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Stabilization of DNA triplexes by dangling aromatic residues ☆

Mio Kubota and Akira Ono*

Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Minami-ohsawa 1-1, Hachioji, Tokyo 192-0397, Japan

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Abstract—Novel nucleoside analogues containing 2′-deoxyinosine and aromatic rings, which are connected by short linker groups, were synthesized and incorporated into oligodeoxyribonucleotides (ODNs). ODNs containing the nucleoside analogues formed stable duplexes and triplexes with target nucleic acids. The stacking interaction between base residues in the nucleoside analogues appears to be a major cause of stabilization.

The stacking interaction as well as hydrogen bond formation between nucleobases have been recognized as the major factors stabilizing duplex formation of nucleic acids.[1] Recently, it has been reported that oligodeoxyribonucleotides (ODNs) containing nucleoside analogues carrying aromatic groups, instead of nucleobases, form stable duplexes without forming hydrogen bonds.[2–6] These results and the studies of selective DNA-polymerase-mediated replication of oligodeoxyribonucletides that contain hydrophobic, non-hydrogen-bonding base pairs[7,8] led to a re-evaluation of the importance of aromatic stacking interactions in duplex formation. However, no study examining the effects of stacking interactions of non-hydrogen-bonding base analogues on triplex stability has been reported even though triplexes as well as duplexes are important motifs for designing biochemical tools.[9] In this report, the synthesis and stabilization of triplex formation as well as duplex formation of ODNs containing new nucleoside–aromatic ring conjugates (**1** and **2** in Fig. 1) is described.

The synthesis of the phosphoramidite building blocks of the corresponding nucleoside–aromatic ring conjugates are shown in Scheme 1. Dinitrophenylation[10] of **3** followed by hydrazine treatment yielded the N1-amino derivative **5**. Condensation of **5** with aromatic rings by amide bond formation gave the nucleoside–aromatic ring conjugates **6** and **7**, which were subsequently converted into the corresponding phosphoramidite building blocks, **8** and **9**. Oligodeoxyribonucleotides (ODNs) containing **1** and **2** were synthesized by the standard protocols on an automated DNA synthesizer and purified using established procedures. Each ODN prepared in this study exhibited a sharp major peak by HPLC analysis. Each ODN was completely hydrolyzed by venom phosphodiesterase and alkaline phosphatese to the corresponding nucleosides, which were analyzed by HPLC (see supporting information).

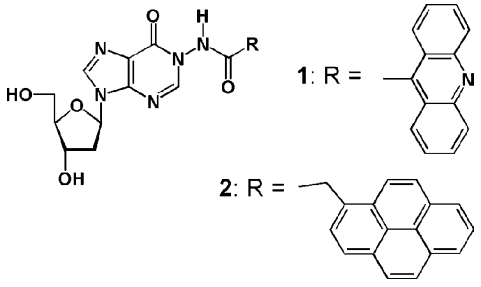

Figure 1. Structures of nucleoside–aromatic ring conjugates.

The thermal stability of the duplexes (Fig. 2) was assessed by determination of the melting temperatures (T_m) (Fig. 2) using UV spectroscopy in appropriate buffers. Typical thermal transition profiles are shown in Figure 3a. The dangling residues were added to the homopyrimidine strands of the control duplex consisting of homopurine and homopyrimidine strands (**D-I** in Fig. 2). Addition of the dAMP residue at the 5′-end of

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* Corresponding author. Tel.: +81-426-77-2545; fax: +81-426-77-2525; e-mail: akiraono@comp.metro-u.ac.jp

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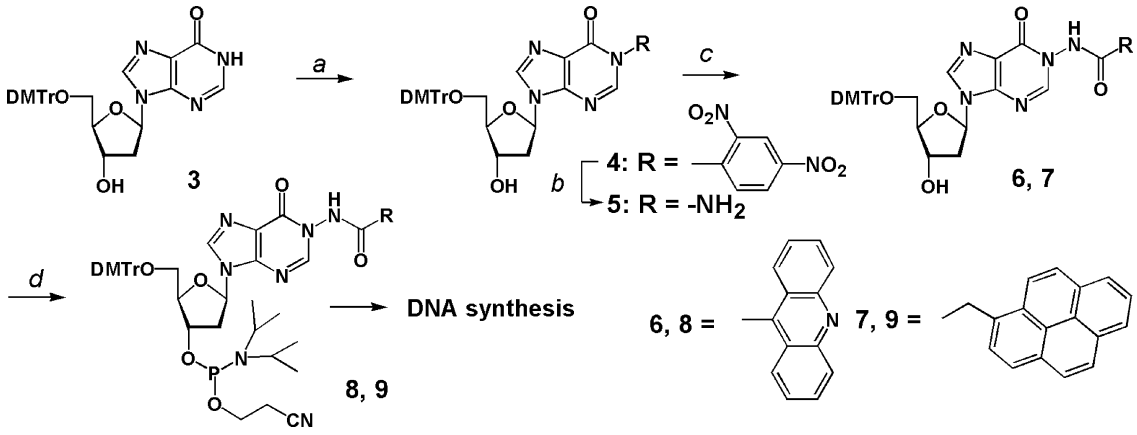

