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Photoinduced electron injection into DNA by *N*-cyclopropyl-1-aminonaphthalene

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ABSTRACT

DNA containing either *N*-cyclopropyl-1-aminonaphthalene (cAN) or *N*-methyl-1-aminonaphthalene (mAN) as a photoinduced electron donor have been developed to investigate the electron injection and charge recombination processes in DNA duplex. Oxidation potentials of the photo-excited aminonaphthalenes (ANs) were estimated to be approximately −3.0 V vs. SCE, which are high enough to induce one-electron reduction of DNA bases. Photoinduced excess electron transfer in the AN-tethered DNA duplexes has been examined using gel electrophoresis, but the apparent electron-transfer efficiencies were almost the same for both of the cAN- and the mAN-tethered DNA. The marginal effect of cyclopropyl substitution on the efficiency of charge separation suggests that charge recombination in the contact radical ion pair is faster and more efficient than cyclopropane-ring opening.

1. Introduction

It has been demonstrated that DNA undergoes reduction by hydrated electrons generated in the radiation-induced ionization of water molecules [1] to afford reductively damaged base structures such as 5,6-dihydrothymine [2,3]. Thus far, many studies have been performed to elucidate the mechanism of DNA damage by radiation-chemically generated hydrated electrons or externally added photoinduced electron donors [2–6]. Site-specific injection of electrons into the DNA duplex has been attempted with the aim of investigating DNA-mediated excess electron transfer reactions; such site-specific injection can be accomplished by incorporating photoinduced electron donors into the DNA strand via covalent linkers [7–27]. In a previous study, we found that electrons injected from an external free donor can induce the reduction of 5,6-thymine glycol to thymine, but those injected from a covalently linked electron donor cannot. This difference can be explained by the rapid charge recombination of the injected electron and the radical cation formed from the internal electron donor. On the other hand, charge recombination between the excess electron and the unanchored donor radical cation is less likely [7]. Recent studies on excess electron transfer in DNA have suggested that back electron transfer occurs rapidly and efficiently, except when deep electron traps exist in the DNA sequences [21–23,26].

In the current study, we have developed DNA duplexes containing *N*-cyclopropyl-1-aminonaphthalene (cAN) as a photoinduced electron donor and investigated the effects of the *N*-cyclopropyl substitution on the apparent electron transfer efficiency through DNA; the radical cation of this donor can undergo spontaneous ring opening. We expected that the modification on the electron donor would not retard back electron transfer, if quite fast and efficient charge recombination process are involved in the reactions. The method using chemical probes to quantify the electron transfer products is useful for determining relative charge-transfer properties of DNA duplexes at a time and would provide complementary data to those obtained from kinetic analyses of the process using time-resolved spectroscopy techniques. *N*-Cyclopropyl-modified DNA bases have been previously used to trap holes migrating along DNA sequences [28–30].

2. Experimental

2.1. General methods

Reagents were purchased from various commercial sources and used without additional purification. NMR spectra were recorded on a JEOL JNM-AL 300 or a JEOL EX 400 spectrometer and chemical shifts are expressed in ppm relative to the residual signals of chloroform (δ = 7.24 in ^{1}H NMR and δ = 77.0 in ^{13}C NMR). Fast atom bombardment (FAB) mass spectrometry was performed with a JEOL JMS-SX 102A mass spectrometer, using nitrobenzyl alcohol as a matrix. Electrospray ionization (ESI) mass spectrometry

