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Efficient DNA-mediated Electron Transport in Ionic Liquids

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ABSTRACT: Electron conductivity of duplex DNA has promising applications in fabricating DNA based bio-sensors and electronic devices for biomimic solar cells. However, in aqueous solution DNA-mediated electron transfer (ET) is often far from ideal for these applications. We reported here in hydrated ionic liquids (IL), electron can propagate through 4 nm of duplex DNA and higher ET efficiency was achieved over longer distance which yielded a noncanonical negative distance decay parameter ($\gamma = -0.02 \text{ \AA}^{-1}$). Fluorescence studies and ET efficiency of duplex DNA in IL-D₂O revealed that the binding of both cationic and anionic species of hydrated IL in DNA minor groove and the exclusion of water from DNA hydration layer significantly improved base-pair stacking of duplex DNA to achieve efficient electric conductivity. As an oxidation reaction of nucleic acids, efficient DNA ET observed here suggested IL could be a promising non-organic and non-aqueous solvent for redox reactions of bio-macromolecules.

KEY WORDS: Ionic liquids; electron transport; conductive biomolecule; DNA minor groove binder; anthraquinone; deuterium water

Ionic liquids (IL) as novel non-organic and non-aqueous solvent possess many remarkable physical and electrochemical properties, such as negligible vapour pressure, high thermal stability, intrinsic conductivity and wide electrochemistry window. Applications of ionic liquids, especially in electrochemistry and battery materials have been substantially explored.^{1,2} Recently, organic/inorganic catalysts were found higher reaction activities in hydrophobic ILs.^{3,4} More interestingly, some ILs are biocompatible and can preserve the structures of biomacromolecules. DNA could maintain duplex structure in hydrated ionic liquids for half a year, showing IL can be an ideal solvent for long term DNA storage.⁵ IL immiscible with water offered an efficient protocol to extract hydrophobic proteins from aqueous buffer.⁶ Further exploration of IL as solvents for biological reactions were rare, though it would extremely beneficial to wide variety of research fields. Few examples focus on enzymatic hydrolysis, including lipases, esterases, proteases, glycosidases, and have shown similar enzymatic activities in hydrated ionic liquids as in conventional solvents.⁴

Redox reactions in biological macromolecules are of great interests in research fields, such as bio-mimic solar cells, water split and nanoscale electric device, etc. DNA mediated electron transport (ET) is a key sequential process following DNA oxidation and the main cause of genomic damage by distant ROS and/or photo-oxidants. The inherent electric conductivity of duplex DNA shown by DNA ET provides the great potentials to apply duplex DNA as a conductive molecular wire. Previous measurements have shown electric conductivity of DNA in various forms, single-molecule, ropes, and films, falls in a large range from semiconductor to superconductor.⁷⁻¹³ In aqueous solution, DNA sequences with consecutive purine bases (adjacent adenines and guanines, also called A-tract and G-tract) are suggested to be more efficient for ET than mixed-base/alternating-base sequences, which might be attributed to narrower HOMO energy gap (ΔHOMO) for coherent ET.¹³⁻²¹ Electron propagation between electron donor and trap over long bridging sequences in duplex DNA (>4 bp, 1.3 nm) might

adopt a hopping mechanism featuring with a shallow distance dependence, which distance decay parameter, γ , can vary from 0.001 to near 0.1 Å⁻¹.²⁰⁻³⁰ The γ value for DNA ET is comparable with or smaller than that of organic films composed of conductive oligomers.³¹ It is interesting to notice that recent study found that several proteins in DNA repair pathway harness DNA ET as chemical signaling pathways, indicating the biological significances of DNA ET.³²⁻³⁵ Nevertheless, efficient ET in aqueous solution requires proper DNA sequence and intact duplex structures.³⁶⁻³⁸ These requisites raise a high threshold for duplex DNA to achieve high ET efficiency, since perturbations to π electron coupling of base pairs, such as mismatches and protein binding would disturb DNA secondary structure or conformation and often significantly hamper ET efficiency through DNA.³⁷⁻⁴⁰ To solve this problem, modified bases have been adopted to narrow Δ HOMO of the bridge bases between the donor and acceptor.^{17,18,41,42} Moreover, ET efficiency of DNA has been improved by DNA secondary structures with extended electron conjugation area, such as G-quadruplex.⁴³ To date, methods for improving ET efficiency without altering the primary and secondary DNA structures are limited. Recent work reported facilitation of ET in aqueous solution under external magnetic field (MF).⁴⁴ Alignment of DNA base pairs by MF might induce ET-active conformation, thus promoting electron propagation. Alternatively, solvent environments would be a pivotal factor to modulate ET efficiency. However, replacing water with D₂O or changing counter ion species resulted in negligible or inhibitory effects on DNA ET.⁴⁵⁻⁴⁷ Alternatively, IL have shown good ability to tune the duplex stability of DNA from both experimental work and molecular stimulation.^{48,49} Inspired by the promising applications of IL as novel solvents for protein and DNA, we considered the possibility for duplex DNA to efficiently conduct electrons in hydrate ionic liquids. Herein, we investigated one electron oxidation of a kinetic electron hole trap, 8-cyclopropylguanine (⁸CPG) by a distant photo-oxidant via DNA ET in hydrated ionic liquids. Significantly efficient DNA ET in IL and the first negative distance decay parameter were

observed over more than 4 nm. The work herein provided a novel view of not only interactions between bio-macromolecules and ionic liquids, but also redox reactions of biological molecules in ionic liquids.

