Volume 244, number 1, 39–42 FEB 06771 February 1989

Parallel stranded DNA with AT base pairing

A.K. Shchyolkina, Yu.P. Lysov, I.A. Il'ichova, A.A. Chernyi, Yu.B. Golova, B.K. Chernov, B.P. Gottikh and V.L. Florentiev

V.A. Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, 117984 Moscow, USSR

Received 2 December 1988

The concentration and temperature dependences of the UV and CD spectra of the oligonucleotide 3'-d(ApTpApTpApTpApTpApTp)-O(CH_2)$_6$O-5'-d(pApTpApTpApTpApTpApT) (eicosamer) in aqueous solution at pH 7 in the presence of 0.5 M NaCl were studied. At less than 10^{-6} M, the eicosamer was shown to form in solution a hairpin with parallel orientation of chains (parallel hairpin). From thermal denaturation profiles [$A_{260}(T)$] the thermodynamic parameters, $\Delta H°$, $\Delta S°$ *and* T_m for parallel hairpin formation were calculated to be -90 ± 8 kJ/mol. -300 ± 20 J · mol^{-1} · K^{-1} and 40.5°C, respectively. The CD spectra of the parallel double helix differed from those of B-form DNA and had characteristic features: decreasing magnitude of the positive maximum at 265 nm and a negative peak at 285 nm.

Oligonucleotide; Parallel hairpin formation

1. INTRODUCTION

It has been shown that phosphate methylated d(T)$_6$ [1] and β-d(GTACGC) with α-d(CATGCG) [2] form a duplex with parallel orientation of the chains (parallel duplex) in solution. Moreover, in a d(CG) crystal obtained from acidic solution, dinucleoside phosphate forms a parallel duplex with G · G and C · CH$^+$ base pairs [3,4].

However, in all cases mentioned, either chemically modified oligonucleotides or a natural dimer but under non-physiological conditions have been studied (recently, two studies [5,6] have demonstrated that parallel duplexes do exist in oligonucleotide solution). In terms of a possible biological function of parallel duplexes, it is important to know whether the oligonucleotides containing natural monomeric units are capable of forming a double helix with parallel stranded chains under physiological conditions.

Correspondence address: V.L. Florentiev, V.A. Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, Vavilova Str. 32, 117984 Moscow, USSR

The present work allows us to answer this question in the affirmative.

We studied the oligonucleotide 3'-d(ApTpApTpApTpApTpApTp)-O(CH_2)$_6$O-5'-d(pApTpApTpApTpApTpApT) (henceforth termed 'eicosamer') that is capable of forming a parallel duplex only intramolecularly (hairpin), thus allowing us to expect readily interpretable and sufficiently unequivocal results.

2. EXPERIMENTAL

The eicosamer 3'-d(ApT)$_5$pO(CH_2)$_6$Opd(ApT)$_5$-3' was synthesized by the phosphotriester method in a solid-phase carrier under a non-automatic regime from protected nucleoside 3'- or 5'-phosphamidites and 1-dimethoxytrityl-6-(*N*-diisopropylaminomethoxyphosphinyl)hexanediol. Decanucleoside nonaphosphate d(AT)$_5$ was obtained by the H-phosphonate method on a Cyclon (New Brunswick Scientific) DNA synthesizer. Partly deblocked 5'-dimethoxytrityloligonucleotides were purified by reverse-phase HPLC on a Nucleosil 7C$_{18}$ column. After detritylation, products were chromatographed on Zorbax-C8.

To record the UV and SC spectra, oligonucleotide solutions in 0.5 M NaCl at pH 7 were used. The solutions were heated to 70°C and cooled to room temperature prior to measurements. The concentration was established by UV spectra (ϵ = 8.8 mM^{-1} · cm^{-1} at 70°C [7]). The concentration of oligonucleotides is given as mol chain/l.

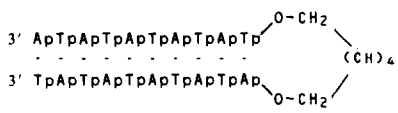
Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

The melting curves were registered on a Beckman (USA) spectrometer at 260 nm in 0.1, 0.5 and 1 cm (path length) cuvettes. The heating rate was constant (0.2 degree/min). CD spectra were recorded in 0.1, 0.2 and 0.5 cm cuvettes on a Jobin Ivon Mark III (France) dichrograph equipped with a cryostat (0–40°C) and thermostatted cell-holder (40–80°C). The accurary in the temperature readings was ±0.5°C.

3. RESULTS AND DISCUSSION

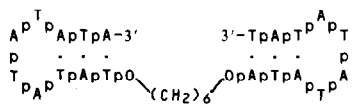
3.1. *Thermodynamics of formation of the parallel hairpin*

The eicosamer can exist in solution as a parallel hairpin, antiparallel dimer (at high concentrations – as a trimer, tetramer, etc.) and double antiparallel hairpin.



