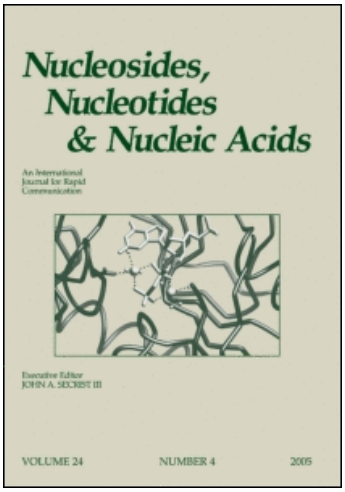

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PARALLEL-STRANDED OLIGONUCLEOTIDES WITH ALTERNATING d(A·isoG)/d(T·C) AND d(A·G)/d(T·m^5isoC) SEQUENCES

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ABSTRACT: Parallel-stranded (ps) DNA hairpins with alternating d(A·isoG)/d(T·C) (designated as ps-t1) and d(A·G)/d(T·m^5isoC) (ps-t2) sequences were studied by means of UV, CD and fluorescence spectroscopy. The thermostability of d(A·G)/d(T·m^5isoC) sequence was close to that of aps d(G·A)/d(T·C). The stability of the ps d(A·isoG)/d(T·C) sequence was even higher than that of a related anti-parallel-stranded (aps) d(G·A)/d(T·C) sequence, being unique for ps DNAs studied so far.

Parallel-stranded (ps) DNA is a family of DNA double helices in which both complementary strands have the same 5'-3' orientation. At neutral pH the reversed W-C A·T, and G·C, as well as G·G and A·A base pairs have been observed experimentally (for reviews see [1, 2]). The ps-DNAs studied so far have been less stable than conventional antiparallel W-C double helices. A variety of non-canonical base pairs in ps-DNA have been predicted by theoretical considerations [1], including a highly stable isoG·C pair with three H-bonds [3]. The first experiments on ps-DNA with isoG·C pairs were reported recently [4,5]. Stable ps-DNA and ps DNA·RNA hybrids having a mixed A·T, isoG·C and m^5isoC·G content were detected [6].

The present study was aimed at the analysis of two ps oligonucleotides incorporating *trans* A·T and either *trans* isoG·C or *trans* m^5isoC·G pairs (Figure 1), including a comparison with a reference aps double helix.

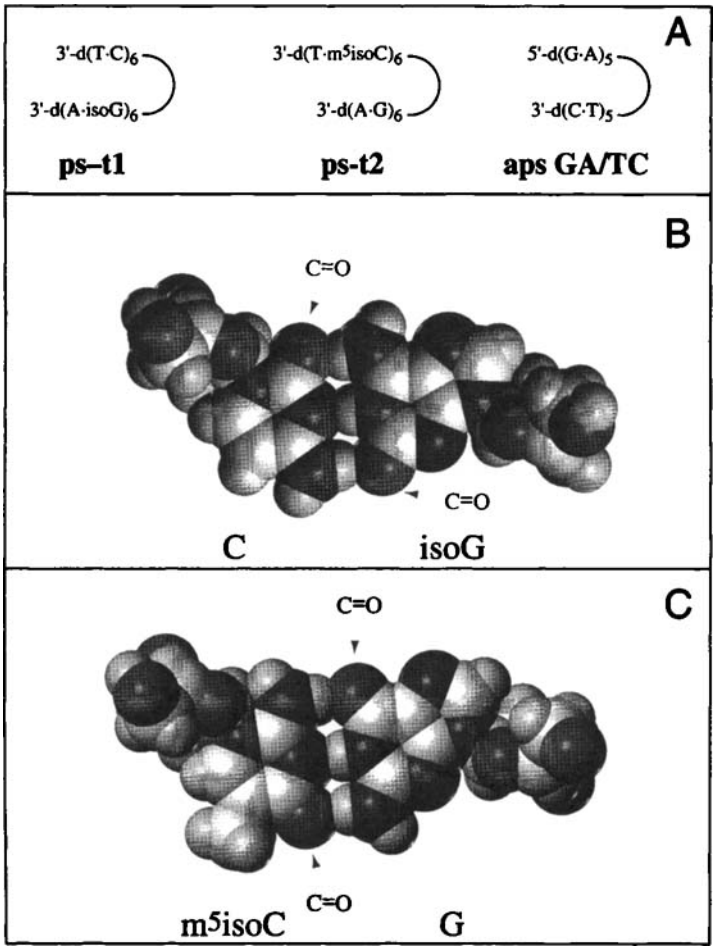

FIGURE 1. Oligonucleotides sequences ps-t1, ps-t2 and aps GA/TC with triethyleneglycol linkers (—) (A) and space filling molecular models of the *trans* isoG·C (B) and *trans* m^{5}isoC·G base pairs (C).

