The B–Z Conformational Transition in Folded Oligodeoxynucleotides: Loop Size and Stability of Z-Hairpins†

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ABSTRACT: The capacity to assume a left-handed conformation and the thermodynamics of loop formation in concentrated aqueous $NaClO_4$ have been investigated for the following palindromic sequences: d-(CGCGCGAAAAACGCGCG) (**A5**), d(CGCGCGTTTTTCGCGCG) (**T5**), d(CGCGCGTACGCGCG) (**TA**), and d(CGCGCGATCGCGCG) (**AT**). The results show that (a) each oligomer assumes a Z conformation upon exposure to increasing $NaClO_4$ concentrations; the salt concentration at the transition midpoint is 1.8 M for both **A5** and **T5** and 3 and 3.5 M for **TA** and **AT**, respectively; (b) in high salt the four oligomers exist, over a wide range of nucleotide concentrations (up to 10^{-3} M) and of temperature (>0 °C), as unimolecular hairpin structures; (c) hairpins **TA** and **AT** exhibit, in buffer A, a lower thermal stability with respect to **A5** and **T5** (ΔT about 16 °C), contrary to what is observed at low ionic strength; (d) on hairpin formation, the enthalpic term is about −52 kcal/mol for the two 17-mers and −38 kcal/mol for the two 14-mers, while the change in entropy is found to be around −150 eu for **A5** and **T5** and −115 eu for **TA** and **AT**. This thermodynamic picture suggests that a two-residue loop for **TA** and **AT**, found at low ionic strength [see preceding paper (Xodo, L. E., Manzini, G., Quadrifoglio, F., van der Marel, G. A., & van Boom, J. H. (1988) *Biochemistry* (preceding paper in this issue)], is substituted by a longer one including two additional residues from a missing dC·dG base pairing at the top of the stem.

DNA sequences with inverted repeats (palindromes), often occurring in control regions of the genome (Rosenberg & Court, 1979; Wells et al., 1980; Muller & Fitch, 1982), can extrude cruciform structures when present in negatively supercoiled plasmids (Panayotatos & Wells, 1981; Lilley, 1980, 1981; Vologodskii et al., 1979). Since this behavior could assume biological relevance, many biophysicists have been induced to investigate the secondary structures of totally or partly palindromic oligodeoxynucleotides, capable in principle of adopting a looped conformation. It has been shown that most of the synthetic sequences studied, which include fully (Scheffler et al., 1968; Wemmer et al., 1985; Marky et al., 1983) and partly (Xodo et al., 1986; Haasnoot et al., 1985, 1986; Germann & van de Sande, 1985; Roy et al., 1986; Summers et al., 1985) matched and mismatched (Orbons et al., 1986) palindromic sequences, can exist in solution as a mixture of two ordered forms identified as the monomeric hairpin loop and the dimeric duplex structures. While several thermodynamic aspects of oligonucleotide loop formation have been investigated for hairpins with a stem in the B conformation (Marky et al., 1983; Haasnoot et al., 1985, 1986; Wemmer et al., 1985; Xodo et al., 1986; Ikuta et al., 1986), the behavior of palindromic sequences in conditions of reduced water activity (obtained by high salt concentration or with alcohol) has not been thoroughly studied. In a previous paper (Xodo et al., 1986) we reported that the 17-mer d-(CGCGCGTTTTTCGCGCG) adopts, in 4.6 M $NaClO_4$ and concentration in the order of 10 μM in strands, exclusively the hairpin structure with the alternating C-G stem in the Z conformation. It was also reported that the closely related d(CGCGCGCGCGTTTTCGCGCGCGCG) does exists, in suitable conditions, as a hairpin with the stem in the Z conformation (Germann et al., 1985). In the light of these findings we have extended our investigation on the B–Z transition to several hairpin structures with a different loop, in terms of both size and composition, but all with the same Z helicogenic alternating guanine–cytosine stem. In this paper we report the thermodynamics of loop formation in concentrated aqueous $NaClO_4$ for some oligonucleotides, in which a segment of adenine or/and thymine residues intervenes in the self-complementary part of the fragments, as illustrated below. The data presented here, combined with those we found for the same molecules in the B conformation [preceding paper in this issue (Xodo et al., 1988)], help to outline a conformational picture of the influence of loop size and loop composition on the overall stability of hairpin structures with a stem in both the B and Z conformation.

