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Large Stabilization of a DNA Duplex by the Deoxyadenosine Derivatives Tethering an Aromatic Hydrocarbon Group

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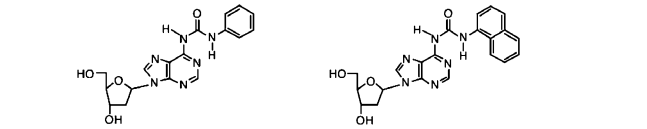
Complementary nucleotide strands can self-assemble in water in the presence of appreciated cations, forming a duplex through stacking of bases as well as hydrogen bonding. Duplexes with a dangling end are widely used to estimate the energy of the stacking interaction,[1,2] because it is believed that the energy contribution from the dangling end results from the stacking interaction between the dangling end residue and the bases of a neighboring base pair. For DNA, stabilization of the core duplex by a single dangling end with free energy contributions from 0.48 to −0.96 kcal mol^{-1} depending on the base type, neighboring base pair, and position of the dangle (5′ or 3′ end) has been reported, and adenine dangling ends are more or equally as stabilizing as other dangling ends.[2] To understand the factors contributing to stacking and to develop nucleotides with enhanced properties, nonnatural residues with an aromatic hydrocarbon group as a single dangling end have been examined.[3] Nevertheless, it is difficult to develop nonnatural aromatic residues that can adopt a conformation suitable for stacking with neighboring bases, and increasing the size of the aromatic hydrocarbon eliminates solubility in water. We report here the synthesis and thermodynamic properties of deoxyadenosine derivatives tethering a phenyl or naphthyl group by means of an amido linker (Scheme 1). The presence of a linker that covalently connects deoxyadenosine with an aromatic hydrocarbon group allows adoption of a conformation suitable for stacking on the end of a DNA duplex, providing free energy contributions equal to or greater than the Watson−Crick A/T base pair (Scheme 2).

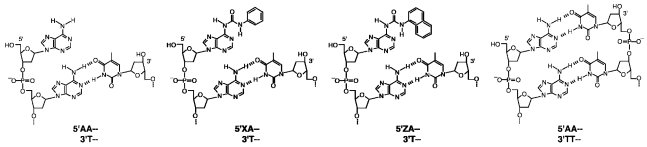
Synthesis of the phenylurea and naphthylurea derivatives of deoxyadenosine was started with 2′-deoxyadenosine (see Supporting Information).[4] Preparation of the oligonucleotides was carried out as was previously reported,[5] and all oligonucleotides were confirmed by MALDI-TOF MS (PE Biosystems Voyager). To evaluate the ability of the deoxyadenosine derivatives to stack on the end of a DNA duplex, we measured the thermodynamic parameters for the formation of DNA duplexes with 5′ or 3′ dangling ends by a self-complementary oligonucleotide (Table 1). All of the duplexes in Table 1 except $(AATGCGCATT)_2$ showed a two-state transition in thermal melting (see Supporting Information).[6] As is shown in Table 1, the single phenylurea or naphthylurea derivative of deoxyadenosine at 5′ ends next to a 5′A/3′T base pair enhanced the core duplex stability by 1.8 and 2.2 kcal mol^{-1} in $\Delta G°_{37}$, respectively, greater than an adenine dangling end (0.9 kcal mol^{-1}). These stabilization energies were comparable to the free energy for the terminal A/T base pair formations of $(AATGCGCATT)_2$

Scheme 1



N^6-(*N*-phenylcarbamoyl)-2′-deoxyadenosine (**X**) N^6-(*N*-naphthylcarbamoyl)-2′-deoxyadenosine (**Z**)

