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DNA Hairpins Containing a Diaminostilbene Derivative as a Photoinduced Electron Donor for Probing the Effects of Single-Base Mismatches on Excess Electron Transfer in DNA

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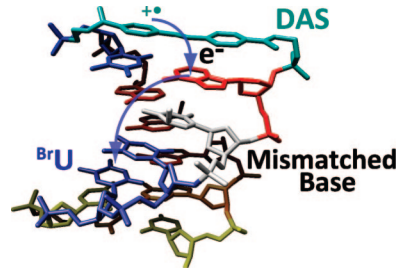
ABSTRACT



To investigate the effects of local structural disorder induced by a single-base mismatch on excess electron transfer (EET) in DNA, a novel hairpin DNA containing diaminostilbene (DAS) as a photoinducible electron donor has been developed. It was clearly demonstrated that EET efficiency depends on the electron injection modes from the electron donors and redox properties of the mismatched bases.

Ultrasensitive detection of single-nucleotide polymorphisms (SNPs) in DNA has been intensively explored because SNPs occur as often as every few hundred to every few thousand base pairs in genomic DNA. Among new technologies for detecting SNPs, electrochemical methods discriminating normal and mismatched base pairs in DNA have been recently developed.[1] Duplex DNA forms well-aligned π-stacking inside the helix, and hole transfer through a π-way mechanism is believed to be affected by structural disorder induced by mismatched base pairs.[2] Single-base mismatches can be detected by noting current differences at electrodes with attached DNA. It has been suggested that electron transfer efficiency through the LUMO (excess electron transfer, EET)[3] of DNA might also be mismatch sensitive.[4]

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In this context, we previously studied EET in DNA containing 5,6-dihydroxy-5,6-dihydrothymidine (thymidine glycol, Tg), which forms a less stable base pair with the complementary 2′-deoxyadenosine, to investigate radiation-induced reductive DNA damage mechanisms and demonstrated that excess electrons could be transported beyond the Tg/A site.[5] The results can be explained by the low electron affinity of Tg, slow decomposition of the corresponding radical anion, and the flexibility of the duplex structures. The third factor might be a critical issue for the investigation of the structural effects of mismatched base pairs on EET in the duplex because exact location of the electron donor cannot be determined. Because of our interest in this aspect and to explore the effect of modified DNA bases on EET in detail, here we developed a novel diaminostilbene (DAS)-capped hairpin DNA and undertook mechanistic investigation of EET through mismatched DNA bases.

Previously, we developed DNA duplexes tethered to phenothiazine (PTZ, $E^*_{ox} = -2.67$ V vs SCE) by a trimethylene linker for the investigation of reductive repair of Tg in DNA.[5] Similar assemblies were employed for quantification of EET from PTZ to an irreversible electron acceptor, 5-bromo-2′-deoxyuridine (^{Br}U),[3g,h] through intervening mismatched base pairs (DS, Figure 1). For comparison, we designed novel electron-donor−DNA conjugates (HP) to study the effects of structural distortion on EET (Figure 1). Similar hairpin structures bearing a stilbenediether ($E^*_{ox} = -2.53$ V vs SCE)[6] or a flavin ($E^*_{ox} = -2.6$ V vs NHE)[3c−e] as photoinducible electron injectors have been reported previously. Our newly developed electron-donor DAS shows absorption in the wavelength range 280−430 nm (Figure 2), has a high excited-state oxidation potential, $E^*_{ox} = -2.73$ V vs SCE,[7] which is comparable to that of PTZ, and does not require any additive for activation of the chromophore.[3c−e] Furthermore, the planar aromatic ring might allow for capping at one end of the duplex, leading to efficient electron injection because of orbital overlap between nucleobases and the chromophore.

