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Long-Range Charge Transfer through DNA by Replacing Adenine with Diaminopurine

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Abstract: A positive charge migrates along DNA mainly via a series of short-range charge transfer (CT) processes between G-C base pairs, which have relatively high HOMO levels. As such, the CT efficiency sharply decreases with the insertion of A-T base pairs between the G-C base pairs. We have previously demonstrated that the CT efficiency through DNA can be dramatically increased by using deazaadenine (Z), an analogue of A, to adjust the HOMO levels of the A-T base pairs closer to those of the G-C base pairs (*Nat. Chem.* **2009**, *1*, 156). In the present study, we have expanded this approach to show that the CT efficiency can also be increased by replacing A bases with diaminopurine (D).

Introduction

DNA consists of two building blocks, adenine-thymine (A-T) and guanine-cytosine (G-C) base pairs. By programming the sequences of these two building blocks, we can now construct various nanometer-sized two- and three-dimensional structures.[1−3] The finding that a positive charge (a hole) generated in DNA migrates along DNA has made it an interesting molecule for the design of nanoelectronic sensors and devices.[4−9] It has been demonstrated that a charge migrates along DNA mainly via a series of short-range charge transfer (CT) processes between G-C base pairs, with their relatively high HOMO levels.[10−23] As a consequence, the CT efficiency sharply decreases with the insertion of A-T base pairs between the G-C base pairs,[5,21] hampering the construction of nanoelectric sensors and devices in which the use of various sequence patterns is indispensable. We have previously reported that the CT efficiency can be drastically increased independent of the G-C content by replacing A-T with Z (deazaA)-T, which has higher HOMO levels closer to those of G-C base pairs than of A-T base pairs.[24] It was also demonstrated that, by replacing A-T base pairs with Z-T base pairs, it is possible to distinguish between matched and mismatched sequences by measuring the CT rate constant through the DNA, which enables the detection of single-nucleotide polymorphisms (SNPs) in various sequence patterns especially effective in A-T-rich DNA. However, though SNP detection requires polymerase chain reaction (PCR) amplification of the genomic region that flanks the SNP site, Z containing DNA can only be amplified for a specific sequence[25] or in the presence of dATP[26,27] during the PCR. In the present study, we have expanded on our earlier work and clearly show that the substitution of A with diaminopurine (D), which can fully replace A during PCR,[28] also dramatically increases the CT efficiency in DNA.

Experimental Section

DNA Synthesis. The cyanoethyl phosphoramidites of *N*-(3-hydroxypropyl)-1,8-naphthalimide and 10-(2-hydroxyethyl)phe-

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nothiazine were synthesized as previously reported.[29−32] All other reagents for DNA synthesis, including the phosphoramidite of D, were purchased from Glen Research. DNA was synthesized on an Applied Biosystems DNA synthesizer, purified by reversed-phase HPLC, and lyophilized. All the naphthalimide (NI)-, phenothiazine (PTZ)-, and D-modified DNA studied here was characterized by MALDI-TOF mass spectrometry (see the Supporting Information, Table S1), and their concentrations were determined by complete digestion with nuclease P1 and AP to 2′-deoxyribonucleosides. Duplex solutions (100 mM NaCl and 10 mM sodium phosphate buffer (pH 7.0)) were prepared by mixing equimolar amounts of the desired DNA complements and gradually annealing with cooling from 80 °C to room temperature.

Melting Temperature Measurements. The thermal denaturation profile was recorded on a JASCO V-530 spectrometer equipped with a Peltier temperature controller (ETC-505T). The absorbance of the DNA sample (at a strand concentration of 2 μM in 100 mM NaCl, 10 mM sodium phosphate (pH 7.0)) was monitored at 260 nm from 10 to 70 °C with a heating rate of 1 °C/min. The T_m value was determined as the maximum in a plot of $\Delta A_{260}/\Delta T$ versus temperature.

Laser Flash Photolysis. The nanosecond transient absorption measurements were performed using the laser flash photolysis technique.[20−24,33−41] Briefly, the third-harmonic oscillation (355 nm, fwhm of 4 ns, 8 mJ/pulse) from a Q-switched Nd:YAG laser (Continuum, Surelight) was used for the excitation light which was expanded to a 1 cm diameter. The light from a xenon flash lamp (Osram, XBO-450) was focused into the sample solution for the transient absorption measurement. Time profiles of the transient absorption in the UV−vis region were measured with a monochromator (Nikon, G250) equipped with a photomultiplier (Hamamatsu Photonics, R928) and digital oscilloscope (Tektronics, TDS-580D). The time profiles were obtained from the average of 16 laser shots. Rate constants were obtained on the basis of three independent experiments.

