with biomaterials have gained many interests. Previous studies reveal that the hydrophobic and cytotoxic effect of CNT hinders further interaction with biological material, thus initial CNT functionalization is needed prior to the integration [16, 66]. Common approaches to promote the CNT biocompatibility have been done using covalent or non-covalent functionalization [67-69]. Covalent functionalization is done by introducing oxygen functional group on either CNT end-group or sidewall through acid oxidation. Although this method can promote CNT water solubility properties and functional group to further facilitate the interaction with biomaterial, alteration of the CNT conductivity as a result of carbon sp^2 bond opening is unavoidable [69]. Non-covalent functionalization has been investigated as an alternative method to preserve the original CNT conductivity. Non-covalent functionalizations are done by wrapping the nanotubes sidewall using polymer [70], surfactant [71], BSA (bovine serum albumin) [72], and biomolecules, such as DNA [73]. The successful biological interaction of non-covalent functionalization MWCNT has been shown by conjugation of protein or carbohydrate with pyrene group [69, 74]. Proof of interaction between photosynthetic reaction center and CNT through non-covalent and covalent functionalization of CNT have been described previously as shown by addition of pyrene or amine linker [75-77].

Using the same principle of electron generation and electron transfer during photosynthesis process, we aim to study the interaction and proof of charge transfer between the CNT and isolated thylakoids in our preparation.

2.6 Graphene

Graphene is a 2D sheet of carbon atom, which is basically a single layer of graphite. The integrity of sp^2 carbon bond between the carbon atoms makes graphene a strong and conductive material. Graphene has been considered as a potential electrode for photovoltaic device due to its conductivity, transparency, and flexibility [43, 78-80]. Common methods to fabricate graphene layers are through mechanical exfoliation of graphite [81, 82], epitaxial growth by using SiC substrate [83, 84], chemical vapor deposition (CVD) [85, 86], and chemical conversion from graphite [87-89]. In the exfoliation method, sticky side of adhesive tape is attached against pure graphite, followed by tape removal to obtain graphene trace. Although this method is easily performed to obtain pure graphene, the resulting random graphene layers are unfavorable.

The chemical conversion method is commonly done to obtain multilayered graphenes. In a chemical conversion process, graphite oxidation forms graphene oxide (GO) to promote water solubility and biocompatibility due to the presence of oxygen containing group (carboxyl and hydroxyl) on the graphene surface [90]. Despite its hydrophilic property, GO is known to have low conductivity due to the presence of oxygen group. Removal of the oxygen group through reduction processes are performed to obtain reduced graphene oxide (rGO). In an attempt to utilize the dispersed rGO, deposited on various substrates through electrostatic

absorption, spin coating, as well as filtration is commonly done[91, 92].It is speculated that stacking between each of the graphene layers during the deposition process may result in reduced surface area for interaction with the materials of interests. Thus, instead of rGO electrodeposition, direct growth of the graphene on substrate using CVD method is preferred to obtain free standing graphene electrode.

2.7 Photosynthetic biocatalyst electron transfer

Previous attempts to generate energy from the biological process have been done using various yeast and bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shewanella putrefaciens* as biocatalysts [6, 93-95]. The electrons generated from the metabolic living reaction are captured and transferred to the electrode. Using similar principle, feasibility of the isolated photosynthetic compartment (thylakoid or chloroplast) or unicellular living photosynthetic organism, such as green algae, cyanobacteria, and photosynthetic bacteria as the biocatalyst for the PEC development have been widely studied [7, 8, 96]. Utilizing photosynthetic unicellular living organism as biocatalyst has gained considerable interests for its ability to generate current at dark and light through a combination of metabolic and photosynthetic processes respectively. In contrast, investigation on isolated photosynthesis compartment enables detailed observations of the process.

Electron transfer between photosynthetic biocatalyst and electrodes can be performed through indirect (mediator) or direct electron transfer (membrane bound protein and conducting nanowire) to the electrode, as shown in the Figure 2.8 [97, 98].

Figure 2.8 Schematic on (A) mediated electron transfer via mediator, direct electron transfer via (B) membrane bound protein, and (C) electronically conducting nanowires

2.7.1 Indirect electron transfer (mediators)

Mediators can either donate or accept electrons from ETC based on their standard redox potential, redox state (reduced or oxidized), and water solubility properties. Hydrophobic mediators have the capability to access PSII components that are buried inside thylakoid membrane, while hydrophilic mediators are known as good electron acceptor for the reducing sites of PSI [17]. The electron transport chain can also be blocked by adding a compound that binds to one of the chain components. For example, DCMU is capable in blocking electron transport from PSII to PSI by occupying the plastoquinone binding site [17, 99]. Common mediators and herbicide used to intercept or block the electron transport chain are shown on Figure 2.9.

Figure 2.9 Redox mediator interception on the electron transport chain (ETC). Dashed lines represent the electron transfer blockage, arrows toward the chain indicate the electron donor, while arrows out of the chain show the electron acceptor. BQ, benzoquinone; DAD, diaminodurene (2,3,5,6-tetramethyl-p-phenylene diamine); DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; DCMU, (3-(3,4-dichlorophenyl)-1,1-dimethylurea); DPC, diphenylcarbazide; DCPIP, dichlorophenol-indophenol; FeCN, ferricyanide; MV, methylviologen; PD, phenylenediamine; PMS, phenazine methosulfate; SM, silicomolybdate; TMPD, N-tetramethyl-p-phenylene diamine

Some mediators are only stable in either oxidized or reduced form. Thus, in an attempt to maintain the redox state, excess of ferricyanide or ascorbate in the preparation medium is required to intercept electrons from the PSII electron acceptor (PD, DAD, and BQ) or the PSI donor (DAD, DCPIP, TMPD) respectively. Although addition of mediator has been proven to increase electron transfer rate between photosynthetic material and electrode, its diffusion rate to penetrate the bound membrane and toxicity limit the practical application.

2.7.2 Direct electron transfer

2.7.2.1 Membrane bound protein

The exposed redox-active protein on the surface is required to promote direct contact and electron transfer to electrode. In isolated reaction center, some proofs of successful direct electron transfer have been established through

interaction between engineered reaction center and electrode, such as oriented PSI through substitution of native PsaD with the engineered PsaD-His₆tag on gold surface [100] and direct interaction between native biocatalyst and electrode [101, 102].

In the isolated thylakoids, recent study shows that photosystem I, photosystem II, plastoquinone, cytochrome *b₆f*, and plastocyanine are involved in direct electron transfer process [103], with addition of positive potential applied at +0.2V vs Ag/AgCl promotes direct electron transfer from thylakoids to electrode due to the capability of plastocyanin to freely diffuse between the stromal and the lumenal side of thylakoid [104].

2.7.2.2 Electronically conducting nanowires

In living unicellular photosynthetic organisms, such as cyanobacteria, mediatorless electron transfer can be done through formation of microbial nanowire (pili) production to enable direct electron transfer based on physical electrical connection without any physical contact [105, 106]. *Synechocystis PCC6803* is a cyanobacteria genus that generates pili when the carbon dioxide supply is limited. Pili have been suggested to facilitate the transfer of excess generated electrons to electrode inside a microbial fuel cell [107, 108].

2.8 Photosynthetic Electrochemical Cell (PEC)

Microbial biocatalyst and electrode are integrated in a microbial fuel cell (MFC) configuration to observe the electron transfer process. The electron transfer between biocatalyst and electrode is indicated by potential and current generation.

Factors that influence the efficiency of electron transfer, such as electrodes, electrolyte, choice of organisms, and the interaction between organisms and the electrode have been described previously [5, 6]. The term fuel is applied to MFC due to the bacteria dependence on carbon source or complex organic substrates to maintain their living processes, in which constant supply of carbon source is needed to preserve the generated current. In contrast, photosynthetic organisms, such as photosynthetic algae and cyanobacteria, can sustain their living processes by using light as energy source. Thus, we will use the term photosynthetic electrochemical cell (PEC) to describe our system.

Electrodes in the PEC design are classified as anode and cathode. Biocatalyst is incorporated on the anode, while the cathode is exposed to the final electron acceptor, such as potassium ferricyanide or oxygen, in a two-chamber or one-chamber PEC. Several studies on the utilization of bacteria metabolic reaction (MFC) or photosynthesis process (PEC) have been done. In the MFCs, electron source is derived from the fermentation of glucose through bacteria metabolism reaction, while in PEC the electrons are generated during the photosynthesis reaction. The generated current or power during device operation is normally used to compare the MFC and PEC performance. As examples, *Shewanella sp.* can generate 0.04mA-1.2mA current [109-111], while 0.4mA-3.5mA current can be obtained using *Escherichia coli* as biocatalyst in MFC [112, 113]. In a similar way, study of the current generation based on illumination has been shown using living blue green algae [8, 114, 115], green algae [116], and isolated spinach chloroplast or thylakoid [7, 117] with reported current generated in the range between 1-350 μ A. The different current outputs between the reported studies are influenced by

several factors, including the chamber configuration (two-chamber or one-chamber), electrode material, presence of mediators, and different electrolyte.

The present work focuses on the fundamental studies of electron transfer based on photosynthesis reaction to the conducting electrodes. Isolated spinach thylakoids are used as the biocatalyst, while ITO, MWCNT, rGO, carbon cloth and free standing graphene electrodes are the conducting materials to be investigated. Photocurrent generation is observed using two-chamber PEC configuration.

CHAPTER 3

Research Methodology

3.1 Chloroplasts and thylakoids isolation

Chloroplasts were isolated from spinach leaves through several centrifugation steps. Spinach leaves (5 g) were ground in 20 mL chloroplast isolation buffer (CIB) containing 300mM sorbitol (Aldrich), 100mM Tris (Bio-Rad), 10mM sodium chloride (Aldrich), 5mM magnesium chloride (MP Biomedics), and 1% (w/v) bovine serum albumin (BSA) (Aldrich) as stabilizer at pH 7.8. Leaf extract was filtered using 100 μ m filter mesh (Aldrich) and centrifuged at 200 $\times$ g for 3 minutes to remove the cell debris. The supernatant was further centrifuged at 1000 $\times$ g for 7 minutes and the isolated chloroplasts were collected as green pellet.

The chloroplasts were subsequently dispersed inside sugar-free environment to induce osmotic shock and expose the thylakoids [118]. Its concentration was determined as the total chlorophyll mass using acetone extraction method [119]. Briefly, isolated thylakoids were diluted 100 times in 80% (v/v) acetone solution followed by centrifugation at 3000 $\times$ g for 2 minutes to retain the supernatant. The concentration, expressed as unit chlorophyll basis, was calculated by measuring the supernatant optical density (OD) at 652nm ($\epsilon_{\text{chlorophyll}} = 36 \text{ L.g}^{-1}.\text{cm}^{-1}$).