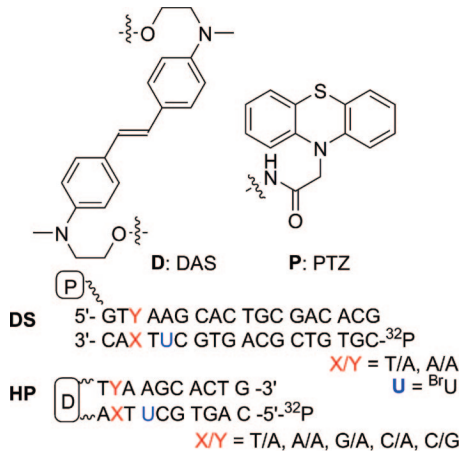

Figure 1. Sequences of oligodeoxynucleotides.

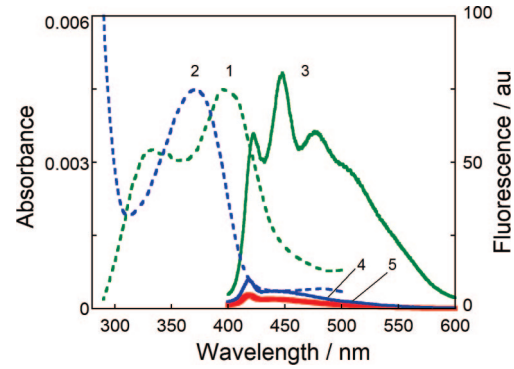

Figure 2. UV absorption (1 and 2) and fluorescence (3−5) spectra of 1 μM diaminostilbene derivative **2** in 0.1% acetonitrile/H_2O (1 and 3) and 1 μM HP [X/Y = T/A (2 and 5) and X/Y = A/A (4)] in phosphate buffer (90 mM phosphate, 10 mM NaCl). Fluorescence spectra were obtained by excitation at 365 nm.

The phosphoramidite of DAS was synthesized from the *t*-butyldimethylsilyl-protected benzaldehyde (**1**) in three steps (Scheme 1). Synthesis of *N*,*N*′-di(2-hydroxyethyl)-diaminostilbene derivative (**2**) from the nonprotected benzaldehyde by McMurry reaction has been reported previously.[8] However, the reported coupling reaction gave multiple products which were hardly isolable by flash chromatography. We thus attempted the synthesis of **2** starting from the benzaldehyde **1**, by which we could obtain **2** as an exclusive product. After dimethoxytrityl derivatization of **2**, the corresponding phosphoramidite derivative (**4**) was obtained by the reaction of **3** with *N*,*N*-diisopropylmethyl phosphonamidic chloride (DIMPACl). The diaminostilbene unit was introduced into DNA using a DNA synthesizer via conven-

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(7) Excited-state oxidation potential was calculated using $E^*_{ox} = E_{ox} - E_{00}$ with the oxidation potential of *N*,*N*,*N*′,*N*′-tetramethyl-4,4′-diaminostilbene (E_{ox}) 0.42 V (Kubota, T.; Uno, B.; Matsuhisa, Y.; Miyazaki, H.; Kano, K. *Chem. Pharm. Bull.* **1983**, *31*, 373–385) and zero excitation energy (E_{00}) evaluated from λ_{00} (= 394 nm) in aqueous solution

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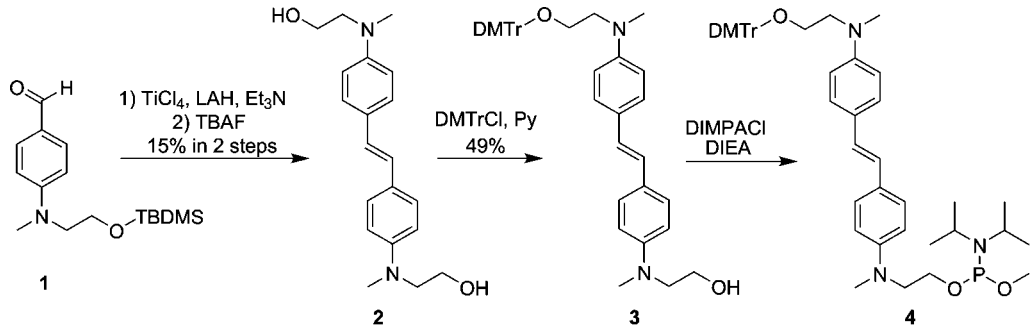
Scheme 1. Synthesis of DAS Phosphoramidite **4**