Results and Discussion

D is an analogue of A in which the C2 hydrogen atom of the purine ring is replaced by an amino group (Figure 1a). The melting temperatures (T_m) measured for a0, a5 (X = Z), and a5 (X = D) are shown in Table 1. While the replacement of A-T with Z-T is slightly destabilizing, replacement with D-T increases the thermostability of the duplex because the 2-amino group of D also participates in hydrogen bonding with T.[42] The HOMO level of D-T was calculated by Nakatani and Saito to be very close to that of G-C;[43] we therefore considered it to be a potential candidate as a charge carrier in DNA.[44,45] To prove that replacing A-T with D-T would increase the CT efficiency, we measured the CT rate through DNA using time-resolved transient absorption measurements. The photosensitizer NI was attached to six consecutive A-T base pairs at one end of the duplex to inject a charge onto the G nearest the NI via CT between A bases upon laser irradiation with a quantum yield of about 2%.[18,19,29] PTZ, which has a lower oxidation potential than G and D,[30] was attached to the other end of the duplex as a hole trap, and the CT through DNA was monitored by the formation of the PTZ radical cation ($PTZ^{\bullet+}$) with a peak around 520 nm (Figure 1a).[20−24] We first tested whether the charge can go through A-T tract sequences with the assistance of D. While the formation of $PTZ^{\bullet+}$ was not observed for a0 due to the slow CT across the five consecutive A-T base-pairs,[21,24] an absorption at 520 nm assigned to $PTZ^{\bullet+}$ emerged upon CT through DNA by replacing the A bases in the A-T tract with D (Figure 2). The CT rate dramatically increased with an increase in the number of replaced D bases. Interestingly, the CT rate increased more than 3 orders of magnitude by replacing all the A bases in the A-tract with D (a5 (X = D)). Though the charge moved slightly more slowly through the DNA replaced with D-T than through the corresponding DNA replaced with Z-T (Table 2, a2, a5 (X = Z)), these results clearly demonstrate that the CT efficiency through DNA can be drastically increased by merely replacing the C2 hydrogen atom of A with an amino group.

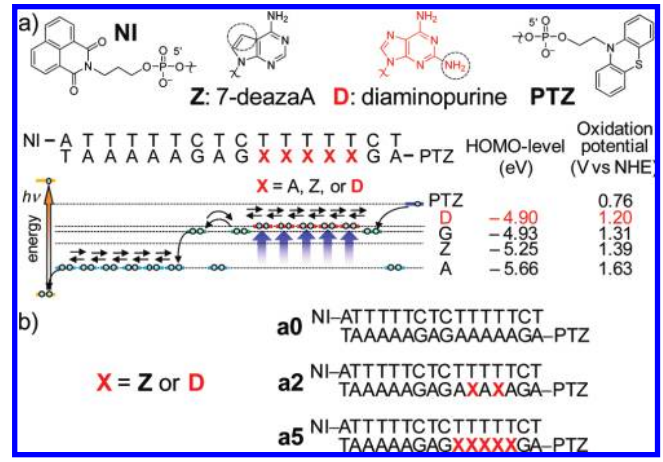

Figure 1. (a) Chemical structures of a photosensitizer naphthalimide (NI), 7-deazaadenine (Z), diaminopurine (D), and a hole trap phenothiazine (PTZ) and a schematic representation for charge injection by CT between A bases and long-range CT through DNA. The blue arrows represent the increase of the HOMO level upon the introduction of a 2-amino group into A. The HOMO levels of the base pairs are the values calculated at B3LYP/6-31G(d).[43] The oxidation potentials of G, D, A, Z, NI, and PTZ are derived from the reported values.[29,30,43] (b) Sequences of DNA used for T_m measurements and CT through the A-T tract.

Table 1. Melting Temperatures of Duplexes (T_m)

DNA	X	T_m (°C)
a0		48.2
a5	Z	41.6
a5	D	52.1

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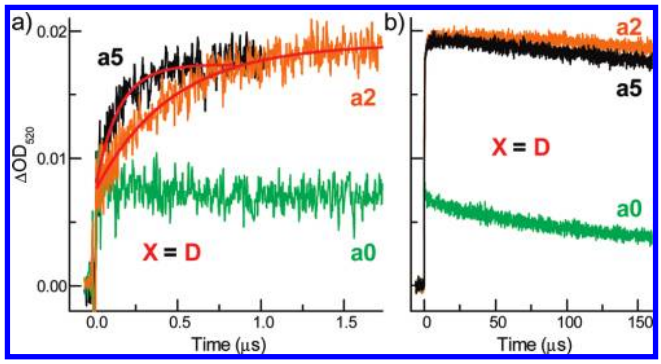

Figure 2. Time profiles of the transient absorption of $PTZ^{\bullet+}$ monitored at 520 nm during the 355 nm laser flash photolysis of DNA a0, a2, and a5 (X = D) on time scales of (a) ~2 μs and (b) ~200 μs. The smoothed red curves superimposed on the experimental data are the single-exponential fit. The sample aqueous solution contained 80 μM DNA and 100 mM NaCl in 10 mM sodium phosphate buffer (pH 7.0) purged with Ar.