3.2 ITO cleaning and preparation

ITO (~1.1 mm ITO thickness and $<10 \text{ } \Omega/\text{cm}^2$ resistance) was purchased from Wintek Technology (S) Pte Ltd. To remove any contaminants, the ITO was sonicated twice in acetone followed by water for 10 minutes. The ITO active area is $\sim 1\text{cm}^2$.

3.3 The effect of distance interaction between thylakoids and electrode

The thylakoids were deposited on the ITO surface using: (1) physical adsorption by leveraging the hydrophilic interaction between an outer lipid bilayer of thylakoids against the hydrophilic ITO surface [120] and (2) electro adsorption by applying +1.5V external charge on the ITO to promote static interaction, as thylakoid membranes were negatively charged at $\text{pH} > 4.3$ [121, 122]. After both techniques were employed, the substrates were dried at room temperature in the dark before use. The investigation on loose interaction was done by dispersing the same thylakoids amount as indicated by chlorophyll concentration. The generated photovoltage and photocurrent were compared to study the effect of different interaction treatments.

3.4 Multiwalled carbon nanotubes (MWCNT) growth and non-covalent functionalization

Vertically aligned multi walled carbon nanotubes (MWCNT) arrays were grown by chemical vapor deposition (CVD) process adapted from the method reported by Zheng and co-workers [123-125]. During MWCNT growing process, pure ethylene (C_2H_4) was used as the carbon source and the forming gas, while

argon (Ar) and hydrogen (H₂) served as carrier gases. The catalysts used in this study were Fe (0.8 nm)/Al₂O₃ (30 nm) on a Si wafer with a 2 μm SiO₂ layer. The Fe layer was deposited by sputtering deposition technique. Typically, MWCNT growth was carried out at 750°C with 200 sccm total flow gas for 10 min growth. The MWCNTs used in the experiment have mixture of semiconducting and metallic CNTs since the CNTs are directly used from the chemical vapor deposition process without further separation process.

Non-covalent functionalization using Triton X-100 (TX-100) surfactant promotes hydrophilic properties of the MWCNT sidewall surface. The hydrophobic tail of TX100 that consists of a long hydrocarbon chain and pyrene groups are anchored to the hydrophobic MWCNT sidewall forming the hydrophobic and π - π interactions against MWCNT sidewall. The exposed hydrophilic tail (polyethylene oxide chain) of TX-100 promoted the MWCNT water solubility property (Figure 3.1).

Figure 3.1 Non-covalent functionalization was formed through hydrophobic and π - π interactions between TX-100 and MWCNT sidewall. Picture was not drawn to scale.

Sonication at 40 Amplitude for 15 minutes followed by centrifugation at $36000\times g$ for 5 minutes were done to obtain soluble MWCNT as supernatant in the form of short MWCNT bundles, with initial concentration was prepared at $12\mu g$ MWCNT/mL in $6\times 10^{-3}\%$ (w/w) TX-100. Stable MWCNT dispersion was indicated by the absence of MWCNT aggregate.

3.5 Deposited MWCNT/ITO preparation and characterization

MWCNT/ITO was prepared through electrodeposition method using electrochemical workstation 660D Potentiostat/Galvanostat (CH Instrument, Inc.). ITO was used as a working electrode while the platinum electrode served as both counter and reference electrodes in a solution containing dispersed MWCNT. MWCNT was electrodeposited on ITO at 0.3mA external current with an approximate surface area of 1cm^2 . Thylakoids were deposited using physical adsorption process by leveraging on the hydrophilic interaction with ITO or MWCNT/ITO. All preparations were dried for approximately 10 minutes at room temperature before use.

The effect of MWCNT deposition time toward photocurrent generation was screened to obtain the optimum MWCNT thickness. Absorbance scan observation directly against the ITO was used to correlate the effect of deposition time with the MWCNT thickness. The linear relationship between deposition time and MWCNT absorbance thickness was plotted at 500nm absorbance value.

3.6 Free standing graphene growth and rGO deposition on carbon cloth surface

Nickel foam substrate loaded into a quartz tube were heated at 1000°C under H₂ (25sccm) and Ar (50sccm) for 10min to clean the surface with ethanol was used as the carbon source. After 20 minutes of chemical vapor deposition (CVD) and cooling process, the nickel substrate was etched using HCl (3M) solution at 80°C to obtain free standing graphene. The graphene was fixed on a glass substrate and used as a working electrode in the subsequent experiments.

Deposited rGO on carbon cloth was prepared through electrodeposition process. Carbon cloth fixed on the glass substrate was used as a working electrode while platinum electrode served as both counter and reference electrodes inside rGO solution. rGO deposition was carried out using addition of 0.3mA external current, as had been established on the previous study in MWCNT/ITO preparation. The effect of rGO deposition time toward photocurrent generation was screened to study the optimum electron transfer rate.

3.7 Electron transfer observation

Electron transfer between thylakoids and electrodes was observed by monitoring the photovoltage and photocurrent generation using two-chamber PEC configuration or based on spectroscopy signals change between the mixture of thylakoids and MWCNT or rGO.

3.7.1 Two-chamber PEC configuration for photovoltage and photocurrent measurements

The electron transfer between thylakoids and electrodes was observed by monitoring the photocurrent generation using two-chamber PEC configuration separated by a proton exchange membrane (PEM) (GasHub). The anode chamber contained 10mM potassium phosphate buffer (pH 7.2) (Merck/Fluka), while the cathode chamber was filled with 50mM potassium ferricyanide (Aldrich). Both chambers were supplemented with 100mM potassium chloride (Merck). Carbon cloth (GasHub) at $\sim 5\text{cm}^2$ was used as both reference and counter electrode in the cathode chamber, while the respective electrode (ITO, MWCNT/ITO, carbon cloth, graphene, and carbon cloth modified rGO) was used as the working electrode inside the anode chamber.

Upon illumination, it was speculated that the electrons generated from the water splitting reaction and reaction center charge separation during the photosynthesis reaction were transferred to the anode electrode followed by the further electron transfer to the cathode chamber. Potassium ferricyanide served as final electron acceptor inside the cathode chamber, while the generated protons during the water splitting reaction travelled across the PEM to oxidize the reduced potassium ferricyanide at cathode chamber. The schematic of two-chamber PEC with ITO as anode and deposited thylakoids was described in Figure 3.2.

Figure 3.2 Schematic of two-chamber PEC with dotted red lines shows the deposited thylakoids on ITO. Detailed enlargement of the red dotted area shows the thylakoid electron transport chain and predicted electron transfer to ITO. Inset: Electron transport pathway is described using dotted lines, while thunderbolts indicate light energy excitation.

The electron transfer between isolated thylakoids and the electrodes was indicated by the photovoltage or photocurrent generation and recorded using electrochemical workstation 660D Potentiostat/Galvanostat (CH Instruments, Inc.). A tungsten halogen lamp (100W Philips A1/215 light bulb) without any additional filter to cover the visible light wavelength (400-800nm) was used as the light source. Light intensity was kept constant at approximately $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ inside the anode chamber using QRT1 Quantitherm (Hansatech). A metal shield was used as a shutter to simulate dark condition at predetermined time points. Photocurrent was recorded under dark saturated open circuit voltage (OCV) conditions, unless otherwise stated.

The protein complexes responsible for interaction and electron transfer were studied in the presence of redox mediators or herbicide. Two representatives of

mediators, DCPIP and SM, were chosen based on different water solubility and standard redox potential to intercept electrons at either PSII or PSI respectively. DCMU blocked the electron transfer from PSII to PSI by occupying the PQ binding site [17, 99]. In an attempt to specify the investigation of PSI site, artificial electron donor of PSI (DCPIP^{red} in the presence of excess ascorbate) and DCMU were added. Direct electron transfer was investigated in the absence of mediator. To justify the photocurrent observed as a result of photosynthesis reaction, control experiments were conducted in the absence of thylakoids.

3.7.2 Detailed observation of working electrode electron transfer

In an attempt to study the detailed electron transfer at the ITO or MWCNT/ITO electrodes, three-electrode configuration submerged inside as described PEC anolyte was used. The ITO or MWCNT/ITO was used as the working electrode, while platinum foil and standard calomel electrode Hg/HgCl (SCE) served as the counter and reference electrodes respectively. Photocurrent observation was studied in the absence of mediator to study the feasibility of electron transfer between thylakoids and ITO or MWCNT/ITO by applying different external potentials. Study also focused on the ITO and MWCNT/ITO electrochemical catalytic toward O₂ and H₂O₂ by using cyclic voltammetry (CV) method at 50mV/s scan rate.

3.7.3 Spectroscopy observation (Raman, UV-Vis absorbance, and fluorescence spectroscopy)

Interaction and charge transfer between thylakoids and dispersed MWCNT or rGO were studied in liquid state by using three spectroscopy techniques, which were: (1) Raman spectroscopy (Reinshaw RM1000, 20mW) using 488nm laser excitation at 1100-2800 cm^{-1} to monitor the MWCNT electronic properties change (D, G, and G' band), (2) UV-Visible spectroscopy (Shimadzu) absorbance scan at 400-700nm range to identify thylakoids stability, and (3) fluorescence spectroscopy (RF5301 Shimadzu Spectrofluorometer) observation at 540-700nm emission wavelength range upon various laser excitation to identify the charge transfer based on fluorescence quenching. The correlation between laser excitation and fluorescence emission was plotted using 2D contour map with a 10nm excitation laser interval from 400-500nm. Each of the spectroscopy signals was compared between isolated thylakoids, MWCNT or rGO, and hybrid thylakoids and MWCNT or rGO under same concentrations. All experiments were performed on mixture of dispersed MWCNT or rGO and thylakoids.

3.8 Characterization method

The morphology of the adsorbed thylakoids was observed using field emission scanning electron microscope (FESEM, JEOL JSM-6700F). The dried thylakoids were sputtered with platinum prior to observation. Optical absorbance was observed from 400 nm to 700nm to monitor the bound protein complexes and light harvesting antennas in the thylakoids.

The as-grown MWCNT arrays were characterized by a field emission scanning electron microscope (FESEM, Carl Zeiss, LEO 1550VP) and the detailed observation of MWCNT was done using transmission electron microscope (TEM, JEOL 2010). After 30-minute sonication in an ultrasonic bath cleaner, a droplet of MWCNT dispersion was casted onto a TEM copper grid and the solvent was evaporated overnight at room temperature.

The morphology of ITO, MWCNT/ITO, carbon cloth, and graphene were observed using field emission scanning electron microscope (FESEM, JEOL JSM-6700F). Absorbance scan was used to monitor the MWCNT thickness on ITO. Linear correlation between MWCNT thickness and deposition time was plotted at A_{500} . The attachment of the thylakoids to the ITO or MWCNT/ITO was monitored spectrophotometrically by scanning its absorbance from 400 nm to 700 nm.