Evaluation of rotational relaxation time. The predominant formation of intramolecular hairpin structures rather than any intermolecular alternative structure was determined by a method based on fluorescence polarization P of bound ethidium bromide [7]. The rotational relaxation time ρ was estimated from the relationship

$$\rho = 3\tau \frac{(1/P_0 - 1/3)}{(1/P - 1/P_0)},$$

where τ is the fluorescence lifetime of bound EtBr at 3.5 °C; P_0, the limiting (extrapolated) value of P at $T/\eta \rightarrow 0$ ($P_0 = 0.42$), η the viscosity of the solution, and T

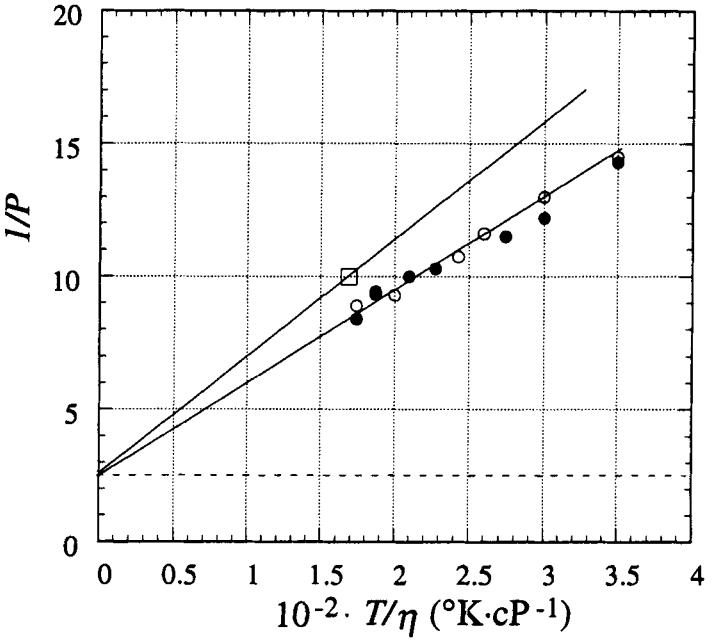

FIGURE 2. Fluorescence polarization of EtBr complexes in 0.1 M NaCl (see text). (●) ps-t1; (O) ps-t2, (❑) 10 bp oligonucleotides.

the absolute temperature. The polarization was measured at temperatures from 3 to 40 °C; the values of T/η at different temperatures were determined from reference curves of $T/\eta(T)$ acquired for the corresponding experimental ionic conditions. The quantum yield of EtBr fluorescence intercalated in ps-t1 and ps-t2 was close to that for aps DNA within experimental error, as judged by the initial slopes of EtBr binding isotherms. We inferred that the fluorescence lifetimes of EtBr intercalated in the ps and aps hairpins were also similar. The concentration of the dye did not exceed 1 per 100 nucleotides in order to avoid energy transfer effects.

The determinations are plotted in the form of $1/P$ versus T/η (Figure 2). The value for the reference 10-bp aps duplex is an average for six duplexes and hairpins of various AT/GC content; the size of the square symbol indicates the range of experimental variation. The ratio of the slope for the ps-t1 and ps-t2 oligonucleotides to that for the 10-bp aps double helix is 1.30±0.05, from which we conclude that ρ of the 12-bp ps-t1 and ps-t2 was ~ 1.3 that of a 10-bp double helix, or 27±2 nsec in 0.1 M NaCl at 3.5 °C. Due to the primary structure (sequence) of the oligonucleotides under study, the demonstration of intramolecular folding and formation of the 12-bp hairpins proves unequivocally the parallel orientation of the two strands in the double helix.