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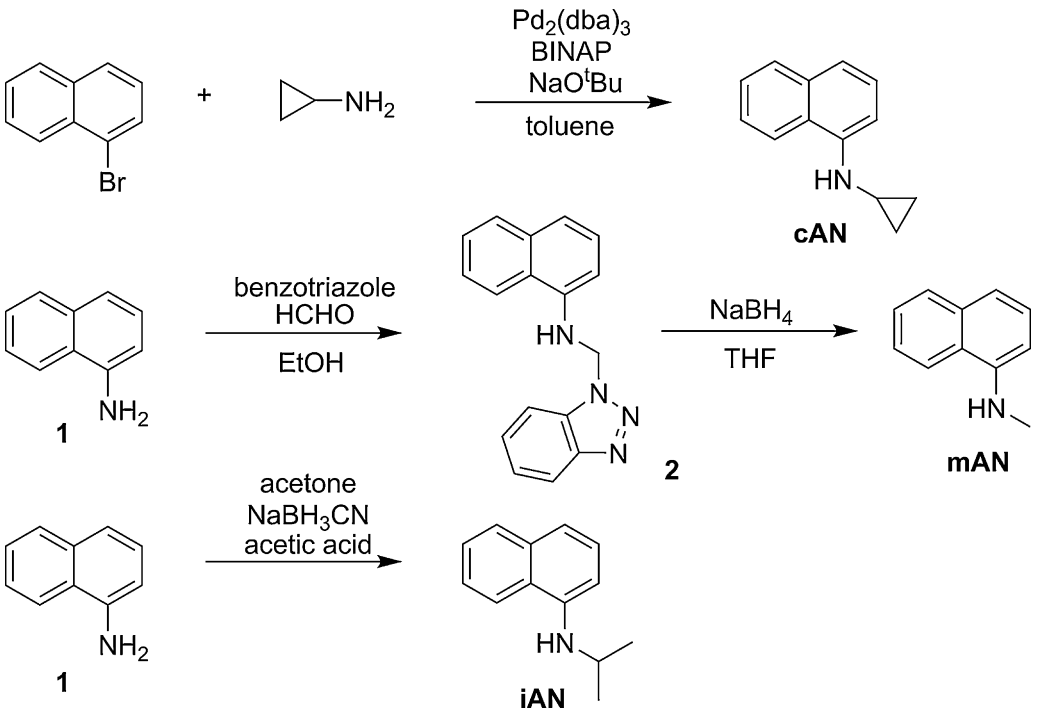


Scheme 1. Synthesis of *N*-alkylaminonaphthalene derivatives (cAN, mAN and iAN).

was carried out with a Bruker Daltonics microTOF spectrometer. DNA synthesis was performed with an Applied Biosystems 3400 DNA synthesizer. Analytical and preparative high-performance liquid chromatographies (HPLCs) were carried out with a Hitachi D2000 HPLC system equipped with a reversed phase column (Inertsil ODS-3, GL Science Inc., ϕ 4.6 mm × 250 mm). Fluorescence measurement was carried out on a Shimadzu RF-5300PC spectrofluorophotometer. UV–visible spectra were obtained using a JASCO V-530 UV/VIS spectrophotometer. Thermal melting of duplex DNA was observed with a JASCO UV–VIS Spectrophotometer V-530 by monitoring absorbance at 260 nm with a heating rate of 1 °C/min. The temperature at the midpoint of the transition was defined as the melting temperature (T_m). Solutions of oligodeoxynucleotides (1 μM) in 10 mM sodium phosphate and 200 mM sodium chloride (pH 7.0) were prepared to determine the melting temperatures. The voltammetric measurements were made under potentiostatic conditions with a three-electrode system consisting of an ALS/CH Instruments 660B electrochemical analyzer (Austin, TX), a platinum wire counter electrode, a platinum working electrode, and a saturated calomel reference electrode (BAS Inc., Tokyo). Aminonaphthalene derivatives (0.5 mM) in phosphate buffered saline (10 mM phosphate, 137 mM NaCl, and 2.7 mM KCl, pH 7.4) containing 1 vol% acetonitrile were used for the AC voltammetry measurements. The laser flash photolysis experiments were carried out with a Unisoku TSP-601 flash spectrometer. A Continuum Surelite-I Nd:YAG (Q-switched) laser with a emission at 355 nm was employed for the photolysis. The probe beam from a Hamamatsu 150 W Xe short arc (CA 263) was guided with an optical fiber scope to be arranged in an orientation perpendicular to the exciting laser beam. The probe beam was monitored with a Hamamatsu R2949 photomultiplier tube through a Hamamatsu S3701-512Q MOS linear image sensor (512 photodiodes). Timing of the exciting pulsed laser, the probe beam, and the detection system was achieved through a Tektronix model TDS 320 double channel oscilloscope that was interfaced to a Dell PC.

2.2. Synthesis (Scheme 1)

2.2.1. N-Cyclopropyl-1-naphthylamine (cAN) [31]

To a solution of 1-bromonaphthalene (1.47 g, 7.09 mmol) and cyclopropylamine (537 mg, 9.38 mmol) in 15 mL of anhydrous toluene were added tris(dibenzylideneacetone)dipalladium(0) (42.8 mg, 0.046 mmol), (±)-BINAP (58.8 mg, 0.094 mmol) and sodium *t*-butoxide (794 mg, 8.26 mmol). The mixture was stirred under an N_2 atmosphere at 80 °C for 4 h. The resulting mixture was then cooled, diluted with chloroform, filtered through Celite and concentrated under vacuum. The crude product was purified by silica gel column chromatography (30:1 chloroform/acetone) to give cAN (1.20 g, 92%) as a yellow oil.