CHAPTER 4

Investigation on Electron Transfer from Isolated Spinach Thylakoids to Indium Tin Oxide (ITO)

4.1 Abstract

The electrons generated through the photosynthetic water splitting reaction have been studied for direct electron transfer under light condition. Isolated thylakoids are incorporated as biocatalyst with indium tin oxide (ITO) as the electrode in a two-chamber photosynthetic electrochemical cell (PEC). The generated current is compared between deposited (physical or electrical adsorption) and dispersed thylakoids. The physical properties of deposited thylakoids are observed using field emission scanning electron microscopy (FESEM) and absorbance spectroscopy techniques. The results show the presence of thylakoid with bound photosystems including light harvesting antennas and other protein complexes. Further investigations reveal that the direction of electron transfer can be tuned by varying the applied potentials to promote the bi-directional photocurrent. The maximum cathodic photocurrent is obtained at -50mV vs Standard Calomel Electrode (SCE), while the maximum anodic photocurrent is shown to increase with increasing positive potential applied. Our observation shows the possibility of either reduction of O_2 or H_2O_2 as the prominent cathodic photocurrent source, while electron transfer from thylakoids to ITO yields the anodic photocurrent.

4.2 Background of the Study

The photosynthetic water splitting reaction driven by light harvesting antenna excitation and reaction centers charge separation has drawn many interests for its potential use in the development of the biological photovoltaic device. Common approaches to exploit the natural photosynthesis process have been reported on light harvesting antenna [126], isolated chloroplast or thylakoid [127], and whole photosynthetic organisms such as photosynthetic algae, cyanobacteria, and purple bacteria [8, 96, 128, 129]. The chloroplast is a specific photosynthesis compartment in plant or algae. The inner membrane of chloroplasts, the thylakoids, contains the photosynthetic protein complexes that make up the electron transport chain (ETC). The ETC includes photosystem II (PSII) that contains the manganese center where the water oxidizing light reaction occurs and electrons are generated, cytochrome *b₆f* complex, and photosystem I (PSI). In isolated thylakoids, upon incorporating the isolated thylakoids and conducting electrodes, the electrons are transferred from the electron transport chain to electrode with or without addition of mediators. While in the intact chloroplast preparation, the generated electrons are used to reduce NADP^+ in dark reaction.

In our study, we focus on investigating the electron transfer between photosynthetic material and electrode as indicated by generated photocurrent in a two-chamber photosynthetic electrochemical cell (PEC). Indium tin oxide (ITO) conducting glass is a common electrode used in photovoltaic devices such as dye sensitized solar cells (DSSC) due to its unique high transparency and conductivity properties. Similarly, the high light transmission in the visible region is optimal for

the photosynthesis process and the high conductivity for efficient electron transfer makes ITO a good candidate as the electrode material in a photosynthetic electrochemical cell (PEC). To utilize the generated electrons from photosynthesis reaction, good contact between photosynthetic material and electrode as the electrons collector is required to reduce medium resistance factor [127]. One of the problems that is associated with close contact interaction between protein and solid state electrode is protein conformational change that may lead to inactivation [130].

Common approaches to promote close contact between proteins and solid surface include chemical surface functionalization and physical forces. One of the problems that is associated with close contact interaction is a protein conformational change which may lead to inactivation [130]. In previous investigations, close contact between photosynthetic proteins and solid surface is achieved through adsorption of isolated thylakoids or isolated PSI on gold electrode surface [102, 127, 131] and entrapment of isolated thylakoid or other sub-cellular plant components using chemical cross linking (glutaraldehyde and bovine serum albumin (BSA)) by forming the covalent linkage between free amino group of thylakoids and albumin with glutaraldehyde or physical treatments (polyvinyl alcohol (PVA)) without any covalent linkage formed [132, 133]. Although entrapment preparation promotes higher stability, lower current generation is observed due to limited water contact to the photosynthesis materials.

Figure 3.2 Schematic of two-chamber PEC with dotted red lines shows the deposited thylakoids on ITO. Detailed enlargement of the red dotted area shows the thylakoid electron transport chain and predicted electron transfer to ITO. Inset: Electron transport pathway is described using dotted lines, while thunderbolts

indicate light energy excitation. describes the schematic of the two-chamber PEC where isolated thylakoids are used as the biocatalyst for electron generation. By using the isolated thylakoids, the current generated can be specified as a result of photosynthesis without any influences of other metabolic reactions occurring inside the cell. The electrons generated during the light reaction are transferred to the cathode chamber and potassium ferricyanide is used as an electron acceptor, while the protons pass through the PEM. The close and loose interaction will be compared to investigate the electron transfer between thylakoids and ITO. The effect of mediators on current production is studied to elucidate their roles in intercepting electrons in the ETC and subsequent electron transfers from the photosynthetic protein to the anode. In an attempt to investigate and isolate the protein complexes responsible for the interaction and electron transfer, redox mediators or herbicide is added inside anode chamber to either intercept or inhibit the electron transport chain. As the mediator representatives, 2,6-dichlorophenol-indophenol (DCPIP) and silicomolybdic acid (SM) are chosen based on different water solubility and standard redox potential to intercept electrons specifically at the PSII and PSI site respectively, while 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) is the chosen herbicide to block the electron transfer from PSII to PSI by occupying the plastoquinone (PQ) binding site as described at Figure 4.1 [17, 99]. Upon DCMU inhibition, reduced DCPIP ($\text{DCPIP}^{\text{red}}$) in the presence of excess ascorbate is added as an artificial electron donor for PSI. The possibility of direct electron transfer between thylakoids and ITO is investigated in the absence of mediator.

Figure 4.1 Electron transport chain (ETC) inside thylakoid membrane with different mediators interception. PS: photosystem, Q and X: primary electron acceptor, PQ: plastoquinone pool, b_6f : cytochrome b_6f , PC: plastocyanin. Arrows toward inside the ETC show the electron donation, while arrows toward outside the ETC indicate the electron shuttled from ETC. Dotted lines show the electron blockage.

In addition, O_2 generation based on water splitting reaction can be used as an electron acceptor at the PSI region in the limited electron acceptor condition in the isolated thylakoids and forms reactive oxygen species (ROS) [134]. The reactive oxygen is produced at PSI either spontaneously or catalyzed by the presence of superoxide dismutase (SOD) forming H_2O_2 and O_2 [135]. It has been studied that inside intact chloroplast, H_2O_2 is neutralized by the presence of ascorbate peroxidase (APX) in the stroma. However, the H_2O_2 scavenging mechanism does not occur in the isolated thylakoids due to the removal of the stromal proteins [136]. The reaction of H_2O_2 production at thylakoid membrane is described using the following equations [137-139]:

The buried H_2O_2 generated inside thylakoids membrane is able to diffuse out to the thylakoid membrane bilayer [138, 140]. Khorobrykh and coworkers show that H_2O_2 generation is proportional to light intensity, where the increase of

illumination results in increased H_2O_2 production [139]. Further study on the effects of mediators and generated H_2O_2 and O_2 toward electron transfer and photocurrent generation in both of one-chamber and two-chamber PEC configuration will be discussed in the following sections.

4.3 Result and Discussion

4.3.1 Protein complexes and light harvesting antennas upon deposition

It is speculated that the isolated thylakoids promote hydrophilic interaction toward ITO after the osmotic shock treatment. However, deposition onto solid surface such as ITO may compromise the structure and function of the photosynthetic complexes. Absorbance scan in the visible light range is performed on the ITO-deposited thylakoids to demonstrate that the light harvesting complexes as part of the photosynthetic complexes are still bound to the electrode.

Distinct absorbance peaks corresponding to chlorophyll *a* (438 nm and 680 nm), chlorophyll *b* (shoulder at 650 nm), and carotenoids (Soret region 450-480 nm) are identified in the spectrum obtained from the substrate with deposited thylakoids (Figure 4.2A). It has been stated that the 680nm absorbance peak indicates that the protein complexes and light harvesting antennas still remain embedded in the thylakoid membrane and are bound to the electrode [141, 142]. The electron micrograph shows well-distributed thylakoids without any aggregation upon adsorption onto ITO (Figure 4.2B). Each of the adsorbed thylakoids may act as an independent micro-electrode and thus contributing to the collective photocurrent.

Figure 4.2 Absorbance scan of physically deposited thylakoids showing absorbance peak at 680nm. (B) FESEM image of physically deposited thylakoids on ITO

4.3.2 The effect of interaction distance

The activity of manganese centers in PSII embedded in the thylakoids upon deposition can be inferred from electron generation during water splitting reaction and reaction center excitation. Deposition of thylakoids on the electrode is hypothesized to promote close interaction and facilitate electron transfer which result in photocurrent enhancement. Open circuit voltage (OCV) indicates the equilibrium condition of the constructed PEC without allowing the electron transfer across the chamber. In this observation, photocurrent is reported under dark saturated OCV condition, unless otherwise stated.

Bi-directional photocurrent generation, denotes anodic photocurrent (positive direction) and cathodic photocurrent (negative direction), can be observed in this study. Anodic photocurrent indicates the electron transfer from thylakoids to ITO, while cathodic photocurrent represents electron transfer from the electrode to the system. Previous studies have described the bi-directional photocurrent generation as a result of different configurations of material work function [143] or based on the presence of artificial electron donor-acceptor under different working

potential applied [144, 145]. Understanding the photocurrent nature is an important factor to obtain efficient PEC design.

The correlation between distance and electron transfer is compared between deposited (electrostatic and adsorption) and dispersed thylakoids using the same concentration. The effect of applied positive charge during thylakoids electrodeposition is studied. In this observation, DCPIP^{ox} is added into the anode chamber as an artificial electron acceptor. Figure 4.3A shows higher potential (~80mV) using deposited thylakoids as compared to the dispersed preparation indicating better electron transfer efficiency which is supported with the photocurrent profile (Figure 4.3B). Same photocurrent and photovoltage generation between electrostatic and adsorption preparation shows the absence of electron transfer enhancement. Based on this observation, adsorption method is used for further observation.

Figure 4.3(A) Open circuit voltage (OCV), (B) Photocurrent in various thylakoids/ITO preparations.

The cathodic photocurrent followed by current decay is observed in the presence of DCPIP^{ox}. It is well-studied that DCPIP intercepts the different site of

PSI complex depending on its redox state. DCPIP^{ox} shuttles the electrons from the PSI primary electron acceptor, while DCPIP^{red} is known as an artificial electron donor to reduce the P700⁺ as described in Figure 4.1[17, 146]. To isolate the PSI photocurrent observation, thylakoids are treated with DCMU (100μM) to block the electron transport from PSII to PSI [147, 148] and DCPIP^{red} as an artificial electron donor to recombine with the PSI hole. In contrast with previous photocurrent observation in the presence of DCPIP^{ox} (Figure 4.3B), the DCMU and DCPIP^{re} treated thylakoids shows the stable cathodic photocurrent generation (Figure 4.4). Based on this result, the cause of the observed cathodic photocurrent decay is due to electron transfer from thylakoids to ITO by using DCPIP^{ox} as the mediator.

