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DNA-based aromatic zipper fastened by an aromatic stacking interaction ☆

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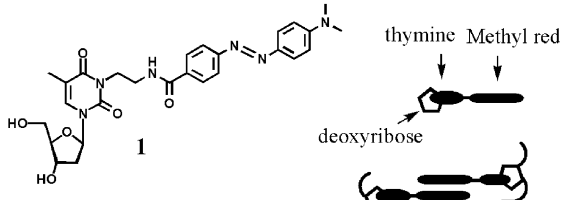
Abstract—A novel thymidine–methyl red conjugate was synthesized and incorporated into oligodeoxyribonucleotides (ODNs). The ODNs containing the conjugate formed a novel nucleic acid-based structural motif, the aromatic zipper, which fastens via the stacking interactions of aromatic residues that are attached to DNA strands. The aromatic zipper can be used to stabilize structural motifs in nucleic acids and their analogues, such as hairpin structures, by fastening their ends.

Stacking interactions and hydrogen bond formation between nucleobases are the major factors that stabilize the duplex formation of nucleic acids.[1] Recently, it has been reported that the addition of nucleoside analogues that carry aromatic groups in place of nucleobases to the ends of oligodeoxyribonucleotides (ODNs) stabilized duplex and triplex formation, despite the fact that these aromatic groups cannot form hydrogen bonds.[2–7] This report describes a novel molecular architecture that consists of two strands of DNA-aromatic ring conjugates, in which the aromatic rings are arrayed along the DNA backbone. The two strands are fastened by a stacking interaction between the aromatic rings, which forms a zipper-like molecular architecture, as illustrated in Figure 1.

Scheme 1 shows a synthetic route for the MR–thymidine conjugate **1** and its phosphoramidite unit **5**. The N3-aminoethylthymidine derivative **2**, which was prepared from thymidine according to the reported method,[8] was coupled with MR, and then deprotected, to generate the MR–thymidine conjugate **1**, which was converted to the nucleoside phosphoramidite unit **5**. ODNs that contained **1** were synthesized on a DNA synthesizer (ABI 392 DNA/RNA synthesizer) using the standard protocol, with slight modifications. The ODNs were subsequently deprotected and purified according to the previously reported methods.[7]



1T	5'-**1**T-3'
$(\mathbf{1})_2$T	5'-$(\mathbf{1})_2$T-3'
$(\mathbf{1})_3$T	5'-$(\mathbf{1})_3$T-3'
$(\mathbf{1})_3$-hpODN-$(\mathbf{1})_3$	5'-$(\mathbf{1})_3$GAATTCTTTTGAATTC$(\mathbf{1})_3$-3'
1-hpODN	5'-**1**GAATTCTTTTGAATTC-3'
hpODN	5'-GAATTCTTTTGAATTC-3'

Figure 1. The structures of thymidine–MR conjugate **1** and the ODNs, and a schematic representation of the aromatic zipper structure (right).

The UV and vis spectra of **1**T, $(\mathbf{1})_2$T, and $(\mathbf{1})_3$T in buffer are shown in Figure 2a. As the number of **1** residues increased, the λ_{max} shifted from 480 to 390 nm and the peak became narrower. This hypsochromic shift and narrowing of the peak suggest that the MR residues

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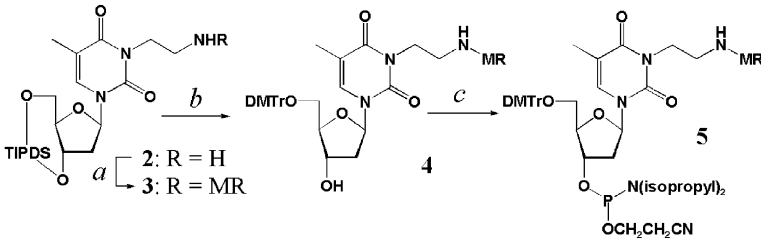

Scheme 1. A schematic representation of the synthesis of thymidine–MR conjugate **1** and its phosphoramidite building block, **5**. Reagents and conditions: (a) *p*-Methyl red, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/HCl; DMF, 66%. (b) (1) TBAF in THF. (2) DMTrCl in pyridine; 85%. (c) Standard procedure.

