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Stabilization of DNA duplex by 2-substituted adenine as a minor groove modifier

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ABSTRACT

2-(1-Naphthalenylethynyl)-2′-deoxyadenosine (^{N}A) was synthesized and incorporated into oligodeoxynucleotides. DNA duplexes containing newly designed 5′-^{N}AT-3′/3′-T^{N}A-5′ base pairs are considerably stabilized than unmodified duplexes by stacking interaction of naphthalene rings in the narrow minor groove as characterized by a new emission at longer wavelength and exciton coupled CD signals.

DNA has attracted considerable attention as a building block of new nanomaterials for potential nanotechnological applications because of their unique helical structures and properties.[1–5] From a supramolecular view point, DNA can be viewed as a versatile scaffold for the attachment of functional molecules.[6–16] In the field of DNA electron transfer chemistry, double-helical DNA, which consists of π-stacked four nucleobases, is known to provide an ideal medium of electron transfer (ET) mediated by a π-stack.[17,18] Various chemical modifications of oligodeoxynucleotides (ODNs) have been developed for the systematic investigation of ET process though DNA containing electron donor and acceptors at given distances.[19] Some studies have also been initiated to modify DNA using base analogs[20,21] in order to enhance ET efficiency for the development of new DNA-based nanotechnological devices. However, the introduction of external molecules onto natural nucleosides (A, T, G, and C) through a linker instead of base analogs possessing different frameworks from natural nucleobases often destabilizes or disturbs the original DNA duplex system to a certain extent. The introduced parts should at least be placed in DNA major or minor groove to maintain the helical structure and the base pairing.[14] Modification at 5-position of pyrimidine bases in the major groove has been more common than that located in the narrower minor groove due to a synthetic reason, and numerous 5-substituted pyrimidine bases have been used for modified DNA.[6–12,22,23] To our knowledge, the accesses to minor groove by appending an external molecule onto the base moiety of natural nucleoside had so far been reported only for A-form RNA.[24]

We report herein that the modification of both complementary strands upon tethering naphthalene to deoxyadenosine at the 2-position though an ethynyl linker effectively lead to a stabilization of the DNA duplex due to the π-stacking between naphthalene moieties in the minor groove without disturbing original B-form DNA. A series of DNA duplexes synthesized for the system was shown in Figure 1a. 2-(1-Naphthalenylethynyl)-2′-deoxyadenosine (^{N}A) was prepared via palladium(0) mediated Sonogashira cross coupling (Supplementary data). After protection of amino group with *N*,*N*-dimethylformamide diethylacetal, protected ^{N}A was converted to phosphoramidite, which was used for ODN synthesis by automated DNA/RNA synthesizer. Aliquots of DNA duplex samples were prepared by annealing equimolar amount of desired DNA complements. The schematic illustration of π-stacked zipper-like block, 5′-^{N}AT-3′/3′-T^{N}A-5′ base pairs in DNA duplex is shown in Figure 1b.

The UV–visible absorption and fluorescence spectra of naphthalene-modified DNA assemblies are shown in Figure 2. The significant red shift of absorption bands of naphthalene upon tethering to DNA through an ethynyl linker results from a strong electron coupling between deoxyadenosine and naphthalene.[25] For duplexes I-c and -d, the absorption bands of naphthalene moieties in the range from 300 to 380 nm showed significant change of the ratio between the two maxima with a spectral shift compared

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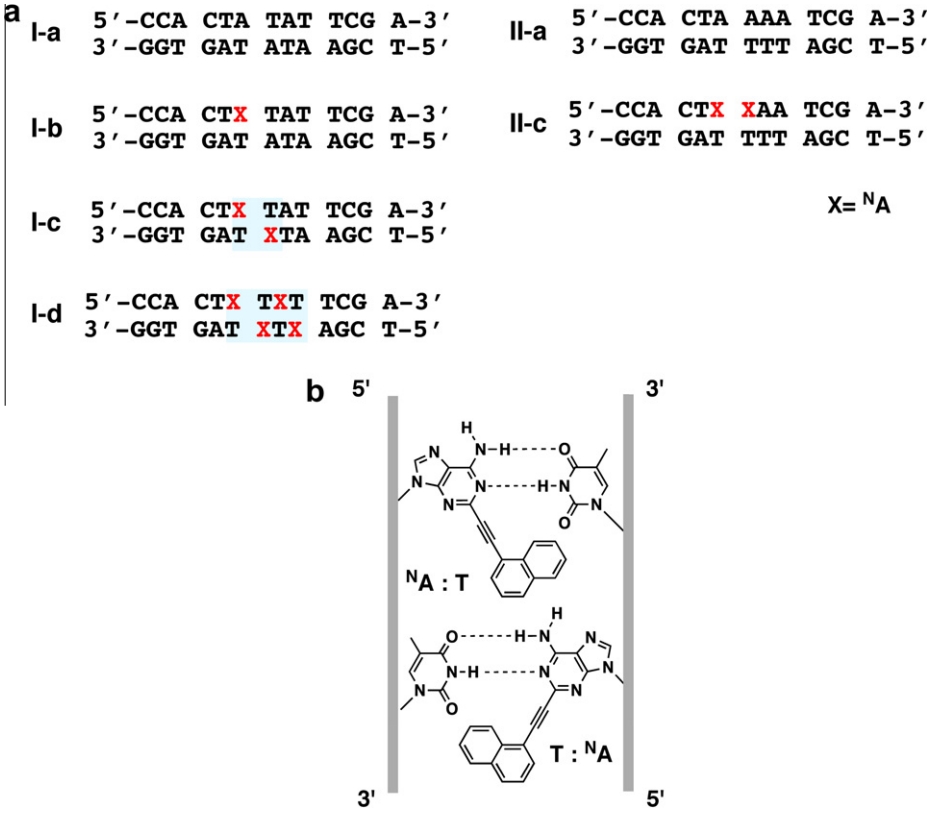