RESULTS

Design of DNA Duplexes for Photo-induced One Electron Oxidation of ${}^8\text{CPG}$

To investigate DNA ET in ionic liquids, a series of 23-mer DNA duplexes were prepared as listed in Table 1. Each duplex, $\text{Q}_n/{}^8\text{CPG}_n$ ($n=1-4$) contained an anthraquinone (Q) as photo-oxidant at the 5'-end of T-rich strands, where anthraquinone was conjugated to a uracil with an alkynyl bond (structure as shown in Figure 1). ${}^8\text{CPG}$, as an electron hole trap, was positioned at the 5'-G of a GG doublet in A-rich strand, and was intervened by consecutive A-T pairs (A-tract) from photo-oxidant. To investigate distance dependence of DNA ET in hydrated ionic liquid, numbers of A-T pairs between Q and ${}^8\text{CPG}$ were increased from four to ten from duplex $\text{Q}_1/{}^8\text{CPG}_1$ to $\text{Q}_4/{}^8\text{CPG}_4$, with increment of AA as one step. Upon photo-excitation of anthraquinone at 350 nm, electron-conjugated uracil was one electron oxidized, and an electron hole was injected into DNA duplexes sequentially.^{50,51} The hole was able to hop along the base-pair stacks and finally cause one electron oxidation of ${}^8\text{CPG}$ in the complementary strand. The fast cyclopropyl ring opening reaction of ${}^8\text{CPG}$ would trap the migrating hole and resulted in decomposition of ${}^8\text{CPG}$, which can then be revealed by enzymatic digestion and HPLC analysis (Figure 1).^{27,52} Decomposition of ${}^8\text{CPG}$ upon photo-irradiation was determined, as described in Eq 2, to reflect the efficiency of DNA ET.

Figure 1. Structures of ionic liquid cations used.

Table 1. Oligonucleotide sequences used to probe DNA CT in ionic liquids

DNA	Sequences ^a
Q ₁ / ⁸ CPG ₁	5'- <i>Q</i> TTTTCCTTTTTTTTAGAGATAG-3' 3'-AAAAAGXAAAAAAATCTCTATC-5'
Q ₂ / ⁸ CPG ₂	5'- <i>Q</i> TTTTTTCCTTTTTTAGAGATAG-3' 3'-AAAAAAAGXAAAAATCTCTATC-5'
Q ₃ / ⁸ CPG ₃	5'- <i>Q</i> TTTTTTTTCCTTTTAGAGATAG-3' 3'-AAAAAAAAGXAAAATCTCTATC-5'
Q ₄ / ⁸ CPG ₄	5'- <i>Q</i> TTTTTTTTTTCCTTAGAGATAG-3' 3'-AAAAAAAAGXAAATCTCTATC-5'
Q ⁸ CPG	5'- <i>Q</i> XTTTTTTTTTCCTTAGAGATAG-3'
T ⁸ CPG ₄	3'-AAAAATAAAAAGXAATCTCTATC-5'
O	5'-TTTTTTTTT O TTTCCTTAGAGATAG-3'
CC ₃	5'-TTTTTTTTTTCCTTTTAGAGATAG-3'
GG ₃	3'-AAAAAAAAGGAAAATCTCTATC-5'
CC ₄	5'-TTTTTTTTTTTCCTTAGAGATAG-3'
GG ₄	3'-AAAAAAAAGGAATCTCTATC-5'

Efficient charge transport through duplex DNA in ionic liquids

DNA ET in hydrated ionic liquid was firstly examined by photo-oxidation of Q₄/⁸CPG₄ in hydrated 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄]) (Figure 2). With the longest bridge length between hole trap and photo-oxidant, Q₄/⁸CPG₄ could accommodate the highest amounts of interactions between DNA bridge and solvent molecules and thus would have the effects of IL pronounced most significantly on hole migration. Significant ⁸CPG decomposition (44%) in 2 M of hydrated [BMIM][BF₄] was observed after irradiation of Q₄/⁸CPG₄ at 350 nm for 45 s. ⁸CPG oxidation was significantly enhanced comparing to that in aqueous solution (PBS, 5%) (Figure 3). A series of control experiments were conducted to validate the efficient ⁸CPG decomposition in hydrated IL was due to the intramolecular electron conductivity of duplex DNA. As shown in Figure 3, negligible ⁸CPG decomposition was observed for either non-irradiated sample (dark, 1%) or irradiated sample in the absence of photo-oxidant (light, 1% for CC₄/⁸CPG₄). These results suggested that ⁸CPG decomposition observed in IL was due to the light-initialized photo-reduction of distant anthraquinone. The possibility was excluded that ionic liquids directly or IL with assistance of light could cause

