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α-DNA VIII: thermodynamic parameters of complexes formed between the oligo-alpha-deoxynucleotides: α-d(GGAAGG) and α-d(CCTTCC) and their complementary oligo-beta-deoxynucleotides: β-d(CCTTCC) and β-d(GGAAGG) are different

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ABSTRACT

The temperature dependence of the formation of a complex between an α-d(CCTTCC) hexanucleotide and its complementary ß-d(GGAAGG) sequence was studied and compared to the formation of the ß-d(CCTTCC):ß-d(GGAAGG) complex. Such α-ß complex is more stable than the regular ß:ß complex. The Tm value for the α:ß complex is 28°C (ΔG° = -7.3 kcal/mole) while Tm=20,1°C (ΔG° = -6.3 kcal/mole) for the ß:ß complex. The stoechiometry of the α:ß complex corresponds to the formation of a 1:1 duplex.

However, when the α- strand is made of α-purines: α-d(GGAAGG), the stability of the α:ß complex, α-d(GGAAGG):ß-d(CCTTCC) is found to be lower (Tm = 13.8°C) than the stability of the regular ß-ß complex, leading to the conclusion that the nature of the α-sequence is important in terms of stability when considering the synthesis of such a sequence for using it as antisense oligonucleotide.

INTRODUCTION:

Among the compounds investigated to control gene expression, various nuclease-resistant antisense oligonucleotide analogues have been synthsized(1-9). These unnatural oligomers which differ from the natural oligomers only by exhibiting the α-configuration at the anomeric carbon atom (C1), present interesting features: (i) they are able to form duplexes with complementary ß- or α- strands, and these duplexes show parallel and antiparallel polarity respectively (4,5,7); (ii) Watson-Crick base pairing specificity is retained (9); (iii) heteroduplexes obtained from hybridization between complementary α- and ß-strands, belong to the B-DNA family (5); (iv) these unnatural α-oligodeoxyribonucleotides are more stable than ß-oligomers against hydrolysis by nucleases (2,8). Furthermore, it has been possible to covalently link an intercalating agent to α-oligodeoxynucleotides and then to form a strong complex with a com-

plementary ß-oligodeoxynucleotide (7,10-12), whose polarity is parallel (7,12) or with a complementary ß-oligoribonucleotide (10,11,13). In addition, the complex formed between poly(rA) and an α-anomeric tetrathymidylate covalently linked to an intercalating oxazolopyridocarbazole dye via its 3'-hydroxyl is more stable than the one formed between poly(rA) and its ß-counterpart (13,14).

These observations led us to study in more detail the thermal stability of the association of α-oligodeoxyribonucleotides with their complementary ß-sequences, since this stability, under biological conditions, is a critical consideration for using such α-oligonucleotides to control gene expression. In this work we report the thermodynamics of double helix formation between hexadeoxyribonucleotides d(CpCpTpTpCpC) or d(GpGpApApGpG) under α- or ß- configuration and their complementary α-anomeric or ß- anomeric sequences, in order to estimate the extent of stabilization brought to the system by one of the strands being in an α- configuration.

MATERIAL AND METHODS.

Synthesis:

The deoxyribonucleotides α-d(CpCpTpTpCpC), ß-d(GpGpApApGpG) and ß-d(CpCpTpTpCpC) were synthesized by a modified phosphotriester method in solution (2,3). HPLC analysis indicated a purity better than 97%. α-d(GpGpApApGpG) was obtained by solid phase synthesis according to the procedure previously described (6). In the text these oligonucleotides are abreviated ß-CCTTCC, α-CCTTCC, α-GGAAGG etc.

General Methods:

Ultraviolet spectra were recorded on a thermostated UVIKON 810 spectrophotometer (KONTRON) and fluorescence on a thermostated SFM25 spectrofluorometer (KONTRON).

CD spectra were recorded on a JOUAN MARK V apparatus interfaced with a Apple IIe microcomputer. For each temperature, a different base line was run. During the experiments, and for temperatures lower than 20°C, nitrogen was continuously circulated through the cuvette compartment.

Cacodylate buffer 10 mM, pH 7.4 with different amounts of NaCl as indicated in the text was used for these experiments and the annealing experiments.