Scheme 1. A schematic representation of the synthesis of the phosphoramidite building blocks **8** and **9**. Reagents and conditions: (a) 2,4-dinitrochlorobenzene, K_2CO_3, DMF, 75%; (b) NH_2NH_2/H_20, K_2CO_3, CH_3CN, 55%; (c) RCOOH, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/HCl, 73–76%; (d) standard procedures.

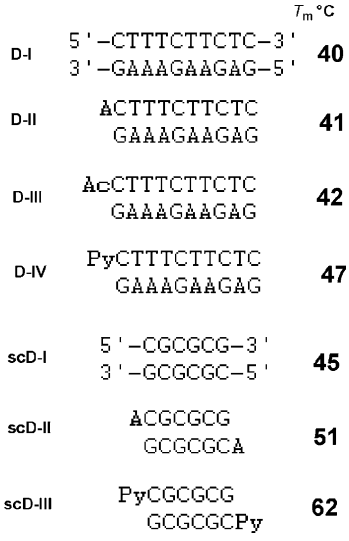

Figure 2. T_ms for the thermal transition of the duplexes. Thermally induced transitions for **D-I**–**D-IV** were measured at 260 nm in buffer (10 mM Na cacodylate, 200 mM NaCl, 20 mM $MgCl_2$; pH 6.6) containing duplex (7 μM), and thermally induced transitions for **scD-I**–**scD-III** were measured in buffer (10 mM Na phosphate, 1 M NaCl, 0.1 mM EDTA; pH 7.0) containing duplex (5 μM).

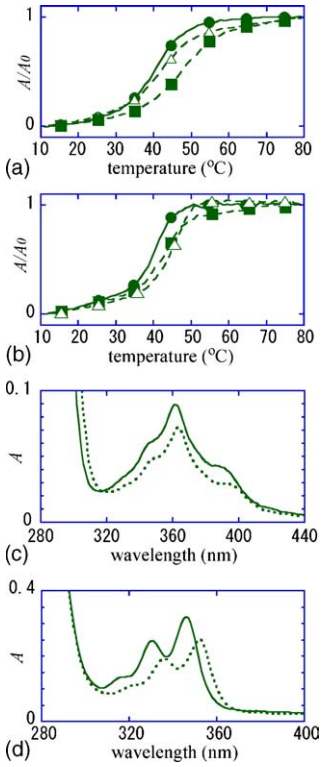

Figure 3. (a) Relative absorbance, $A/A_0 = [(A_{t\,°C} - A_{10\,°C})/(A_{80\,°C} - A_{10\,°C})]$ at 260 nm versus temperature. (●) **D-I**, (△) **D-III**, (■) **D-IV**. (b) Relative absorbance, $A/A_0 = [(A_{t\,°C} - A_{10\,°C})/(A_{80\,°C} - A_{10\,°C})]$ at 260 nm versus temperature. (●) **T-I**, (△) **T-III**, (■) **T-IV** (c) and (d) Hypochromic and bathochromic shift of λ_{max} of the aromatic rings according to the formation of duplexes **D-IV** (c) and triplexes **T-III** (d). The spectra were measured at 70 °C (above T_m) (—) and 10 °C (below T_m) (- - -) in the same buffers used for thermal transition experiments.

the control duplex did not significantly stabilize duplex formation (**D-II**). In contrast, addition of conjugates **1** and **2** efficiently stabilized duplex formation as shown by the increased T_m values (**D-III**, **D-IV**).

The effects of dangling nucleotides on duplex stability should be sequence dependent. For example, it has been reported that the sequence, 5′-d(ACGCGCG)-3′, which has the dangling dAMP residue at the 5′-end of the self-complementary sequence was largely stabilized (**scD-II** in Fig. 2) compared to the control sequence (**scD-I**).[2] Thus, the stabilization effect of pyrene conjugate **2**, which most effectively stabilized the homopyrimidine–homopurine duplex formation, was re-examined using the self-complementary sequence and it was found that the addition of conjugate **2** to the self-complementary sequence greatly stabilized the duplex formation (**scD-III**). Consequently, it was inferred that the addition of the conjugate at the dangling position can stabilize a wide range of duplexes in addition the duplexes studies here.