tional phosphoramidite chemistry. Slight modifications were made for the coupling/oxidation protocols. The coupling time was extended to 16.7 min for the coupling of diaminostilbene phosphoramidites. Because diaminostilbene undergoes oxidative degradation by aqueous iodine, (2*R*,8a*S*)-(+)-(camphorylsulfonyl)oxaziridine was used as an alternative oxidizing agent.[9] Incorporation of damaged base structures such as Tg into the same strand was unsuccessful because the synthesized strands were decomposed during purification by HPLC for unknown reasons, and thus we prepared sequences containing single-base mismatches between DAS and ^{Br}U (Figure 1).

Table 1. Electron-Transfer-Induced DNA Strand Cleavage at the 5′-Adjacent Base of ^{Br}U as Determined by Polyacrylamide Gel Electrophoresis[a]

	strand cleavage (%/h) (T_m/°C)			
X/Y	DS		HP	
T/A	0.9	(64.6)	47	(80.3)
A/A	5.8	(62.8)	32	(73.5)

[a] Initial strand cleavage rates were obtained by fitting cleavage curves (% scission product plotted against total material) to first-order exponential curves.

Thermal stabilities of the duplexes were evaluated by thermal-melting temperatures (T_m, Table 1). The prepared HP with a sequence common to those of DS (5′-TYAAG-CACTG/3′-AXT^{Br}UCGTGAC) formed thermally stable hairpin structures, even with the single-base mismatch A/A inserted in the sequence (T_m = 73.5 °C for HP(A/A), 62.8 °C for DS(A/A)). Upon photoexcitation of the chromophores in the DNA, the fluorescence from DAS was efficiently quenched and the fluorescence from PTZ in DS was moderately quenched (Supporting Information). The fluorescence intensity of DAS in HP was only slightly dependent on the identity of the intervening base (X = T or A) (Figure 2). These observations suggest the photoexcited DAS in the hairpin injects an electron to the terminus A/T base pair more efficiently than PTZ in DS.

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UV irradiation (365 nm) of DS and HP under an N_2 atmosphere and subsequent piperidine treatment at 90 °C yielded DNA fragments generated as a consequence of irreversible electron capture by ^{Br}U and subsequent strand cleavage at the 5′-adjacent nucleotide (Figure 3, Table 1).[5]

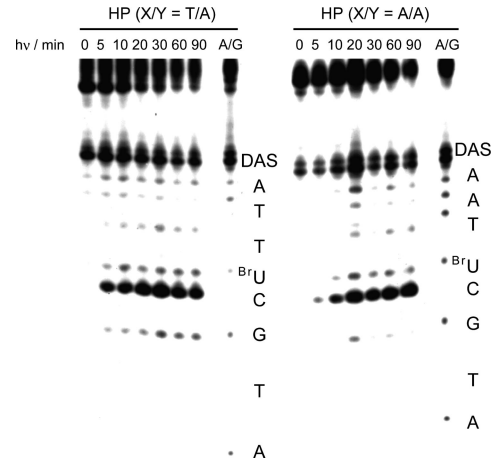

Figure 3. Polyacrylamide gel images of DNA hairpins HP. DNA (1 μM) in NaCl (90 mM) and phosphate buffer (10 mM, pH 7) were photoirradiated (365 nm, 4 °C) for 0−90 min under an N_2 atmosphere, followed by piperidine treatment (90 °C, 20 min). Strand cleavage bands at both the 5′- and 3′-sides of DAS were always observed after the piperidine treatment. A/G indicates A+G Maxam−Gilbert sequencing lane.

Low yield of strand cleavage in DS is probably due to the low absorption coefficient at 365 nm and poor electron injection from PTZ that is tethered to the duplex by a flexible linker. This observation is inconsistent with the results of fluorecence quenching above. An interesting finding was that EET efficiency through A/A in DS (5.8%/h) was much better than that through the A/T base pair (0.9%/h). In view of thermal stability of the duplexes, several reasons might account for these observations. Structural disorder around the mismatch may enable electron injection beyond the intervening bases. In addition, A might be redox-chemically insulating because of its low electron affinity and thus does not irreversibly trap excess electrons.