Parallel hairpin

Antiparallel dimer



Double antiparallel hairpin

If we restrict ourselves to considering only the open chain (M), parallel hairpin (H) and antiparallel dimer (D), which seems to be reasonable for sufficiently low concentrations of oligonucleotide, then

$$M \overset{K_1}{\rightleftharpoons} H \qquad 2M \overset{K_2}{\rightleftharpoons} D$$

$$K_1 = \frac{[H]}{[M]} \qquad K_2 = \frac{[D]}{[M]^2}$$

and

$$K^{eff} = \frac{[H] + 2[D]}{[M]} = K_1 + \frac{-(1+K_1) + \sqrt{(1+K_1)^2 + 8K_2C_0}}{2}$$

Therefore, at the oligonucleotide concentration satisfying the condition $8K_2C_0 << (1+K_1)^2$, the experimental results reflect to a high degree of accuracy the intramolecular process (formation of the parallel hairpin). The stability within the experimental error of melting curves on further dilution of the sample may serve as an experimental criterion for reaching sufficiently low concentrations of oligonucleotide.

Indeed, the eicosamer melting curves at 0.9×10^{-6}, 0.55×10^{-6} and 0.5×10^{-6} M are seen to coincide within the experimental error and differ significantly from the melting curves for higher concentrations of oligonucleotide (fig.1). The choice between the parallel and double antiparallel hairpin was made based on the melting curves of the decamer $d(AT)_5$ which is capable of forming an antiparallel hairpin only via the intramolecular mechanism. One can see from fig.2 that the decamer's melting curve principally differs from the corresponding eicosamer melting curve at low concentrations and probably reflects disturbance of the stacking interaction.

From the thermal denaturation profiles by non-

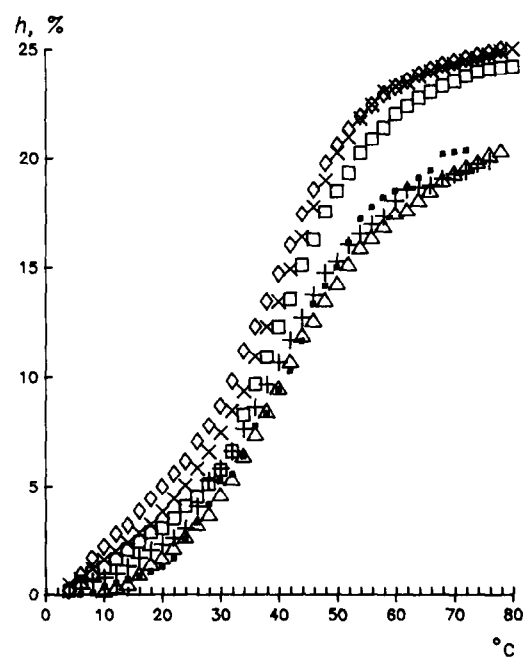


Fig.1. Thermal denaturation profiles of eicosamer at 0.5×10^{-6} (■), 0.55×10^{-6} (+), 0.9×10^{-6} (△), 3.6×10^{-6} (□), 1.9×10^{-5} (×) and 0.8×10^{-4} (◇).

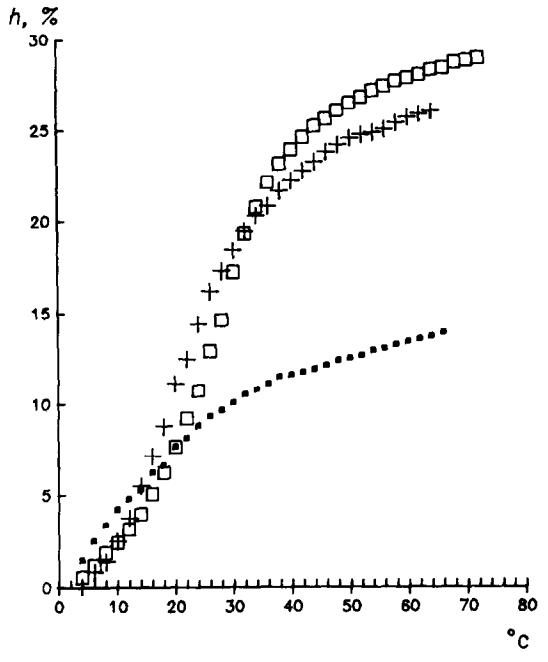


Fig.2. Thermal denaturation profiles of decamer $d(AT)_5$ at 1.1×10^{-6} (■), 1.0×10^{-5} (□) and 1.1×10^{-4} M (+).

