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Semiconducting Polymer Nanobiocatalysts for Photoactivation of Intracellular Redox Reactions

Yan Lyu, Jingqi Tian, Jingchao Li, Peng Chen, and Kanyi Pu*

Abstract: An organic semiconducting polymer nanobiocatalyst (SPNB) composed of a semiconducting polymer core conjugated with microsomal cytochrome P450 (CYP) has been developed for photoactivation of intracellular redox. The core serves as the light-harvesting unit to initiate photoinduced electron transfer (PET) and facilitate the regeneration of dihydronicotinamide adenine dinucleotide phosphate (NADPH), while CYP is the catalytic center for intracellular redox. Under light irradiation, the semiconducting core can efficiently catalyze the generation of NADPH with a turnover frequency (TOF) 75 times higher than the reported nanosystems, ensuring the supply of the cofactor for intracellular redox. SPNB-mediated intracellular redox thus can be efficiently activated by light in living cells to convert the model substrate and also to trigger the bioactivation of anticancer drug. This study provides an organic nanobiocatalytic system that allows light to remotely control intracellular redox in living systems.

Specific control of biochemical reactions in living systems is of great interest because it represents a promising strategy to decipher the related biological functions and can potentially lead to new therapeutic modalities. Although physical (light and thermal),[1] chemical (small molecular inhibitors),[2] and biological (second messengers)[3] signals can be used to control biochemical reactions, light has the advantages of affordable cost, low toxicity, flexible tunability, and fine spatiotemporal resolution and is most desired for non-invasive remote regulation.[4] Among different biochemical reactions, intracellular redox plays a pivotal role in transferring energy, biosynthesizing metabolites, bioactivating drugs, and detoxifying xenobiotics within cells.[5] Inspired by natural photosynthesis, optical agents including organic dyes, quantum dots, carbon nitrides, carbon dots, and peptide nanotubes have been used as artificial photocatalysts to activate redox via photoinduced electron transfer (PET) in solutions.[6] However, the activation process of these nanomaterials usually requires cytotoxic transition-metal components to enhance charge separation[7] or mediators to facilitate electron transfer.[8] Particularly, organic dyes not only depend on these metallic components,[9] but also have relatively poor photostability (Supporting Information, Figure S1).[10] Owing to these limitations, existing optical agents have not been demonstrated for photoactivation of intracellular redox.

Semiconducting polymer nanoparticles (SPNs) have emerged as versatile optical agents for biological applications ranging from molecular imaging[11] to phototherapy.[12] Owing to their good biocompatibility and high photothermal conversion efficacy, SPNs can serve as light-responsive modulators to regulate heat-related biological functions including activation of protein ion channels[13] and gene transcription.[14] Furthermore, SPNs have efficient light-harvesting properties and their band gaps can be fine-tuned by the precursor polymers, making them effective as the electron donors for PET.[15] Recently, SPNs have also been used to capture sunlight to accelerate the electron-transport rates in photosystem, enhancing the photosynthesis of chloroplasts.[16] These studies proved that SPNs could be promising for remote activation of intracellular reactions.

Herein we report the development of a completely organic semiconducting polymer nanobiocatalyst (SPNB) and demonstrate its application for in situ photoactivation of intracellular redox in living cells. SPNB is composed of a water-soluble SPN and microsomal cytochrome P450 (CYP), which serve as the light-harvesting PET initiator and the catalytic center, respectively. Microsomal CYP, the most important P450-containing system in eukaryotic cells, is an endoplasmic reticulum attached protein cluster mainly containing CYP and cytochrome P450 reductase (CPR).[17] Because CYPs conduct oxidation by indirect utilization of electrons transferred from the cofactor dihydronicotinamide adenine dinucleotide phosphate (NADPH) via CPRs, the intracellular redox reaction efficacy is significantly determined by the external availability of NADPH. Within SPNB, the SPN can harvest light and initiate PET to efficiently reduce nicotinamide adenine dinucleotide phosphate (NADPH) to NADPH, guaranteeing the adequate electron supply for CYP-catalyzed oxidation. Thus, SPNB enables in situ activation of intracellular redox under light irradiation.

To find the ideal PET initiator, four semiconducting polymers (SPs) including poly(9,9-dioctylfluorenyl-2,7-diyl) (PFO), poly(9,9-dioctylfluorenyl-2,7-diyl)-alt-(benzo[2,1,3]thiadiazol-4,7-diyl) (PBTFT), poly[2,7-(9,9-dioctylfluorene)-alt-4,7-bis(thiophen-2-yl)benzo[2,1,3]thiadiazole] (PFODDTB) and poly[2,6-(4,4-bis(2-ethylhexyl)4H-cyclopenta[2,1,2-b:3,4-b']dithiophene)-alt-4,7(2,1,3' -benzothiadiazole)] (PCPDDBT) (Figure 1b) were used to prepare SPNs through nanoprecipitation in the presence of an amphiphilic copolymer, polystyrene-block-poly(acrylic acid) (PS-b-PAA).

The resulted SPNs had the carboxyl groups on the surface...
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visible-light absorption, SPN-PFO with the highest reduction potential however showed the lowest charge separation efficacy among four SPNs.

