Thermodynamic behaviour of the heptadecadeoxynucleotide d(CGCGCGTTTTTCGCGCG) forming B and Z hairpins in aqueous solution

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Received 12 May 1986; Accepted 30 May 1986

ABSTRACT

UV and CD data of the partially self-complementary heptadecadeoxynucleotide d(CGCGCGTTTTTCGCGCG), obtained as a function of temperature, salt and strand concentration, show that : a) at low NaCl and strand concentration the oligomer exhibits, on increasing the temperature, a biphasic thermal profile which is indicative of two structural transitions, from dimeric duplex to hairpin and from hairpin to coil; b) the loop stabilizes enthalpically both B and Z hairpin structures with respect to the corresponding unconstrained hexamer d(CGC6CG) by a few Kcal/mol; c) the oligomer undergoes a B-Z transition which appears to be complete, at 0°C, when induced by NaClO₄; by contrast the B-Z transition induced by NaCl does not reach completeness even at salt saturation. The independence of the denaturation temperature, at high salt conditions, on the oligomer concentration indicates that the Z structure is present also in the hairpin.

INTRODUCTION

Over the past few years, as the solid-phase synthesis of DNA fragments became a common practice in many laboratories (1-4), a great number of studies on the conformation and dynamics of synthetic oligodeoxynucleotides have been carried out(5-13). Short sequences with the ability to fold back into hairpin structures have recently received considerable attention (14-21). Hairpins are important secondary structures because they represent common features in both t-RNAs and r-RNAs. Moreover it has been demonstrated that inverted repeat sequences in superhelical DNA can extrude into cruciform structures (22,23) which seem to be implicated at both transcription and replication level (24). As part of our investigation of the conformational dynamics of alternating purine/pyrimidine oligodeoxynucleotides, in particular with respect to the B-Z transition (25-27), we have recently studied a few sequences which can form hairpin structures characterized by a loop of unpaired nucleotide residues. In this contribution we report the thermodynamic behaviour in aqueous solution as well as the ability of the hairpin stem to assume a left-handed conformation in high salt conditions of the heptadecadeoxynucleotide d(CGCGCGTTTTTCGCGCG).

MATERIALS AND METHODS

d(CGCGCGTTTTTCGCGCG) was synthesized following a modified solid phase phosphotriester method (1-4). The purity of the oligomer was checked by polyacrilamide-gel electrophoresis (28). The gel (20x15x0.1 cm) contained 20% acrylamide, 7M urea, 50mM Tris.HCl (pH=8.3), 1mM EDTA and 0.07% ammonium persulfate. Electrophoresis was carried out at room temperature and at constant voltage of 600 V, 30 V/cm. Bromophenol blue dye was used as a marker. Electrophoresis bands were stained plunging the gel in a solution of

stains—all dye dissolved in formamide–water (1:1). The oligomer exhibited clearly a single band confirming its purity.

Absorbance spectra and absorbance versus temperature profiles measured at 270 nm were recorded using a Cary 219 Spectrophotometer equipped with a thermostatted cuvette holder and a Heake F3 thermostat provided with a temperature programmer Heake P6 10. Melting curves were obtained by increasing the temperature at the rate of 0.3 °C/min. The oligomer concentration ranged from 6 to 36 μM in single strands (assuming an average extinction coefficient per base of $7000 \text{ M}^{-1}\text{cm}^{-1}$). All the solutions at different ionic strength contained equal concentrations of Tris.HCl (pH=7.4) and NaCl or NaClO_4 . Samples were degassed immediately before use by stirring the solutions under vacuum.

CD spectra were obtained using a Jasco J500A dichrograph equipped with a thermostatted cell holder. The dichrograph was connected with a Jasco DP-500N data processor.