Table 2. Time Constants (τ) and Rate Constants for CT through DNA (k_{ht})

DNA	X	τ^a (μs)	$k_{ht}{}^a$ (s^{-1})
a0		>200	$<5 \times 10^3$
a2	Z	0.22[b]	4.5×10^6 [b]
	D	0.46 ± 0.03	$(2.2 \pm 0.1) \times 10^6$
a5	Z	<0.05[b]	$>2 \times 10^7$ [b]
	D	0.14 ± 0.01	$(6.9 \pm 0.7) \times 10^6$
L	Z	0.45	2.2×10^6 [b]
	D	11 ± 1	$(9.1 \pm 0.1) \times 10^4$
L-1	Z	32	3.1×10^4 [b]
	D	110 ± 10	$(9.1 \pm 0.2) \times 10^3$
L-5	Z or D	>200	$<5 \times 10^3$
LAC	Z	45	2.2×10^4 [b]
	D	59 ± 2	$(1.7 \pm 0.1) \times 10^4$
LGT	Z	41	2.4×10^4 [b]
	D	110 ± 20	$(8.7 \pm 0.8) \times 10^3$

[a] The charge transfer rate through DNA ($1/\tau$) was determined from the single-exponential fit of the time profile of the formation of $PTZ^{\bullet+}$. Rate constants were obtained on the basis of three independent experiments. [b] Derived from the reported values.[24]

The CT also occurred over 100 Å through a G-C and A-T mixed nonrepeating sequence by replacing the A-T base pair with a D-T base pair (Figure 3, L (X = D)). The CT rate was approximately 20 times slower than that of the corresponding DNA replaced with the Z-T base pairs (Table 2, L (X = Z)). The slower CT rate observed for DNA with D-T base pairs may be partly explained by the charge localization at the DD site, similar to that seen in the GG sites,[46] because of the higher HOMO level of the D-T base pair compared with that of the G-C base pair (vide infra). Consistent with the results obtained for DNA with Z-T base pairs, the CT rate significantly decreased in response to converting one D-T base pair to an A-T base pair L-1 (X = D), and the CT rate become too slow to be observed by converting five D-T base pairs to A-T base pairs L-5 (X = D), showing that a fast CT through a mixed DNA sequence can only be achieved by replacing the A-T base pairs with either Z-T or D-T base pairs.

We also investigated the effect of the mismatch on the CT rate. The presence of a D-C mismatch (LAC) or G-T mismatch (LGT) in the DNA caused a considerable decrease in the CT rate (Figure 3c). The CT rate constants obtained for the DNA with a mismatch were on the same order for DNA with Z-T and the D-T base pairs, showing that CT across the mismatch