Figure 1. (a) Sequences of the naphthalene-modified ODNs synthesized in this study. (b) Schematic illustration of the base pairs, 5′-^{N}AT-3′/3′-T^{N}A-5′ in DNA duplex.

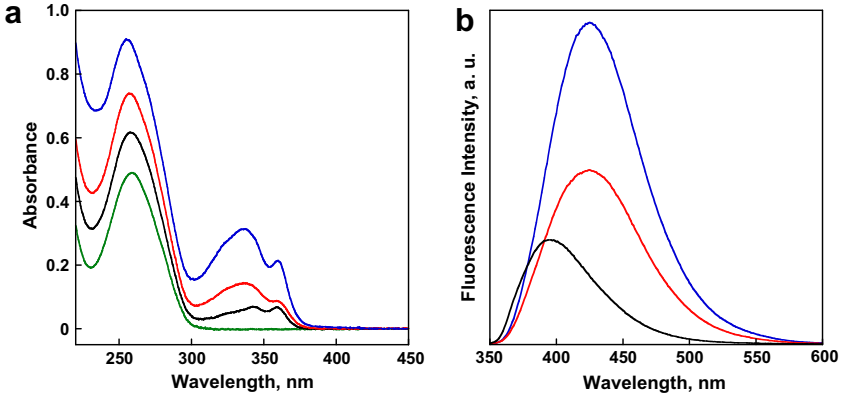

Figure 2. (a) Absorption spectra of ODN I-a (green line), I-b (black line), I-c (red line), and I-d (blue line); 2.0 μM duplex, 50 mM phosphate buffer, pH 7.0, 100 mM NaCl. (b) Fluorescence spectra of ODN I-b (black line), I-c (red line), and I-d (blue line); 2.5 μM duplex, 50 mM phosphate buffer, pH 7.0, 100 mM NaCl. Excitation wavelength is 335 nm.

to the duplex I-b. This spectral change suggests the presence of π–π interaction between two naphthalene moieties on the different strands. Fluorescence spectra of ODN I-b–d are shown in Figure 2b. The doubly modified duplex I-c exhibits an excimer-like intense fluorescence that is 31 nm red-shifted with respect to the singly modified duplex I-b. Duplex I-d showed a similar fluorescence maximum ($\lambda_{fl\cdot max}$ = 425 nm) as duplex I-c with a higher intensity.[26]

The melting temperatures (T_m) of DNA assemblies obtained by monitoring the characteristic DNA duplex absorption at 260 nm are given in Table 1 together with their wavelength of fluorescence maxima. The doubly modified duplex I-c was found to be more stable (ΔT_m = +5.5 °C) than unmodified duplex I-a. The T_m value of duplex I-d possessing four ^{N}A in total was also higher than that

Table 1
Melting temperatures (T_m) and wavelength of fluorescence maxima for DNA duplexes[a]

Duplex	T_m (°C)	ΔT_m (°C)	$\lambda_{fl\cdot max}$ (nm)
I-a	45.3	–	–
I-b	42.6	−2.7	394
I-c	50.8	+5.5	425
I-d	47.6	+2.3	425
II-a	46.6	–	–
II-c	39.3	−7.3	–

[a] Experimental conditions: [DNA duplex] = 2.0 μM (for T_m mesuaurements), or 2.5 μM (for fluorescence spectra mesurements) in 50 mM sodium phosphate, pH 7.0, 100 mM NaCl.

of the unmodified duplex (ΔT_m = +2.3 °C), although the T_m value was lower than that of duplex I-c. On the other hand, duplex II-c, which has two modifications in the same strand was less stable (ΔT_m = −7.3 °C) than the corresponding unmodified duplex. The increase in T_m values of DNA duplexes containing naphthalene-modified deoxyadenosine in both strands (I-c and -d) suggests that π-stacking between naphthalene moieties in the minor groove enhanced duplex stabilization.