Conversely, electron transfer in HP sequences showed that EET through mismatch A/A base pairs was lower (32%/h) than that through T/A base pairs (47%/h) (Figure 3, Table 1). This could be explained by the reduction of electronic interaction between the nucleobases, as is often discussed in the mismatch effects on hole transfer reactions in DNA.[2] Photoirradiation was also examined under N_2O, which is known to be a hydrated-electron scavenger.[10] The effect of hydrated electrons was only limited since slight decreases in the yield to similar extents were caused for both DS and HP (Supporting Information). This result implies that excess electrons mainly injected from DAS or PTZ into DNA and migrate inside the duplexes of HP and DS, which is not affected by the types of the donor conjugations.

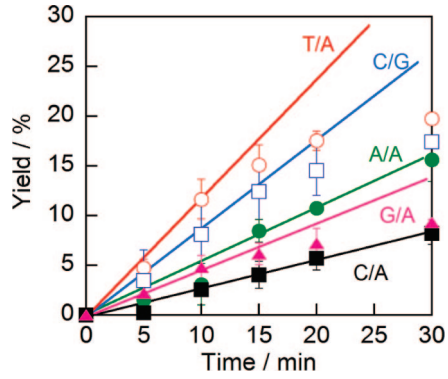

Figure 4. Electron-transfer-induced DNA strand cleavage at the 5′-adjacent base of ^{Br}U as determined by polyacrylamide gel electrophoresis. HP (1 μM, X/Y = (○) T/A, (□) C/G, (●) A/A, (▲) G/A, (■) C/A) in NaCl (90 mM) and phosphate buffer (10 mM, pH 7) were photoirradiated (365 nm, 4 °C) under an N_2 atmosphere, followed by piperidine treatment (90 °C, 20 min). Straight lines show initial fragmentation rates which were obtained by fitting cleavage curves to first-order exponential curves.

Figure 4 shows quantitative analysis of EET-induced strand cleavage for some selected HP sequences. EET through mismatched base pairs is always less efficient than full-matched sequences. It has been demonstrated that cytosine (C) in duplex DNA could be an electron trap because proton transfer to the corresponding radical anion ($C^{-\bullet}$) from the complementary G might be competitive to electron transfer between the nucleobases.[11] Our observation that EET through C-containing base pairs (C/G and C/A) was less efficient than that through other sequences clearly supports the mechanism. Considering that EET through C/A was suppressed to some extent, it is anticipated that $C^{-\bullet}$ acquires a proton not from the complementary A[12] but from the surrounding water to generate the neural radical ($CH^{\bullet}$).

In view of the linker length between the DNA and PTZ in DS, direct electron transfer to ^{Br}U might be less likely, unless the structures are drastically disordered. It has been shown that conformational changes could enhance hole migration in DNA,[13,14] and a similar mechanism could be applicable to EET through mismatched base pairs. However, the opposite EET sensitivities of our two types of the probes toward mismatch structures may suggest that the formation of electron-transfer active conformations is not a predominant factor in the present systems. Instead, electrons might be injected from PTZ in DS onto a nucleobase beyond the mismatch site because of the flexible linker and further migrate forward until being trapped by ^{Br}U. On the other hand, HP bearing mismatched bases can form duplex structures, and thus such direct electron injection should not be possible in HP. It is not difficult to imagine that the mismatch reduces the chance of back electron transfer to the PTZ-radical cation.

The results obtained in this study demonstrate that DNA-mediated EET efficiency can be quantified by the use of the DAS-hairpin DNA. In particular, DAS is highly reducing toward DNA bases including A ($E_{red} = -2.76$ V vs SCE),[15] which enable us to evaluate relationships between local structures of the duplexes and the EET reactivities. Investigation of EET through varieties of mismatched base pairs, including modified DNA bases, is now in progress in our group to understand the effects of EET on DNA damage formation induced by ionizing radiation exposure to DNA.

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Supporting Information Available: Experimental procedures, ^1H and ^{13}C NMR, ESI MS data, fluorescence spectra, and polyacrylamide gel electrophoresis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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