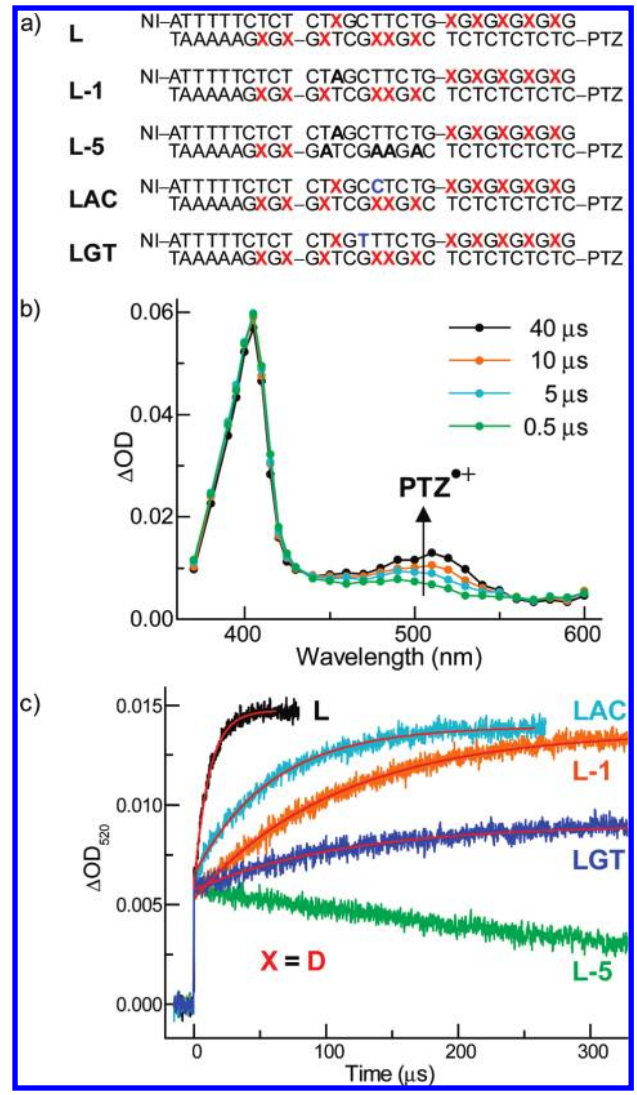

Figure 3. CT through G-C and A-T mixed nonrepeating sequences over 100 Å assisted by D. (a) Sequences of DNA. (b) Transient absorption spectra of L (X = D) obtained at 0.5, 5, 10, and 40 μs after the 355 nm laser flash excitation. (c) Time profiles of the transient absorption of $PTZ^{\bullet+}$ monitored at 520 nm during the 355 nm laser flash photolysis of L, L-1, L-5, LAC, and LGT (X = D). The smoothed red curves superimposed on the experimental data are the single-exponential fit. The sample aqueous solution contained 80 μM DNA and 100 mM NaCl in 10 mM sodium phosphate buffer (pH 7.0) purged with Ar.

is the major rate-determining step in these DNA sequences and that it likely reflects the disorder of the DNA structure caused by the mismatch.[23,47−53] These results clearly show that it is possible to distinguish between matched and mismatched sequences by measurement of their CT rates through DNA in which A-T base-pairs are replaced with either Z-T or D-T base-pairs.

To investigate whether a charge localizes at the DD site, the effect of a DD site on the charge recombination processes in

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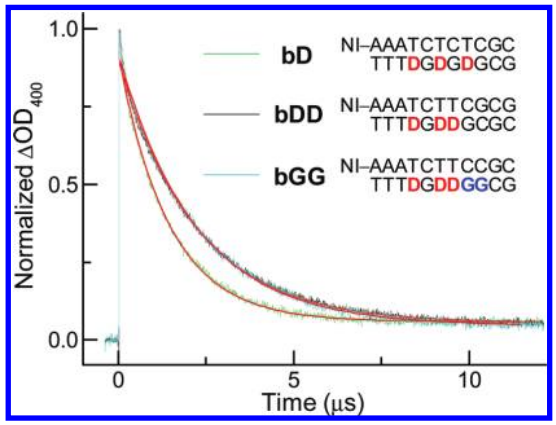

Figure 4. Time profiles of the transient absorption of $NI^{\bullet-}$ monitored at 400 nm during the 355 nm laser flash photolysis of an Ar-saturated aqueous solution of NI-modified DNA. The smoothed red curves superimposed on the experimental data are the single-exponential fit from which the lifetime of the charge-separated state (τ') was determined. The sample aqueous solution contained 80 μM DNA and 100 mM NaCl in 10 mM sodium phosphate buffer (pH 7.0) purged with Ar.

DNA was investigated. Without the hole trap PTZ at the other end of DNA, photoirradiation of NI results in a charge separation and charge recombination as shown in Figure 4.[37] Compared to DNA bD ($\tau' = 1.4$ μs), the presence of a DD site resulted in a slight increase in the lifetime of the charge-separated state (bDD; $\tau' = 2.2$ μs), showing that a charge partially localizes at the DD site distal from NI. On the other hand, the presence of an additional GG site did not affect the charge recombination dynamics in DNA (bGG; $\tau' = 2.2$ μs). Therefore, a charge localizes at the DD site rather than at the GG site in DNA consisting of G-C and D-T base pairs due to the higher HOMO level of the D-T base pair compared with that of the G-C base pair.[43] On the basis of the similar system except using stilbene-4,4′-dicarboxamide as a photosensitizer, Lewis and co-workers reported that the presence of a GG site causes a significant increase of the lifetime of the charge-separated state during the charge separation and recombination processes in DNA.[54−56] Thus, the depth of a DD hole trap in DNA consisting of G-C and D-T base pairs is much shallower compared to that of a GG hole trap in DNA consisting of G-C and A-T base pairs. This can be explained by the smaller HOMO energy difference between D-T and G-C base pairs than that between G-C and A-T base pairs.[43]

In summary, by replacing A-T base pairs with the D-T base pairs, we have further proven the concept that high CT efficiency through DNA can be achieved much less dependent on the G-C content by raising the HOMO levels of the A-T base pairs, making them closer to those of the G-C base pairs. In addition to the synthetic method,[24] D will offer us an additional choice in constructing DNA with increased CT efficiency on the basis of the PCR replication and amplification of DNA sequences of interest such as genomic DNA containing SNPs.

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Supporting Information Available: UV absorption spectra and thermal denaturation curve monitored during the T_m measurements and MALDI mass spectral data of all DNA studied in this investigation. This material is available free of charge via the Internet at http://pubs.acs.org.

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