Annealing Experiments:

Annealing experiments were done on a UVIKON 810 spectrophotometer (KONTRON), interfaced with an IBM PC compatible microcomputer. The temperature control was through a HUBER PD415 temperature programmer connected to a refrigerated water bath (HUBER Ministat). Cuvettes were 1 cm pathlength quartz cells and nitrogen was continuously circulated through the cuvette compartment. Prior to the experiments, the sequences to be studied were mixed together and allowed to incubate at 80°C for a length of time sufficient to allow the optical density of the mixture to be perfectly stable (about 1 hour). Digitized absorbance values and temperatures were stored by the computer for subsequent plotting and analysis. In a typical experiment, the computer collected absorbance and temperature every 20 sec while the temperature variation was 0.5°C/min. This typically resulted in more than 450 points for an annealing curve ranging from 80°C to 0°C. Control experiments were run using a two compartments cuvette in order to determine the temperature variation of the sum of the absorbances of two compounds without any annealing.

The results were fitted on the same microcomputer with a non linear least squares program based upon the Marquardt algorithm (15,16) and using the method described by Turner et al (17). The oligomers being non self-complementary, the changes in enthalpy, ΔH°, and entropy, ΔS°, for the reaction are related to α, the fraction of strands in the double-strand state through: $\Delta H^\circ - T\Delta S^\circ = -RT\mathrm{Log}[2\alpha/(1-\alpha)^2 Ct]$ were Ct is the total strand concentration (each strand at a concentration Ct/2). The program fitted the annealing curves with temperature dependent absorptions for both the helix and the sum of the separated single strands assuming that these absorptions are linear functions of temperature and with apparent enthalpy and entropy for the transition. In order to allow an easier comparison between the annealing profiles corresponding to different oligomers, the normalized absorbance was plotted as a function of temperature. Absorbances were normalized to the absorbance at 70°C.

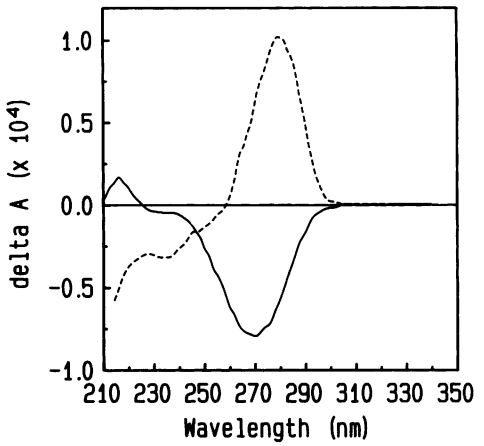

Figure 1: CD spectra of α-CCTTCC (——) and ß-CCTTCC (----) in cacodylate buffer 10 mM pH 7.0, NaCl 0.1 M, at 4°C.

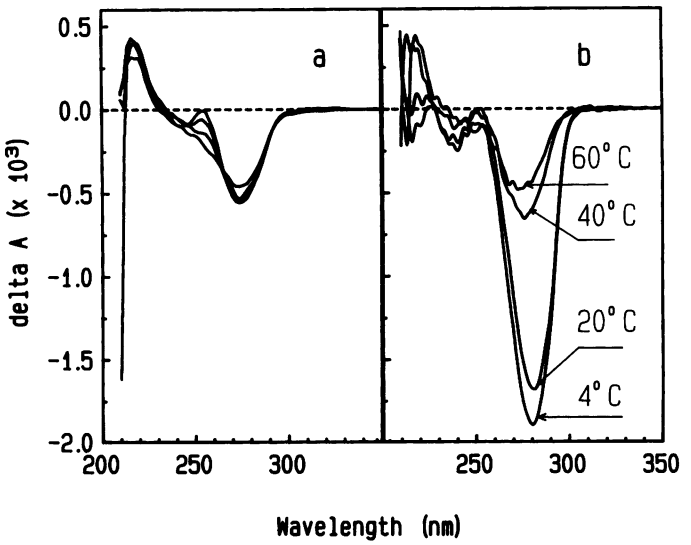

Figure 2: CD spectra of a 1:1 mixture of α-CCTTCC and ß-GGAAGG as a function of temperature in cacodylate buffer 10 mM pH 7.0, NaCl 0.1 M (panel b).
The sum of the spectra of α-CCTTCC alone and ß-GGAAGG alone in the same conditions are shown in panel a.

Mixing curves and fluorescence measurements:

Mixing curves for α-ß and ß-ß hybrids were obtained by varying the ratio of each component, keeping the total concentration of strand constant. The total strand concentration was 1.2 10^{-5} M in 0.01M cacodylate buffer, pH 7 and 1M NaCl. Ethidium bromide was added at a concentration of 9.13 10^{-5} M. The fluorescence of the mixtures was then measured at 2°C and 60°C respectively, the fluorescence of the free dye in the same conditions being set up all along the experiment at 20. The excitation wavelength was 520 nm and the emission wavelength was 610 nm.