${}^8\text{CPG}$ decomposition in the absence of electron migration through the bridging duplex DNA. Furthermore, a mixture of duplex Q_4/GG_4 and $\text{CC}_4/{}^8\text{CPG}_4$, in which photo-oxidant and electron hole trap were placed in the separate duplexes as a so called cross control, and were irradiated in hydrated $[\text{BMIM}][\text{BF}_4]$. Negligible ${}^8\text{CPG}$ decomposition was observed in this control experiment, which confirmed that no diffusible oxidants or interduplex charge transport were involved here. Since DNA ET is highly sensitive to sequence integrity, DNA duplex $\text{Q}_4/\text{T}{}^8\text{CPG}_4$ (Table 1) containing a T-T mismatch on "ET bridge" was irradiated in hydrated IL. ${}^8\text{CPG}$ decomposition was significantly attenuated (14%) compared to the full-match sequence $\text{Q}_4/{}^8\text{CPG}_4$ (44%), indicating that ET in hydrated $[\text{BMIM}][\text{BF}_4]$ was within each individual duplex. Hence, DNA mediated electron hole transport in hydrate IL was an intraduplex process, which was the same as the process occurring in aqueous solution. Furthermore, figure S1 showed that the yields of ${}^8\text{CPG}$ decomposition increased linearly with the concentration of $[\text{BMIM}][\text{BF}_4]$ up to 2 M. The dependence on IL concentration indicated that the electron conductivity of duplex DNA was elevated by the presence of ionic liquids.

Figure 2. Experimental setup to investigate DNA mediated CT in ionic liquid. Photo-irradiation initiates charge transport along DNA duplex; oxidation of 8CPG is revealed by HPLC analysis; anthraquinone (Q) and ${}^8\text{CPG}$ (X) were adopted as photo-oxidant and kinetic hole trap, respectively.

Figure 3. Escalation of ⁸CPG decomposition from Q₄/⁸CPG₄ in [BMIM][BF₄] is due to DNA mediated CT. ⁸CPG decomposition in Q₄/⁸CPG₄ under various conditions: in PBS buffer without [BMIM][BF₄] (PBS); with 2 M [BMIM][BF₄]; without irradiation (dark); irradiated without Q (light); Q and ⁸CPG were separated in two duplexes (cross); with a T-T mismatch in duplex between Q and ⁸CPG (mismatch). All samples had the same solution composition (except for PBS sample): 20 μM DNA in 20 mM PBS, 50 mM NaCl, 2 M [BMIM][BF₄].

Electron conductivity of DNA is more efficient in ionic liquids than aqueous solution

The process of charge transport could be divided into three stages, as electron hole injection, electron hole migration, and electron hole trapping.⁵³ The above experiments clearly showed that ⁸CPG decomposition, as an overall product of the three stages of DNA ET, was much more efficient in hydrated ionic liquid than in aqueous buffer. However, IL effects on each stage were not revealed directly by the above ⁸CPG reaction. To this end, we designed an oligonucleotide Q⁸CPG (Table 1), in which photooxidant Q and hole trap ⁸CPG were placed adjacent to each other without the intervening bases. Hence, no electron hole migration was involved due to the lack of base pairs as the bridging pathway for electron propagation, especially in single stranded oligonucleotides. One electron oxidation between Q and ⁸CPG was studied by irradiating single stranded Q⁸CPG and duplex Q⁸CPG/GG₄ for 18 min in aqueous solution and 2 M hydrated [BMIM][BF₄], respectively. The decomposition of ⁸CPG in Q⁸CPG

would be a sole outcome from the direct combination of electron hole injection and trapping stages. In contrast to duplex $Q_4/{}^8CPG_4$, oxidative decomposition of 8CPG in single stranded $Q{}^8CPG$ was attenuated, from 47% in aqueous solution, to 30%, in 2 M hydrated $[BMIM][BF_4]$ (Figure 4). However, negligible decomposition of 8CPG was observed after photoirradiation of duplex $Q{}^8CPG/GG_4$, which presumably is due to the ultrafast radical charge recombination when Q and 8CPG was adjacently stacked in duplex DNA (data not shown). This result suggested the inhibitory effect of $[BMIM][BF_4]$ on a direct electron hole injection-trapping process. Hence electron hole migration was the only stage that was facilitated by hydrated ionic liquids to achieve efficient electron conductivity in duplex DNA.

Figure 4. Decomposition percentage of 8CPG (Y) in $Q{}^8CPG$ in aqueous solution (PBS) and 2 M $[BMIM][BF_4]$ (IL) after irradiation at 350 nm for 18 min.