As a control, in the absence of thylakoids, DCPIP^{red} and DCMU alone show no photocurrent generation (data not shown), thus justifying the current observed as a result of photosynthetic reaction. Similar studies on photocurrent generation using an isolated PSI biocatalyst are shown in the presence of mixed oxidized-reduced DCPIP or potassium ferricyanide as mediators [102, 149].

Figure 4.4 Photocurrent generation of thylakoids treated with DCMU and DCPIP^{red}.

4.3.3 Identification of protein complex responsible for interaction and charge transfer

Protein complex responsible for the electron transfer is studied upon addition of redox mediators (SM and DCPIP) to intercept the ETC. The photocurrent profile upon treatment using various mediators is shown in Figure 4.5. The presence of SM induces conformational change by exposing the plastoquinone to the artificial electron acceptors [150-152] and results in anodic photocurrent based on electron transfer to the ITO. In the absence of mediator, cathodic photocurrent is still generated indicating direct electron transfer. This observation shows that the direction of electron transfer and the photocurrent intensity can be tuned by varying the different electron interception from ETC using various mediators. In the absence of thylakoids, negligible photocurrent generation is observed for each mediator applied.

Figure 4.5 Photocurrent of deposited thylakoids in the presence of various mediators using 10s illumination interval.

In an attempt to elaborate the site of electron transfer at either PSII or PSI site, herbicide (DCMU) is added in the presence of DCPIP^{ox}. The DCPIP^{ox}

intercepts the ETC at the DCMU sensitive region. Upon DCMU treatment in the presence of DCPIP^{ox}, stable cathodic photocurrent is detected, in contrast with the previous photocurrent observation in Figure 4.3B. Interestingly, similar cathodic photocurrent is observed between DCPIP^{ox}+DCMU and in the absence of mediator, showing that the generated cathodic photocurrent is insensitive toward DCMU addition and prominently occurs at PSI site.

4.3.4 The effect of applied potentials

To study the effect of applied potentials, a three-electrode configuration system in one-chamber electrochemical cell is used. Bi-directional photocurrent generation, denoted as anodic photocurrent (positive direction) and cathodic photocurrent (negative direction), can be observed upon different applied potentials in the absence of mediator. The negative applied potentials promote excess negative charge and facilitate the electron transfer from the ITO (cathodic photocurrent), while positive potentials reduce the thermodynamic electron barrier for direct electron transfer from the thylakoids to the ITO (anodic photocurrent).

Cathodic photocurrent is observed upon applying negative bias potentials, and the highest cathodic photocurrent as indicated by saturated change in photocurrent magnitude is observed upon addition of $\sim -50\text{mV}$ vs SCE (Figure 4.6A). The reversed photocurrent polarity from cathodic to anodic is a result of direct electron transfer based on plastocyanin diffusion to electrode upon applying $\sim +125\text{ mV}$ vs SCE (Figure 4.6B) [104].

Figure 4.6 (A) Photocurrent over different applied potentials showing the anodic and cathodic photocurrent generation without mediator. Experiment was performed in a single chamber. ITO electrode size is 1 cm². (B) The plotted generated photocurrent vs applied potential showing reversed photocurrent polarity at ~+125mV vs SCE.

The presence of O₂ or H₂O₂ gases as photosynthetic products is hypothesized to be possible sources for cathodic photocurrent. In intact chloroplasts, the generated H₂O₂ is neutralized by the presence of ascorbate peroxidase (APX) in the stroma, however H₂O₂ scavenging mechanism does not occur in the isolated thylakoids due to the removal of stromal proteins [136]. In isolated thylakoids, electron acceptors are limited, hence O₂ generated by water splitting can be used as electron acceptors at PSI, forming H₂O₂ as reactive oxygen species (ROS)[134]. To proof the reduction of O₂ or H₂O₂ as a possible sources of cathodic photocurrent generation, cyclic voltammetry is performed in the absence or presence of thylakoids.

In the absence of thylakoids, the cathodic current is increased gradually upon H₂O₂ addition and a peak shifted is observed (Figure 4.7A), which is consistent with previously published results [153, 154]. Other than H₂O₂ reduction, the presence of O₂ has been suggested to contribute to the observed cathodic current

[153]. To remove the residual O_2 content, the electrolyte is bubbled with N_2 and decrease in cathodic current background is monitored. In the presence of thylakoids, peaks in CV curve at $-0.5V$ and $+0.5V$ vs SCE are observed, indicating thylakoids attachment on electrode and an increase of $300nA$ in cathodic photocurrent is detected upon illumination at $\sim -0.5V$ vs SCE (Figure 4.7B). Presence of light catalyzes O_2 and H_2O_2 generation as the photosynthetic by-product, and the negative potential promotes rapid H_2O_2 or O_2 reduction on ITO, thus results in higher cathodic photocurrent generation

The presented CV data supports the possibility of generated O_2 and H_2O_2 from the photosynthetic light reaction which serves as possible sources of the observed cathodic photocurrent. Detailed study on H_2O_2 or O_2 reduction of the adsorbed thylakoids on ITO is not further discussed in the present study.

Figure 4.7 CV of ITO (A) in the presence of various H_2O_2 concentrations and absence of thylakoids, inset shows a peak shifted upon increasing H_2O_2 concentrations (B) in the presence of thylakoids.

4.4 Conclusion

In this work, we investigated electron transfer in isolated thylakoids during photosynthetic reaction by comparing the photocurrents under different conditions. The photoelectrochemical study reveals higher photocurrent generation in the deposited thylakoids and in the presence of DCPIP^{ox} as compared to dispersed thylakoids and the absence of mediator. The electrostatic interaction shows the absence of photocurrent enhancement as compared to normal adsorption. In an attempt to analyze the electron donation site, further investigation is done by introducing various redox mediators (SM and DCPIP) or herbicide (DCMU) to either shuttle or block the electron from different site of electron transport chain depending on their standard redox potential and water solubility properties. Application of varying applied potentials results in a bi-directional photocurrent. Reduction of H₂O₂ or O₂ on the electrode surface is speculated to be possible sources of the observed cathodic photocurrent, while the anodic photocurrent is due to direct electron transfer from the thylakoid to the ITO as the result of direct electron transfer from the thylakoid to the ITO based on plastocyanin diffusion.

CHAPTER 5

Interaction and Charge Transfer of Isolated Thylakoids and Multiwalled Carbon Nanotubes

5.1 Abstract

Electrons and charge separation based on photosynthetic light reaction has gained many interests in an attempt to fabricate biological photovoltaic devices. In plant cells, photosynthesis occurs at specific compartment called chloroplasts, where thylakoids are enclosed. The integration of the natural photosynthetic materials with conducting solid surface electrode opens the possibility for novel solar-powered device. Among the various materials used as electrodes, carbon based material is attractive for its low-cost production, high conductivity, and mechanical strength. Incorporation of thylakoids with multiwalled carbon nanotubes (MWCNT) is expected to increase charge transfer due to its large surface area. Interaction and charge transfer between isolated thylakoids and MWCNT is the main focus of this work. Chloroplast was isolated from spinach leaves follow by osmotic shock treatment to release the thylakoids inside. Pristine MWCNT is dispersed in Triton X-100 (TX-100) as surfactant to retain its electronic property through non-covalent interaction. Three spectroscopy techniques (Raman, UV-Vis, and fluorescence) are used to monitor the interaction and possibility of charge transfer between thylakoids and MWCNT properties upon mixing. The Raman and UV-Vis observations suggest close interaction between the thylakoids, including the protein and light harvesting antennas, and the MWCNT. Approximately 40-50%

fluorescence quenching observed using single wavelength excitations indicates the charge transfer occurred within the hybrid system.

5.2 Background of the Study

Electrons and charge separation based on photosynthetic light reaction has gained many interests in an attempt to fabricate biological photovoltaic devices. Chloroplast is a light harvesting complex in higher-plant that is able to utilize light energy through excitation of light harvesting antennas and reaction center to generate electron and hydrogen [155]. It consists of the lipid bilayer membrane containing thylakoids stacks in which water splitting occurs at photosystem II (PSII) manganese center. Several works have been done to integrate the photosynthesis material, including the isolated photosynthetic compartment (chloroplast or thylakoid) [127], isolated light harvesting antenna [156], or unicellular photosynthetic organisms [8, 96, 128, 129] with conducting materials in an attempt to generate electricity upon illumination through photosynthesis water splitting reaction and charge separation. To specify the observation on the feasibility of photosynthesis reaction, isolated thylakoids from spinach leaves are used as the representative of photosynthetic material in this work.

Carbon nanotubes (CNT) are potential material to fabricate photovoltaic device electrode due to its unique characteristics such as low-cost production, high conductivity and mechanical strength [52, 53]. The major drawback on the nanotubes bio-application lies in its hydrophobic properties [157]. Direct interaction between carbon nanotubes and biomaterial is known to harm biological cells due to the nanotubes hydrophobic properties, carbon toxic effect, and possibility on

nanotubes penetration into the cell membrane that results in cell death [158, 159]. Thus, modification of the nanotubes surface is required to facilitate interactions with the isolated thylakoids. Common ways to induce CNT water solubility are obtained through covalent functionalization using strong acid oxidation to induce oxygen containing group or non-covalent functionalization using polymer, DNA, or surfactant through π - π interaction toward CNT sidewall [70, 71, 160]. Although covalent functionalization may result in the formation of carboxylic moieties that can be used for further interaction with biomaterial, it is known that the original CNT electronic property is altered due to the sp^2 carbon bond opening [69]. Thus, non-covalent functionalization is preferable to retain the original CNT electronic properties.

The present work describes the fundamental study of interaction and charge transfer between multiwalled carbon nanotubes (MWCNT) and isolated spinach thylakoids. MWCNT is chosen due to its metallic and high conductivity properties. In an attempt to justify the charge transfer phenomenon, detailed observation of the charge transfer phenomenon using different spectroscopy methods are described in the following studies.

5.3 Results and Discussion

5.3.1 Grown MWCNT and MWCNT-thylakoids characterization

The uniform length of vertically grown MWCNT on Si substrate is monitored under FESEM, with 100 times higher magnification exposes the high density MWCNT (Figure 5.1A-B). Based on the TEM observation, the inner and

outer diameters of individual MWCNT are measured at 5 and 8nm respectively (Figure 5.1C).