RESULTS AND DISCUSSION

Complex formation:

The formation of a complex between an α-CCTTCC deoxynucleotide and its complementary ß-sequence has been followed in cacodylate buffer 10 mM, pH 7 and NaCl 100 mM and at a strand ratio equal to 1:1, using circular dichroism.

The CD spectrum of the α-CCTTCC hexanucleotide alone, at low temperature, is shown in Figure 1. It is caracterised by a negative band at 270 nm. In the same conditions, the ß-CCTTCC hexanucleotide showed a positive band which is slightly shifted (5 nm) toward higher wavelength. The difference in the sign of the signal comes from the enantiomery between the α- and the ß-hexanucleotides, while the difference in the position of the maximum could result from a difference of base stacking caused by changes in the sugar conformation and/or in the sugar-base torsion angle as already shown for an α-$(Tp)_7T$ oligonucleotide (18).

The formation of a complex between α-CCTTCC and ß-GGAAGG was indicated by a strong enhancement of the negative signal at 280 nm (Fig 2). At higher temperatures (60°C and 40°C), the CD spectra of the mixture of α-CCTTCC and ß-GGAAGG are the same as the sum of the spectra of the two components (Fig 2a). For lower temperatures, 20°C and 4°C, a slight displacement of the negative band from 275 nm to 280 nm, together with an amplification of the signal were observed, leading to a strong difference between the spectra of the α-CCTTCC and ß-GGAAGG mixture

and that of the sum the spectra of the components. This indicates the formation of a complex between the α-CCTTCC sequence and its complementary α- or ß- sequence thus corroborating what has been already shown through NMR studies in the case of the duplex α-CCTTCC: ß-GGAAGG (4). The spectra of the 1:1 mixture of α-CCTTCC and ß-GGAAGG at 4°C or 20°C are about the same indicating that most of the annealing takes place for temperatures between 20°C and 40°C.

In figure 3 are represented the CD spectra corresponding to a mixture of α-GGAAGG with its complementary ß-sequence; ß-CCTTCC. In this case, the formation of the complex could only be observed at 4°C, while above 20°C, no evidence of annealing appeared. Thus the stability of the complex formed between an α-CCTTCC sequence and its ß-complementary sequence appears to be considerably higher than that of the complex formed between the ß-CCTTCC sequence and its α-complementary sequence.

Stability of the complexes

UV Temperature variation curves, under identical experimental conditions, of the three duplexes studied are shown in Fig 4. The ß-GGAAGG : α-CCTTCC duplex is the most stable of the three (Tm=28°C) and its degree is 7° higher than that of ß-GGAAGG : ß-CCTTCC formed with ß-CCTTCC (Tm = 20.1°C). The free energies, associated with these transitions differ by about 1 kcal/mole of strand: -7.3 kcal/mole and -6.3 kcal/mole respectively. The hypochromicity and the slopes of the annealing are quite comparable indicating that we are not faced to formation of many hybrids due to the anomerism of the α-strand. The annealing curves were analysed by using a two state model with linear base lines (19,20) and the derived thermodynamic parameters, ΔH° and ΔS°, are shown in table 1. Our estimate of the thermodynamics for the annealing of ß-CCTTCC with ß-GGAAGG can be compared to the value predicted from the library published in the litterature (21). Our estimate of ΔG° for the ß:ß duplex at 25°C: -6.3 kcal/mole is in excellent agreement with the -6.3 kcal/mole predicted. However the observed ΔH°(-39.1 kcal/mole) is lower than the predicted ΔH° (-44.5 kcal/mole). This discrepancy could result from the treatment which assumes that the thermodynamics of complex formation is temperature independent.