2.2.2. N-(Benzotriazol-1-ylmethyl)-1-naphthylamine (**2**) [32]

To a solution of 1-naphthylamine (**1**, 1.77 g, 12.4 mmol) and benzotriazole (1.56 g, 13.1 mmol) in 40 mL of ethanol was added 37% aqueous formaldehyde (1.0 mL, 12.3 mmol). The solution was stirred under an N_2 atmosphere at room temperature for 1 h. White solid obtained after evaporation was used for the next reaction without purification.

2.2.3. N-methyl-1-naphthylamine (mAN) [32]

To a solution of compound **2** (1.77 g, 12.4 mmol) in 40 mL of tetrahydrofuran was added sodium borohydride (2.31 g, 61.0 mmol) over a 2-h period at 20 °C with vigorous stirring. The mixture was kept well-stirred for another 3 h. Solvent was removed under reduced pressure. The residue was extracted with ethyl acetate, washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by silica gel column chromatography (10:1 hexane/ethyl acetate) to give mAN (777 mg, 40%) as a pale pink oil.

2.2.4. N-isopropyl-1-naphthylamine (iAN) [33]

To a solution of 1-naphthylamine (**1**, 1.44 g, 10.0 mmol) and sodium cyanoborohydride (629 mg, 10.0 mmol) in 10 mL of acetone was added acetic acid (2.0 ml, 35.0 mmol) over a 5-min period.

The mixture was stirred at 0 °C for 2 h under an N_2 atmosphere. The solvent was removed under reduced pressure. The residue was neutralized with $NaHCO_3$ and extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by silica gel column chromatography (10:1 hexane/ethyl acetate) to give iAN (1.75 g, 94%) as a colorless oil.

*2.2.5. Ethyl (N-naphthalen-1-yl-amino)acetate (**3**)*

A solution of 1-naphthylamine (**1**, 2.03 g, 14.2 mmol) and ethyl chloroacetate (1.92 g, 15.7 mmol) in 5 mL of ethanol was stirred at 80 °C for 14 h under an N_2 atmosphere. The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (60:1 chloroform/acetone) to give **3** (1.61 g, 50%) as a red oil. ^{1}H NMR ($CDCl_3$) δ 7.90–7.93 (m, 1H), 7.79–7.83 (m, 1H), 7.44–7.49 (m, 2H), 7.27–7.37 (m, 2H), 6.47 (dd, 1H, J = 6.34, 0.98 Hz), 5.03 (broad, 1H), 4.22–4.32 (m, 2H), 4.04 (2H), 1.32 (3H); ^{13}C NMR δ 171.0, 142.2, 134.2, 128.5, 126.3, 125.8, 124.9, 123.4, 120.0, 118.1, 104.4, 61.3, 45.9, 14.1; HR-MS (FAB-pos) m/z calcd. for C14H16N1O2: 230.1181; found 230.1186 ($M+H^+$).

*2.2.6. Ethyl (N-cyclopropyl-N-naphthalen-1-yl-amino)acetate (**4**)*

To a solution of compound **3** (646 mg, 2.82 mmol), (1-ethoxycyclopropoxy)trimethylsilane (1.32 g, 7.56 mmol) and sodium cyanoborohydride (440 mg, 7.00 mmol) in 1.5 mL of anhydrous methanol was added acetic acid (1.23 g, 20.4 mmol). The mixture was stirred at room temperature for 18 h under an N_2 atmosphere [34]. The solvent was removed under reduced pressure. The residue was extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography (10:1 hexane/ethyl acetate) to give **4** (485 mg, 64%) as a bright red oil. ^{1}H NMR ($CDCl_3$) δ 7.87–7.90 (m, 1H), 7.61–7.63 (m, 1H), 7.36–7.41 (m, 2H), 7.21–7.28 (m, 3H), 3.89–3.94 (m, 4H), 2.97 (sept, 1H), 1.00 (t, 3H, J = 7.20 Hz), 0.48–0.52 (m, 2H), 0.36–0.40 (m, 2H); ^{13}C NMR δ 171.1, 147.2, 134.4, 128.9, 128.3, 125.2, 125.4, 125.6, 123.4, 123.2, 119.2, 60.1, 56.9, 33.4, 14.0, 8.5; HR-MS (FAB-pos) m/z calcd. for C17H20N1O2: 270.1494; found 270.1500 ($M+H^+$).