The duplex-stabilizing mode of dAMP and the conjugates should be different. From the typical DNA duplex

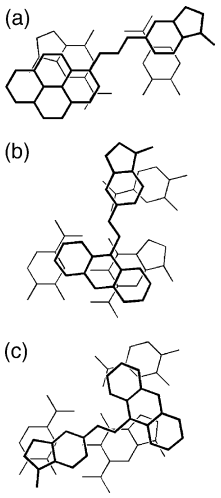

Figure 4. Illustrations of possible 5′-end stacking geometry for the conjugates. The conjugates placed at the 5′-ends adjacent to C-G base pair and C^+-C-G base triad. (a) Dangling **2** at the 5′-end of duplex **D-IV**. (b) Dangling **1** at the 5′-end of the third strand of triplex **T-III**. (c) Dangling **1** at the 5′-end of the duplex part of triplex **T-IV**.

structure[1] it has been inferred that dangling nucleotides such as dAMP stabilize the duplex formation mainly by the intra-strand-stacking interaction. In contrast, the conjugates at the dangling position stabilize the duplex formation by both of intra-strand stacking (for example, the stacking between hypoxanthine and cytidine in **D-III**) and inter-strand stacking (for example, the stacking between the aromatic ring and guanine in **D-III**) as illustrated in Figure 4. The reason that the dangling dAMP residue did not significantly enhance the stability of the duplex **D-II** is probably because the intra-strand-stacking effect through the homopyrimidine strand was weak,[1] consequently additional intra-strand stacking does not confer any additional stability. The situation was totally different for **D-IV**. The pyrene ring of **2** at the dangling position may interact with the guanine base of the homopurine strand. Since the stacking ability of purine bases is much higher than that of pyrimidine bases,[1] **D-IV** was significantly stabilized by the additional stacking interaction.

This inter-strand-stacking ability of conjugates **1** and **2** is likely to be crucial in stabilizing triplex formation (Fig. 5). T_m values in Figure 5 were corresponding to the dissociation of the triplexes directly to the single strands, without passing through the duplexes, since the triplexes were more stable than the corresponding duplexes in the solution containing the divalent cation in acidic pH (20 mM $MgCl_2$; pH 5.0) (see supporting information). The addition of a dAMP residue at the 5′-end of the third strand showed no detectable effect on the thermal dissociation profile of the control triplex **T-I** (Fig. 3b). However, addition of conjugates **1** and **2** at the dangling position of the third strand significantly stabilized the triplex formation (Fig. 5, **T-III** and **T-VI**). Interestingly, addition of the conjugates to the 5′-end of the homopyrimidine strand in the duplex part also stabilized the triplex formation (**T-IV** and **T-VII**) and the stabilizing effects of the conjugates attached at both the third

		T_m °C
T-I	5'–CTTTCTTCTC–3' 5'–GAAAGAAGAG–3' 3'–CTTTCTTCTC–5'	40
T-II	ACTTTCTTCTC GAAAGAAGAG CTTTCTTCTC	40
T-III	AcCTTTCTTCTC GAAAGAAGAG CTTTCTTCTC	45
T-IV	CTTTCTTCTC GAAAGAAGAG CTTTCTTCTCAc	47
T-V	AcCTTTCTTCTC GAAAGAAGAG CTTTCTTCTCAc	52
T-VI	PyCTTTCTTCTC GAAAGAAGAG CTTTCTTCTC	42
T-VII	CTTTCTTCTC GAAAGAAGAG CTTTCTTCTCPy	43
T-VIII	PyCTTTCTTCTC GAAAGAAGAG CTTTCTTCTCPy	46

Figure 5. T_ms for the thermal transition of triplexes. Thermally induced transitions for **T-I**–**T-VIII** were measured at 260 nm in buffer (10 mM Na cacodylate, 200 mM NaCl, 20 mM $MgCl_2$; pH 5.0) containing triplex (7 μM).

strand and duplex ends are synergetic, greatly enhancing the stability of the triplexes (**T-V** and **T-VIII**). The large aromatic surface formed by the two aromatic residues (hypoxanthine and acridine or pyrene) of the conjugate could overlap with the surface of the base triad as illustrated in Figure 4, and the stacking interaction between the large aromatic surfaces could significantly stabilize the triplex formation. The hypochromic[11,12] and bathochromic shift of the absorption λ_{max} of the aromatic residue with duplex and the triplex formation provides evidence of aromatic stacking interactions between the aromatic residues and base-pairs or base-triads (Fig. 3c and d).

The results are the first reported example of stabilizing the triplex formation using dangling residues and indicate that the large aromatic surfaces of the conjugates consisting of nucleoside and aromatic rings can enhance the stability of triplexes as well as duplexes in cases where both intra- and inter-strand-stacking interactions between the aromatic rings and base-pairs or base-triads are possible. This rationale may prove useful in the design of biochemical probes for antisense, antigene, and decoy[13] methodologies.

Acknowledgements

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