Materials and Methods

Oligodeoxynucleotides. The oligodeoxynucleotides d-(CGCGCGTTTTTCGCGCG) (**T5**), d(CGCGCGAAA-AACGCGCG) (**A5**), d(CGCGCGTACGCGCG) (**TA**), d-(CGCGCGATCGCGCG) (**AT**), and d(CGCGCG) were synthesized following a modified phosphotriester method as described elsewhere (van der Marel et al., 1981; van Boom et al., 1982). The samples were purified by gel permeation chromatography using a Sephadex G-50 resin, with elution of the column with a solution of 0.5 M triethylammonium bicarbonate (TEAB). The samples' purity was checked by 20% polyacrylamide gel electrophoresis in denaturating conditions (Frank & Koster, 1979).

UV Spectroscopy. Absorbance spectra and absorbance versus temperature profiles were recorded at 270 and 247 nm,

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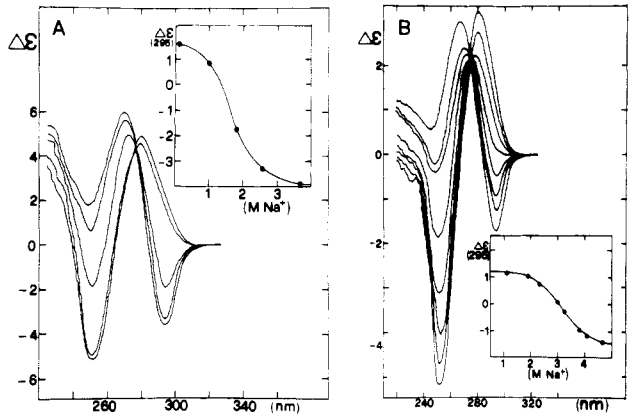

FIGURE 1: (A) Circular dichroism spectra of d(CGCGCGAAAAACGCGCG) at increasing amounts of $NaClO_4$ in 0.1 M Tris·HCl (pH 7.4), 5 °C, and a nucleotide concentration of 0.24 mM (phosphate). The insert shows the change in the ellipticity at 295 nm as a function of $NaClO_4$ concentration (B) Circular dichroism spectra of d(CGCGCGTACGCGCG) at increasing amounts of $NaClO_4$ in 0.1 M Tris·HCl (pH 7.4), 5 °C, and nucleotide concentration of 0.7 mM (phosphate). The insert shows the change in the ellipticity at 295 nm as a function of $NaClO_4$ concentration.

as described in the preceding paper. Melting curves were obtained by increasing the temperature at a rate of 0.1 °C/min. The oligomer concentration ranged from 0.03 to 0.3 mM (phosphate), assuming as extinction coefficients the average values obtained on the basis of 14 000 M^{-1} cm^{-1} for purines and 7000 M^{-1} cm^{-1} for pyrimidines. Measurements were carried out in 0.1 M Tris·HCl and 4.6 M $NaClO_4$, pH 7.4 (buffer A), and/or 0.1 M Tris·HCl and 6 M $NaClO_4$, pH 7.4 (buffer B). Each melting profile was recorded also on cooling the samples (at a rate of 0.3 °C/min) in order to check the reversibility of the process, which proved to be good.

Circular Dichroism Measurements. Circular dichroism (CD) measurements were made with a Jasco 5000 A dichrograph, equipped with a thermostated cell holder and connected with a Jasco DP 500 N data processor. CD spectra of oligonucleotide solutions [0.5 mM (phosphate)] were recorded by using either a 1-cm or a 0.5-cm path length cell. Typically, prior to recording, each spectrum was accumulated four times and the base line was subtracted. The B–Z transitions were induced by adding weighted amounts of dried $NaClO_4$ directly into the cuvette. The spectra were recorded after the cell thermostation was carefully checked.

Analysis of the Data. The thermodynamic parameters for the hairpin–coil conformational equilibrium have been obtained from melting profiles analysis (see Results). Data were corrected for volume expansion of the solvent as a function of temperature. The best fits of the transition were made by using a nonlinear least-squares program [algorithm of Marquardt: Bevington (1969)] in a VAX hardware system.