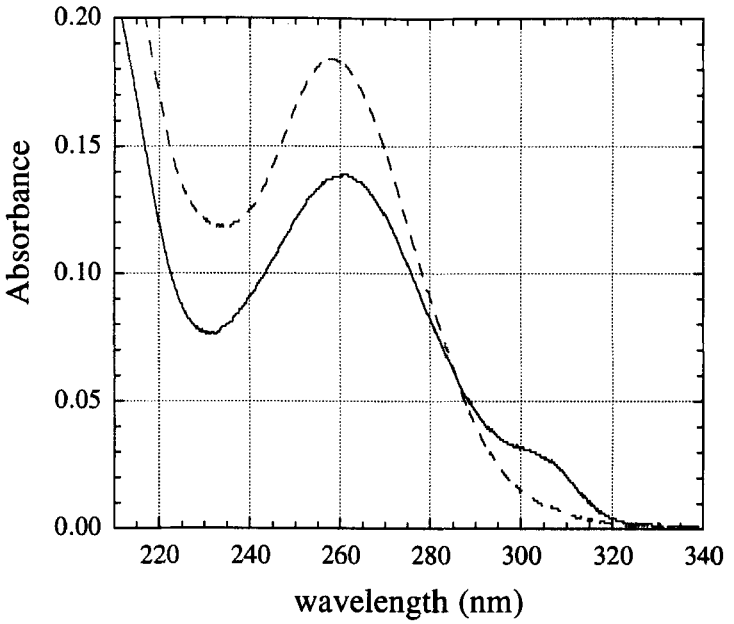

FIGURE 3. UV absorption spectra of ps-t1 (—) and ps-t2 (---) in 0.1 M NaCl, 10 mM Na-phosphate buffer, pH 7, 0.2 mM EDTA, 25 °C.

UV absorption and circular dichroism (CD) spectra. UV and CD spectra of the ps DNAs were measured (Figures 3, 4). The UV spectrum of the ps -t1 displays a shoulder at ~ 315 nm, intrinsic for the isoG base [4]. The CD spectra have characteristic positive bands at ~280 nm and 190 nm and a negative band centered at 260 nm (Figure 4). The CD spectral signatures are specific for these sequences of ps-DNA.

Ethidium bromide intercalation as a structural probe. We studied the binding of the intercalator EtBr to the ps ps-t1 and ps-t2 hairpins in order to probe for features of secondary structure. The titration with EtBr was monitored by fluorescence emission at 610 nm, thereby detecting only fully intercalated EtBr molecules having a high fluorescence quantum yield. The EtBr binding isotherms are presented in Figure 5 (points).

The parameters of EtBr binding to the hairpins were determined using a statistical mechanical treatment for binding ligands with exclusion length l to a finite lattice [8]. The most important thermodynamic quantity is the average number of ligands bound per base pair, r, which can be expressed in the form