Figure 5.1(A) FESEM image of vertically grown MWCNT (B) 100x magnification of as grown MWCNT, (C) Individual MWCNT under Transmission Electron Microscopy (TEM) observation, inset: 10x higher magnification.

The entangled MWCNT bundles are hydrophobic. An attempt to introduce hydrophilic properties through non-covalent functionalization is chosen in this work. Sodium dodecyl sulfate (SDS), dodecyl-benzene sodium sulfonate (NaDDBS), Tween-20/60/80, cetyltrimethyl-ammonium bromide (CTAB), and Triton X-100 (TX-100) are common surfactants used to disperse the carbon nanotubes. In order to promote the biocompatible interaction between MWCNT and biomaterial, non-ionic surfactant is preferable because it retains the biomaterial stability compared to the ionic surfactant. Aside from promoting the hydrophilic properties, several studies have reported the effectiveness of TX-100 (non ionic surfactant) in dispersing MWCNT as compared to other surfactants used [161, 162]. Thus, we choose the non-ionic TX-100 surfactant to promote the hydrophilic properties of MWCNT surface while at the same time preserving the thylakoids stability.

The hydrophobic tail of TX-100 consists of a long hydrocarbon chain and pyrene group is anchored to the hydrophobic MWCNT sidewall through

hydrophobic and π - π interactions while the exposed hydrophilic tail (polyethylene oxide chain) promotes water solubility. Dispersed MWCNT after TX-100 functionalization and sonication process reveals the short MWCNTs (Figure 5.2A) while MWCNT and thylakoids morphology after interaction is shown at Figure 5.2B. The isolated thylakoids are shown to have rough surface areas with an approximate diameter of 2-7 μ m. Upon mixing with thylakoids, the free individual MWCNTs are hardly visualized suggesting the MWCNT adsorption on the thylakoids.

Figure 5.2FESEM image of (A) MWCNT, (B) hybrid of MWCNT and thylakoids.

5.3.2 Raman spectroscopy to study MWCNT electronic properties

Raman spectroscopy is a common instrument used to study the electronic properties of carbon material due to the non-invasive effect toward the material[61, 62].Investigation on electron or charge transfer is performed at first order (1100-1800 cm^{-1}) and second order (2600-2800 cm^{-1}) range of MWCNT electronic properties to identify the change in MWCNT electronic properties upon interaction with the thylakoids.

Observation of the MWCNT Raman signal upon 488nm laser excitation reveals three prominent peaks at $\sim 1350\text{cm}^{-1}$, $\sim 1590\text{cm}^{-1}$, and $\sim 2700 \text{ cm}^{-1}$ which

correspond to the characteristic of the D, G, and G' band of the MWCNT respectively [62]. D band indicates the defects and disorder of graphite structure, while G and G' band represent sp^2 carbon bond scattering [163-165]. In the isolated thylakoids, Raman signal is detected as the excitation signal of light harvesting antennas according to the laser wavelength excitation used, in which only carotenoid signal ($\sim 1150\text{cm}^{-1}$ and $\sim 1520\text{cm}^{-1}$) can be detected using 488nm laser excitation since higher laser energy is needed to excite chlorophyll a and chlorophyll b signal [166, 167].

Interaction between thylakoids and MWCNT is justified by the appearance of both signals in the hybrid system (Figure 5.3). Similar thylakoids Raman spectrum in the native state at $1100\text{-}1800\text{cm}^{-1}$ region as compared to the hybrid MWCNT-thylakoids indicates the preserved thylakoids stability (Figure 5.3A). The absence of peak shift of MWCNT D, G, and G' band in the hybrid (Figure 5.3A and B) is hypothesized to be due to local charge transfer on the nanotubes sidewall that is overwhelmed with the presence of inner tube signals or based on the insufficient charge transfer to induce the Raman signal change.

Figure 5.3 Raman spectroscopy signal for MWCNT, thylakoids, and MWCNT-thylakoids. (A) Observation at $1100\text{-}1800\text{ cm}^{-1}$ and (B) Observation at $2600\text{-}2800\text{ cm}^{-1}$.

5.3.3 Bound protein complexes and light harvesting antennas upon direct interaction with TX-100 and MWCNT

The optimization of TX-100 concentration as MWCNT dispersing agent is required to preserve the thylakoids stability. As a surfactant, TX-100 is capable to release proteins embedded in the thylakoid lipid bilayer by replacing lipid compound with the surfactant molecule. Stable thylakoids preparation, which is indicated by the bound protein complexes and light harvesting antennas, is monitored by the presence of 680nm absorbance peak [141, 142]. Both PSI and PSII contain light harvesting antenna complexes, which consist of the chlorophyll a, chlorophyll b, and carotenoid. In the bound complexes, the excitation energy of chlorophylls a and chlorophyll b are directly funneled to the respective photosystem reaction centers, hence inhibits their individual absorbance profiles [142, 168].

In this study, thylakoids absorbance spectrum inside various TX-100 concentrations is compared. Absorbance scan is plotted on visible light spectrum (400-700nm) since the light harvesting antennas have optimum light absorption at 420nm and 680nm[166, 169].Figure 5.4A, shows that native thylakoids preparation retains its 680nm absorbance peak (arrow) at $\leq 6 \times 10^{-3}\%$ (w/w) TX-100 concentration. Further increasing TX-100 concentration ($\geq 0.1\%$ (w/w)) exhibits ~10nm absorbance blue-shift shows the unbound light harvesting antennas. This observation is in accordance with previous studies that reports the thylakoids stability upon incubation with $\leq 7 \times 10^{-3}\%$ (w/w) TX-100[168, 170, 171].

Dispersed MWCNT absorbance spectrum is notified by gradual absorbance decrease from the UV spectrum toward relatively flat absorbance at visible

spectrum [172, 173]. The preserved 680 nm peak (arrow) on both thylakoids and MWCNT absorbance profiles suggests that both protein complexes and light harvesting antennas remain intact upon direct interaction with MWCNT (Figure 5.4B).

Figure 5.4 Absorbance spectrum of (A) isolated thylakoids in various TX-100 concentrations (B) isolated thylakoids, MWCNT, and hybrid thylakoids-MWCNT.

Previous observation on Raman (Figure 5.3) and absorbance (Figure 5.4) signals show no indication of charge transfer between thylakoids and MWCNT. Thus, further study onto monitor the charge transfer is done using fluorescence spectroscopy.

5.3.4 Charge transfer based on fluorescence quenching

Fluorescence spectroscopy is used to monitor the charge transfer between thylakoids and MWCNT. A fluorescence quenching in the hybrid system suggests electronic interaction and charge transfer [174-176]. Based on thylakoids absorbance profile, two distinct energy levels in the blue region (~ 420 nm) and the red region (~ 680 nm) are observed. The resulted fluorescence of thylakoids is noted

due to the presence of embedded light harvesting antennas, such as chlorophyll and carotenoid.

Screening on different laser wavelength specified for each light harvesting antenna (chlorophyll a, chlorophyll b, and carotenoid) are done at 425nm, 470nm, and 488nm excitation. Upon excitation, fluorescence emission peak at ~680nm is observed (Figure 5.5A). Approximately of 40-50% fluorescence quenching is detected after MWCNT adsorption, suggesting a charge transfer between thylakoids and MWCNT (Figure 5.5B). Similar quenching is observed at different laser wavelengths, indicating that the optimum charge transfer has been obtained.

Figure 5.5(A) Thylakoids fluorescence under different excitation wavelength, (B) Fluorescence quenching in the hybrid system.

Bound protein complexes are indicated by low intensity fluorescence signal since the energy is transferred through the electron transport chain and ATP formation. In contrast, unbound light harvesting antennas exhibit high fluorescence signal as a result of fast return of the excited electron to its original energy level. Screening on the effect of the TX-100 concentrations reveals that TX100 at concentration $\geq 10^{-2}$ % (w/w) releases the embedded light harvesting antennas as

indicated by four times higher fluorescence intensity and shifted fluorescence peak. The presence of individual chlorophyll a (670nm) or chlorophyll b (650nm) signals are detected upon TX-100 concentration increase (Figure 5.6).

Figure 5.6Thylakoids stability over different TX-100 concentration upon (A) 425nm, (B) 450nm, (C) 470nm, (D) 488nm laser excitation.

The stable bound protein and light harvesting antennas over 24 hour incubation is preserved as indicated by the retained 680 fluorescence peak in the absence of light at room temperature inside 6x10⁻³% (w/w) TX-100 (Figure 5.7).The 1.5 times fluorescence increase after 3 and 6 hours storage is hypothesized to be due to swelling phenomenon of the TX-100 adsorption into thylakoids as compared to the initial preparation (0 hour) with the observed ~50% fluorescence decrease upon 24 hour incubation is hypothesized as a sign of protein degradation.

The thylakoids fluorescence profile upon 425nm laser wavelength excitation is taken as the representative.

Figure 5.7 Stability over prolonged time incubation 425nm.

The correlation between laser excitation and fluorescence emission is plotted using 2D contour map. TX-100 exhibits fluorescence emission signal at 580-640nm as a result of its phenyl group [177]. In monomeric state, TX-100 fluorescence emission is commonly observed at ~300-340nm [178, 179]. Our observation shows TX-100 fluorescence emission at 580-640nm upon excitation using 440-470nm laser. The emission shift is noted as a result of fluorescence delay along with concentration increase, as previously described on aromatic hydrocarbon fluorescence emission in solution [180, 181]. Further observation shows the dependence on TX-100 fluorescence emission wavelength and intensity according to different concentrations prepared (Figure 5.8).

Figure 5.8 2D contour map of (A) 0% w/w TX-100, (B) 0.01% w/w TX-100, (C) 0.05% w/w TX-100, and (D) 0.1% w/w TX-100.

The TX-100 fluorescence emission is quenched when it is used as MWCNT dispersing agent as a result of charge transfer between TX-100 and MWCNT (Figure 5.9A and B) [177, 182]. The thylakoids incubated in TX-100 exhibit fluorescence emission at ~660-690nm with the highest fluorescence detected at ~680nm (Figure 5.9C). The absence of fluorescence quenching of TX-100 shows absence of charge transfer between thylakoids and TX-100. Upon mixing with MWCNT, efficient charge transfer is monitored at the whole wavelengths excitation with approximately 40-50% fluorescence quenching is detected (Figure 5.9C and D).

Figure 5.9 2D contour map of (A) TX-100, (B) MWCNT, (C) Thylakoids, and (D) MWCNT-thylakoids.

Based on the described results, we have successfully shown the proof of direct interaction and charge transfer between MWCNT and isolated spinach thylakoids using different spectroscopic techniques, although the detail mechanisms of charge transfer could not be explained yet. To understand the detail charge transfer mechanism, we will integrate the dispersed MWCNT on conducting solid electrode (ITO) to further study the charge transfer mechanisms based on photocurrent generation (will be discussed in Chapter 6).

