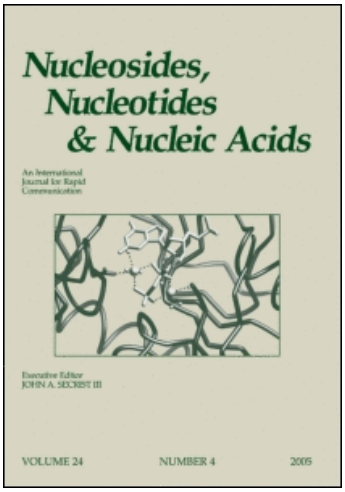

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Fluorescent 2-Pyrimidinone Nucleoside in Parallel-Stranded DNA

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ABSTRACT

Stretches of parallel-stranded (ps) double-helical DNA can arise within antiparallel-stranded (aps) Watson-Crick DNA in looped structures or in the presence of sequence mismatches. Here we studied an effect of a pyrimidinone-G (PG) base pair on the stability and conformation of the ps DNA to explore whether P is useful as a structural probe.

INTRODUCTION

It has been proposed that DNA can form regions with parallel-stranded orientation upon binding of protein or other ligands, and that these regions may participate in the regulation of gene function.[1–3] A specific *trans*GC base pair scheme in ps-DNA has been established by FTIR and UV spectroscopy[4] and later confirmed

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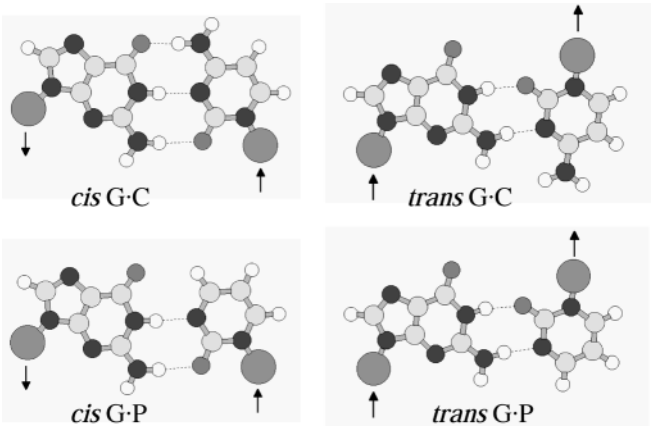

Figure 1. Schemes of *cis*GC and *cis*GP base pairs in antiparallel-stranded DNA and of *trans*GC and *trans*GP base pairs in parallel-stranded DNA.

with NMR[5] (Fig. 1). The scheme implies that the P base analogue has the potential for substituting for C in the sheared *trans*GC pair. This base analogue has distinctive UV absorption and fluorescence spectral properties.[6,7] The specific aim of this study was to elucidate whether 2-pyrimidinone (P) can serve as a probe for monitoring ps-DNA formation and for distinguishing between ps- and aps-DNA.

RESULTS AND DISCUSSION

2-Pyrimidinone phosphamidite was prepared and the oligonucleotide 5′-ATCPCTATAG-3′ was synthesized by Syntol (Moscow). The following duplexes were studied at concentrations of 4 or 8 μM in 0.5 M LiCl, 10 mM Tris HCl buffer, pH 7.6:

psP1 5′-ATC**P**CTATAG-3′
5′-TAGGGATATC-3′

apsP1 5′-ATC**P**CTATAG-3′
3′-TAGGGATATC-5′

psN1 5′-ATCCCTATAG-3′
5′-TAGGGATATC-3′

apsN1 5′-ATCCCTATAG-3′
3′-TAGGGATATC-5′

The presence of a PG base pair strongly destabilized the aps duplex (Fig. 2A) as well as the ps duplex, though the latter was affected to a somewhat lesser extent (Fig. 2B). The helix-coil transition enthalpy ΔH decreased by 86 kJmol^{-1} due to the substitution of P for C in a CG bp of the aps duplex. The corresponding energetic penalty for the ps duplex was ~49 kJmol^{-1}. These values were significant and raised the question as to whether the P base was paired to guanine and stacked with the neighboring bases or, alternatively, if P was flipped out of the structure. To address this question, we studied the fluorescence of the P-containing oligonucleotides. The quenching of the P fluorescence in aps-P1 and ps-P1 (excitation at 303 nm, emission