$$r = N^{-1} \cdot \frac{\partial \ln \Xi_N}{\partial \ln C_1}$$

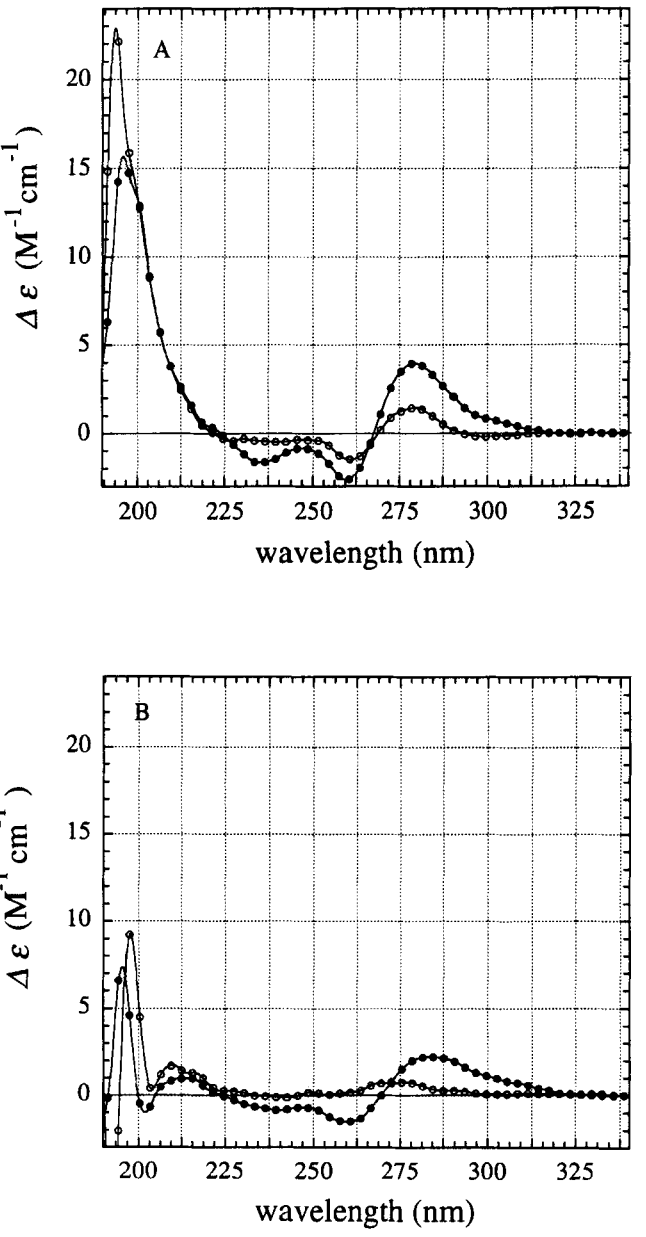

FIGURE 4. Circular dichroism (CD) spectra of ps-t1 (●) and ps-t2 (O) at 15 °C (A) and 94 °C (B) in 10 mM Na-phosphate buffer, pH 7, 0.2 mM EDTA.

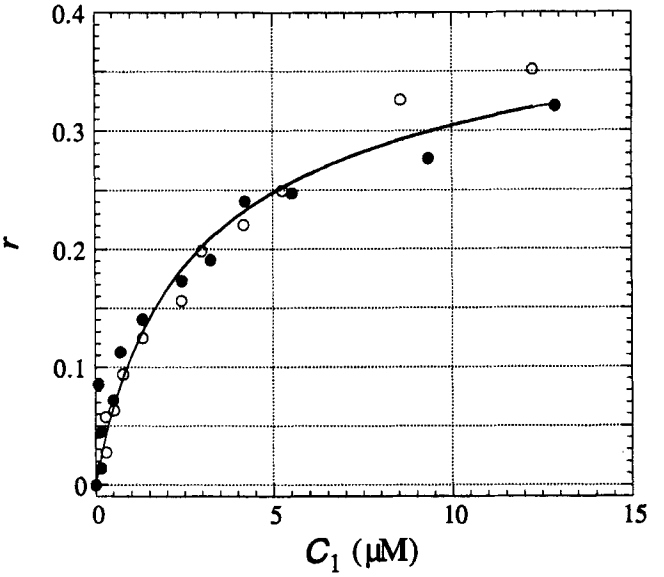

FIGURE 5. EtBr binding isotherm to ps-t1 (●) and ps-t2 (O) in 0.1 M NaCl, 25°C. Solid curves are the best theoretical fits (see text). C_f [free EtBr].

where N is the oligonucleotide length in base pairs, Ξ *is* the grand canonical partition function, and C_1 is the molar concentration of free ligand. If there is no interaction between the bound ligand molecules

$$\Xi_N = \sum_{q=0}^{[N/l]} \frac{[N-q(\tau-1)]!}{[N-q\tau]!q!} \cdot (KC_1)^q$$

where K is the association constant. For N = 12 and l = 2 the resulting equation for the binding isotherm is

$$r = 0.08 \cdot \frac{KC_1\left(11+90KC_1+252K^2C_1^2+280K^3C_1^3+105K^4C_1^4+6K^5C_1^5\right)}{1+11KC_1+45K^2C_1^2+84K^3C_1^3+70K^4C_1^4+21K^5C_1^5+K^6C_1^6}$$

The best fit (Figure 5) yielded a value for the apparent association constant K of $(1.8\pm0.2)\cdot10^5$ M^{-1}.

Unlike ps-DNA with GA/GA sequences [9], both ps-t1 and ps-t2 supported EtBr intercalation readily. We conclude that these ps double helices exhibit an extensive flexibility supporting intercalation of up to 5-6 EtBr molecules per 12 base pairs, with an association constant close to that for aps DNA under the prevailing experimental conditions.

