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Formation of a duplex structure by an α-octathymidylate: circular dichroism studies

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The conformation of an octathymidylate synthesized with the α-anomer of thymidine has been studied as a function of temperature using circular dichroism. At high ionic strength a sigmoidal melting profile indicates the formation of a double-stranded structure. This duplex structure is stabilized by addition of $MgCl_2$. The thermodynamic parameters ΔH and ΔS corresponding to the formation of the duplex structure are determined by analysis of the melting curves.

Oligonucleotide, α-; CD; Thermodynamic parameter

1. INTRODUCTION

The natural nucleic acids are built with nucleotide units in which the base has the β-anomeric configuration with respect to the sugar moiety. However, it is noteworthy that a few natural nucleoside derivatives in which the glycosidic linkage exhibits the anomeric α-configuration have been described by Sequin [1], and that some synthesized α-nucleosides exhibit biological activities [2,3]. Due to their greater resistance towards nucleases than their β-analog, the interest in using α-oligodeoxynucleotides to control gene expression has been growing during the past few years [4]. It has been shown that α-oligonucleotides covalently linked or not to an intercalating agent recognize their complementary β-oligomer [4–10]. Recently we have shown that the unnatural octadeoxynucleotide α-$(Tp)_7T$ forms a duplex structure with its complementary β sequences either from the ribo or deoxyribo series [11]. The evidence of a self recognition of α-oligonucleotides generating a stable antiparallel duplex structure has been obtained by Morvan et al. [10] using NMR experiments. The aim of the present work is to demonstrate, using the CD technique, that an α-octathymidylate forms a stable self-complementary duplex structure while with its β-analog in aqueous solution no T/T base pairing was observed. We report some thermodynamic parameters of this duplex formation.

2. MATERIALS AND METHODS

α-Thymidine was purchased from Sigma. Its purity was checked by thin layer chromatography. The α-oligothymidylate α-$(Tp)_7T$ was synthesized by the phosphotriester method exactly as described for β-oligonucleotides [4,5].

CD measurements were carried out using Jobin-Yvon Mark III dichrograph in cells with pathlength ranging from 0.1 to 1 cm. The temperature of the cell was adjusted with a circulating water bath and held constant to ± 0.5°C. Concentration used to calculate CD amplitude of the oligonucleotide is that of nucleotide unit.

The Van 't Hoff transition enthalpy and entropy variations were obtained from a nonlinear least squares analysis of the temperature dependence of the CD signal using a two states model [12].

The expression of the CD signal at the temperature T for an association reaction involving structures formed from the same sequences is:

$$\Delta\epsilon(T) = f\Delta\epsilon_d + (1 - f)\Delta\epsilon_s$$

In this expression f is the fraction of single strand in the duplex state and $\Delta\epsilon_s$ and $\Delta\epsilon_d$ are the CD signals of the single- and double-strand species, respectively, and they are assumed

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to be linear functions of temperature ($\Delta\epsilon_s = aT + b$ and $\Delta\epsilon_d = a'T + b'$). f is related to the changes in enthalpy ΔH, and entropy ΔS by the expression:

$$K = f/[2(1-f)^2C] = \exp(-\Delta H/RT + \Delta S/R)$$

where C is the total strand concentration.

Because of the low T_m values, whatever the experiments, we could not discern the linear region below transition. So, we fitted with a flat prebaseline ($a' = 0$) the melting curve obtained with the most available temperature range (1.4 mM nucleotide concentration in the presence of 43 mM $MgCl_2$), and we analysed the other melting profiles with this prebaseline. The choice of prebaseline between a reasonable upper and lower limit affected the Van 't Hoff enthalpy and entropy variation data by about 15% and the T_m value by about 1°C.