The photocatalytic abilities of these SPNs were then studied and compared in terms of reducing NADP+ to NADPH. The generation of NADPH was monitored by measuring the absorption at 340 nm every 12 min during light irradiation. At each timepoint, the amounts of generated NADPH followed the order: SPN-PFBT > SPN-PFODBT > SPN-PCPDTB > SPN-PFO (Figure 2c). After 1 h light irradiation, the generated NADPH catalyzed by SPN-PFBT was 1.3-1.9-, and 2.8-fold higher than that by SPN-PFODBT, SPN-PCPDTB, and SPN-PFO, respectively. The turnover frequencies (TOFs) were estimated to be 133.6 h⁻¹, 105.3 h⁻¹, 68.8 h⁻¹, and 48.6 h⁻¹ for SPN-PFBT, SPN-PFODBT, SPN-PCPDTB, and SPN-PFO (Figure 2d), respectively. As compared with the reported nanomaterials such as peptide nanotubes (≤1.78 h⁻¹), these SPNs not only had much higher TOFs but also exhibited comparable or even better kinetic performance to catalyze NAD(P)H generation despite being without a redox transition metal (Pt or TiO₂), mediator ([Cp*Rh(bpy)H₂O]²⁺), and enzyme usage (Supporting Information, Figure S8). The order of photocatalytic abilities of these SPNs to generate NADPH was consistent with that of their charge separation efficacies. Note that among these four SPNs, SPN-PFBT was the best photocatalyst, mainly because of its highest charge separation efficacy and the relatively more negative reduction potential.

Next, SPN-PFBT and SPN-PFODBT were chosen to activate microsomal CYP-catalyzed redox towards the substrate, 7-benzyloxy-4-trifluoromethyl coumarin (BFC), in solutions. The reaction efficacy was monitored by the fluorescence increase as the oxidation of non-fluorescent BFC produced the fluorescent product, 7-hydroxy-4-trifluoromethyl coumarin (HFC). After 1 h light irradiation, the fluorescence increment for the SPN-PFBT activated CYP system was 1.46-fold higher than that for SPN-PFODBT (Figure 3a), while BFC or BFC with microsomal CYP alone barely showed fluorescence increase. Moreover, the fluorescence increases in the presence of SPNs alone were 3 to 4.3 times lower than their corresponding enzymatic systems, proving that the generation of HFC was mainly attributed to the redox catalyzed by SPN-activated microsomal CYP. Moreover, in terms of CYP, the enzymatic catalytic efficencies (kcat/Km) were improved by 283- and 33-fold for SPN-PFBT (0.26 μm⁻¹ min⁻¹) and SPN-PFODBT (0.03 μm⁻¹ min⁻¹) respectively, compared with that of the wild-type (WT; 0.00092 μm⁻¹ min⁻¹) (Figure 3b; Supporting Information, Figure S9 and Table S2).

The SPN-PFBT-mediated photocaotivation of microsomal CYP-catalyzed redox was tested against nifedipine, a drug used to block calcium channels to treat high blood pressure and primarily metabolized by microsomal CYP to the deactivated form (dehydro-nifedipine). After light irradiation for 20 min in the presence of microsomal CYP and SPN-PFBT, most nifedipine (HPLC retention time, T₀ = 19.3 min) was oxidized to dehydro-nifedipine (T₀ = 15.7 min; Figure 3c), as confirmed by electrospray ionization-mass spectrometry (ESI-MS) analysis (Supporting Information, Figure S10). In contrast, dehydro-nifedipine was marginally generated if one component of SPN, enzyme and light was absent. Note that the peak at T₀ = 18.8 min was attributed to the light-induced degradation because of the light sensitivity of nifedipine. Thus, SPN-PFBT was able to activate microsomal CYP-catalyzed redox against both model and clinically-relevant substrates.

Because of the good cytocompatibility of SPNs (Supporting Information, Figure S11), SPN-PFODBT was used for intracellular regulation. SPNB was synthesized by conjugating to microsomal CYPs via a carbodiimide coupling reaction between carboxyl groups of the nanoparticles and amine groups of the enzymes. The increased diameter measured by DLS and slower migration rate of electrophoresis confirmed the successful bioconjugation (Figure 4b; Supporting Information, Figure S12). The resulting nanoparticles were delivered into the living liver hepatocellular cells (HepG2) and the in situ photocatalysis of SPNB-catalyzed intracellular redox was assessed using BFC as the substrate. The minimal overlap for the emission spectra of the generated HFC and SPN-PFODBT ensured the fluorescence tracking of both components.

After being cultured with SPNB overnight, HepG2 cells were incubated with BFC for 2 h and then exposed to light irradiation with the supply of NADP⁺ for 30 min before fluorescence cell imaging. The SPNBs and the product (HFC) were indicated as pseudo red and green, respectively. Upon irradiation, the green fluorescence in SPNB-treated cells significantly increased by 1.7-fold compared with that without irradiation, and was 42.5-fold higher than that in the control cells without SPNB-treatment regardless of irradiation (Figures 4c,d; Supporting Information, Figure S13). Note that SPNB-treated cells had higher green fluorescence than the control cells without light irradiation. This was caused by the increased amount of microsomal CYP in living cells contributed by SPNB that enhanced the intrinsic oxidation of BFC in living cells. These data confirmed that the SPNB-catalyzed intracellular redox could be activated following the proposed mechanism in Figure 4a: upon light irradiation, the...
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Communications

**Biocatalysts**

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An organic nanobiocatalyst composed of a semiconducting polymer core conjugated with microsomal cytochrome P450 (CYP) has been developed. It can undergo an intracellular redox reaction that is non-invasively triggered by light in living cells.

**Biokatalysatoren**

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