5.4 Conclusions

Interaction and charge transfer between isolated thylakoids and MWCNT are studied using various spectroscopy techniques (Raman, UV-Vis, and fluorescence). Direct interaction between the thylakoids and MWCNT is shown by appearance of both signals in the Raman and absorbance observation. Further observation on the effect of TX-100 shows that addition of 6×10^{-3} % (w/w) TX-100 promotes stable MWCNT dispersion and bound protein complexes inside thylakoids based on the appearance of 680nm peak at both absorbance and fluorescence observations. An approximate of 40-50% fluorescence quenching upon mixing the thylakoids and MWCNT suggests the efficient charge transfer occurred.

CHAPTER 6

Carbon-nanotube Modified Electrode in a Photosynthetic Electrochemical Cell

6.1 Abstract

The photosynthetic water splitting reaction has been considered as one of the potential energy sources due to the electron generation in the presence of light. This study explores photocurrent generation based on electron transfer between isolated spinach thylakoids and carbon nanotube-modified indium tin oxide (ITO) conducting glass electrode. The measurements are conducted in a two-chamber photosynthetic electrochemical cell (PEC). The photosynthetic complexes are bound to the electrode as demonstrated by the appearance of 680nm chlorophyll peak. Electron transfer is determined based on photocurrent generation. Multi-walled carbon nanotube (MWCNT) deposition on ITO promotes higher electron transfer rates as shown by higher current generation compared to bare ITO. The cathodic photocurrent suggests that the prominent direction of electron transfer occurs from the electrodes to the system. The reduction of generated O_2 or H_2O_2 during photosynthesis is the source of cathodic photocurrent. The deposited MWCNT stimulates higher reduction rate.

6.2 Background of the Study

Photosynthetic electron generation has drawn many interests in the development of biological photovoltaic device. Electrons produced during light reaction are transferred to a conducting electrode and results in photocurrent. Previous studies reveal the capability of photosynthetic material, including living unicellular photosynthetic organisms and isolated photosynthetic compartments as biocatalyst for photocurrent generation [8, 127, 183]. Several investigations on isolated chloroplasts photocurrent generation have been reported using photosynthetic electrochemical cells (PEC) [133, 184]. In this work, we focus on electrode modification to increase the photocurrent generation, in which the feasibility of indium tin oxide (ITO) as a potential electrode is investigated.

ITO has high transparency in the visible light spectrum that optimizes light utilization and is a good electrode candidate for PEC. It is commonly used as an electrode in photovoltaic devices (PVs) [43-45]. In Chapter 4, we have demonstrated direct electron transfer between thylakoids to ITO as shown by photocurrent generation in the absence of mediator. However, the generated photocurrent is relatively low compared to common PV cells. In an attempt to improve the ITO performance, we hypothesize that modification of the ITO surface by integrating conducting material will result in a higher photocurrent. Multi-walled carbon nanotubes (MWCNTs) have large surface areas that improve the interaction between ITO and thylakoids. The hydrophobic property of MWCNTs has been shown to cause toxic effects toward the biological sample [159], hence its functionalization is required. MWCNT functionalization methods are performed either covalently or non-covalently [67-69]. Covalent functionalization alters the

nanotubes physical properties and results in conductivity change due to sp^2 carbon bonds opening [185, 186]. To preserve the MWCNT electronic properties and the biological sample activity, it is preferred to perform non-covalent functionalization on the MWCNT. For example, conjugation of proteins or carbohydrate with pyrene group promotes interaction with the carbon nanotubes wall through π - π interaction and preserves the biological activity [74, 185]. Covalent functionalization of carbon nanotube with N_α, N_α -bis(carboxymethyl)-L-lysinehydrate (ANTA), 1-ethyl-3-[3'-(dimethylamino)propyl]carbodiimide (EDC), and N-hydroxysuccinimide (NHS) promote specific interaction with His-tagged enzyme [187].

We use two-chamber PEC separated by a proton exchange membrane (PEM) to investigate the electron transfer between thylakoids and ITO or MWCNT/ITO electrodes. Thylakoids are used as the photosynthetic biocatalyst to limit the photocurrent observation to the photosynthetic reaction without any interference from other metabolic reactions. To enhance the biocompatible interaction with thylakoids, MWCNTs are solubilized in non-ionic surfactant, Triton X-100 (TX-100). There are two possibilities on the electron transfer direction between thylakoids and electrode: (1) electron transfer from the thylakoids to the electrode based on water splitting reaction (shown as anodic photocurrent, positive current polarity) and (2) electron transfer from the MWCNT to reduce the generated H_2O_2 or O_2 (cathodic photocurrent, negative current polarity). In the present study, we will focus on the effect of MWCNT deposition on the photocurrent generation.

6.3 Results and Discussion

6.3.1 Electrode characterization and thylakoids stability

The electron transfer rate to surfaces depends on the interaction between the electron donor and the electrode surface. The interaction, however, is also dependent on the morphology of the surface. Relatively smooth ITO surface limits the active surface area of interaction between ITO and the thylakoids. In an attempt to increase the active surface area, MWCNT is deposited on the ITO surface through the electrodeposition process. The dispersed MWCNT is electrodeposited on the ITO surface by applying 0.3mA external current toward ITO. ITO is used as working electrode, while platinum foil serves as both counter and reference electrodes in this process.

It is known that different nanomaterial surface morphology can be obtained by varying the electrodeposition condition and the presence of molecules in the electrolyte. For example, rough surface morphology has been observed when MWCNT was co-deposited in the presence of Cu^{2+} ions at 5A dm^{-2} current, while relatively smooth surface is retained at $\leq 1\text{A dm}^{-2}$ current addition [188]. The deposition of MWCNT is seen as the formation of the dark area on the ITO surface (Figure 6.1A). Detail observation under FESEM reveals the nano-agglomerate MWCNT with approximately 100-200nm diameter in the presence of TX-100 (Figure 6.1B). This observation agrees with published studies in which it has been demonstrated that deposited MnO_2 or tin methanesulfonate (SnCH_3SO_3) forms agglomerates on the Pt surface in the presence of TX-100 [189, 190]. The adsorbed thylakoids vesicles (“blebs”) on deposited MWCNT are shown by FESEM (Figure 6.1C). The size of the “blebs” (1-2 μm) is much smaller compared to those that are

deposited on bare ITO ($\sim 10\ \mu\text{m}$) as shown in Chapter 4.

Figure 6.1 (A) Photograph of MWCNT deposited on ITO (upper part: ITO; lower part: ITO with deposited MWCNT). Electron micrograph of (B) deposited MWCNT on ITO for 2 minutes and (C) adsorbed thylakoids vesicles on the deposited MWCNT surface.

The effect of deposition time on MWCNT thickness is investigated by performing absorbance scan directly against ITO. Optimal MWCNT thickness is an important parameter to obtain an efficient electron transfer. Absorbance scan from 200-700nm has been performed to investigate the characteristic of dispersed and deposited MWCNT. The optimal range to monitor the thickness of the MWCNT layer on the ITO is in the visible wavelength. Typical absorption spectrum of dispersed nanotubes in solution is shown by decrease of absorbance scan from UV to visible light range (Figure 6.2) [172, 173]. Two distinct peaks at 220 nm and 275 nm in our dispersed MWCNT preparation are the characteristics of MWCNT and TX-100, respectively. TX-100 contains benzene ring that absorbs UV light [180, 181]. The decrease of TX-100 absorbance peak in the dispersed MWCNT solution compared to the TX-100 at the same concentration is an evidence of interaction and charge transfer between MWCNT and TX-100 [191]. Upon deposition of MWCNT on ITO, its distinct absorbance peak is masked by high ITO absorbance [192].

Figure 6.2 Deposited and dispersed MWCNT absorbance spectrum

Due to high absorption properties of ITO glass substrate on the UV range, only absorbance scans at visible light range can be performed [192]. Longer electrodeposition time induces thicker MWCNT layer as shown by the increase of absorbance (Figure 6.3A). The correlation between deposition time and absorbance at 500 nm is shown to be linear (Figure 6.3A, inset) suggesting that specific MWCNT thickness can be achieved by tuning the deposition time.

Binding of thylakoids to ITO or MWCNT/ITO electrodes are demonstrated by the appearance of 680 nm peak (Figure 6.3B, arrow). This absorbance peak is noted as the characteristic of the chlorophyll-bound molecules in the photosystem, suggesting that the protein stability is retained after direct interaction with the respective electrodes [141, 142].

Figure 6.3 UV-Visible absorbance spectra of (A) MWCNT/ITO at different deposition time and (B) thylakoid membrane deposited on ITO and MWCNT/ITO surfaces.

6.3.2 Photoelectrochemical measurement

The electron transfer activity between thylakoids and electrode is indicated by the photocurrent generation. The photocurrent is observed under dark saturated open circuit potential (OCV). Similar photocurrent observation methods have been described by the previous studies [43, 77]. The dark saturated potential in the system is measured at approximately -0.4V and -0.75V vs pseudoreference electrode (carbon cloth) for the ITO and MWCNT/ITO electrodes respectively, which are equivalent to $\sim +0.16\text{V}$ and -0.3V vs SCE potential applied to the working electrode.

Upon illumination of the experimental set-up, photocurrent is observed to be increase with increased thickness of MWCNT indicating more efficient electron transfer within the system. The MWCNT/ITO electrode obtained by two-minute deposition time yields photocurrent that is approximately 400% higher compared to ITO (Figure 6.4). Deposition time of 3 minutes shows current decline. The decrease in photocurrent is due to longer electron distance or competition of adsorbed light that can be utilized by photosynthesis reaction. Similar observations have been

reported elsewhere [193, 194]. In the absence of thylakoids, negligible generated photocurrent using both electrode preparations demonstrating that the observed current is a result of photosynthetic reaction (data not shown). At the optimum MWCNT deposition time of 2 minutes, the highest photocurrent obtained using the experimental set-up is ~ 200 nA which is in the same order of magnitude as current generated in the mediatorless microbial fuel cell (MFC) [195]. The generated cathodic photocurrent shows that the favourable electron transfer direction is obtained from the electrodes to the system. The same photocurrent profile is observed for both ITO and MWCNT/ITO electrode.

Figure 6.4 Photocurrent profile upon illumination at 10s interval. The curves were adjusted to 0 minute deposition reading.

Understanding the electron transfer direction allows the suitable design for more efficient photocurrent generation. ITO work function in a vacuum is reported to range from 4.4 to 4.9eV [38-40], while MWCNT work function varies between 4.3 to 4.9eV [196, 197] depending on its preparation method. These work functions are equivalent to -0.2 to +0.4V for MWCNT and -0.1 to +0.4V for ITO vs normal

hydrogen electrode (NHE), respectively [198]. Similar work function between ITO and MWCNT indicates that increase in photocurrent generation is mainly due to the larger surface area interaction. Figure 6.5A shows the hypothetical electron transfer pathway within the system. We hypothesize that direct electron transfer (anodic photocurrent) from thylakoids to the electrode occurs at PSI (trace 1,2), while the back electron transfer (cathodic photocurrent) is obtained based on the H_2O_2 or O_2 reduction (trace 3,5).