Scheme 2



($-\Delta\Delta G°_{37} = 1.9$ kcal mol^{-1}). Intriguingly, the stabilization by the aromatic hydrocarbon groups originates from the entropy term, implying preorganization of the DNA strand in the single-stranded state[7] or contributions from desolvation. The deoxyadenosine derivatives at 5′ ends of $(TGCGCA)_2$ also increased the duplex stability by 2.9 and 3.3 kcal mol^{-1}, respectively, which were greater than these derivatives next to 5′A/3′T. It is noteworthy that these free energy contributions were much larger than the terminal A/T base pair formations of $(ATGCGCAT)_2$ ($-\Delta\Delta G°_{37} = 1.7$ kcal mol^{-1}). Because the prediction parameters for an adenine dangling end on 5′A/3′T and 5′T/3′A base pairs are the same (−0.51 and −0.50 kcal mol^{-1} in $\Delta G°_{37}$, respectively),[2] the phenyl and naphthyl groups next to 5′T/3′A interact more efficiently than the same groups next to 5′A/3′T. Addition of a second dangling residue to the 5′ dangling nucleotide increased the duplex stability, while such stabilization was not observed for an adenine dangling end.[8] The duplex with the highest stability in Table 1 is $(AZATGCGCAT)_2$, which provides a $\Delta G°_{37}$ that is 4.3 kcal mol^{-1} greater than that of $(ATGCGCAT)_2$. In contrast, a single phenylurea derivative of deoxyadenosine at 3′ ends enhanced the duplex stability to the same degree as adenine (0.7−0.8 kcal mol^{-1}), and the second dangling residue did not increase stability. This observation demonstrates the lesser ability of the phenyl group to stack on the 3′ end of the duplex. On the other hand, stabilization by a single naphthylurea derivative of deoxyadenosine at 3′ ends was slightly greater (−1.0 kcal mol^{-1}), and the second dangling residue increased the duplex stability. As compared to the XX or ZZ dangling end, a smaller stabilization energy was provided by the addition of an adenine to the 3′ X or Z dangling end, respectively, demonstrating the stacking interaction between the hydrocarbon groups in the XX and ZZ dangling end. This observation is different from that at the 5′ end, suggesting the position-dependent manner. Table 1 also shows that the stabilities of $(XTGCGCAT)_2$ and $(ZTGCGCAT)_2$ are 0.5−0.6 kcal mol^{-1} greater than that of $(ATGCGCAT)_2$. The results imply

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Table 1. Thermodynamic Parameters for the Self-Complementary Duplexes[a]

sequence	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (kcal mol^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	$\Delta\Delta G°_{37}$[b] (kcal mol^{-1})	T_m[c] (°C)
ATGCGCAT[d]	−62.0	−171	−9.3	0	54.3
AATGCGCATT[e]	−75.8	−208	−11.2	−1.9	61.2
ATGCGCAA	−49.1	−131	−8.5	0.8	56.3
AATGCGCAT[d]	−64.6	−175	−10.2	−0.9	60.9
AAATGCGCAT[d]	−65.0	−176	−10.3	−1.0	61.3
XATGCGCAT	−53.9	−138	−11.1	−1.8	72.5
XXATGCGCAT	−63.5	−165	−12.2	−2.9	72.8
AXATGCGCAT	−71.1	−187	−12.9	−3.6	72.5
ZATGCGCAT	−56.8	−146	−11.5	−2.2	72.7
ZZATGCGCAT	−64.9	−168	−12.9	−3.6	76.2
AZATGCGCAT	−70.4	−183	−13.6	−4.3	76.9
ATGCGCATA[d]	−63.1	−171	−10.0	−0.7	60.1
ATGCGCATAA[d]	−64.0	−173	−10.3	−1.0	61.3
ATGCGCATX	−56.2	−148	−10.1	−0.8	64.1
ATGCGCATXX	−60.5	−161	−10.6	−1.3	64.8
ATGCGCATXA	−66.2	−181	−10.1	−0.8	59.5
ATGCGCATZ	−53.1	−138	−10.3	−1.0	66.8
ATGCGCATZZ	−64.3	−166	−12.6	−3.3	74.8
ATGCGCATZA	−59.7	−156	−11.4	−2.1	70.1
TGCGCA	−47.7	−129	−7.6	1.7	50.3
XTGCGCA	−55.7	−145	−10.5	−1.2	67.0
XTGCGCAT	−47.6	−122	−9.8	−0.5	67.0
ZTGCGCA	−55.7	−144	−10.9	−1.6	69.7
ZTGCGCAT	−44.4	−111	−9.9	−0.6	70.0

[a] The parameters were determined by T_m^{-1} versus log(C_t) plot and curve fittings.[8] The average errors in $\Delta H°$, $\Delta S°$, and $\Delta G°_{37}$ by the two methods are ±4.7%, ±4.7%, and ±3.4%, respectively. All experiments were done in a buffer containing 1 M NaCl, 10 mM Na_2HPO_4, 1 mM EDTA at pH 7.0. Underline indicates the nucleotides not forming a canonical Watson−Crick base pair. [b] $\Delta\Delta G°_{37} = \Delta G°_{37} - \Delta G°_{37}$(ATGCGCAT). [c] T_m is calculated at 100 μM. [d] Data are from ref 8. [e] Because accurate parameters could not be obtained due to a non-two-state transition, the values indicated are the predicted ones according to the nearest-neighbor parameters.[5]

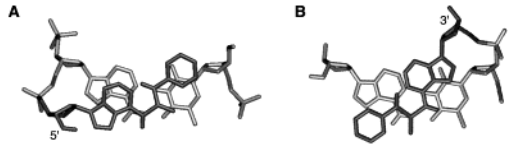

Figure 1. Illustrations for the phenylurea derivative of deoxyadenosine (black) stacking on the neighboring 5′A/3′T base pair (gray) at the 5′ end (A) and 3′ end (B) of a B-DNA duplex.

that the X and Z pair with thymine in the duplexes. However, their enthalpy terms (−47.6 and −44.4 kcal mol^{-1}) are comparable to those of $(TGCGCA)_2$ and $(ATGCGCAA)_2$. Obviously, more data are required to confirm the base pair formation.

