DNA Technology

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Sequence-Dependent Photocurrent Generation through Long-Distance Excess-Electron Transfer in DNA

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Abstract: *Given its well-ordered continuous π stacking of nucleobases, DNA has been considered as a biomaterial for charge transfer in biosensors. For cathodic photocurrent generation resulting from hole transfer in DNA, sensitivity to DNA structure and base-pair stacking has been confirmed. However, such information has not been provided for anodic photocurrent generation resulting from excess-electron transfer in DNA. In the present study, we measured the anodic photocurrent of a DNA-modified Au electrode. Our results demonstrate long-distance excess-electron transfer in DNA, which is dominated by a hopping mechanism, and the photocurrent generation is sequence dependent.*

DNA has attracted attention as a biomaterial for charge transfer (CT) media since the first study examining DNA conductivity.[1] Recently, electrochemical studies on CT in DNA have involved the development of DNA sensors, which are highly sensitive to DNA structures such as mismatches and lesions that perturb the π stacking between base pairs in vitro.[2] Furthermore, as observed in state-of-the-art organic solar cells, photon-to-electron conversions in π-stacking multichromophores have provided a new mode for signal transduction in DNA sensors based on photoelectrochemistry.[2c] Additionally, DNA may be used as a scaffold for building a one-dimensional chromophore array that can conduct electrons efficiently.[3] From more fundamental and biological perspectives, an understanding of oxidative CT, i.e. hole transfer (HT), is important for explaining remote DNA damage.[4] By contrast, reductive CT, i.e. excess-electron transfer (EET), can be regarded as a key process for repair of damaged DNA such as T–T lesions.[5] Thus, understanding the mechanism of CT in DNA provides important biological insight, which may be useful for improving current devices.

For studies of CT in DNA, photoelectrochemical methods using a DNA-modified electrode are powerful because of the high sensitivity and mild conditions involved, which maintain the structure of DNA, such as its orientation.[2c,6] To date, several groups including us have studied cathodic photocurrent generation in DNA-modified electrodes to investigate the sequence dependence of HT in DNA.[7] However, to the best of our knowledge, long-distance EET in DNA has not yet been examined using these methods. For EET in DNA, thymine (T) and cytosine (C) are considered to be electron carriers from an energetic perspective.[8,9] Although the sequence dependence of EET in DNA has been clarified by using product analysis methods[9a–c] and laser flash photolysis of donor–DNA–acceptor systems with short sequences,[9d,e] long-distance EET in DNA has not been observed, and ambiguity remains. In this work, we prepared three kinds of ethylenedioxythiophene trimer (3E)-modified DNA oligomers (ATn, CT6, and GT6; Figure 1) to evaluate sequence-dependent photocurrent generation resulting from long-distance EET in DNA.

DNA oligomers were synthesized and confirmed by MALDI-TOF MS and HPLC analysis (Table S1 and Figure S1 in the Supporting Information). For all DNA oligomers, the formation of a B-type duplex structure under the experimental condition was confirmed by circular dichroism (CD) and melting temperature (T_m) measurements (Figures S2, S3 and Table S1). Steady-state absorption spectra of all of the DNA oligomers (Figure S4) showed absorption bands for 3E (390 and 410 nm) as well as for the nucleotides (around 260 nm). As shown in Figure 1b, the DNA oligomers were activated by cleaving the disulfide linkage with dithiothreitol. Subsequently, upon immersing an Au electrode into the DNA solution, the activated DNA oligomers were immobilized on the surface of the Au electrode to generate a DNA-modified electrode (for details, see the Supporting Information). To determine the surface coverage of DNA on the electrode, we performed chronocoulometry with $[Ru\text{-}(NH_3)_6]^{3+}$ as a redox label, as established by Tarlov et al.[10] The surface coverage of the DNA oligomers ranged from 2.4 pmol cm^{-2} to 3.6 pmol cm^{-2} (Table S1). Moreover, DNA modification on the electrodes was confirmed by differential pulse voltammetry (DPV), which showed an oxidation peak at around

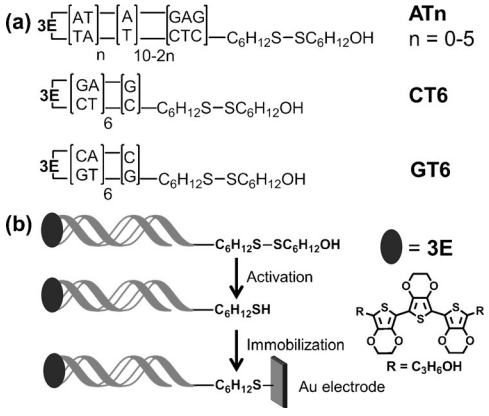

Figure 1. a) The DNA oligomers used in this study. 3E is trimer of ethylenedioxythiophene b) Preparation of the DNA-modified electrode.