3. RESULTS

3.1. *Temperature influence of α-(Tp)₇T in NaCl*

Fig.1 shows the effect of temperature variation on the CD spectrum of α-(Tp)$_7$T at a concentration of 0.45 mM in 0.1 M NaCl. The decrease of the temperature induced an increase of the intensity of the CD signal of α-(Tp)$_7$T and a red-shift of the band located around 265 nm. At the highest temperature (80°C) we remarked a persistent negative band centered at 262 nm and a positive CD signal below 240 nm. A general nonlinear variation of CD signal with temperature was observed (fig.2). It was clear that in our experimental conditions the oligonucleotide α-(Tp)$_7$T had no thermically stable structure. However, the shape of the fusion curves suggested a sigmoidal profile characteristic of the double-to-single strand transitions. To check this hypothesis we increased, on the one hand, the NaCl concentration (from 0.1 to 1 M in an α-(Tp)$_7$T solution at 0.39 mM) and, on the other hand, the nucleotide concentration (from 0.39 mM to 1.4 mM in 0.1 M NaCl). In both cases we observed an increase of the CD signal (data not shown). The fusion curves obtained were shifted to higher temperatures and revealed largely a sigmoidal profile (fig.2).

3.2. *Melting of α-(Tp)₇T in MgCl₂*

In order to investigate the suspected T-T basepair formation, we performed similar experiments in the presence of the divalent ion Mg^{2+} which is known to stabilize generally the double helix of DNA against conversion to the coil form by binding to phosphate [13]. At low temperature (−2°C) in 0.1 M NaCl and 43 mM $MgCl_2$ we noted that whatever nucleotide concentrations used, either 0.45 mM or 1.4 mM, the highest negative band was centered at 268 nm and the smallest at 224 nm, the amplitude of the CD signal being slightly greater with the highest nucleotide concentration (data not shown). We remarked that both CD spectra were larger than those obtained in

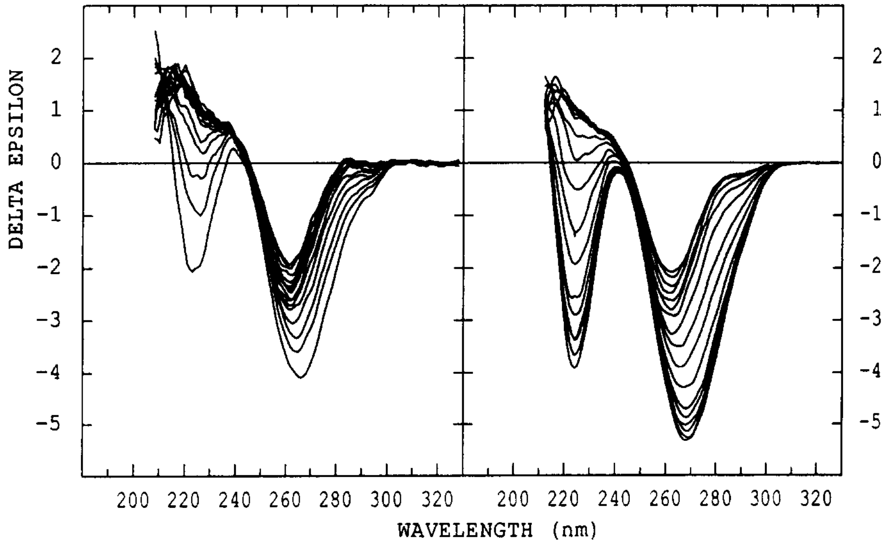

Fig.1. CD spectra of α-(Tp)$_7$T as a function of temperature in 10 mM sodium cacodylate, pH 7. The CD amplitude decreased in order of increasing temperature from −2°C to 80°C. Left: thymine concentration: 0.45 mM in 0.1 M NaCl. Right: thymine concentration: 1.40 mM in 0.1 M NaCl and 43 mM $MgCl_2$.