2.2.7. (N-Cyclopropyl-N-naphthalen-1-yl-amino)acetic acid succinimidyl ester (cAN-suc)

To a solution of compound **4** (485 mg, 1.80 mmol) in 3.5 mL of 1,4-dioxane was added 1 M aqueous sodium hydroxide (2.5 mL, 2.5 mmol). The solution was stirred at room temperature for 8 h. The solvent was removed under reduced pressure. The residue was acidized with saturated aqueous citric acid and extracted with ethyl acetate, washed with brine, dried over Na_2SO_4 and concentrated under vacuum. Obtained colorless oil was used for the next reaction without purification. To the product in 5 mL of anhydrous dimethyl sulfoxide were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (578 mg, 3.02 mmol), 1-hydroxybenzotriazole (397 mg, 2.93 mmol). After 30-min continuous stirring at 0 °C, *N*-hydroxysuccinimide (362 mg, 3.15 mmol) was added to the solution. The reaction mixture was stirred at room temperature for another 24 h. The reaction mixture was quenched by adding water, extracted with ethyl acetate, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography (50:1 chloroform/acetone) to give cAN-suc (185 mg, 31% in 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.94–7.97 (m, 1H), 7.81–7.84 (m, 1H), 7.39–7.60 (m, 5H), 4.38 (s, 2H), 3.11 (sept, 1H, J = 3.30 Hz), 2.76 (s, 4H), 0.71–0.78 (m, 2H), 0.58–0.60 (m, 2H); ^{13}C NMR δ 168.9, 166.1, 146.1, 134.6, 128.8, 128.5, 125.8, 125.7, 125.6, 124.4, 122.8, 119.7, 54.8, 33.4, 25.5, 8.71; HR-MS (FAB-pos) m/z calcd. for C19H19N2O4: 339.1345; found 339.1340 ($M+H^+$).

*2.2.8. Ethyl (N-methyl-N-naphthalen-1-yl-amino)acetate (**5**)*

Starting with the ethyl ester **3** and formaldehyde, the same procedure as for **4** gave (methyl-naphthalen-1-yl-amino)acetic acid ethyl ester **5** (yield 94%) as a bright red oil. ^{1}H NMR ($CDCl_3$) δ 8.23 (dd, 1H, J = 7.56, 0.73 Hz), 7.82 (dd, 1H, J = 9.03, 1.95 Hz), 7.37–7.56 (m, 4H), 7.19 (d, 2H, J = 9.03 Hz), 4.21 (q, 2H, J = 7.08 Hz), 3.93 (s, 2H), 3.04 (s, 3H), 1.26 (t, 3H, J = 7.08 Hz); ^{13}C NMR δ 170.8, 148.7, 134.8, 128.6, 128.3, 125.7, 125.5, 125.4, 123.6, 123.4, 115.9, 60.6, 58.7, 41.7, 14.2; HR-MS (FAB-pos) m/z calcd. for C15H18N1O2: 244.1337; found 244.1331 ($M+H^+$).

2.2.9. (N-Methyl-N-naphthalen-1-yl-amino)acetic acid succinimidyl ester (mAN-suc)

Starting with **5**, the same procedure as for cAN-suc gave mAN-suc as a brown oil. ^{1}H NMR ($CDCl_3$) δ 8.19 (dd, 1H, J = 8.08, 1.47 Hz), 7.83 (dd, 1H, J = 8.52, 1.65 Hz), 7.39–7.61 (m, 5H), 4.26 (s, 2H), 3.08 (s, 3H), 2.70 (s, 4H); ^{13}C NMR δ 169.1, 165.9, 147.5, 134.6, 128.4, 125.9, 125.8, 125.5, 125.4, 125.3, 124.1, 123.1, 55.8, 41.5, 25.3; HR-MS (FAB-pos) m/z calcd. for C17H17N2O4: 313.1188; found 313.1195 ($M+H^+$).

2.3. Synthesis of aminonaphthalene-tethered oligodeoxynucleotides

Oligodeoxynucleotide containing an amino-linker at their 5′-terminus (ODN 1) was synthesized at a 1 μmol scale (500 Å CPG column) on an Applied Biosystems (ABI) 3400 DNA synthesizer using a standard phosphoramidite chemistry. The C3-amino-linker phosphoramidite was purchased from Glen Research (5′-Aminomodifier C3-TFA) and used following the procedure provided by the supplier. To the succinimidyl ester derivative (cAN-suc or mAN-suc, 3.0–7.0 mg) in acetonitrile (200 μL) were added ODN 1 bearing the C3-amino linker (200 μM, 200 μL) and 100 μL of saturated aqueous $NaHCO_3$, and the reaction mixture was incubated at 37 °C for 24 h. Purification of the conjugates was carried out by the use of HPLC. ESI-MS (negative): calcd. for cAN–ODN 1 (C196H242N76O106P18) [$(M-4H)^{4-}$]: 1479.0; found 1478.8; calcd. for mAN–ODN 1 (C194H240N76O106P18) [$(M-4H)^{4-}$]: 1472.5; found 1472.5.