Inverse Distance Dependence of 8CPG Decomposition in Hydrated Ionic Liquids

Having confirmed that escalation of 8CPG decomposition in $[BMIM][BF_4]$ was due to ionic liquids facilitated electron migration through duplex DNA. Further investigation on distance dependence of ET yields was conducted to study the IL effect over various lengths of electron migration. In a series of DNA duplexes, $Q_n/{}^8CPG_n$ ($n=1-4$), electron hole trap, 8CPG , and photo-oxidant, anthraquinone, were separated by bridging A-tract with four to ten AT base pairs. As

shown in Figure S2, upon irradiation for 45 s in PBS solution, $^{8\text{CPG}}$ decomposition (Y) decreased from 14% to 5% with bridge length elongated from 20.4 Å to 40.8 Å. Natural logarithm of Y was plotted against distance between photo-oxidant and $^{8\text{CPG}}$ (r) in Figure 5. Distance dependence of DNA ET was obtained by fitting data in Figure 5 to Eq 1.²³

$$\ln Y = \ln A - \gamma \times r \quad \text{Eq 1}$$

where γ is the decay parameter.^{53,54} In aqueous solution, $\gamma = 0.02 \text{ Å}^{-1}$, indicated that ET yield decay along with migration length. The oscillatory periodicity of charge transport yield with increasing intervening adenines was consistent with previous findings.^{27,55} Maximum $^{8\text{CPG}}$ oxidation was achieved when five and nine intervening base pairs were positioned between Q and $^{8\text{CPG}}$ in $\text{Q}_1/^{8\text{CPG}}\text{G}_1$ and $\text{Q}_3/^{8\text{CPG}}\text{G}_3$, respectively. The periodic oscillation of distance dependence was consistent with conformation-gated domain hopping mechanism.⁵⁵ However, when same exploration was conducted in hydrated ionic liquid, distance dependence of ET yield in 2 M [BMIM][BF₄] did not show the typical decay of $^{8\text{CPG}}$ decomposition as in aqueous solution. In $\text{Q}_1/^{8\text{CPG}}\text{G}_1$, $^{8\text{CPG}}$ closest to Q achieved the lowest decomposition (18 %), while $^{8\text{CPG}}$ decomposed with highest efficiency (44%) in $\text{Q}_4/^{8\text{CPG}}\text{G}_4$ in which $^{8\text{CPG}}$ was placed furthest away from Q (Figure S2). In hydrated ionic liquid, the oscillation dependence of ET yields was barely observable with much attenuated amplitudes and opposite phase as that in PBS. Distance decay parameter γ obtained in hydrated IL was a negative value, as -0.02 Å^{-1} . Increasing ET yield over longer adenine tract between photo-oxidant and electron hole trap was seldom observed in previous studies of aqueous solution. Although the reason for the unique inversed distance dependence in [BMIM][BF₄] required further study, better pronounced IL facilitation on DNA ET was observed over longer migration distance which accommodated more interactions between IL species and DNA bridge.

Figure 5. Distance dependence of CT yield in $Q_n/{}^8CPG_n$ ($n=1-4$) in aqueous and 2 M hydrated [BMIM][BF₄]. Y : decomposition of 8CPG ; r : distance between photo-oxidant and 8CPG .

Effects of Hydrated Ionic Liquid Cation and Anion Species on Charge Transport

DNA ET in $Q_4/{}^8CPG_4$ was further investigated in hydrated ionic liquids other than [BMIM][BF₄] in order to confirm efficient DNA mediated ET in hydrated IL was not limited to [BMIM][BF₄]. Two ionic liquids with different cations, 1-butyl-1-methylpyrrolidinium tetrafluoroborate ([BMP][BF₄]) and 1-ethylpyridinium tetrafluoroborate ([EtP][BF₄]), were investigated to reveal the effects of cations on DNA ET (Figure 2); while [BMIM][Cl] and [BMIM][Ac] were investigated for anion effects. As shown in Figure 6, yields of DNA ET in ionic liquids were sensitive to their cationic species. Higher ET yield of $Q_4/{}^8CPG_4$ was observed in [BMP][BF₄] (8%) and [EtP][BF₄] (30%) than that in PBS. [EtP]⁺ with aromatic cation as [BMIM]⁺ showed higher ET yields than that in [BMP]⁺ which contains alkylated cyclic pyrrolidinium cation. Better facilitation from aromatic cations could plausibly relate to stronger hydrophobic interaction with DNA base pairs. Hence, IL cations could show distinct effects on DNA ET due to the difference in their structures.

Figure 6. $^{8\text{CP}}\text{G}$ decomposition in $\text{Q}_4/^{8\text{CP}}\text{G}_4$ with various ionic liquids.

Remarkably, unlike previous literatures which only focus on cation interactions with DNA,^{48,49,56} we found that the anion effects on electron conductivity of duplex DNA in ionic liquids was not negligible. ET yields were dramatically attenuated from 44% to less than 2% in [BMIM][Cl] and were not detectable in [BMIM][Ac], suggesting that the effects of ionic liquid on charge transport was also strongly modulated by the anion species. This finding was the first time to observe the anion effects on chemical reactions of oligonucleotides. Positively charged cations of ionic liquid was assumed to be the dominant species in ionic liquids to interact with DNA via electrostatic attractions and H-bonds, while anion contributions to the interactions were often considered to be much less intense. However, our experiment suggested that charge transport was associated with both ion species, especially related to the cation and anion composition of ionic liquids.