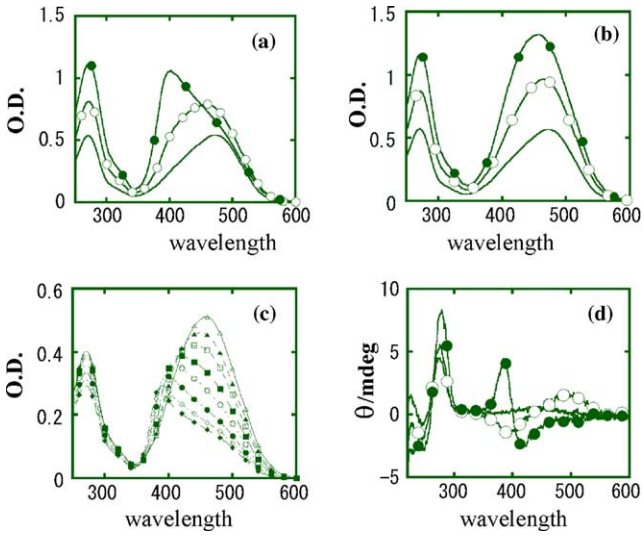

Figure 2. (a) UV and vis spectra of (solid line) **1**T, (open circles) $(\mathbf{1})_2$T, and (closed circles) $(\mathbf{1})_3$T in 10 mM sodium phosphate (pH 7.0) plus 100 mM NaCl. (b) UV and vis spectra of (solid line) **1**T, (open circles) $(\mathbf{1})_2$T, and (closed circles) $(\mathbf{1})_3$T in water. (c) UV and vis spectra of $(\mathbf{1})_3$T in water (open triangles), 10 mM sodium phosphate (pH 7.0) (closed triangles), and with NaCl concentrations of 10 mM (open squares), 40 mM (closed squares), 100 mM (open circles), 360 mM (closed circles), 800 mM (open lozenges), and 1330 mM (closed lozenges). (d) CD spectra of (solid line) **1**T, (open circles) $(\mathbf{1})_2$T, and (closed circles) $(\mathbf{1})_3$T in 10 mM sodium phosphate (pH 7.0) plus 100 mM NaCl.

formed a cluster in which the dyes were highly ordered and assembled along the vertical axis of the day plane, in a so-called H* aggregate.[9–11] In contrast, no such absorption changes due to H* aggregate formation were observed in water (Fig. 2b). As shown in Figure 2c, the UV and vis spectra of 5′-$(\mathbf{1})_3$T-3′ changed gradually as the salt concentration increased. The circular dichroism (CD) spectra of **1**T, $(\mathbf{1})_2$T, and $(\mathbf{1})_3$T in buffer are shown in Figure 2d. A positive-to-negative transition in the Cotton effect around 400 nm was induced as the number of residues of **1** increased. Since MR residues have vis absorption near 400 nm, the induction of the CD band suggests that the MR residues in $(\mathbf{1})_3$T interact tightly. It is well known that DNA duplex formation is stabilized as the salt concentration increases, since salts neutralize the repulsion between the negative charges on the sugar-phosphate backbones of the DNA strands.[1,12] Analogously, duplex formation of $(\mathbf{1})_3$T was stabilized and a zipper-like structure was formed in the presence of salts, which may tighten the stacking interaction, since a number of (six) MR residues were packed in the duplex structure.

