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THERMODYNAMIC PARAMETERS OF THE BINDING OF AN OLIGO-α-THYMIDYLATE TO ITS COMPLEMENTARY OLIGO-β-ADENYLATE SEQUENCES

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The temperature dependence of the formation of a double-stranded complex between an oligo-α-thymidylate and its complementary sequence, β-riboadenylate or β-deoxyriboadenylate, was studied using circular dichroism and compared to the duplexes formed using an oligo-β-thymidylate. Analysis of the melting profiles allowed us to determine the thermodynamic parameters (ΔH, ΔS and ΔG) associated with the formation of the various duplexes. The order of the stability was found: α-dT:β-dA ≈ α-dT:β-rA > β-dT:β-dA > β-dT:β-rA.

During recent years it has been demonstrated that the use of anti-sense oligodeoxynucleotides as modulators of gene expression at the mRNA level was an alternative approach to antiviral chemotherapy (1-6). In vivo, one of the difficulties encountered in the use of synthetic anti-sense RNAs is their sensitivity to nucleases present in cells. Because of their greater stability the unatural α-oligonucleotides as opposed to β-oligonucleotides have been used (7-12). The α-oligodeoxynucleotides studied form duplexes with complementary β-strands. Recently, Paoletti et al (11) reported the thermodynamic data of double helix formation between an α-d(CCTTCC) and its complementary β-d(GGAAGG) sequence and compared to the formation of β:β complex . They concluded that such α:β duplex is more stable than β:β duplex. In previous work (12), we reported the results of circular dichroism studies on the interaction of the oligo(α-thymidylate) α-(Tp)$_7$T with its complementary sequences containing β-nucleosides β-r(Ap)$_7$rA and

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β-d(pA)$_8$. We demonstrated: i) as its β-analog the α-octathymidylate is able to form duplexes with its ribose or deoxyribose complementary β-strands r(Ap)$_7$rA and d(pA)$_8$ respectively; ii) binding of α-(Tp)$_7$T to its complementary β-strands is stronger than binding of β-(pT)$_8$ to its complementary β-strands. In order to estimate the extent of stabilization brought to the system by the α-strand we carried out a thermodynamic investigation of the thermal stability of the association of α-(Tp)$_7$T and β-(pT)$_8$ with their complementary β-sequences. We report in this work the corresponding thermodynamic data of double helix formation, the changes in enthalpy ΔH, in entropy ΔS and free energy ΔG, obtained from fits of dichroic melting curves using a two-state model (13-15).

MATERIALS AND METHODS

Circular dichroism measurements were carried out with a Jobin-Yvon Mark III dichrograph. The temperature of the cell was adjusted with a circulating water bath and held constant ± 0.5°C. The α-oligothymidylate, α-(Tp)$_7$T was synthesized by phosphotriester method as previous described (16). The oligonucleotides β-r(Ap)$_7$rA, β-d(pA)$_8$ and β-(pT)$_8$ were purchased from P.L Biochemicals. All measurements were performed under identical experimental conditions: 1M NaCl, 10mM Na cacodylate, pH 7.0.

The Van't Hoff transition enthalpy and entropy variations were obtained from a non linear least squares analysis of the temperature dependence of the CD signal at a wavelength where the variations of the CD amplitude of the oligomers alone were as small as possible to minimize the inaccuracy in the calculation (fig 1). To allow an easier comparison between the various melting profiles the intensity of the CD signal was normalized at 0°C.

The expression of the CD signal at the temperature T for an association reaction involving structures formed from complementary 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 sum of the separated single-stranded and of the double-stranded species, respectively. f is related to the changes in enthalpy ΔH,and entropy ΔS by the expression:

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

where C_t is the total strand concentration. Every melting curve but the β-(pT)$_8$:β-r(Ap)$_7$rA profile was fitted assuming that the

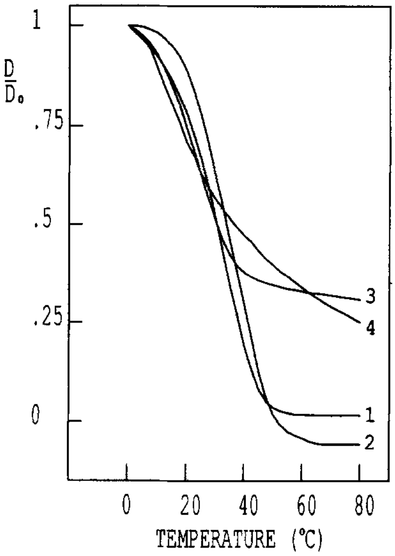

Figure 1: Delta Epsilon normalized at 0°C vs temperature for 1A:1T complexes.
(1): α-$(Tp)_7T$:β-$d(pA)_8$ at 270 nm.
(2): α-$(Tp)_7T$:β-$r(Ap)_7rA$ at 234 nm.
(3): β-$(pT)_8$:β-$d(pA)_8$ at 243 nm.
(4): β-$(pT)_8$:β-$r(Ap)_7rA$ at 250 nm.
Total strand concentrations: 0.086 mM.
All solutions contained 10 mM sodium cacodylate pH 7.0, and 1 M NaCl.