The circular dichroism (CD) spectra of duplexes at the wavelength shorter than 300 nm indicated that the overall B-form DNA double strand structures were retained (Fig. 3). On the other hand, positive Cotton effects of I-c and -d in the region of 300–400 nm can be attributed to the strong exciton coupling between naphthalenes in the narrow minor groove and indicates right-handed and π-stacked arrangement of naphthalene moieties.[27]

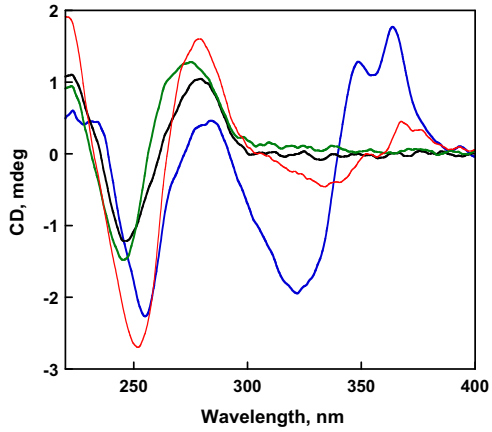

Figure 3. CD spectra of ODN I-a (green line), I-b (black line), I-c (red line), and I-d (blue line); 2.5 μM duplex, 50 mM phosphate buffer, pH 7.0, 100 mM NaCl.

The calculated structures (MacroModel, Amber*) of naphthalene-modified duplexes are shown in Figure 4. Naphthalene moieties of one strand and the other strand are shown in blue and green, respectively. A single naphthalene moiety was located in the minor groove along DNA duplex in duplex I-b (Fig. 4a). It should be noted that the single modification in the minor groove does not destabilize the DNA duplex to a large extent (ΔT_m = −2.7 °C) as compared to the modification of 5-position of pyrimidine base by 1-ethynylnaphthalene in the major groove.[28] The second naphthalene moiety on the other strand of the duplex I-c can form π–π stacking with the first one like a piece of zipper (Fig. 4b), although the minor groove of duplex II-c does not seem to provide enough space for two consecutive naphthalene moieties in the same strand (Fig. 4d). The second π-stacked zipper-like block by introduction of four ^{N}A can be fitted in the minor groove of DNA (Fig. 4c). These results are consistent with the assumption that the π-stacking between multiple naphthalenes in the narrow minor groove of DNA results in an effective enhancement of duplex stability. The present study clearly indicated that introduction of 5′-^{N}AT-3′/3′-T^{N}A-5′ base pairs in duplex induced a remarkably large thermal stability as characterized by an excimer-like new emission at 425 nm.

In summary, we have devised a system for enhancing the stability of DNA duplex using naphthalene-modified adenine–thymine base pairs. Applications of chemically modified DNA have recently attracted much attention for designing nanomaterials. Since substitutional functional groups may be easily introduced on naphthalene moiety of the deoxyadenosine, such AT base pairs with

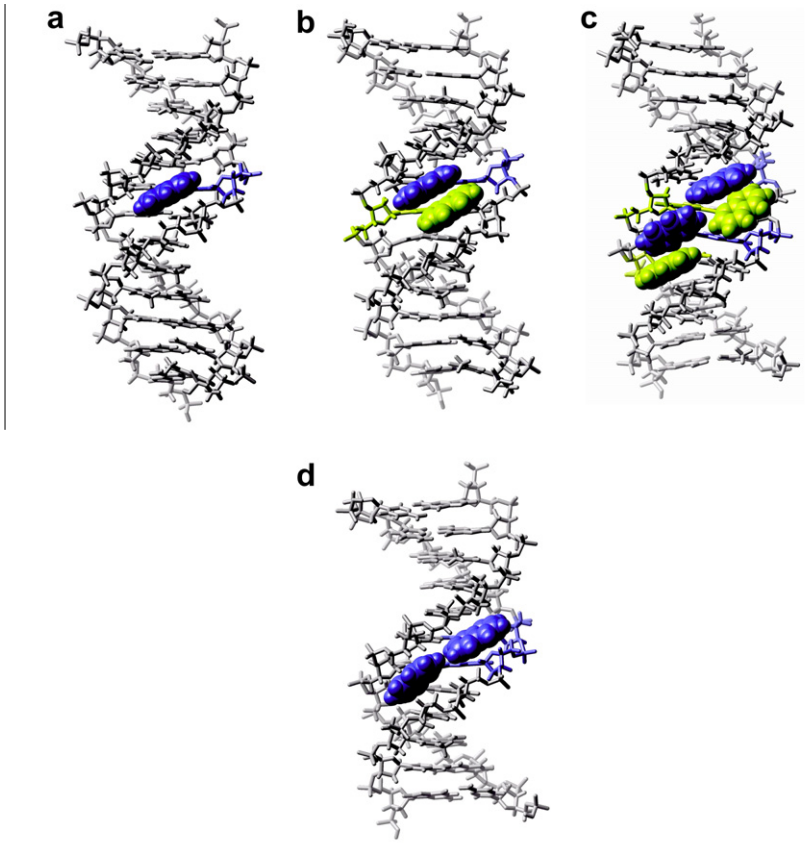

Figure 4. Structures of (a) I-b, (b) I-c, (c) I-d, and (d) II-c calculated by MacroModel, Amber*.

enhanced thermal stability would be quite useful for constructing DNA nanomaterials and photonics with an enhanced ET efficiency.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.104.

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