RESULTS AND DISCUSSION

Thermally induced transitions

Fig.1 shows a typical thermal profile obtained recording the variation of the oligomer absorbance at 270 nm with temperature in 0.1 M NaCl, 0.1 M Tris.HCl and at strand concentration of 36 μM . Although it may not appear immediately evident, a biphasic thermal profile is generally present, since a major absorbance variation at higher temperatures is preceded by a smaller but unequivocal one at lower temperatures. This feature is best noticed in the corresponding derivative curves, which are reported in Fig. 2 for several different oligomer concentrations. These two absorbance changes are indicative of two thermally induced transitions: the first (from now on referred to as transition I), occurring at lower temperatures, is characterized by a small hyperchromic variation and its semitransition temperature ($T_{1,m}$) increases with the

Fig. 1) Absorbance (at 270 nm) versus temperature profile for d(C6C6C6TTTTTC6C6C6) in Tris.HCl 0.1 M, NaCl 0.1 M (pH=7.3) and at strand concentration of 36 μM .

oligomer concentration; the second (from now on referred to as transition II) is characterized by a higher hyperchromic change and its $T_{II,m}$ is independent on the oligomer concentration. The reversibility of the melting process has been checked recording a few thermal profiles on cooling the sample at a rate of $0.3^{\circ}/\text{min}$; transition II appeared fully reversible and transition I exhibited a very slight hysteresis indicating a minor deviation from equilibrium in the process. The thermal behaviour has been explored also at different NaCl concentrations, in the range 0.01–0.3 M. The $T_{II,m}$ increases with the ionic strength as shown in Fig. 3, becoming higher than 90°C and therefore difficult to detect at NaCl concentration higher than 0.3 M. Transition I is clearly resolved at NaCl concentrations higher than 0.05 M, and its $T_{I,m}$ increases with ionic strength. These evidences, in analogy with similar ones described in the recent literature (14,17), suggest the following considerations:

- transition I, being dependent on the oligomer concentration, involves an interstrand process, whereas transition II, which is concentration independent, reflects an intrastrand process;
- the UV spectrum at 95°C (after transition II had occurred) is clearly indicative of the single stranded coil (being almost coincident with the spectrum obtained with the stoichiometric mixture of the constituent monomers)(data not shown);

Fig. 2) Differential melting curves of $d(CGCGCTTTTCGCGC)_n$ Tris.HCl 0.1M, NaCl 0.1M (pH=7.3) four different strand concentrations : a) $36 \mu\text{M}$; b) $26 \mu\text{M}$; c) $16 \mu\text{M}$; d) $6 \mu\text{M}$.

Fig.3) Plot of the semitransition temperature $T_{II,m}$ versus $\ln[Na^+]$ for d(CGCGCGTTTTTCGCGCG) at strand concentration of 16 μM .

c) the increase of both transition temperatures with salt concentration suggests that in both cases the pre-transition structure has a higher charge density than the post transition one (29,30).

These considerations lead to the identification of transition I with that from a duplex structure (D_2) to a hairpin structure (H), and of transition II with the denaturation of the hairpin to the coil state (C) (scheme 1):

Fig.4) Plot of the reciprocal semitransition temperature $T_{1,m}$ versus $\ln C_p$ for d(CGC6C6TTTTTC6C6C6) in Tris.HCl 0.1 M, NaCl 0.1 M (pH=7.3).

Thermodynamic Analysis

On the basis of the equilibria illustrated in Scheme 1, a thermodynamic analysis has been performed in order to estimate the enthalpic and entropic changes involved in both transitions. With regard to transition II the magnitude of the hyperchromic effect has allowed a van't Hoff analysis of the denaturation profile on the assumption that the ratio between the coil and hairpin concentrations, which is in turn the equilibrium constant $K_{II} = (C)/(H)$, can be sufficiently approximated by the quantity $(A - A_H)/(A_C - A)$, being A the effective absorbance, A_H and A_C the absorbances of the hairpin and of the coil form, respectively, at each given temperature in the range of the transition. The thermodynamic parameters have been obtained by van't Hoff plots ($\ln K_{II}$ versus $1/T$). Linear plots have been obtained. The mean values of the enthalpy and entropy changes for transition II determined on the basis of the denaturation curves at several oligomer concentrations are reported in Table I. In the case of transition I a similar treatment of the data has not been performed, since the hyperchromic effect involved is too small. However, an estimate of the enthalpy change has been obtained through the analysis the dependence of $T_{1,m}$ on the oligomer concentration (14). Fig. 4 shows the plot of $\ln C_p$ versus $1/T_{1,m}$ and the related thermodynamic parameters are reported in Table I.