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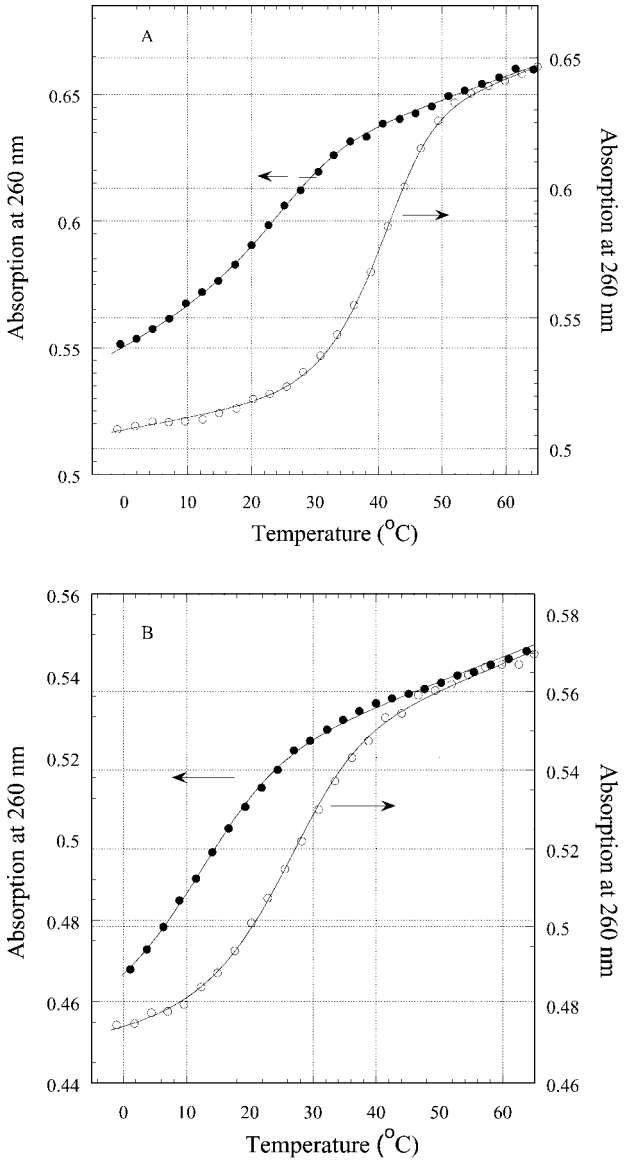

Figure 2. Thermal denaturation curves of the ps and aps duplexes monitored by absorbance at 260 nm. (A) aps-P2 (•), aaps-N1 (•), (B) ps-P1 (•), ps-N1 (•). Solid curves are the best fits to two-state model. Curves were taken at a constant heating rate of 0.8°C/min. Concentration of duplexes was 4 μM, samples contained 0.5 M LiCl, 10 mM Tris HCl buffer, pH 7.6.

at 370 nm) was indicative of a stacked state of the fluorophore in these structures, i.e., a configuration less exposed to water. It is noteworthy that in the ps-P1 duplex, the P fluorescence was less quenched and the base appeared to be more accessible to water than in aps-P1. This effect is attributable to the different geometries of the

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Table 1. Calculated parameters of formation of antiparallel duplexes.

		Total internal energy	Bond energy	VdW energy	H-bonds	Electrostatic interactions
5′-ATC**C**CTC-3′	7nuclAmber	−779.65	23.27	−524.56	−154.0	−870.61
3′-TAG**G**GAG-5′	CG bp only	−83.52	3.38	−198.63	−25.81	18.14
5′-ATC**P**CTC-3′	7nucl Amber	−707.17	23.49	−517.12	−147.19	−817.84
3′-TAG**G**GAG-5′	PG bp only	−9.81	3.11	−192.18	−19.13	77.13

Energy parameters are given in $kJmol^{-1}$. Contributions of CG and PG base pairs including their interaction with the neighboring base pairs are presented as "CG bp only" and "PG bp only".

ps-DNA and aps-DNA grooves. The quenching of the P fluorescence in both duplexes confirmed the pairing of P with G and to its stacking with the neighboring bases.