To further validate that anions of ionic liquid influenced the interaction between cations and DNA duplexes, DNA ET in $\text{Q}_4/^{8\text{CP}}\text{G}_4$ was investigated in a mixture of [BMIM][Cl] and NaBF_4 to *in situ* prepare [BMIM][BF₄] as hydrated IL. DNA ET was inhibited in either [BMIM][Cl] or NaBF_4 individually. DNA duplex structure was significantly destabilized in NaBF_4 solution and 2 M of [BMIM][Cl] with significantly large decrease in melting temperature, 17 °C (Table S1). With increasing NaBF_4 ratio to [BMIM][Cl] from 1:9 to 1:1, oxidative decomposition of $^{8\text{CP}}\text{G}$ in $\text{Q}_4/^{8\text{CP}}\text{G}_4$ was recovered from 2% to 14% as shown in Figure S3. The yield increment

of DNA ET was a result from a restoration of proper interactions between cationic/anionic species and duplex DNA, even though the cation and anion interactions were generated as an *in situ* ionic liquid.

Effect of Water Molecule on DNA-mediated electron transfer in Ionic Liquid

Previous studies have shown that water molecule could have significant influence on dissociation of cation and anion in IL and consequentially affect the interaction among ion species and biomolecules.⁵⁷⁻⁶³ Electron propagation through duplex $\text{Q}_3/{}^8\text{CPG}_3$ in hydrated $[\text{BMIM}][\text{BF}_4]$ was examined in either H_2O or D_2O . ${}^8\text{CPG}$ decomposition was slightly increased in D_2O -PBS solution (16%) compared to H_2O -PBS (12%) as shown in Figure 7. Though the theory of proton assisted hole transfer suggested replacing H_2O with D_2O might inhibit ET in aqueous buffer,^{45,46,64} the fast kinetic hole trap ${}^8\text{CPG}$ did not undergo proton transfer with paired guanine upon one-electron oxidation.^{27,52} Thus oxidation reaction of ${}^8\text{CPG}$ was unlikely significantly affected by deuterated water directly. However, the isotope effect was much more pronounced in the presence of ionic liquids. Nearly 50% increment of ${}^8\text{CPG}$ decomposition was observed when the hydration molecules were switched from H_2O (35%) to D_2O (58%). Combining with the effects of cationic and anionic IL species on DNA-mediated electron transfer, this isotopic effect of water indicated that the association between $[\text{BMIM}]^+$ and BF_4^- , and/or between DNA and IL may involve water molecules in hydrated ionic liquids.

Figure 7. Decomposition percentage of $^{8\text{CP}}\text{G}$ in $\text{Q}_3/^{8\text{CP}}\text{G}_3$ in PBS and 2M hydrated [BMIM][BF₄] with H₂O or D₂O.

Interactions of Ionic Liquids with duplex DNA

To understand the mechanism of efficient DNA-mediated electron transfer in hydrated IL, further studies on the interactions between ionic liquid species and duplex DNA were conducted. To determine the duplex stability in hydrated ionic liquid, **O/GG4** (Table 1) with a thiazole orange (TO) positioned in the A-tract via covalent linkage was applied to monitor DNA structure changes during thermal denaturation. Covalently tethered TO was reported to be a useful probe to study the structural dynamics of DNA due to the correlation between the fluogenic signal and aromatic stacking of TO within DNA.^{65,66} As shown in Table S1, melting temperatures of **O/GG4** showed no more than slightly increase in hydrated ionic liquids with BF₄⁻ as anion species. Whereas, T_m of **O/GG4** could not be observed in [BMIM]Cl or [BMIM]Ac. Significant decrease in thermal stability indicated **O/GG4** could not maintain stable duplex structure in these two hydrated IL under room temperature, which could account for the loss of efficient ET in hydrated [BMIM][Cl] and [BMIM][Ac].

4',6-diamidino-2-phenylindole (DAPI) is an ideal indicator which specifically binds minor grooves of duplex DNA. Therefore, fluorescence indicator displacement (FID) assay was performed by titration of various ionic liquids to DAPI-DNA complex to reveal ionic liquid

interaction with DNA minor grooves. As shown in Figure S4, percentage of DAPI displaced increased by titration with ionic liquids suggesting binding of ionic liquid molecules to DNA minor groove, which was consistent with previous finding.⁴⁸ [BMIM][BF₄], [EtP][BF₄] had similar binding affinities to minor groove with DC₅₀ around 0.7 M. Larger DC₅₀, 1.0 M, was obtained in [BMP][BF₄]. Once aromatic five member ring was switched to non-aromatic ring, minor groove binding affinity of [BMP][BF₄] was attenuated. This could be attributed to the large steric hindrance and less hydrophobicity of pyrrolidinium compared to aromatic cations. Furthermore, in Figure S5, CD spectrum of duplex **CC₄/GG₄** in 0-2 M [BMP][BF₄] showed slightly decrease of the negative peak at 245 nm, suggesting the mild alteration of deoxyribose-phosphate backbone by electrostatic interaction between duplex and IL. On the other hand, duplex DNA showed negligible alteration on CD peak at 282 nm in hydrated ionic liquids, which suggested that the binding of ionic liquid cations in the minor groove applied little disturbance to DNA base stacking.