2.4. Photolysis and polyacrylamide gel electrophoresis.

Oligodeoxynucleotides were 5′-^{32}P-labeled with [γ-^{32}P]ATP (Perkin Elmer, 370 MBq/mL) and T4 polynucleotide kinase (Nippon Gene). The labeled mixtures were subsequently centrifuged through MicroBio-Spin 6 or 30 columns (Biorad) to remove excess unincorporated nucleotide. Complementary ODNs (1 μM, respectively) were annealed in phosphate buffer (10 mM sodium phosphate, 90 mM NaCl, pH 7.0) by heating to 90 °C, followed by slow cooling to room temperature. The samples (20 μL) in 1.5-mL eppendorf tubes were exposed to UV light with a LAX-100 Xe lamp (Asahi Spectra) through a UTVAF-50S-36U glass filter (Sigma Koki) at 4 °C. The DNA samples were precipitated by adding 10 μL of herring sperm DNA (1 mg/mL), 5 μL of 3 M sodium acetate (pH 5.2), and 400 μL of ethanol, and then chilling at −20 °C. The precipitated DNA were dissolved in 10vol% piperidine, heated at 90 °C for 20 min, and then dried under reduced pressure. The radioactivities of the samples were assayed using an Aloka 1000 liquid scintillation counter (Aloka), and the dried DNA pellets were resuspended in loading buffer (8 M urea, 40% sucrose, 0.025% xylene cyanol, 0.025% bromophenol blue). All reaction mixtures, along with a Maxam-Gilbert G+A sequencing reaction, were heat-denatured at 90 °C for 3 min and quickly chilled on ice. The samples

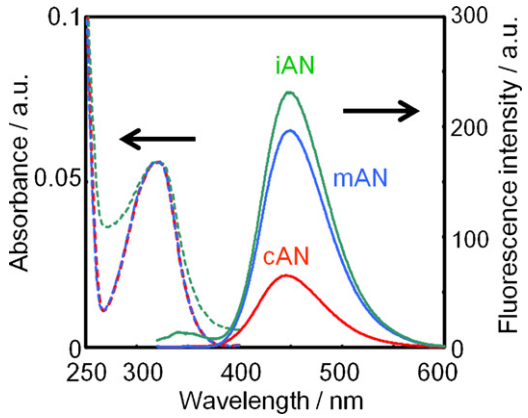


Fig. 1. UV-absorption (dotted lines) and fluorescence (solid lines, λ^{ex} = 312 nm) spectra of cAN, cAN, and iAN in deoxygenated aqueous buffer containing 0.1% acetonitrile.

(2.0 μL, $(5-10)\times10^3$ cpm) were loaded onto a 20% polyacrylamide (acrylamide–bisacrylamide 19:1) gel containing 7 M urea, electrophoresed at 1700 V for approximately 60 min, transferred to a cassette, and stored at −80 °C with Fuji X-ray films (RX-U). Cleavage of the labeled strand was quantified by autoradiography using ATTO Densitograph software (version 3.0). Individual yields were calculated relative to each total band intensity. The averages of three independent measurements for each sample were indicated.

3. Results and discussion

3.1. Photophysical properties of aminonaphthalene derivatives

Before synthesizing cyclopropylaminonaphthalene-tethered DNA, we investigated photophysical properties of three 1-aminonaphthalene (AN) derivatives in aqueous solution. *N*-Cyclopropyl-1-aminonaphthalene cAN, *N*-methyl-1-aminonaphthalene mAN, and *N*-isopropyl-1-aminonaphthalene iAN were synthesized according to previously reported procedures [31–33]. Fig. 1 shows the UV-absorption and fluorescence spectra of the naphthalene chromophores in 0.1% acetonitrile–water. The spectra of all the derivatives were similar with λ_{max} at approximately 320 nm (for absorption) and λ^{fl}_{max} at approximately 440 nm (for fluorescence). As shown in Fig. 2, the ground-state oxidation potentials of ANs were determined to be +0.38 V vs. SCE (cAN), +0.27 V (mAN), and +0.30 V (iAN) by employing AC

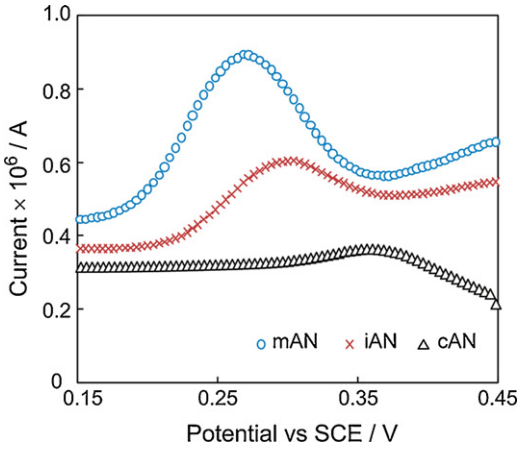


Fig. 2. AC voltammographs of (△) cAN, (◯) mAN, and (×) iAN measured in phosphate buffered saline (10 mM phosphate, 137 mM NaCl, and 2.7 mM KCl, pH 7.4) containing 1 vol% acetonitrile.