variations of $\Delta\epsilon_d$ and $\Delta\epsilon_s$ with temperature are linear. In the case of β-$(pT)_8$:β-$r(Ap)_7rA$ complex because of no convenient pre-baseline and too large variations of the CD signal of the oligomers alone (particularly β-r(Ap)7rA) the program fitted the corresponding melting profile with a flat pre-baseline and $\Delta\epsilon_s$ values experimentaly obtained by studying the variations of the CD signal of the compounds separately.

RESULTS AND DISCUSSION

The calculated thermodynamic parameters are listed in table 1. Our estimate of thermodynamic data for the β-$(pT)_8$:β-$d(pA)_8$ duplex formation can be compared to the values expected from the data published by Breslauer (17). The estimations of ΔH (-42.6 Kcal/mole) and ΔS (-122 ue) are lower than predicted -63.7 Kcal/mole and -168 ue respectively. Lately, Paoletti et al (11) observed a similar inequality between the data obtained by means of melting curve analysis and Breslauer data. Several reasons can be advanced to explain this discrepancy. First, our determination is derived from treatment of the CD data according

Table 1: Thermodynamic parameters of double-helix formation for α-$(Tp)_7T$:β-$d(pA)_8$, α-$(Tp)_7T$:β-$r(Ap)_7rA$, β-$(pT)_8$:β-$d(pA)_8$ and β-$(pT)_8$:β-$r(Ap)_7rA$ in 10 mM Na cacodylate buffer pH 7.0 and 1 M NaCl

	-ΔH Kcal/mole	-ΔS ue	-ΔG Kcal/mole
α-$(Tp)_7T$:β-$d(pA)_8$	43.6	121	7.5
α-$(Tp)_7T$:β-$r(Ap)_7rA$	40.4	110	7.6
β-$(pT)_8$:β-$d(pA)_8$	42.6	122	6.2
β-$(pT)_8$:β-$r(Ap)_7rA$	38.5	114	4.5

ΔG represents the free energy at 25 °C.
Estimated precision in data is about ±10%.

to a two-state model. however if "intermediate states" are involved, the ΔH calculated from optical data due to be lower than the value calculated from the calorimetric data (18). Second, the treatment assumes that the duplex formation is thermodynamically temperature independent, this assumption is probably not verified (19,20). Third, it is likely to occur an A-T fraying process at the ends of the duplex reducing the total stacking interaction. However, it is useful to note that our estimate of ΔG (-6.2 Kcal/mole) which is more reliable than ΔH and ΔS values is in acceptable agreement with the -7.3 Kcal/mole predicted. The thermodynamic data we have obtained allow us to evaluate the relative thermodvnamic stabilities of the various duplexes according to the kind of sequence. The free enery change values indicate: i) duplexes formed with the α-anomer containing oligothymidvlate are more stable than those formed with its β-analog; ii) the α-$(Tp)_7T$:β-$d(pA)_8$ duplex is as stable as α-$(Tp)_7T$:β-$r(Ap)_7rA$; iii) the β-$(pT)_8$:β-$d(pA)_8$ duplex is more stable than β-$(pT)_8$:β-$r(Ap)_7rA$. The order of the stability is α-dT:β-dA ≈ α-dT:β-rA > β-dT:β-dA > β-dT:β-rA.

Although, ΔH and ΔS values are less reliable than ΔG values, thev merit a comment. The most negative enthalpy change (favorable) and negative entropy change (unfavorable) occur for formation of the duplexes containing ß-d(pA)$_8$. The α-(Tp)$_7$T:ß-d(pA)$_8$ has a more favorable enthalpy change and a less favorable entropy change than α-(Tp)$_7$T:ß-r(Ap)$_7$rA; the net effect is similar values of the free energy change for both complexes; there is a compensation between the enthalpic and entropic contributions in the stability of the duplexes. The duplex ß-(pT)$_8$:ß-d(pA)$_8$ has a more favorable enthalpy change and a less favorable entropy change than ß-(pT)$_8$:ß-r(Ap)$_7$rA, but in this case the net result is a more favorable free energy change for the ß-(pT)$_8$:ß-d(pA)$_8$ complex.

The thermodynamic parameters determined in this paper as well as those determined in reference 11 should be useful for further refining rules for the design of anti-sense oligonucleotides using α-oligonucleotides.

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