It is reported that the amido linker portion of the self-assembled dendrimers by ureido-*s*-triazine derivatives forms a hydrogen bond with an intramolecular ring nitrogen atom in the triazine group, forming a planar configuration in water.[9] In the same fashion, the amido linker of the deoxyadenosine derivatives may interact with N1 of adenine, and the aromatic hydrocarbon group stacks on the interstrand base of a neighboring base pair. Assuming the configuration above, the phenylurea derivative may adopt a base pair-mimic geometry as illustrated in Figure 1, that is consistent with our thermodynamic observations. The phenyl group at the 5′ end of B-DNA overlaps with the interstrand base of a neighboring base pair, but there is less overlap at the 3′ end. With the naphthylurea derivative of deoxyadenosine, stacking with the neighboring base pair may be possible at the 3′ end as well as at the 5′ end. From the configuration shown in Figure 1, we may also expect that the stacking increases when the neighboring base pair is 5′T/3′A.

The base-pair mimic nucleotides developed here stabilized the DNA duplex equally or more than Watson−Crick A/T base pairs. Incorporation of the single deoxyadenosine derivative as a dangling end residue increased the DNA duplex stability up to 2.2 kcal mol^{-1} per modification, which is greater than an increase in stability reported for pyrene as a DNA base of the 5′ dangling end (1.7 kcal mol^{-1} per modification).[3a] The deoxyadenosine derivatives at helical termini providing large stabilization energy may be useful for stabilizing a hybridization with designed DNA. Also, because the configuration of the amido linker determines the position of the aromatic hydrocarbon group mediated by noncovalent interactions, the derivatives might also be applied as an environmental response material.

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Supporting Information Available: Synthetic procedures of the phenylurea and naphthylurea derivatives of deoxyadenosine, their calculated and measured mass data, and melting curves for the duplexes with the 5′ single dangling end next to the 5′A/3′T base pair (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) Protection of hydroxyl groups of 2′-deoxyadenosine was performed by reaction with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) to give the bis-TBDMS derivative in 73% yield. The compound was reacted with phenyl isocyanate at room temperature, and the phenylurea derivative was obtained in 77% yield. Removal of the TBMDS groups on 3′ and 5′-hydroxyl groups by treatment with tetrabutylammonium fluoride gave N^6-(*N*′-phenylcarbamoyl)-2′-deoxyadenosine (**X**) in 93% yield. Each compound was purified by column chromatography on silica gel (CH_2Cl_2/MeOH). Protection of the 5′-hydroxyl group of nucleoside X with a 4,4′-dimethoxytrityl (DMT) group (89%) and phosphitilation of the 3′-hydroxyl group with cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphoramidite (82%) yielded the phosphoramidite derivative which can be readily used for automated DNA synthesis.[5,8,10] Synthesis of N^6-(*N*′-naphthylcarbamoyl)-2′-deoxyadenosine (**Z**) was achieved using the same process. Synthesis of the phenyl and naphthyl derivatives was confirmed by identification with ^{1}H NMR (Varian INOVA 400 NMR) and ESI MS (Finnigan Mat LCQ) (see Supporting Information).

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(6) Melting experiments were conducted on Shimadzu 1650 and 1700 spectrophotometers, and the heating and cooling rates were 0.5 and 2 °C/min, respectively. At ~7 μM, the melting temperatures (T_m's) of $(XATGCGCAT)_2$ and $(ZATGCGCAT)_2$ monitored at 260 nm were 58.0 and 60.0 °C, respectively, both higher than that of the octanucleotide duplex of $(ATGCGCAT)_2$ (45.3 °C), and much higher than that of adenine dangling ends of $(AATGCGCAT)_2$ (48.3 °C). The melting curve for $(ZATGCGCAT)_2$ monitored at 325 nm revealed a decrease in absorption when the duplex was dissociated, and its T_m was identical to that at 260 nm. These observations imply that the aromatic hydrocarbon group stacks efficiently on the 5′ end of the duplex and the dangling residues are not frayed before the cooperative transition. The non-two-state transition for $(AATGCGCATT)_2$ might have originated from a hairpin-loop formation.

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