Figure 3 shows the thermal denaturation profiles of solutions that contain different concentrations of $(\mathbf{1})_3$T. The melting temperature, T_m, increased with increasing concentrations of $(\mathbf{1})_3$T and salts (Fig. 3a and b). Generally, the T_m of the transitions induced by the denaturation of certain structures in the single-stranded state is independent of strand concentration, while the T_m of dissociations of duplexes is dependent on strand concentration.[13,14] Therefore, the results of the thermal denaturation experiments corroborate our hypotheses that $(\mathbf{1})_3$T forms duplex structures in the presence of salts, and that the stacking of the MR residues in the duplexes becomes tighter as the salt concentration increases. It is noteworthy that such a short ODN ana-

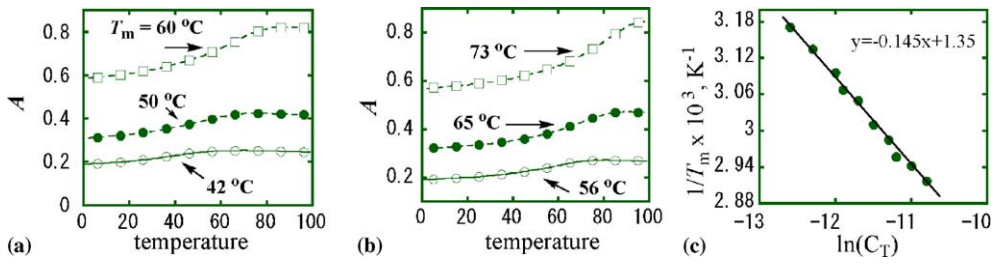

Figure 3. *A* (absorbance at 450 nm) versus temperature (°C) calculations. (a) Each solution contained the appropriate concentration of $(\mathbf{1})_3$T in buffer containing 10 mM sodium phosphate (pH 7.0) and 50 mM NaCl. 3.5 μM (open circles), 7.0 μM (closed circles), and 14 μM (open squares) $(\mathbf{1})_3$T. (b) Each solution contained the same buffer plus 100 mM NaCl. (c) van't Hoff plot of $1/T_m$ versus $\ln C_T$, where T_m is the melting temperature and C_T is the total concentration of strands. The line shown is best least squares fit to the data. The T_m values were measured in a solution that contained 10 mM sodium phosphate (pH 7.0) and 50 mM NaCl.

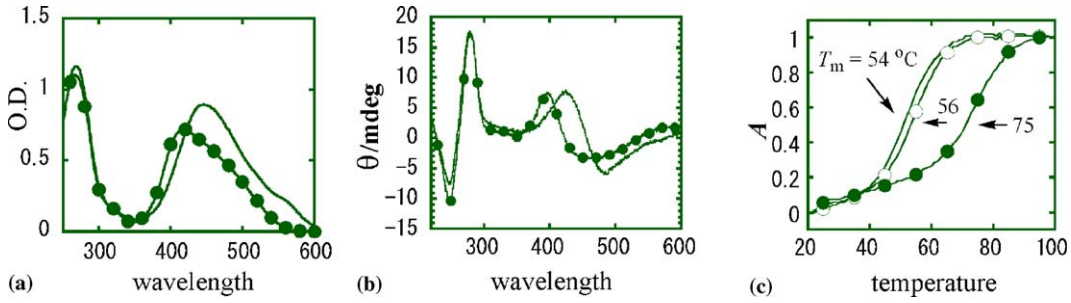

Figure 4. (a) UV and vis spectra of $(\mathbf{1})_3$–**hpODN**–$(\mathbf{1})_3$ in water (solid line), and in 10 mM sodium phosphate (pH 7.0) plus 200 mM NaCl (closed circles). (b) CD spectra of $(\mathbf{1})_3$–**hpODN**–$(\mathbf{1})_3$ in water (solid line), and in 10 mM sodium phosphate (pH 7.0) plus 200 mM NaCl (closed circles). (c) Relative absorbance, $A = [(A_{t\,°C} - A_{20\,°C})/(A_{100\,°C} - A_{20\,°C})]$ at 450 nm versus temperature for the mixtures. Each solution contained 3.3 μM ODN in 10 mM Na phosphate (pH 7.0) plus 50 mM NaCl. (solid line) **hpODN**; (open circles) **1**–**hpODN**; and (closed circles) $(\mathbf{1})_3$–**hpODN**–$(\mathbf{1})_3$.