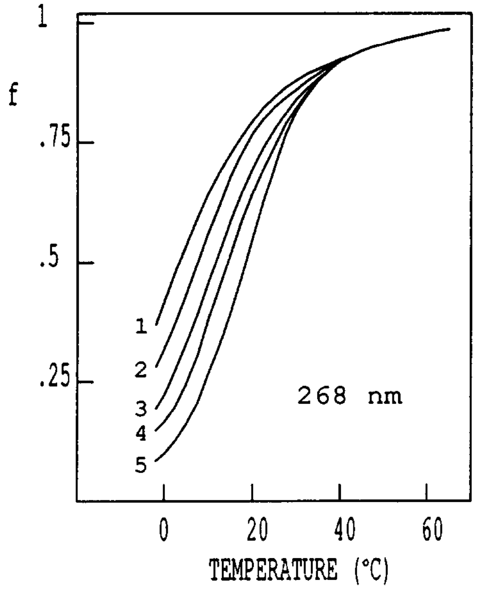

Fig.2. Delta epsilon of α-$(Tp)_7T$ normalized at 80°C. Thymine concentrations: (1) 0.45 mM in 0.1 M NaCl; (2) 1.40 mM in 0.1 M NaCl; (3) 0.39 mM in 1.0 M NaCl; (4) 0.45 mM in 0.1 M NaCl and 43 mM $MgCl_2$; (5) 1.40 mM in 0.1 M NaCl and 43 mM $MgCl_2$. Other conditions were those of fig.1.

the presence of NaCl salt alone. The melting curves exhibited clearly sigmoidal profiles reflecting cooperative melting processes (fig.2).

3.3. *Analysis of equilibrium melting curves*

The melting curves have been analysed by using a two-states model with a flat prebaseline according to the manner exposed in section 2. The average values of $\Delta H \cdot \Delta S$ and T_m are listed in table 1.

Table 1

Thermodynamic parameters of double-helix formation

NaCl salt (M)	MgCl salt (mM)	Base concentration (mM)	$-\Delta H$ (kcal/mol)	$-\Delta S$ (cal/[K·mol])	T_m (°C)
0.1	–	0.45	22	62	0
0.1	–	1.4	28	85	5
1.0	–	0.39	26	72	10
0.1	43	0.45	31	89	13
0.1	43	1.4	34	101	18

The other conditions were 10 mM sodium cacodylate, pH 7. Estimated precision in ΔH and ΔS is about ± 15%. Estimated precision in T_m is ± 1°C

The salt dependence of the thermodynamic values reflects the importance of the interaction between the phosphate groups of each strand in the duplex form. The stability is increased somewhat at the highest salt concentration by more favorable enthalpy and entropy variations. Using the data in table 1, we calculated predicted contribution of TT/TT base pairs to the stability of the double helix. In 1 M NaCl we obtained an enthalpy change of about −3.7 kcal/mol for the TT/TT stacking interaction. This value is lower than those found for usual nearest-neighbor Watson-Crick interactions (from −5.6 kcal/mol with GA/CT to −11.9 kcal/mol with CG/GC, in 1 M NaCl [14]).

4. CONCLUSION

So far, thymine-thymine base pairing was observed in the crystal structures of 3′,5′-di-*o*-acetylthymidine [15] and *cis*-thymidine-3′,5′-*N,N*-dimethylphosphoramidate [16]. It was found that parallel T-T pairing occurs at hexanucleotide level in aqueous solution, provided that phosphate groups are methylated [17]. The sigmoidal melting profiles of α-$(Tp)_7T$ indicate a helix-coil transition of a double-stranded conformation. Our results show clearly thymine-thymine base pairing formation provided that the nucleoside unit is an α-nucleoside. The fact that the thermodynamic data are influenced by the ionic strength of the medium and the presence of the divalent ion Mg^{2+} shows the importance of the interstrand phosphate-phosphate repulsive interactions.

The low value of enthalpy change associated at the disruption of the interaction in the neighboring base pairs TT/TT found, reveals a weak stacking interaction. It is recognized that the principal source of the stability of a duplex form is the base stacking which is strongly influenced by the hydration of the molecule. It is highly likely that between α-oligonucleotide and β-oligonucleotide there is a variation in the geometries and that α-$(Tp)_7T$ adopts a conformation type in which repulsions of hydrophobic groups by water lead to an attractive interaction of the α-base weaker than with natural bases. Other reasons can explain the low value of ΔH found. First, our determination is derived from treatment of the CD data in an all-or-none manner; however, if 'intermediate states' are involved, the ΔH calculated from the optical data may be lower than the value calculated from the calorimetric data [18]. Second, it is possible that the terminal base pairs are involved in a fraying process reducing the enthalpic contribution assigned to internal TT/TT base stack. Nevertheless, our thermodynamic data represent a first evaluation which will be refined by other experimental techniques.

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