Formation of novel double-stranded structures formed by oligodeoxyribonucleotides carrying aromatic groups

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ABSTRACT
A novel thymidine-methyl red conjugate was synthesized and incorporated into oligodeoxyribonucleotides (ODNs). Each thermal denaturation profile of solutions of the ODNs showed a sharp transition curves. The melting temperatures corresponding to the transition curves became higher as the salt concentrations of the solutions increased. These results may indicate the formation of duplex structures in which two strands are connected via the stacking interactions of aromatic residues that are attached to DNA strands.

INTRODUCTION
Stacking interactions and hydrogen bond formation between nucleobases are the major factors that stabilize the duplex formation of nucleic acids. 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. This report describes a synthesis of ODNs containing novel thymidine-methyl red conjugate 1 (Fig. 1) and formation of novel molecular architecture that consists of two strands of ODN containing 1, 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 may form a zipper-like molecular architecture, as illustrated in Figure 1.

RESULTS AND DISCUSSION
Synthesis of a thymidine-methyl red conjugate 1 and incorporation of 1 into ODNs. A synthetic scheme for a novel thymidine-methyl red conjugate 1 and a corresponding phosphoramidite unit is shown in Scheme 1. According to the reported procedures, thymidine was converted into N3-(2-aminoethyl)thymidine derivative 3. Coupling of methyl red and the amino group of 3, followed by the deprotection of the silyl protecting group yielded the thymidine-methyl red conjugate 1 which was converted...
Scheme 1. Synthesis of the thymidine-methyl red conjugate 1 and a corresponding phosphoramidite unit 6. a; reference 3.  b; reference 3.  c; methyl red, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, hydrochloride, 66% from 2. d; TBAF in THF. e; DMTpCl, pyridine, 85% from 4. f; established procedures, 80%.

into the corresponding phosphoramidite unit by the established procedures. ODNs containing 1 were synthesized (Applied Biosystems 392 DNA/RNA Synthesizer), deprotected, purified by the established procedures. The synthesized ODNs are compiled below.

\[ 5'-1T-3' \quad 5'-{(1)_2T}-3' \quad 5'-(1)_3T-3' \quad 5'-(1)_2T-3' \]

**UV, Vis and CD spectra.** The UV and Vis spectra of 1T, (1)_2T, and (1)_3T in buffer are shown in Figure 2a. As the number of 1 residues increased, the \( \lambda_{\text{max}} \) shifted from 480 to 390 nm and the peak became narrower. This hypochromic shift and narrowing of the peak suggest that the MR residues formed a cluster in which the dyes were highly ordered.\(^{[4]}\) The circular dichroism (CD) spectra of 1T, (1)_2T, and (1)_3T in buffer are shown in Figure 2b. 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 (1)_3T interact tightly.

**Thermal denaturation of ODNs containing 1.** The melting temperature, \( T_m \), increased with increasing concentrations of (1)_3T and salts (data not shown). Generally, the \( T_m \) of dissociations of duplexes is dependent on strand concentration. Therefore, the results of the thermal denaturation experiments corroborate our hypotheses that (1)_3T 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. The zipper-like structure can be used to stabilize higher nucleic acids and their analogues for biochemical applications.\(^{[5]}\)

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**REFERENCES**


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**Figure 2.** a) UV and Vis spectra of - (solid line) 1T, - (open circles) (1)_2T, and - (closed circles) (1)_3T in 10 mM sodium phosphate (pH 7.0) plus 100 mM NaCl. b) CD spectra of - (solid line) 1T, - (open circles) (1)_2T, and - (closed circles) (1)_3T in 10 mM sodium phosphate (pH 7.0) plus 100 mM NaCl.