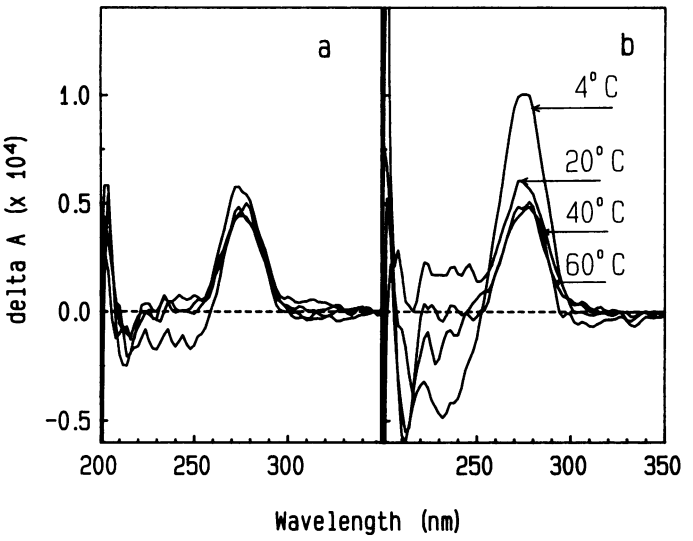

Figure 3: CD spectra of a mixture of α-GGAAGG and ß-CCTTCC (1:1 as strand ratio) as a function of temperature (panel b).
The sum of the spectra corresponding ta α-GGAAGG alone and ß-CCTTCC alone are shown in panel a. Same experimental conditions as in Figure 2.

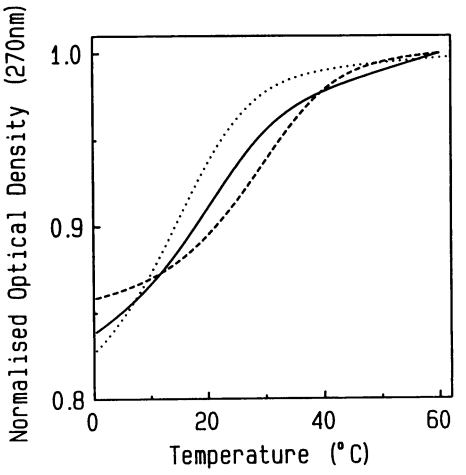

Figure 4: Normalized absorbance at 270 nm vs. temperature for ß-CCTTCC:ß-GGAAGG (———), α-CCTTCC:ß-GGAAGG, (— — —) and ß-CCTTCC:α-GGAAGG (·····). Total strand concentrations: 10^{-5}M. All solutions contained 10 mM cacodylate buffer pH 7.0, and 1.0 M NaCl.

This assumption is probably not verified as long as stacking of single strands states, which is important for ß-GGAAGG, is temperature dependent (22,23). Nevertheless, comparison between the thermodynamics derived for annealed ß-GGAAGG : ß-CCTTCC and for annealed ß-GGAAGG : α-CCTTCC allows us to conclude that the hetero complex ß-α is more stable than the natural ß-ß complex and that the ΔH° and ΔS° corresponding to each transition are of the same order of magnitude. As shown in Figure 5, the maximum of stabilisation is reached when the complexes are formed in 0.5 M NaCl since highering the ionic strength from 0.5 M NaCl to 1M NaCl stabilises the ß-ß complex when the stability of the corresponding α-ß complex is not changed in these conditions.

If α-GGAAGG was annealed with ß-CCTTCC, the results are different. The annealing curve (Fig. 4) shows a higher induced hypochromicity when compared to the hypochromicity corresponding to the ß-GGAAGG:α-CCTTCC complex, 18.5% and 13.5% respectively, and a lower temperature of half formation, 13.8°C compared to 28°C in the previous case. This finding corroborates the observed CD spectra for the ß-CCTTCC : α-GGAAGG complex. The estimates of the thermodynamics, in this case, indicate that the destabilisation is mainly due to an enthalpic factor (Table 1), ΔH° for the α-GGAAGG : ß-CCTTCC complex being equal to -84 kcal/mole compared to -37 kcal/mole even though the entropy factor is slightly more favorable: -94 e.u. compared to -101 u.e. This result indicates that the nature of the nucleotides in the α strand is not indifferent toward the stabilisation of an α-ß complex, a homopurine α- strand being unfavorable for the formation of a complex with a ß- complementary strand. It has been reported (9) that in conditions of NMR studies, the Tm corresponding to the annealing of an α-d(TCTAAAC) sequence with the ß-complementary sequence: d(AGATTTG) was about the same as the corresponding ß-ß duplex: Tm = 33°C. If actually, the duplex of α-purines with ß-pyrimidines is less stable and the duplex of α-pyrimidine with ß-purine is more stable than the corresponding ß-ß duplex, the balance in the TCTAAAC sequence between purines and pyrimidines would lead to a stability of the α-ß duplex expected to be about the same as the stability of the ß-ß duplex.