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0.42 V corresponding to the generation of a distinct radical cation of 3E ($3E^{\bullet+}$; Figure S5).[11]

Photocurrent measurements of the DNA-modified electrode were performed using a xenon lamp (300 W) equipped with a band-pass filter ($\lambda > 400 \pm 10$ nm) to excite 3E only. To avoid changes in DNA morphology, a potential of 200 mV vs. Ag/AgCl was applied.[6] A stable anodic photocurrent appeared upon irradiation of the DNA-modified Au electrode (Figure S6). Moreover, we found that the DNA oligomers were stable during the photocurrent measurements for more than 100 s (Figure S6) under these conditions.[6] The photocurrents generated are given in Figure 2.

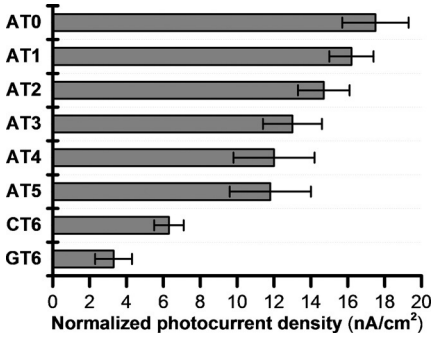

Figure 2. Normalized photocurrent density of the DNA-modified electrode. Error bars represent the standard deviation from five experiments.

As seen in Figure 2, the normalized photocurrent density (I_P) showed significant sequence dependence. In ATn, the I_P clearly decreased as the number of alternating A:T base pairs (n) increased. This tendency agrees with those of our previous study based on laser flash photolysis.[9d] By contrast, significant suppression of I_p was observed with both CT6 and GT6, thus indicating that excess-electron hopping between T residues was affected by the insertion of a C or G residue.[9e]

A schematic energy diagram of photocurrent generation from the DNA-modified electrodes is shown in Figure 3. Photoexcitation of 3E yielded a charge-separated state, in other words, excess-electron injection from the singlet excited 3E (^{1}3E*), to T. The injected excess electron migrated through T and was then trapped by the Au electrode to yield a photocurrent. $3E^{\bullet+}$ was reduced by ascorbic acid (AA) as a sacrificial electron-donating agent to regenerate 3E. The redox levels of the components are presented in Figure 3.[8,11,12] The significant sequence dependence of the photocurrent generation suggests that T-to-T hopping is a dominate mechanism for EET in DNA. Laser flash photolysis studies indicated that fast charge recombination

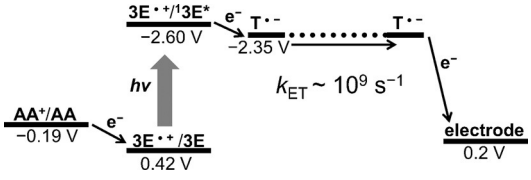

Figure 3. Energy diagram of photocurrent generation in the DNA-modified Au electrode in the presence of ascorbic acid (AA). Potential vs. Ag/AgCl.

is a major factor that leads to low EET efficiencies.[9d,e] Here, this issue was solved by AA, which regenerates 3E from $3E^{\bullet+}$ for long-distance EET in DNA.

In Table 1, the relative quantum yields of EET estimated from I_P are summarized. The rate constant of EET (k_{ET}), which corresponds to the reciprocal of the lifetime of the

Table 1: Normalized photocurrent density (I_P) and the relative quantum yield of EET (Φ_{rel}) in DNA-modified electrodes.

DNA	I_P[a] [nA cm^{-2}]	Φ_{rel}[b]	DNA	I_P[a] [nA cm^{-2}]	Φ_{rel}[b]
AT0	17.5 ± 1.8	1.00	AT4	12.0 ± 2.2	0.69
AT1	16.2 ± 1.2	0.93	AT5	11.9 ± 2.2	0.68
AT2	14.7 ± 1.4	0.84	CT6	6.3 ± 0.8	0.36
AT3	13.0 ± 1.6	0.74	GT6	3.3 ± 1.0	0.19

[a] Normalized to coverage of DNA oligomers on the electrode of 1 pmol cm^{-2}. [b] Yield of EET relative to that of AT0 based on I_P.