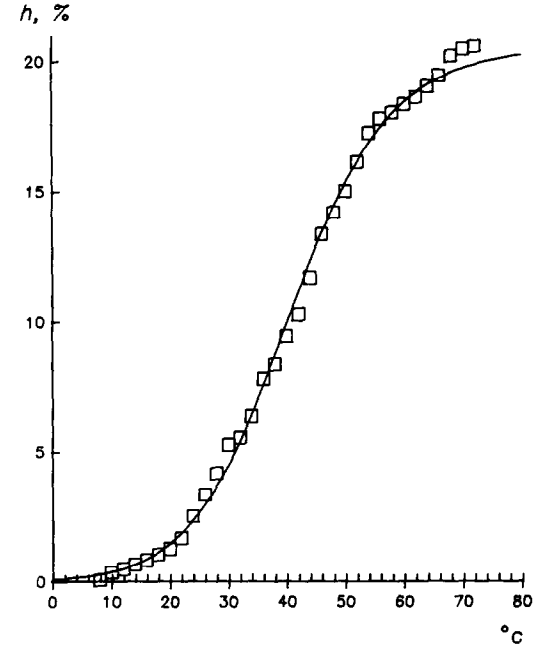


Fig.3. Experimental data on eicosamer melting (0.5×10^{-6} M) and the thermal denaturation profile calculated by thermodynamic parameters.

linear regression, the thermodynamic parameters for formation of the parallel hairpin were calculated to be $\Delta H = -90 \pm 8$ kJ/mol, $\Delta S = -300 \pm 80$ J·mol^{-1}·K^{-1} and $T_m = 40.5$°C. Fig.3 compares the experimental eicosamer melting curves with the theoretical melting curve calculated using these data.

3.2. *CD spectra of the parallel hairpin*

The CD spectrum of the parallel hairpin (eicosamer spectrum at minimal temperature and concentration) differs significantly from the B-form spectrum (decamer spectrum at maximal concentration and minimal temperature). It can be seen from fig.4 that the greatest differences are observed in the long-wavelength (>260 nm) region. A certain decrease of the maximum at 267 nm is accompanied by the appearance of the negative effect at 283 nm. To what extent the shape of this CD spectrum correlates with precisely parallel types of base pairing will be shown by further studies.

Regarding this point, the data obtained allow us to conclude that the parallel helix, at least with the

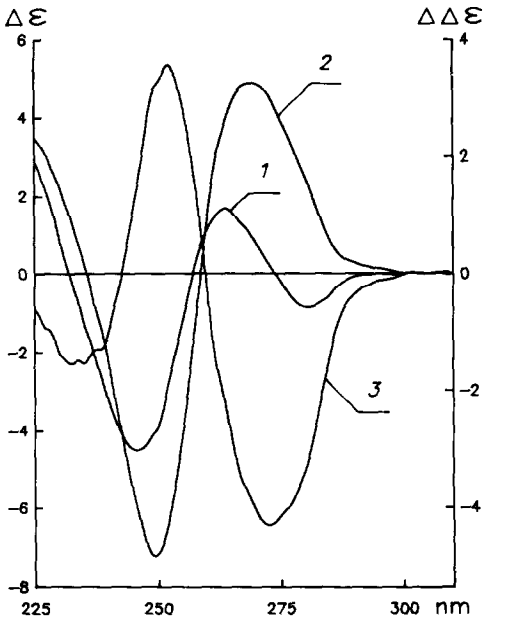


Fig.4. CD spectrum of the parallel hairpin (eicosamer, 0.5×10^{-6} M, 1°C) (1), that of the antiparallel hairpin duplex [$d(AT)_5$, 1.1×10^{-4} M, 1°C] (2) and their difference spectrum (3).

A · T type of base pairing, can be realized in oligonucleotide solution under physiological conditions. Simultaneously, the parallel helix is less profitable thermodynamically at physiological temperatures (~37°C). Therefore, if a particular nucleotide sequence has to choose between a parallel and antiparallel pairing with an equal number of complementary pairs, all other conditions being equal, the antiparallel helix would be preferred.

REFERENCES

[1] Koole, M.A., Van Gendren, M.H.P. and Buck, H.M. (1987) J. Am. Chem. Soc. 109, 3916–3921.
[2] Morvan, F., Rayner, B., Imbach, J.-L., Lee, M., Hartley, J.A., Chang, D.-K. and Lown, J.W. (1987) Nucleic Acids Res. 15, 7027–7044.
[3] Cruse, W.B.T., Egert, E., Kennard, O., Sala, G.B., Salisbury, S.A. and Visvamitra, M.A. (1983) Biochemistry 22, 1833–1839.
[4] Coll, M., Solans, X., Font-Altaba, M. and Subirana, J.A. (1987) J. Biomol. Struct. Dyn. 4, 797–811.
[5] Van de Sande, J.H., Germann, M.W., Pon, R.T., Ramsing, N.B., Elhorst, W.V., Kitzing, E., Clegg, R.C. and Jovin, T.M. (1988) Science 341, 1321–1325.
[6] Ramsing, N.B. and Jovin, T.M. (1988) Nucleic Acids Res. 16, 6659–6676.
[7] Chen, P.S., Toribata, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758.