The time-resolved fluorescence decay was performed on duplex **O/GG₄** to further investigate the microenvironment of base pair stacking once duplex DNA was placed in hydrated ionic liquids. The fluorescence lifetime (τ) of TO covalently tethered to **O/GG₄** was sensitive to the conformation of flanking base pairs and hence became an ideal parameter to monitor the subtle alterations in base pair stacking in duplex DNA. τ of **O/GG₄** was 1.74 ns in aqueous PBS, which was consistent with an intercalated TO molecule in duplex DNA.⁶⁷ In hydrated ionic liquids of [BMIM][BF₄], [BMP][BF₄] and [EtP][BF₄], longer fluorescence lifetime of TO in **O/GG₄** was observed (τ =2.00-2.17 ns) and indicated that TO achieved a better stacking within the base pair array (Figure S6). Furthermore, the similar τ values were observed for the three hydrated ILs and suggested that the interactions between duplex DNA and IL species of [BMIM][BF₄], [BMP][BF₄] and [EtP][BF₄] may reach comparable magnitudes on the dynamic conformation of base pair stacking.

DISCUSSION

We observed significant efficient electron conductivity of duplex DNA in hydrated ionic liquids, such as [BMIM][BF₄], and [EtP][BF₄]. Though ionic liquid is an electrolyte, facilitation of charge transport is not due to the inherent conductivity of ionic liquid or intermolecular charge transport. [BMIM][BF₄] enhanced ET efficiency over the range from 2.0 nm to 4.1 nm. Since photo-oxidation of single stranded **Q^{8CP}G** which merely allowed hole injection and hole trapping steps, showed reduced, rather than increased, ^{8CP}G decomposition in [BMIM][BF₄], ionic liquids mainly facilitated electron propagation through base pair array within duplex DNA. Electron migration in duplex DNA revealed inverse distance dependence of ^{8CP}G decomposition in hydrated IL with more ^{8CP}G decomposition over a longer length of DNA bridge. All these findings suggested the interaction modes between IL and DNA were critical to accomplish efficient electron conductivity in DNA.

Investigation of duplex structure by CD spectra suggested that strong electrostatic interaction might exist between DNA phosphate backbones and IL cations. Furthermore, the binding of ionic liquids to DNA minor groove was confirmed by DAPI displacement assay. Binding of ionic liquids with proper composition of cation and anion species showed negligible alteration on secondary structure of duplex DNA. Aromatic cations in IL, such as [BMIM]⁺ and [EtP]⁺ showed strong hydrophobic interactions with DNA base pairs in minor grooves. More remarkably, distinct efficiencies of DNA-mediated electron transfer in hydrated imidazolium IL with different types of anion species suggested not only cation, but anions also contribute to the interactions between IL and duplex DNA. Hydrophobic anion, such as BF₄⁻, would strongly associate with imidazolium cations via H-bonding through water molecules, while Cl⁻ and Ac⁻ as more basic anions would tend to form H-bonding directly with nucleobases.^{57,58,68} With indirect modulation via H-bonds through water molecules and BMIM⁺, BF₄⁻ has limited interactions with duplex DNA and hence [BMIM][BF₄] only slightly compromised the thermal

stability of duplex DNA. However, the association of cations was not conspicuous for the more basic hydrophilic anions, such as Cl^- and Ac^- , and the corresponding hydrated ionic liquids were featured more towards individual ions in aqueous solution.^{48,59,69} Without strong association with anions, both $[\text{BMIM}]^+$ cations and Cl^-/Ac^- anions would severely penetrate DNA duplex through minor groove and strongly destabilize duplex structure, resulting T_m of duplex DNA in hydrated $[\text{BMIM}][\text{Cl}]$ and $[\text{BMIM}][\text{Ac}]$ below room temperature. Therefore, the binding of IL to DNA minor groove was strongly regulated together by IL cation and anion species. Anions with high affinity to imidazolium cation would apparently assist cations to achieve appropriate binding modes and affinity to duplex DNA, and as a result, facilitate electron transfer through duplex DNA.

Furthermore, time-resolved fluorescence decay assay is useful to reveal the microenvironment changes of base pair stacking within DNA duplex in hydrate ionic liquids. As shown in Figure S6, elongated fluorescence lifetime of O/GG₄ in the presence of IL indicated that binding of IL to DNA minor groove resulted in a limited rotation of the TO central methine bridge. This could be an indirect proof of restricted dynamic motions and less flexible stacking of DNA base pairs by IL. Rigid base stacking in IL might result in either higher percentage of or longer lifetime of duplex DNA residing in ET-active conformation domains transiently formed over A-tract bridge, which would allow more efficient charge transport and show the inverse distance dependence of ⁸CPG decomposition in hydrated IL. Longer DNA bridge would allow higher numbers of IL molecules to bind duplex DNA along minor grooves of double helix and cause an additively larger effects on locking base pair stack into ET-active conformation, which resulted in more efficient electron migration over longer stem of duplex DNA. In hydrated IL, though DNA ET still showed oscillatory efficiencies with a similar period as in aqueous solution, the amplitude was greatly attenuated. Since fast ET and back-electron-transfer (BET) could attenuate the amplitude of the periodic distance dependence when coherent ET

outcompetes incoherent hopping along electron migration, dampened oscillatory amplitude observed in hydrated IL would suggest A-tract achieved more coherent ET in ET-optimal conformation upon binding of IL molecules.^{70,71}