A number of considerations can be made about transition II. The melting experiments clearly show a greater thermal stability of the hairpin structured heptadecamer with respect to that of the corresponding duplex of d(CGC6C6) whose denaturation temperature is concentration dependent but always lower than that of the heptadecamer(31). We have tried to assess if this stabilization has an enthalpic contribution beyond the entropic one due to the constraint imposed by the loop. The comparison of the denaturation enthalpy changes measured for the hairpin (58 ± 1 Kcal/mol) and for the duplex of d(CGC6C6) (51 ± 1 Kcal/mol)(31), indicates that the presence of the loop stabilizes enthalpically the hairpin with respect to the "stem only" by 7 Kcal/mol. The origin of this contribution could be attributed to: a) the stacking between the thymidine residues in the loop and the flanking cytosine and guanine bases paired in the stem, as already seen in the case of the dodecamer

Table I
Thermodynamic data for d(CGC6C9TTTTTC6C6C6)

[NaCl] (M)	Olig.conc. $\mu\text{M}/\text{strand}$	Melting temp. Trans I Trans II °C °C		ΔH_{VH} (a) Kcal/mol. Trans. I	ΔH_{VH} (b) Kcal/mol. Trans. II	ΔS (u.s.) Trans. II
0.01	8 4		76 76			
0.04			81			
0.1	6 16 26 36	30 38 43 46	83 82 83 83	23	59 57 58	165 160 163
0.2	6	37	87		58	165
0.3	5.8	42	87		55	153

(a) From the concentration dependence of $T_{1,m}$; (b) From the shape of melting curves

d(C6C6TATAC6C6) (17), or b) the suppression of the "fraying end" effect for the C6 base pair next to the loop.

With regard to transition I the enthalpy change (20 ± 2 Kcal/mol) is mainly attributable to the structural changes in the thymidine stretch. This suggests that in the dimeric duplex the central thymidine residues, rather than constituting a bulge, are likely to be appreciably stacked and possibly paired by hydrogen bonds.

CD Data and B-Z transition

The ability of the heptadecamer to assume a left handed Z conformation in high salt conditions has been investigated by recording the CD spectra at a number of increasing salt concentrations. Different salts have been used to induce the transition. Fig. 5 shows the CD spectra obtained at 0°C at several NaClO_4 concentrations. The insert shows the CD signal changes at 253 and 295 nm as the salt concentration is increased. It is evident that the oligomer undergoes a B to Z transition, and that the transition is complete at around 4 M salt. On the contrary the B-Z transition prompted by NaCl appears to be not complete, as illustrated in Fig. 6, even at saturated conditions. An estimate based on the known CD spectra of the hexamer d(CGC6C6)₂ in the two limit conformations, and taking into account the small contribution attributed to the thymidine residues, provides for the Z structure fraction a value of about 0.6 (A comparison of the Δ values obtained at high salt concentrations in NaCl and NaClO_4 is misleading due to the marked reduction of the ellipticity values of the Z-form in the presence of the latter salt). An important point is to establish if the Z structure is present as a bimolecular duplex or as a hairpin. An answer to this question was obtained by studying the effect of the temperature on the CD spectrum in nearly saturated NaCl solution for two oligomer concentrations differing by an order of magnitude ($C_1=90 \mu\text{M}$, $C_2=8 \mu\text{M}$ in single strands, respectively). The relative CD thermal profiles are practically indistinguishable with a cooperative transition in the range 70–90°C and with a midpoint at around 80°C, independent on the oligomer concentration data not shown). Such a finding clearly

Fig. 5) CD spectra of $d(C6C6C6TTTTTC6C6C6)$ at increasing amounts of $NaClO_4$ in Tris.HCl 0.1 M (pH=7.3) and at strand concentration of $6.9 \mu M$. Insert shows the change in the ellipticity at 253 nm and 295 nm as a function of $NaClO_4$ concentration.