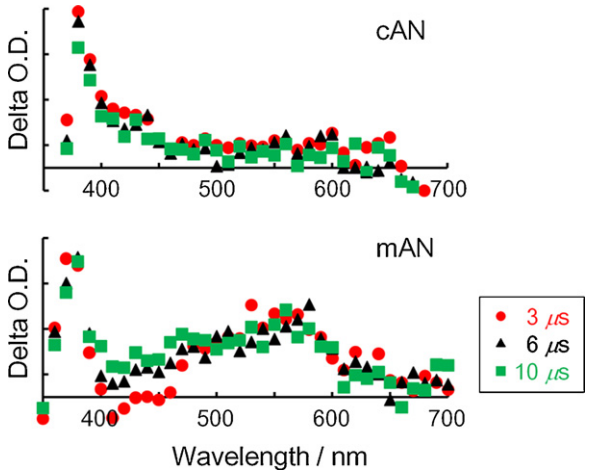


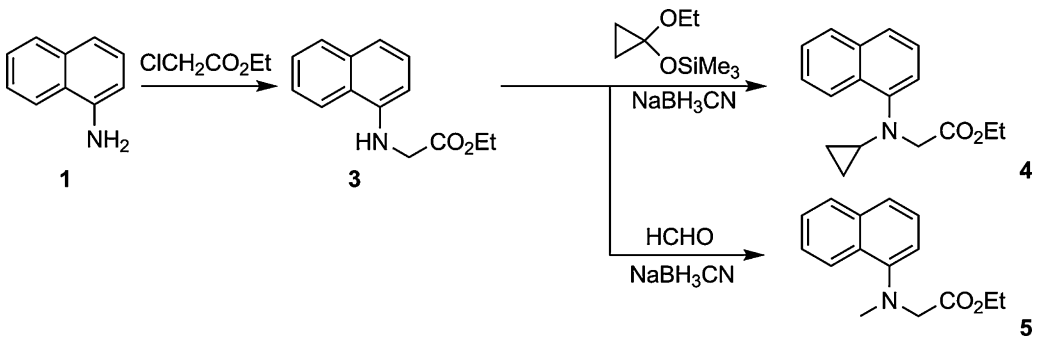
Fig. 3. Transient absorption spectra of the intermediates as observed after the 355-nm laser photolysis of cAN (upper) and mAN (lower) (0.1 mM) in the presence of 1,4-dicyanobenzene (1 mM) in Ar-saturated acetonitrile.

voltammetry [35]. Excited state oxidation potential (E^*_{ox}) can be calculated using $E^*_{ox} = E_{ox} - E_{0-0}$ with the oxidation potential of 1-aminonaphthalene (E_{ox}) and zero excitation energy evaluated from λ_{0-0} (=371 nm) in aqueous solution. The excited-state oxidation potentials of ANs were thus calculated to be approximately −3.0 V, which is sufficiently high for thymidine (E_{red} = − 2.35 V) and 2′-deoxycytidine (E_{red} = − 2.48 V) reduction [22]. Indeed, fluorescence intensities of ANs monitored in the presence of thymidine decreased as increasing the concentration of thymidine (data not shown), which suggests that photo-excited ANs can induce one-electron reduction of thymidine. It seemed that bulkiness of the substituent on the amino group have little effect on their reducing properties, thus we proceeded the following experiments for cAN and mAN derivatives.

Ring opening of the cyclopropyl radical cation of cAN in aqueous buffer was observed by nanosecond laser flash photolysis (Fig. 3). Excitation of mAN with a 355-nm Nd:YAG laser in the presence of 1,4-dicyanobenzene as an electron acceptor resulted in the formation of transient species (λ_{max}; around 580 nm) within 1 μs after the pulse. This absorption spectrum is quite similar to that observed for the 1-aminonaphthalene radical cation [36] and hence, it can be assigned to the relatively stable mAN radical cation (mAN$^{+\bullet}$). On the other hand, no such broad absorption at 580 nm was detected in the photolysis of cAN. Dynamic absorption spectra of the ring-opening products have not been reported so far, but it is proposed that spontaneous ring opening of the cyclopropylamine radical cation affords an iminium carbon radical as an intermediate. Considering that the spectra obtained after excitation showed no remarkable changes in the time region of 0.1–100 μs, the observed spectra might be assigned to the iminium carbon radical or more stable final products. It is thus estimated that decomposition of cAN radical cation (cAN$^{+\bullet}$) proceeds with a rate constant of $k > 10^7$ s^{-1}.