Recently, higher rate of hole transport was reported for DNA-lipid complex in chloroform and suggested that reduced water interaction maybe favorable to DNA ET.⁷² In hydrated IL, DNA molecules are under a dehydrated condition compared to that in aqueous buffer, since ionic liquid can exclude water molecules from the hydration layer surrounding DNA duplexes. Studies have shown that water molecules might play a key role in hydration of ionic liquids.⁵⁷⁻⁶⁰ Cations of ionic liquids with hydrophobic anions, like [BMIM][BF₄], associate with the anions via H-bonding using H₂O as medium. Hence such ionic liquids may not dissociate to free cations and anions even after extensive dilution by water.⁵⁷⁻⁶⁰ These studies are consistent with our observation here that IL effects on DNA ET were modulated by both cations and anions of ionic liquids. The fact that only IL with hydrophobic anions but not hydrophilic Cl⁻ and Ac⁻, can accelerate DNA ET, implied the involvement of water molecules in the mechanism. IL-assisted DNA ET were also better pronounced in deuterated water due to the stronger H-bonds formed within network among D₂O, IL and DNA. Invasion of IL ions into hydration layer and limitation of water interactions would reduce the flexibility of duplex DNA and promote the possibility of base pair array to stay in ET-active conformation, which might result in elevated efficiency in DNA-mediated charge transport. The current results are consistent with theoretical work,^{48,56} suggesting that depletion of water from DNA and binding ionic liquids to the minor groove of duplex DNA could be the two major reasons accounting for the assistances of IL to DNA mediated ET.

CONCLUSION

In summary, higher yields of DNA ET were observed in hydrated ionic liquid. Contrary to the decay of yields over longer duplex DNA in aqueous buffer, DNA ET over 4.1 nm showed

inverse distance dependence in hydrated [BMIM][BF₄], with an oscillating enhancement of ET efficiency along an A-tract bridge. Facilitation of ET efficiency is closely associated with cation and anion species of ionic liquid. ILs with aromatic cations ([BMIM]⁺ and [EtP]⁺) have better facilitation on electron transfer efficiency than those with non-aromatic cations ([BMP][BF₄]). Hydrophobic anion [BF₄]⁻ modulated the binding of ionic liquid cations to DNA minor groove, while ionic liquids with hydrophilic anions (Cl⁻, Ac⁻) disrupt duplex structure and inhibit ET. Binding of ionic liquid to DNA minor groove was not limited to the cation component, but also involved the anions. Additionally, D₂O-[BMIM][BF₄], resulted in better pronounced ⁸CPG decomposition than H₂O-[BMIM][BF₄], suggesting that tight binding to minor groove to exclude water molecules might account for the facilitation effects from ionic liquids. Our study has revealed novel phenomena of the interactions between highly charged DNA molecules and ionic liquids, and promoted reactivity of bio-redox reactions in hydrated ionic liquids. Elevation of DNA ET in hydrated ionic liquid not only provides a novel strategy to achieve efficient biomolecular electronic wire for nanoscale devices, but also broadens knowledge in the redox reaction of biological macromolecules in novel ~~green~~ solvents with non-organic/non-aqueous features.

METHODS

Oligonucleotides Preparation

All oligonucleotides were synthesized on a MerMade 4 DNA synthesizer (BioAutomation). Phosphoramidites of natural nucleotides and anthraquinone-5-ethynyl-dU (Q) were purchased from Glen Research and Berry & Associates. ⁸CPG phosphoramidite was prepared by following the protocol developed at our laboratory.²⁷ Thioazole orange (O) tethered oligonucleotide was prepared as reported.⁷³ To ensure high coupling yield, elongated coupling time (5 min) was adopted for incorporating unnatural bases and fluorophore into DNA oligonucleotides. The

synthetic DNA oligonucleotides were cleaved and deprotected via incubation in concentrated NH_4OH at 75 °C for 17 h or AMA (1:1 v/v mixture of concentrated NH_4OH and 40% aqueous methylamine) at 37 °C for 2 h, followed by HPLC purification twice and the molecular weights were verified by ESI-MS (Sangon Biotech, Shanghai). Concentration of DNA oligonucleotides was determined by UV-Vis absorption at 260 nm (Shimadzu UV-1800 UV-Vis spectrophotometer).

General Methods for Photo-irradiation

40 μM of duplex DNA (40 mM PBS, 100 mM NaCl, pH 7.4) were annealed by heating at 90 °C for 5 min and cooling to room temperature in 2.5 h. To the DNA solution was added 4 M IL aqueous solution to obtain the DNA sample in IL for photo-irradiation (final concentration: 20 μM DNA in 20 mM PBS, 50 mM NaCl, and 2 M IL). Ionic liquids used in experiments were purchased from Sigma-Aldrich and used as delivered. As shown in Figure 1, ILs included 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF₄], 1-ethylpyridinium tetrafluoroborate [EtP][BF₄], 1-butyl-1-methylpyrrolidinium tetrafluoroborate [BMP][BF₄], 1-butyl-3-methylimidazolium acetate [BMIM][Ac] and 1-butyl-3-methylimidazolium chloride [BMIM][Cl].