During the photosynthesis light reaction process, O_2 is generated from the water splitting reaction. H_2O_2 is liberated through a two-step reaction. The reaction includes reduction of O_2 to superoxide ($\text{O}_2 + \text{e}^- \rightarrow \text{O}_2^-$, E° : -0.16V vs NHE) followed by oxygen radical reduction ($2\text{O}_2^- + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O}_2 + \text{O}_2$, E° : +0.94V vs NHE) in the absence of other electron acceptors than O_2 which constitutes about 10% of the total electron flow from water [134, 137-139]. Further reduction of the generated O_2 or H_2O_2 at the interface between thylakoids and electrodes (ITO or MWCNT/ITO) may occur upon application of initial potentials to the respective electrodes. Several routes of O_2 reduction on the electrode surface have been widely studied including the generation of H_2O , H_2O_2 , HO_2^- , OH^- , and O_2^- depending on the electrode type, electrolyte pH, and potentials applied [199, 200]. Previous studies have described that prominent O_2 reduction on the nanotubes surface are based on the peroxide pathways to generate HO_2^- or H_2O_2 according to the following reactions [200, 201]:

Since the H_2O_2 is also generated during light reaction, we also consider the

possibility of the H_2O_2 reduction on the electrode surface as the source of cathodic photocurrent, in accordance with the following equation:

In neutral condition ($\sim\text{pH } 7.0$), two steps O_2 electroreduction to generate H_2O_2 is favorable. Thus our hypothetical cathodic photocurrent is speculated based on the reactions (2) or (3), since the anolyte used in the preparation has a neutral pH ($\text{pH } 7.2$). All of the redox potentials are reported vs NHE

Figure 6.5(A) Work function schematic, showing the possible pathways of electron transfer upon electron excitation in PSI Dotted line represents an electron transport blockage by DCMU. Trace 1 and 2 indicate the possible electron transfer route from the thylakoids to the electrode (anodic photocurrent), while trace 3-5 show the electron back transfer to reduce the generated H_2O_2 and O_2 based on the O_2 and

H₂O₂ reductions (cathodic photocurrent). (B) Photocurrent generation in the gradual addition of DCMU (0-400 μ M). (C) Photocurrent generation in the absence of DCMU. Illumination is given at 10s interval.

In an attempt to justify the site of electron transfer at either PSI or PSII, control experiment using DCMU as herbicide is added using MWCNT/ITO as the working electrode. DCMU blocks the electron transfer from PSII to plastoquinone pool and results in approximately 70% reduction of the photocurrent as compared to the untreated thylakoids (Figure 6.5B). The retained photocurrent is still observed upon addition of 400 μ M DCMU in the system although previous studies highlighted the complete blockage of electron transfer from PSII to PSI upon addition of 10-20 μ M DCMU [147, 148]. Control experiment using similar preparation in the absence of DCMU shows slower photocurrent decay as compared to the DCMU addition (Figure 6.5C). In the absence of thylakoids, DCMU does not contribute to photocurrent generation, indicating that the decrease in the observed photocurrent is not caused by DCMU interaction with the electrode.

Besides inhibiting the PSII reducing site, addition of DCMU is shown to have a direct inhibition effect toward PSII oxidizing site [202]. Further prolonged illumination of treated thylakoids using DCMU results in the degradation of carotenoid as the protective agent followed by loosely bound chlorophyll and eventually leads to the PSII inactivation [203, 204], while PSI remains active through its cyclic electron transport [203, 205]. The photocurrent decrease upon DCMU treatment is speculated as a result of less H₂O₂ or O₂ generation due to the abolishment of PSII water splitting activity. To justify the contribution of H₂O₂ and

O₂ in the cathodic photocurrent, we will compare the catalytic activity of the electrodes (ITO and MWCNT/ITO) in the absence of adsorbed thylakoids.

6.3.3 The effect of H₂O₂ and O₂ toward cathodic current growth

The effect of deposited MWCNT on ITO toward H₂O₂ and O₂ are studied using cyclic voltammetry (CV) method in the absence of thylakoids. Figure 6.6 shows the correlation between cathodic current and various H₂O₂ concentrations. As-defined analyte is used in the CV observation, using 50mV/s scan rate.

In the forward scan direction, an approximately two times higher cathodic current is observed using MWCNT/ITO as compared to the ITO using the same H₂O₂ concentration upon negatively applied potential. The higher conductivity and surface area due to the presence of the deposited MWCNT are the prominent factors to facilitate H₂O₂ reduction as compared to the ITO electrode. The more negative applied potential to the MWCNT/ITO electrode drives efficient H₂O₂ or O₂ reduction. This observation supports our previous higher photocurrent generation at Figure 6.4.

Figure 6.6 Correlation of cathodic current toward H₂O₂ concentrations on (A) ITO, (B) MWCNT/ITO

In addition to the H_2O_2 effect, the observation on the O_2 effect between two electrodes is observed. The electrolyte is pre-treated using N_2 bubbling to eliminate the initial O_2 content. Decrease in the cathodic current generation upon N_2 bubbling (Figure 6.6) shows the MWCNT/ITO sensitivity toward O_2 in comparison with our previous observations in the absence of H_2O_2 . The CV observation confirms that the cathodic photocurrent generated in our system is based on the O_2 or H_2O_2 reduction on the electrode.

Figure 6.7 Comparison between ITO and MWCNT cathodic current upon N_2 pre-treatment. Potential is reported vs SCE electrode.

6.4 Conclusions

We show the photocurrent generation using isolated thylakoids as biocatalyst inside a two-chamber PEC. Screening of two electrodes, ITO and MWCNT/ITO, toward photocurrent generation is the main focus of this study. The MWCNT/ITO exhibits higher photocurrent ($\sim 400\%$) compared to ITO which is

attributed to the larger active surface area that interacts with the thylakoids. The decrease of photocurrent at deposition time longer than 2 minutes is speculated to be due to the longer distance that the electrons have to travel to reach ITO. The addition of DCMU blocks the electron transport from PSII to plastoquinone pool resulting in reduced observed photocurrent. Observed photocurrent decrease upon thylakoids treatment using DCMU justifies the contribution of the PSII in the photocurrent measurement. Further observation of the electron transfer direction suggests the predominant electron transfer from the MWCNT/ITO electrode to the system. We speculate the cathodic photocurrent generation as a result of H_2O_2 or O_2 reduction. Higher conductivity, higher surface area interaction, and more negative potential applied on the MWCNT/ITO electrode may be the important factors to increase the cathodic photocurrent generation in our PEC observation.

CHAPTER 7

Free Standing Graphene and Modified Carbon Cloth Electrodes based on the Photosynthetic Electrochemical Cell

7.1 Abstract

The electrons generated during water splitting reaction have been considered as a possible clean energy source through the development of photosynthetic photovoltaic device. In this work, we investigate the feasibility of several carbon materials including free standing graphene, reduced graphene oxide (rGO) and carbon cloth as the working electrode in two-chamber PEC. The proof of direct charge transfer between isolated thylakoids and carbon electrodes is shown based on the anodic photocurrent generation. The untreated carbon cloth exhibits higher photocurrent generation compared to the untreated free standing graphene, which is hypothesized to provide better interaction between thylakoids and carbon cloth surface. To increase the biocompatibility and conductivity of carbon cloth for higher photocurrent, rGO is incorporated on the carbon cloth surface using electrodeposition method. Upon rGO deposition, thylakoids are adsorbed on the electrode surface. The results show no significant photocurrent increment when rGO/carbon cloth was used as the electrode compared to the untreated carbon cloth, likely because of the thermodynamic constraint of electron generation during the photosynthesis light reaction.

7.2 Background of the Study

The efficient electron generation driven by light energy source during photosynthesis has been widely studied as a potential candidate for the development of clean renewable energy source [102, 127, 206]. Electron generated during photosynthetic reaction is channeled through thylakoid electron transport chain to the electrode, resulting in photocurrent generation. However, the low current generation limits its practical application. This work focuses on the screening of different electrode materials including free standing graphene electrode, carbon cloth, and reduced graphene oxide as potential electrode.

Graphene is a single layer of graphite which has been considered as a potential electrode material for photovoltaic device due to its unique characteristics, such as high conductivity, biocompatibility, transparency, and flexibility [43, 78-80]. However, its hydrophobic property hinders further biological interaction with photovoltaic biocatalysts. Formation of graphene oxide (GO) through oxidation of graphite increases hydrophilicity due to presence of oxygen containing group (carboxyl and hydroxyl) that makes GO dispersible in water [90]. Despite its water solubility property, GO is not a good conductor due to the disruption of the sp^2 carbon bonding into sp^3 bond with conductivity around 1 and 5×10^{-3} S/cm. Reduction of GO to reduced graphene oxide (rGO) to remove oxygen group and recover its sp^2 bond may increase its conductivity into 8500 S/m. Electrostatic adsorption, spin coating, and filtration are common methods to deposit the soluble rGO on the solid surface for further application [91, 92]. During the deposition process stacks of graphene layers can form, and result in less active surface area interaction with biomaterial. To overcome the stacking problem, direct growth of

graphene using chemical vapor deposition (CVD) method on substrate is applied to obtain free standing graphene electrode [207, 208].

In this study, we exploit the feasibility of isolated thylakoids from spinach leaves as representative photosynthetic biocatalyst. The electron transfer efficiency between thylakoids and several different electrodes (carbon cloth, free standing graphene, and carbon cloth modified rGO) is screened based on photocurrent generation. Direct interaction between thylakoids and the electrodes is achieved through physical adsorption techniques.

7.3 Results and Discussion

7.3.1 The morphology of carbon cloth and graphene electrode

Free-standing graphene has a macroporous structure with approximate size of 400-500 μm , while carbon cloth has a tighter structure with distance between each carbon fabric is around 10-20 μm (Figure 7.1A-B). The tight hydrophobic structure of carbon cloth hinders interaction between thylakoids and the inner site of carbon cloth. FESEM images show the smooth and wrinkled graphene surface as compared with small pores on the carbon cloth surface(Figure 7.1C-D).

Figure 7.1(A) graphene electrode and (B) carbon cloth, (C) and (D) 100 times higher magnification on graphene and carbon cloth electrodes respectively.

The adsorbed thylakoids are clearly shown on both electrode surfaces (Figure 7.2A-B). Damaged thylakoids can be detected on the carbon cloth surface, likely because of the sharp nanoscale features of carbon cloth that that may further disrupt the thylakoids.