3.2. Electron injection from photo-excited ANs onto DNA

To synthesize AN-tethered oligodeoxynucleotides, succinimidyl esters of cAN and mAN were prepared from 1-aminonaphthalene (Scheme 2). Ethyl ester derivatives (**4** and **5**) obtained by reductive amination were derivatized into succinimidyl esters by following a standard procedure. The resulting succinimidyl esters were incubated with oligodeoxynucleotide containing a 5′-amino linker (ODN 1, Fig. 4) at ambient temperature to afford cAN- (cAN–ODN 1) or mAN- (mAN–ODN 1) tethered DNA. Complementary strand containing 5-bromouracil (BrU) as the electron-transfer probe in



Scheme 2. Synthesis of *N*-alkylaminonaphthalene derivatives (**4** and **5**).

the sequence (ODN 2) was synthesized using a DNA synthesizer. The thermal stabilities of the duplexes were evaluated on the basis of their thermal melting temperatures (T_m). T_m of the unmodified ODN 1/ODN 2 duplex was 67.5 °C, which was close to that of cAN–ODN 1/ODN 2 (68.6 °C) and mAN–ODN 1/ODN 2 (68.4 °C). The stabilization effect by ANs indicates that the naphthalene rings directly interact with DNA, although binding mode of the chromophores is not clear at this point. It has been reported that anchored aminonaphthalene partly stabilizes duplex DNA [18,20].

For a quantitative analysis of the excess electron transfer in DNA, the 5′-terminus of ODN 2 was ^{32}P-labeled, and subjected to photochemical analysis. To avoid undesirable photo-excitation of DNA bases and BrU, UV-light through a glass filter (320–390 nm) was employed for the photoinduced electron-injection experiment. The duplexes (cAN–ODN 1/ODN 2 and mAN–ODN 1/ODN 2) in a N_2 atmosphere were exposed to UV-light, then treated with piperidine at 90 °C. Because oxygen molecules could quench excess electrons on the DNA duplex, we examined the experiments under N_2-saturated conditions. The base-catalyzed hydrolysis process yielded DNA fragments those correspond to the products obtained when the DNA strand was cleaved at the nucleobase adjacent to the 5′-end of BrU (Fig. 5). Electron capture by BrU affords a uracil 5-yl radical, which in turn abstracts a hydrogen from the 5′-adjacent deoxyribose. The resulting intermediates are considered to be alkali-labile [18,37]. The intensity of the cleavage bands increased with the irradiation time, suggesting that electrons could be injected into the DNA duplexes by photo-excitation of the AN derivatives. In the absence of either AN or BrU, strand cleavage at the corresponding sites was negligible (see Supplementary data, Fig. S1). As can be seen in Fig. 5, the yields of the electron-transfer fragments from cAN–ODN 1/ODN 2 and mAN–ODN 1/ODN 2 were almost equal (5.4 ± 0.4%/30 min for cAN–ODN 1/ODN 2, 6.6 ± 0.6%/30 min for mAN–ODN 1/ODN 2). If the rapid cyclopropane-ring-opening of cAN competes with the charge recombination between the injected electrons and the AN radical intermediates, the yields of electron-transfer products should be higher for cAN–ODN 1/ODN 2 than mAN–ODN 1/ODN

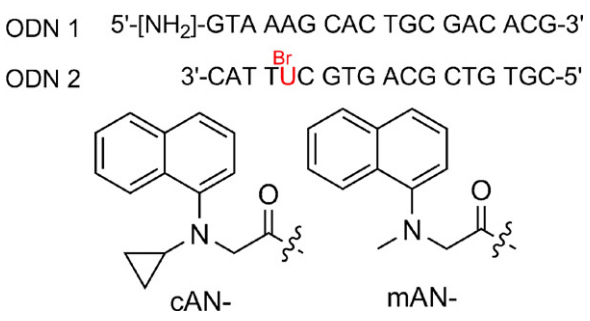


Fig. 4. Sequences of oligodeoxynucleotides.