DNA sample aliquots (30 μL) were transferred to 1.5 mL microtubes and irradiated at 350 nm for 45 s, unless indicated otherwise. A 450 Watt Illuminator (HORIBA Jobin Yvon) with a monochromator and a 320 nm long-pass filter was used for all the photo-irradiation experiments. After irradiation, DNA samples were diluted with deionized water (70 μL) and transferred to dialysis units (Thermo Scientific, #69553, 2K MWCO). Ionic liquid was removed by overnight dialysis. And then, samples were dried by lyophilization and digested to nucleosides by alkaline phosphatase (New England Biolabs) and phosphodiesterase I (i-DNA biotechnology) via incubation at 37 °C for 18 h. The resulting nucleosides were analyzed by HPLC (column and method details). Oxidative decomposition of ⁸CPG (Y) was determined by:

$$Y = \left(1 - \frac{A_{irr}}{A_{non-irr}}\right) \times 100\% \quad \text{Eq 2}$$

where A_{irr} and $A_{non-irr}$ are normalized peak area of ^{8CP}G in irradiated and non-irradiated samples from HPLC trace (peak areas were normalized to that of dT in the same trace). Standard deviation over three repeated experiments was used as error of the data.

FID Assay

Fluorescence indicator displacement (FID) assay was performed on a Shimadzu RF-5301PC spectrofluorophotometer. The concentration of DNA duplex was kept as 1.5 μM with 20 mM PBS, 50 mM NaCl, pH 7.4. The DNA samples were heated to 90 $^{\circ}\text{C}$ for 5 min and cooled down to room temperature in 2.5 h. To DNA was added 2.67 molar equivalent 4',6-diamidino-2-phenylindole (DAPI, 4 μM) according to binding the stoichiometry. Ionic liquids were titrated to DNA (from 0 to 2 M) to displace the binding dye. The samples were mixed for 30 min at 25 $^{\circ}\text{C}$ before measurement. Emission spectrum of DNA-DAPI (excitation at 358 nm) was recorded. Three repeated experiments were conducted for each data point. DAPI fluorescence decrease percentage (f) was corrected by the control:

$$f = \frac{I - I_{DNA}}{I_{DNA} - I_{DNA}} \times 100\% \quad \text{Eq 3}$$

where I_{DNA} , I and $I - DNA$ are integrations of DAPI fluorescence (370-600 nm) in DNA+PBS, DNA+PBS+IL and PBS+IL; f is percentage of DAPI that is displaced from duplex DNA by ionic liquid. DC_{50} , the concentration of ionic liquid needed to displace 50% DAPI from DNA minor groove, was obtained from the FID curve.

Circular Dichroism

DNA samples (100 μL) in various hydrated IL were prepared by the same protocols as described in photo-irradiation assay. Circular dichroism spectra of each DNA sample were obtained by averaging five repeated measurements on a JASCO J-1500 CD spectrometer with scanning rate at 50 nm/min.

Fluorescence Melting of DNA Duplexes

The thermal stability of DNA was studied on a Roche real-time PCR cycler (LightCycler 480 II) with 465/510 nm filter set. Samples (20 μ L) containing 1.5 μ M DNA, 20 mM PBS, 50 mM NaCl, pH 7.4 with/without ionic liquids were annealed as described in photo-irradiation assay. Fluorescence denaturing curves of duplexes, O/GG₄, in various hydrated IL were monitored from 35 °C to 90 °C with a ramp of 0.6 °C/min. Melting points of DNA duplexes were determined as the maximum of the first derivative of the denaturing curves and averaged by three repeated records.

Fluorescence Lifetime

The time-resolved fluorescence decay assay was performed on a time-correlated single photon counting (TCSPC) spectrofluorimeter (FluoroCube, Horiba Jobin Yvon) with a pulsed diode laser (NanoLED-470L, Horiba Jobin Yvon). Samples containing duplex DNA O/GG₄ (3 μ M, with 20 mM PBS, 50 mM NaCl) and 2 M corresponding ionic liquid were excited at 466 nm and the fluorescence emission of O was monitored at 530 nm. Horiba Jobin Yvon Datastation software was used to analyze the results with consideration of the reduced chi-square value and the randomness of the weighted residuals. The fluorescence decay curves were fitted to multiple exponential decay function with χ^2 values around 1. The mean fluorescence lifetime τ was calculated as $\tau = \sum \tau_i^2 A_i / \tau_i A_i$, ($i = 1, 2, 3$).

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Author contributions

S.X., Z.M., and F.S. designed the experiments, analysed the data and wrote the manuscript. J-R. W. and G.D. contributed to the synthesis of TO-DNA. X.W., E.K.L.Y. measured life-time

of TO-DNA in ionic liquids. All authors discussed the results and commented on the manuscript.

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SUPPORTING INFORMATION

Reagents and materials; Table S1 of melting temperatures of duplex DNAs; Figures S1-S6 of IL concentration effects, charge transport yields, IL effects on dynamic structures of duplex DNA.

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Efficient DNA-mediated Electron Transport in Ionic Liquids

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The enhancement of ET efficiency in hydrated ionic liquid.

Ionic liquid, as a sustainable storage media for biomolecules, may help to further understand the principle of electron transport in nucleic acids.