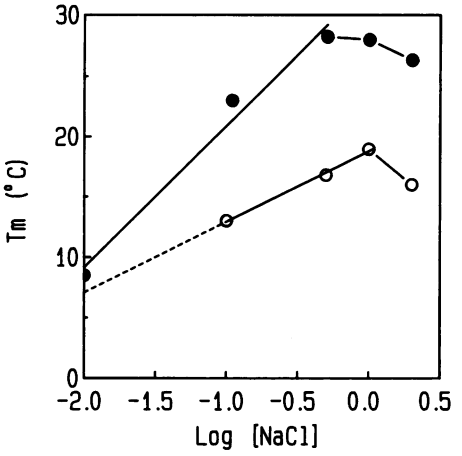

Figure 5: Salt dependence of the Tm for ß-CCTTCC:ß-GGAAGG (-o-) and α-CCTTCC:ß-GGAAGG (-●-).

Stoichiometry of the complex:

Mixing curves based upon the binding of ethidium bromide(EthBr) (24) to a mixture of d(CCTTCC) and d(GGAAGG) and which corresponds to the combinations, ß:ß, α:ß and ß:-α is shown on figure 6. The fluorescence of the complexes culminates around 50% of d(GGAAGG), at a temperature of 4°C. At 60°C, only the fluorescence of the free dye is observed and no more complex is de-

Table 1: Thermodynamics for single-strand to double-helix transition for ß-CCTTCC:ß-GGAAGG, α-CCTTCC:ß-GGAAGG and ß-CCTTCC:α-GGAAGG in cacodylate buffer 10 mM pH 7.0 and NaCl 1M. ΔG° represents the free energy at 25°C.

	ΔH° (kcal/mol of duplex)	ΔS° (u.e.)	ΔG° (kcal/mol of duplex)	tm (°C)
ß-CCTTCC ß-GGAAGG	-39.1 ± 3	-110 ± 10	-6.3 ± 2	20.2
α-CCTTCC ß-GGAAGG	-37.5 ± 0.9	-101 ± 3	-7.3 ± 1	28.1
ß-CCTTCC α-GGAAGG	-34.0 ± 0.8	-94 ± 3	-6.0 ± 0.9	13.8

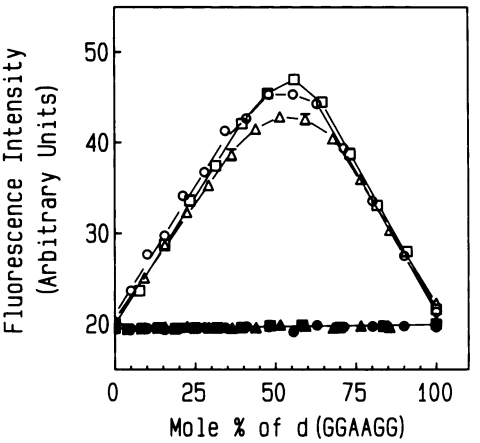

Figure 6: Mixing curve titration of CCTTCC + GGAAGG followed through fluorescence of bound ethidium. All solutions contained 10 mM cacodylate buffer pH 7.0 and 1.0 M NaCl. The fluorescence of free ethidium was set to 20.
ß-GGAAGG + ß-CCTTCC at 4°C (—o—) at 60°C (—●—)
ß-GGAAGG + α-CCTTCC at 4°C (—△—) at 60°C (—▲—)
α-GGAAGG + ß-CCTTCC at 4°C (—□—) at 60°C (—■—)

tectable. Whatever the anomeric configuration of the strands, the plots are about the same, indicating that the stoichiometry of the complex formation is the same. In addition, the enhancement of the fluorescence which results from the binding of ethidium bromide to the α-ß or the ß-α complex is about the same as the enhancement resulting from the binding of the dye to a regular ß-ß complex. This result is in accordance with the finding that the overall structure of an annealed α-ß duplex is not quite different from the structure of the corresponding ß-ß duplex (5,9).

CONCLUSION:

The annealing profiles, as a function of temperature, of hybrids formed between a ß-hexamer and its complementary α- sequence were studied and compared to the corresponding ß-ß complex. As previously shown (1), it appears that the α-d(CCTTCC) can form a duplex with the ß-d(GGAAGG) sequence. This duplex is more stable than the regular ß-d(CCTTCC):ß-d(GGAAGG) duplex, leading to a higher tm: 28°C and 20.1°C respectively, in 1M

NaCl. The reverse situation, in which an α-d(GGAAGG) sequence is associated to ß-d(CCTTCC) leads to a duplex less stable than the corresponding ß-ß duplex : tm = 13.8°C and 20.1°C respectively. This finding suggests that α-purine sequences could represent unfavorable factors in the duplex formation between α- and ß-strands.

The contribution of α-purines to an overall destabilisation of the α:ß duplex has been confirmed by using an α- sequence synthesised with the four different bases (unpublished results), and the respective contribution of α-A and α-G to this destabilisation will be reported in due course.

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