logue forms stable duplex structures in solutions of relatively low salt concentrations. In solutions that contain 1.0 M NaCl, for which many of the thermodynamic values for unmodified oligonucleotides have been reported in the literature,[13] the $(\mathbf{1})_3$T duplex does not dissociate at temperatures near 100 °C.

The free energy parameters for DNA duplex formation represent the equilibrium position between the single- and double-stranded forms.[14,15] The following van't Hoff thermodynamic parameters[16] were obtained from the van't Hoff plots of $1/T_m$ versus $\ln(C_T)$: $\Delta H°$ (kcal mol^{-1}) = −13.7; $\Delta S°$ (cal deg^{-1} mol^{-1}) = −18.5; and $\Delta G°_{37}$ (kcal mol^{-1}) = −8.0 (Fig. 3c). As expected, the enthalpy term for the duplex formation of $(\mathbf{1})_3$T was smaller than the value for duplex formation of unmodified DNA,[13] since the $(\mathbf{1})_3$T duplex was stabilized only by the π–π stacked contacts of the aromatic rings, and no hydrogen bonds were formed. In contrast, the entropy term was more favorable for duplex formation of $(\mathbf{1})_3$T compared to unmodified DNA.[13] The tight binding of relatively hydrophobic MR residues with aromatic zipper formation, that is, cluster formation of MR residues, may be highly favored in entropic terms, like the hydrophobic interactions observed in protein structures.[16]

The hypsochromic shift and peak narrowing of the UV and vis spectra of $(\mathbf{1})_3$–**hpODN**–$(\mathbf{1})_3$ that were observed following the addition of salts to the solution (Fig. 4a) were similar to those observed for $(\mathbf{1})_3$T (Fig. 2c). Moreover, the induction of a positive-to-negative transition in the Cotton effect near 400 nm following the addition of salts was observed in the CD spectra of $(\mathbf{1})_3$–**hpODN**–$(\mathbf{1})_3$ (Fig. 4b), which was analogous to the CD spectra of $(\mathbf{1})_3$T (Fig. 2d). These results suggest that a zipper-like structure forms between the $(\mathbf{1})_3$ residues that are attached to both the 3′- and 5′-ends. Since **hpODN** forms a hairpin structure,[17] $(\mathbf{1})_3$–**hpODN**–$(\mathbf{1})_3$ should form a hairpin structure that is clipped by the zipper-like structure at the end of the stem. The hairpin structure of **hpODN** was stabilized by the addition of $(\mathbf{1})_3$ residues, as shown by the difference in the thermal denaturation profiles of $(\mathbf{1})_3$–**hpODN**–$(\mathbf{1})_3$ and **hpODN** (Fig. 4c). Since the T_m of **1**–**hpODN** was only slightly higher than that of **hpODN**, the possibility that aromatic stacking between the MR residue and the base pair at the end of the stem lesion (duplex lesion) stabilized the hairpin structure could be excluded. Consequently, the zipper-like structure behaves as a zipper that can stabilize structural motifs, such as hairpins, duplexes, and nucleic acid analogues for biological and medicinal use, by fastening their ends.

The leucine zipper, which is a structural motif found in proteins, behaves like a zipper that connects peptides via hydrophobic interactions involving leucine-rich sequences.[18] In this report, we propose a new structural motif, the aromatic zipper, which is fastened by an aromatic stacking interaction. The aromatic zipper can be used to stabilize higher nucleic acids and their analogues for biochemical and medicinal applications, and may have application as a novel structural architecture for nano- and bio-nano material development.

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