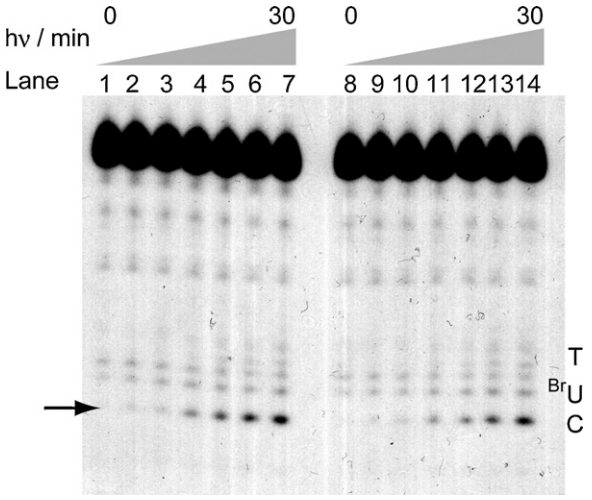
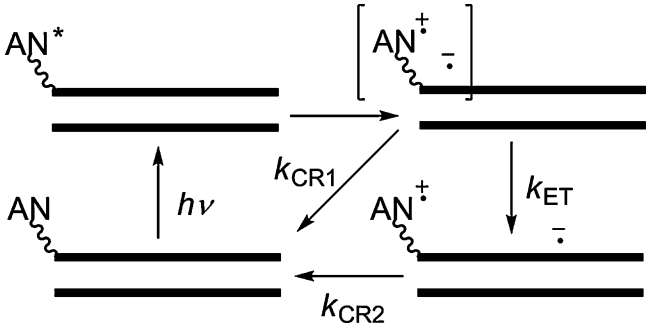


Fig. 5. Polyacrylamide gel electrophoresis image of (lanes 1–7) cAN–ODN 1/ODN 2 and (lanes 8–14) mAN–ODN 1/ODN 2. Duplexes (1 μM) in NaCl (90 mM) and phosphate buffer (10 mM, pH 7.0) were UV-irradiated at 4 °C for 0–30 min under an N_2 atmosphere, followed by piperidine treatment (90 °C, 20 min). The arrow indicates the fragment bands generated as a result of electron capture by BrU.

2. Thus, our results suggest that very fast back electron transfer is involved in the reaction pathways.

Photo-excitation of the AN moiety in DNA results in the formation of a contact radical ion pair of AN and a nucleobase (Scheme 3). It has been proposed that some of the injected electrons migrate along the duplex via a thermally activated hopping mechanism at ambient temperature [9,12,38]. These negative charges may move back to the radical cation of AN ($AN^{+\bullet}$), unless they are irreversibly trapped by BrU. Release of Br^- from the radical anion of BrU ($BrU^{-\bullet}$) proceeds with a rate constant of $k \sim 10^8\ s^{-1}$ [39], which might be slower in duplex DNA due to stabilization of the excess electron by hydrogen bonding interaction between $BrU^{-\bullet}$



Scheme 3. Photoinduced charge separation and recombination processes in the DNA conjugates.

and the complementary adenine [40]. As has been demonstrated in a recent research [21–23,26], charge recombination in a contact ion pair (k_{CR1}) occurs rapidly (within a few picoseconds), while back electron transfer from a charge separated state (k_{CR2}) may be slow enough to compete with the ring opening of $cAN^{+\bullet}$. Rate constant for excess electron transfer between each base pair (k_{ET}) has been estimated to be $\sim 10^8\ s^{-1}$ [10,26]. In view of the marginal effect of the ring opening in cAN-tethered DNA as shown above, it is presumable that charge recombination in the contact ion pair is quite efficient. This is supported by the fact that the distance between the AN and BrU units has a negligible effect on the apparent electron transfer efficiency, as has been observed in the experiments performed using DNA containing a 2-basepairs-longer bridge sequence (see Supplementary data, Fig. S2). There is some controversy about quantification of hole migration through DNA by product analysis, because relative amounts of the charge transfer products sometimes do not reflect charge transfer efficiency in DNA, especially when the rate constants for product formation are not sufficiently high. On the other hand, we have shown that the fast charge recombination process could lower apparent excess electron transfer efficiency by quantitating the reaction products, and our result supports previous findings by ultrafast spectroscopic studies [21–23,26].

4. Conclusion

In summary, we have synthesized cAN-tethered oligodeoxynucleotides to investigate the mechanism of excess electron transfer in DNA. Almost no effect of cyclopropyl substitution on the forward electron transfer to BrU suggests that charge recombination in the contact-ion-pair state is more efficient than the further hopping of electrons along the duplex. We believe that the results of this study would inspire the design of new DNA materials that facilitate efficient electron transport.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2011.01.025.

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