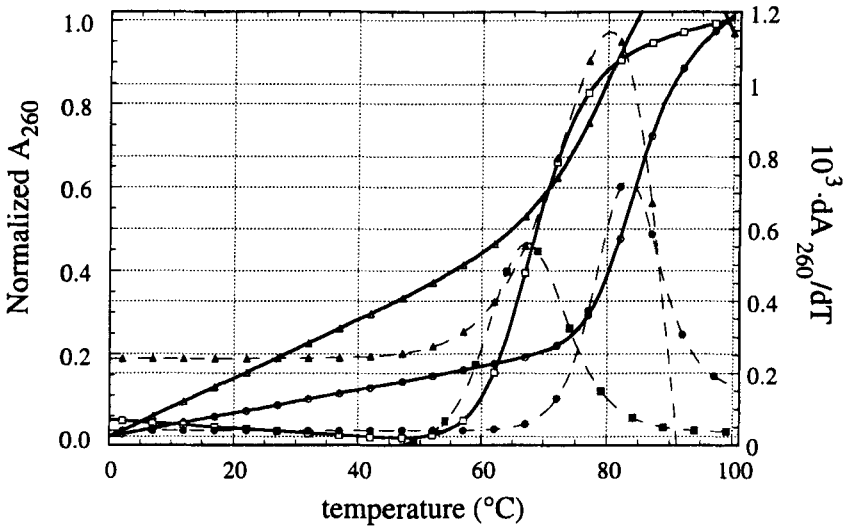

FIGURE 6. Thermal denaturation curves of ps-t1 (O), ps-t2 (Δ) and control 10 bp aps GA/CT (❑) hairpins. The dashed derivative curves d(Absorption)/dT are marked with the corresponding filled symbols.

Thermodynamics of the helix-coil transition. The thermal denaturation profiles of ps-t1 and ps-t2 hairpins are given in Figure 6. A considerable premelting hyperchromism was observed for the ps-t2 hairpin , the source of which is under study and will be discussed elsewhere. The thermal denaturation curves of ps-t1 and ps-t2 were analyzed using a two-state model for the helix-coil transition (Figure 6). Transition enthalpies, entropies and transition midpoints were determined as parameters by fitting theoretical curves to experimental points (Table). Both the enthalpy and entropy of ps-t2 formation were smaller than for ps-t1, and the calculated free energies of the hairpin formation indicated a significantly greater thermodynamic stability of ps-t1 than of ps-t2. For comparison, the transition enthalpies and free energies of formation calculated for a single base stacking in ps-t1, ps-t2 and the aps GA/TC sequence are presented in the Table. In spite of the somewhat lower stability of *trans* A·T pairs in ps-t1 and ps-t2 compared to conventional Watson-Crick *cis* A·T pairs in aps GA/TC (for example, see[1]) the free energy of formation per single base stacking were calculated to be greater for the two ps oligonucleotides. In the alternating ps AT/GC context the *trans* isoG·C base pair appeared to be more energetically advantageous than *trans* m^5isoC·G pairs and, strikingly, than even canonical Watson-Crick *cis* G·C pairs. The observed ranking order of base pair stability *trans* isoG·C > *trans* m^5isoC·G > *cis* G·C correlates well with molecular mechanics calculations [6].

TABLE Thermodynamics parameters for the formation of parallel-stranded hairpins ps-t1 and ps-t2

Oligonucleotide	ΔH, kJ/mol	ΔS, kJ/mol·°K	T_m °C	Δh, kJ/mol bp	$\Delta g^{37\,°C}$ kJ/mol bp
ps-t1	-310±10	-0.87±0.03	83.5±0.4	-28.2±0.9	-3.4±0.1
ps-t2	-172±8	-0.48±0.03	87.8±0.2	-15.6±0.7	-2.0±0.1
aps GA/TC	-236±8	-0.69±0.02	67±0.2	-26.1±0.1	-2.1±0.1

The high stability of the ps-t1 double helix is unique for ps DNAs studied so far, exceeding that of the corresponding aps GA/TC sequence, thereby implying a potential for ps d(A·isoG)/d(T·C) sequences in antisense technology.

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