shows that this denaturation is an intramolecular process, analogous to the one observed at low salt concentration. The structure that undergoes the denaturation process is therefore the hairpin, or, better the hairpins since the CD spectra show the presence of not much dissimilar amounts of both B and Z helices in saturated NaCl. A small variation in the CD signal is found also in the low temperature range (0 to $40^\circ C$). Since the 253 nm band becomes slightly more negative and the 295 nm one is slightly less negative on increasing the temperature in this range, this behaviour is likely due to a small shift toward B form in the B-Z equilibrium. This finding is in agreement with the behaviour of $d(CG)_{n=3,4}$ oligomers, as evidenced by NMR (32) and CD (31) measurements. A qualitatively similar result was obtained when the melting temperature of the hairpin in 5 M $NaClO_4$ (the solvent in which the hairpin is present mainly in the Z-conformation) was followed by both UV and CD measurements at different oligonucleotide strand concentrations. Also in this case $T_{11,m}$ is independent on the oligomer concentration and, as expected, the melting temperature is markedly depressed by the helix destabilization effect exerted by the ClO_4^- anion (33).

Fig.6) CD Spectra of $d(CGCGCGTTTTTCGCGCG)$ at increasing amounts of NaCl in Tris.HCl 0.1M(pH=7.3) and at strand concentration of $9 \mu M$. Insert shows the change in ellipticity at 253 nm and 295 nm as a function of NaCl concentration.

A precise evaluation of the enthalpy change in $NaClO_4$ 5 M, associated to transition II (from Z-hairpin to coil) was difficult to obtain due to the small difference in hyperchromicity between the two states. However the average value over three different melting profiles at different oligonucleotide strand concentrations has been found to be 55 ± 2 Kcal/mol. Comparing this value with the enthalpy change associated with the denaturation of the Z-form duplex $d(CGCGCG)_2$ in 5 M $NaClO_4$ (49 ± 1 Kcal/mol), it turns out that the loop stabilizes enthalpically the hairpin also when its stem is in the Z conformation.

CONCLUSIONS

This work, based on optical and chiroptical measurements, shows that the heptadecadeoxynucleotide $d(CGCGCGTTTTTCGCGCG)$ exists in aqueous solution at low strand concentration as an equilibrium between two structured conformations: the dimeric duplex and the monomeric hairpin, not differently from analogous oligomers reported in the recent literature (14-17,20,21). At 0.1 M NaCl the dimer is predominant at lower temperature and higher strand concentrations and the equilibrium is shifted towards the hairpin on increasing the temperature and/or decreasing the strand concentration. The hairpin, in both B and Z conformations, appears stabilized by the loop with respect to the duplex of $d(CGCGCG)$ not only through an entropic but also enthalpic contribution. Since the transition from the dimeric duplex to the hairpin proceeds

probably through a cruciform structure, as already proposed (16), work is in progress in our laboratory in order to obtain also kinetic data on this process by means of T-jump experiments with the aim of getting more information about the energetics of this transition. Van de Sande et al. (21) have recently provided experimental evidence, mainly on the basis of the electrophoretic mobility, that the 24-mer sequence d(CGCGC6C6C6TTTTTC6C6C6C6) is able to assume a hairpin structure and have argued that the hairpin is maintained also when high salt conditions promote the B-Z transition of the double helix stem. The spectroscopic data presented in this paper for the heptadecadeoxynucleotide d(C8C8C8TTTTTC8C8C8) demonstrate indeed the stability of a Z-stem hairpin conformation, corroborating the evidences reported earlier (21). It is interesting to note that the ability of the hairpin to assume a left-handed conformation is influenced by the nature of the co-ion (ClO_4^- versus Cl^-), being ClO_4^- more effective than Cl^- .

ACKNOWLEDGMENTS

This work has been carried out with the financial contribution of Consiglio Nazionale delle Ricerche and of Ministero della Pubblica Istruzione.

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