CD spectra of the four duplexes were taken at 3°C with the aim of verifying whether the duplex conformation was markedly affected by the presence of a PG bp (data not shown). The changes in CD signal upon insertion of a PG bp in place of a CG bp reflect the spectral properties of the P base analogue, but do not provide evidence for a significant distortion of secondary structures in the aps-P1 and ps-P1 duplexes compared to those of aps-N1 and ps-N1.

What is an origin of the destabilization of the aps and ps duplexes due to the PG base pair? In order to address this problem, we carried out quantum mechanical calculations of the energy parameters for aps duplex formation. Energy minimizations of the two 7-bp aps duplexes having 5′-d(ATCPCTA)-3′ or 5′-d(ATCCCTA)-3′ strands were performed. The low energy conformations of the two duplexes were similar. The difference of the total internal energies was ~74 kJ mol^{-1} (Table 1), a value close to the experimental difference in the formation enthalpies of aps-N1 and aps-P1. This fact may be fortuitous, inasmuch as the internal energies of different molecules cannot be compared directly. However, it is noteworthy that the energy of electrostatic interactions in the GP-containing duplexes contributes the greatest amount to the energy penalty, implying that a charge distribution in P different from that of C may be responsible. The presence of only two H-bonds in *cis*PG bp of aps-P1 instead of the conventional three H-bonds of aps-N1 additionally destabilizes aps-DNA.

CONCLUDING REMARKS

The insertion of a PG bp in place of a CG bp in CPC/GGG context is accompanied by the following effects: (i) absence of conformational changes of either aps or ps duplex detectable by CD measurements or molecular modeling; (ii) less quenching of the fluorescence of P upon formation of a ps duplex than upon formation of aps duplex; (iii) significantly reduced stability of the ps-DNA and particularly of the aps-DNA upon substitution of the PG bp for the CG bp. From molecular mechanics

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calculations we propose that the destabilization may derive primarily from unfavorable electrostatic interactions of 2-pyrimidinone in comparison to cytosine. The lower stability of PG-bp containing duplexes may severely limit the possible use of this base analogue as a structure-specific probe.

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REFERENCES

1. Ramsing, N.B.; Jovin, T.M. Parallel stranded duplex DNA. NAR **1988**, *16* (14A), 6659–6676.
2. Rippe, K.; Kuryavyi, V.V.; Westhof, E.; Jovin, T.M. *Structural Tools for the Analysis of Protein-Nucleic Acids Complexes. Advances in Life Sciences*; Lilley, D.M.J., Heumann, M., Such, D., Eds.; Birkhäuser Verlag: Basel, 1992; 81–107.
3. Tchurikov, N.A.; Chernov, B.K.; Golova, Y.B.; Nechipurenko, Y.D. Parallel DNA: generation of a duplex between two Drosophila sequences in vitro. FEBS Lett. **1989**, *257* (2), 415–418.
4. Mohammadi, S.; Klement, R.; Shchyolkina, A.K.; Liquier, J.; Jovin, T.M.; Taillandier, E. FTIR and UV spectroscopy of parallel-stranded DNAs with mixed A*T/G*C sequences and their A*T/I*C analogues. Biochemistry **1998**, *37* (47), 16529–16537.
5. LeProust, E.M.; Pearson, C.E.; Sinden, R.R.; Gao, X.L. Unexpected formation of parallel duplex in GAA and TTC trinucleotide repeats of Friedreich's ataxia. J. Mol. Biol. **2000**, *302* (5), 1063–1080.
6. Connolly, B.A.; Newman, P.C. Synthesis and properties of oligonucleotides containing 4-thiothymidine, 5-methyl-2-pyrimidinone-1-beta-D(2′-deoxyriboside) and 2-thiothymidine. Nucl. Acids Res. **1989**, *17* (13), 4957–4974.
7. Gildea, B.; McLaughlin, L.W. The synthesis of 2-pyrimidinone nucleosides and their incorporation into oligodeoxynucleotides. Nucl. Acids Res. **1989**, *17* (6), 2261–2281.

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