injected excess electron on DNA, was estimated from Φ_{rel} and the k_{ET} value for AT0, which was calculated from a previously reported value (see Table S2). The observed k_{ET} values reflect the hopping rate of excess electrons. For example, in the case of AT5, on the basis of the random walk model (Equation S1 in the Supporting Information), the T-to-T hopping rate (k_{hop}) can be estimated as 1.5×10^{11} s^{-1}, which agrees with our previously reported value (1.1×10^{11} s^{-1}) for interstrand EET in alternating A:T sequences.[9d] The consistency in the rate constants obtained from laser flash photolysis and the photoelectrochemcal technique indicates that migration of a localized nucleobase radical anion based on structural fluctuation is plausible for EET in DNA, besides the migration of a polaron.[9c,d,13]

Dramatically lower Φ_{rel} and k_{ET} values were obtained for CT6 and GT6. In Figure 4 a–d, the present observations are explained based on hopping and tunneling mechanisms. For EET in CT6 and GT6, tunneling indicates ET from T to T over an energy barrier formed by C or G. Even for these, forward and backward ET processes are included in the apparent EET, thus, the ET rate from T to T has to be determined with a random-walk equation. As shown in Figure 4 a, C was assumed to be an electron carrier in CT6 because of slight differences in the reduction potentials

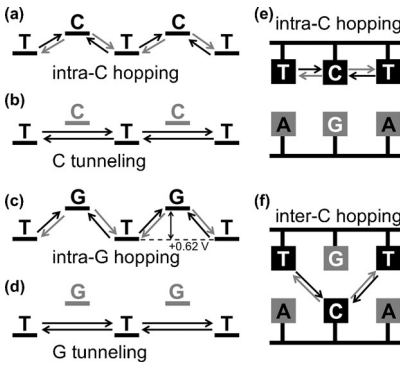

Figure 4. a–d) Proposed schematic energy diagram of EET in CT6, where C acts as a carrier (a) or a spacer (b), and in GT6, where G acts as a carrier (c) or a spacer (d). e, f) an illustration of EET in CT6 (e) and GT6 (f) through a hopping mechanism.

between T and C (0.09 V).[8] However, the upward electron hopping may affect the efficiency of EET in CT6, thus resulting in lower Φ_{rel} and k_{ET} values than those of AT0. In addition, C can be assumed to be a spacer (energy barrier) for tunneling, as shown in Figure 4b. For GT6 (Figure 4c), upward electron hopping from T to G is unlikely owing to the large energetic difference (0.62 V),[8] which excludes the possibility that G acts as an electron carrier. By contrast, the assumption of tunneling through G is plausible (Figure 4d). However, similarly to intrastrand EET in CT6, interstrand EET in GT6 is also possible (Figure 4e,f). We found that the Φ_{rel} value for GT6 (0.19) was smaller than that for CT6 (0.36) by a factor of 0.53 because of insufficient interaction between the LUMOs of nucleobases. The estimated factor is similar to our present results for interstrand and intrastrand EET from AT5 through a hopping mechanism (0.68) and almost consistent to our previous results obtained by laser flash photolysis for interstrand and intrastrand EET via an A:T sequence through a hopping mechanism (0.50).[9d] C can thus be an electron carrier for EET through a hopping mechanism when a single G:C base pair is inserted between consecutive T residues, although multiple G:C pairs in Ts completely terminate EET in DNA through proton transfer from G to $C^{\bullet-}$ in the $G:C^{\bullet-}$ base pair.[9e]

Given that long-distance CT in DNA is a fundamental process not only in biology but also in materials sciences, the estimated stepwise hopping rate (ca. $10^{10-11}\,s^{-1}$, see the Supporting Information) must be converted into charge mobility (μ). We found that the evaluated rate corresponded to approximately $10^{-3}\,cm^2V^{-1}s^{-1}$, which is higher than that of HT (ca. $10^{-5}\,cm^2V^{-1}s^{-1}$).[14] Therefore, with respect to bioelectronics, EET is more promising for use in applications than HT, which is related to DNA damage.[4] These results also provide new insight for the development of improved optoelectronic devices, including DNA sensors.

To the best of our knowledge, this is the first time a photoelectrochemical technique has been applied to study the sequence dependence of long-distance EET in DNA (40.8 Å). In addition, we found that photocurrent generation in DNA-modified Au electrodes is dominated by sequence-dependent EET in DNA, which is caused by the difference in reduction potential and π stacking of the nucleobases. We expect that this photoelectrochemical technique will facilitate investigations into EET in DNA and its further application.

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