Figure 7.2 Electrode characterization upon thylakoids adsorption on (A) graphene and (B) carbon cloth.

The electroactivity of untreated graphene and carbon cloth electrodes in the absence of thylakoids is determined through cyclic voltammetry (CV) in a 3-electrodes electrochemical cell with graphene or carbon cloth as working electrode, platinum foil as counter electrode, and SCE reference electrode. Potassium ferricyanide (50mM) and potassium chloride (100mM) solution in the absence of thylakoids are used as electrolytes and probe respectively, at a scan rate of 50mV/s. The $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions in potassium ferricyanide is easily reduced/oxidized at carbon electrodes, while potassium chloride is added to suffice the ionic strength for the electron transfer process [209].

Upon scanning, two distinct current peaks (anodic and cathodic) are observed. Anodic current describes the electron flow from the electrolyte to the electrode based on the oxidation of Fe^{2+} to Fe^{3+} into oxidation sweep from -0.3V to the 0.7V vs SCE electrode. Anodic current is denoted as positive polarity, while cathodic current is shown as a negative current in this study showing the reduction of Fe^{3+} . Higher current generation together with closer peaks separation between each of anodic and cathodic peaks observed using graphene electrode as compared to carbon cloth indicates higher electroactivity of graphene (Figure 7.3).

Figure 7.3 Cyclic voltammetry (CV) of carbon cloth and graphene electrodes respectively. The potential is reported vsSCE.

7.3.2 Electron transfer between thylakoids and carbon electrodes

The stability of thylakoids preparation upon integration with carbon material is studied based on photocurrent generation. Absence of redox mediator is used to investigate the feasibility of direct electron transfer.

In this work, the photocurrent generation of thylakoids using free standing graphene and carbon cloth is compared under the same thylakoids concentration. Both graphene electrode and carbon cloth are fixed on the transparent glass surface prior to use. Photocurrent is recorded under dark saturated open circuit potential, which is approximately at -0.4V toward pseudoreference electrode of carbon cloth in the absence of mediator. Although previous CV observation reveals the higher catalytic activity of graphene electrode, our photocurrent observation shows the higher photocurrent generation using carbon cloth (Figure 7.4).

Figure 7.4 Photocurrent generation between as graphene electrode and as purchased carbon cloth without any initial treatment. Illumination is given at 10s interval.

In contrast to our observation, a previous study has revealed a higher current generation using graphene electrodes as compared to the planar carbon cloth in the microbial fuel cell configuration (MFC) [207]. As described in the previous study, the initial electrodes pre-treatments using conducting polymer treatment promotes better interaction between the positively charged electrode and the negatively charged bacteria and results in closer interaction and higher electron transfer rate in graphene electrode.

Our results suggest that the adsorption method may not be effective to promote close interaction between graphene electrode and thylakoids due to the hydrophobic properties of the untreated graphene. Thus, pre-treatment of graphene electrode may be needed to increase the electron transfer rate and the generated photocurrent.

7.3.3 Charge transfer between rGO and thylakoids

The initial study of thylakoids stability and the proof of charge transfer between rGO and thylakoids are shown using absorbance and fluorescence observation. Bound protein complexes and light harvesting antennas of thylakoids are indicated by the appearance of 680nm absorbance peak (Figure 7.5, arrow) [141, 142].

Figure 7.5 Absorbance scan of (A) rGO, (B) thylakoids, (C) rGO and thylakoids.

The charge transfer is shown using fluorescence spectroscopy. As previously discussed on the Chapter 5 fluorescence study, the charge transfer between thylakoids and rGO can be described as the fluorescence quenching upon mixing with ~ 40% fluorescence quenching is observed (Figure 7.6). Control experiment of rGO shows the absence of fluorescence.

Figure 7.6 2D contour map of (A) isolated thylakoids, and (B) mixture of thylakoids and rGO.

7.3.4 Photocurrent using modified carbon cloth electrodes

Electrode biocompatibility, conductivity, and large surface area interaction is an important issue to ensure the efficient electron transfer. To enhance the carbon cloth performance, reduced graphene oxide (rGO) is electrodeposited on the carbon cloth surface.

The electron transfer rate from thylakoids to electrodes is compared using different deposition time. It is known that the amount of deposited rGO layer can be tuned by varying the deposition time and applied current during electrodeposition process. In this study, we observe the effect of prolonged deposition time on the carbon cloth toward photocurrent generation. The photocurrent is recorded under the respective saturated dark open circuit potential.

Figure 7.7 Photocurrent generation of thylakoid deposited on carbon cloth and r-GO modified carbon cloth. Illumination is given at 10s interval.

As shown on Figure 7.7, only ~10% photocurrent enhancement is observed after 10 minutes deposition compared to bare carbon cloth. We speculate that the optimum electron transfer between thylakoids and the untreated carbon cloth electrode is constrained by the thermodynamic of photosynthesis reaction inside the thylakoids.

7.4 Conclusion

We show the direct electron transfer between the thylakoids and carbon electrodes based on the anodic photocurrent generation using two-chamber PEC. The untreated carbon cloth exhibits higher photocurrent generation compared to the untreated graphene electrode indicating better close contact interaction. Further modification of the carbon cloth with rGO shows ~10% photocurrent increase upon 10 minutes electrodeposition time. It is speculated that the thermodynamic

constraint of the photosynthesis light reaction limits the amount of the generated electrons that can be transferred to the electrode.

CHAPTER 8

Conclusions and Future Works

8.1 Concluding Remarks

The remarkable ability of photosynthesis reaction to generate electrons during the water splitting reaction and reaction center charge separation upon illumination has gained many interests as a potential material for the development of biological photovoltaic device. Despite its advantages, detailed study of the electron transfer between photosynthetic material and electrode has not been widely explored.

The works presented in this thesis are focused on the investigation of electron transfer in the photosynthesis reaction to the conducting electrode surface. In an attempt to obtain highly efficient electron transfer, the basic understanding of external and internal factors that affect the electron transfer between the photosynthetic material and electrode is required. Several external parameters, including the mediator interception into thylakoids ETC, electrode modification, and different potentials applied have been explored. The internal parameter lies on the thermodynamic constraint of maximum electrons generation during the photosynthesis light reaction.

Isolated thylakoids from spinach leaves are used as the representative of photosynthetic material in order to specify the investigation on the light effect toward electron transfer and generated photocurrent. ITO, MWCNT, MWCNT/ITO, free standing graphene, rGO, carbon cloth, and rGO/carbon cloth are chosen as

several electrode materials of interest. The rate of electron transfer between the isolated thylakoids and electrode is studied through photocurrent generation in a two-chamber PEC.

Our results show that the distance of interaction is an important factor to increase the electron transfer rate between the thylakoids and electrode. Observation on the effect of mediators shows the bi-directional photocurrent (anodic and cathodic), indicating that the generated photocurrent can be tuned or increased by modifying the electron site interception. Further observation reveals the anodic photocurrent as a result of electron transfer from the thylakoids to the electrode, while the cathodic photocurrent is speculated to be due to the reduction of the generated O_2 or H_2O_2 as the photosynthetic by-product (Chapter 4).

In an attempt to increase the generated current in the absence of mediator, modification on ITO electrode to promote higher conductivity and active area interaction is done by incorporating MWCNT on ITO. Preliminary observation on the direct interaction and charge transfer between isolated thylakoids and functionalized MWCNT is studied on Raman, absorbance, and fluorescence spectroscopy. The approximate 50% fluorescence quenching is an indication that active charge transfer occurred (Chapter 5). Photocurrent observation of the MWCNT/ITO shows the four times higher photocurrent increase upon 2 minutes deposition time as compared to the unmodified ITO (Chapter 6).

The feasibility of carbon electrode is tested using free standing graphene electrode, carbon cloth, and rGO. Higher photocurrent is generated using carbon cloth which is speculated due to better interaction as compared to graphene electrode. No further photocurrent increase can be observed upon the integration of

rGO on carbon cloth. We hypothesize that the thermodynamic constraint of electron generation during the photosynthesis light reaction has been obtained (Chapter 7).

8.2 Future Directions

The overall results presented in this thesis describe the capability of thylakoids as biocatalyst for the photovoltaic device. However, some further considerations and improvements to increase the stability and photocurrent generation are needed in future studies.

The present study leverages on the hydrophilic interaction between thylakoids and electrode surface by using physical adsorption technique. Although promising photocurrent generation has been shown, the possible multilayer formation as a result of hydrophilic interaction between thylakoids during the adsorption process may reduce the electron transfer efficiency. In an attempt to increase the generated photocurrent, additional investigation on novel immobilization strategy to improve the interaction between each of the isolated thylakoids and electrode surface is required to increase the electron transfer.

Although the presented results show promise in using isolated thylakoids as biophotovoltaic material, protein degradation and the absence of regenerative mechanisms renders a major challenge for extended application. The reactive oxygen species (ROS) generated as the photosynthesis by-product is known as a major factor that promotes the damage of the D1 protein which is responsible for the water splitting reaction.

Based on the detail fundamental study of photosynthesis reaction presented in this study, further exploration on the feasibility of using living photosynthetic

organisms, such as blue green algae (cyanobacteria) or green algae (chlorella) as the biocatalysts for the PEC will pose an interesting solution to the stability problem encountered in the thylakoid-based system. The presence of regenerative mechanisms inside the living cells and the reproduction capability may prolong the PEC lifetime compared to the isolated thylakoids used.

Although living organisms possess long-term stability compared to isolated thylakoids, the challenges of using living organisms are mainly due to the multitude of metabolic reactions and the presence of cell membrane. By using thylakoids, electrons generated by the photosynthesis reaction with reduced influences from other reactions can be isolated and is supported by the well-established thylakoids electron transport chain for further study on the electron transfer. By contrast, in living organisms, the associated photosynthesis, respiration, and metabolic reactions hinder detailed observation of the exact current driven electron source. In addition, the presence of cell membrane in the living organism inhibits direct electron transfer to the electrode, since the proteins responsible for the electron transfer is enclosed within the membrane. Although the redox mediator is commonly added to intercept the generated electrons, the loss of electron transfer efficiency occurs during the change of reduction/oxidation state of the mediators. While, in the thylakoid membrane, although photosynthetic protein subunits are still bound inside the lipid bilayer, osmotic shock treatment has been performed to expose photosystem I subunit which may be responsible for direct electron transfer and thus enhances the generated current and electron transfer efficiency.

Based on the challenges and advantages of the living organisms, more studies and investigations need to be done to explore both systems. The isolated

photosynthetic compartment (thylakoids) will elucidate the generated electrons based on photosynthesis reaction, while the living organisms will provide better stability for the prolonged study used. Our results presented in this thesis provides basic understanding and insights to the photocurrent generation using living organisms in the future

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