Results

The B–Z Transition. The CD spectrum for each of the four oligomers (**T5**, **A5**, **TA**, **AT**) was measured as a function of increasing amounts of $NaClO_4$. Two representative sets of such spectra, one relative to the 17-mer **A5**, the other to the 14-mer **TA**, are reported in Figure 1. The inserts show the change of the CD signal at 295 nm as the concentration of salt increases. $NaclO_4$ induces a cooperative B to Z transition in both 17-mers **A5** and **T5** (Xodo et al., 1986), very similar to the one occurring in poly(dG–dC) (Pohl & Jovin, 1972): the salt concentration at the midpoint is 1.8 M $NaClO_4$. In the case of the tetradecamers **TA** and **AT** more stringent conditions are required in order to have the B to Z transition

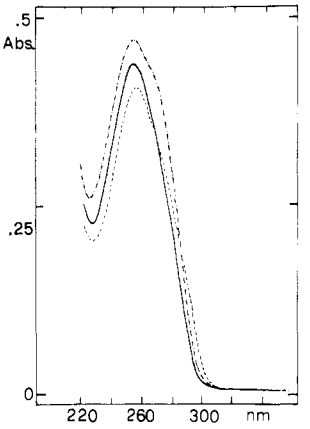

FIGURE 2: UV spectra of d(CGCGCGTACGCGCG) in various conformations: (a) (–·–) random coil (4.6 M $NaClO_4$, 0.1 M Tris·HCl, pH 7.4, and T = 95 °C); (b) (—) right-handed (0.1 M $NaClO_4$, 0.1 M Tris·HCl, pH 7.4, and T = 5 °C); (c) (- -) left-handed (4.6 M $NaClO_4$, 0.1 M Tris·HCl, pH 7.4, and T = 5 °C).

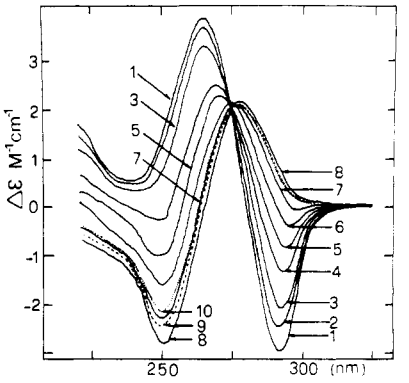

FIGURE 3: Circular dichroism spectra of d(CGCGCGTACGCGCG) in 4.6 M $NaClO_4$, 0.1 M Tris·HCl (pH 7.4), and a nucleotide concentration of 0.21 mM (phosphate) at different temperatures: (1) 5 °C; (2) 20 °C; (3) 30 °C; (4) 40 °C; (5) 45 °C; (6) 50 °C; (7) 55 °C; (8) 60 °C; (9) 70 °C; (10) 85 °C.

reach completion. Indeed, as shown by the insert of Figure 1A, the 14-mer **TA** is characterized by a transition midpoint at 3 M $NaClO_4$. Similarly, for the 14-mer **AT** the concentration of $NaClO_4$ at the semitransition point has been found to be 3.5 M salt (data not shown). The B to Z transition was also followed by UV spectroscopy. Absorbance spectra at both low and high ionic strength for the 14-mer **TA** are illustrated in Figure 2. An isosbestic point is present at 270 nm, and the shift from the B to the Z conformation is accompanied by a hypochromic effect at wavelengths below 270 nm. The effect of the temperature on the CD spectrum at 4.6 M $NaClO_4$ for each of the four oligomers has been investigated. In figure 3 the spectrum of **TA** is shown at six different temperatures covering its denaturation range (see next section). The increase of the temperature slightly shifts the equilibrium from the Z to the B structure prior to denaturation (Figure 3, curve 8), a behavior already reported in the literature for some oligomers (Holak et al., 1984; Tran-Dinh et al., 1984, Manzini et al., 1987) and for poly(dG–dC) at 2.5 M NaCl (Behe et al., 1985). In order to assess the extent of this Z to B conversion, we analyzed the CD spectra taking into account the three forms (B, Z, coil) present in solution in the temperature range covered (0–90 °C). Hence at each temperature we assumed that

$$\Delta\epsilon^{295} = \Delta\epsilon_B{}^{295} f_B + \Delta\epsilon_Z{}^{295} f_Z + \Delta\epsilon_C{}^{295} f_C \quad (1)$$

$$1 = f_B + f_Z + f_C \quad (2)$$

the f_i's being the molar fractions of the B, Z, and coil con-

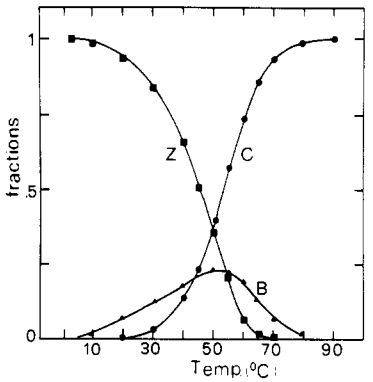

FIGURE 4: Fractions of the Z, B, and coil (C) conformations of d(CGCGCGTACGCGCG) in 4.6 M $NaClO_4$, 0.1 M Tris·HCl (pH 7.4), and a nucleotide concentration of 0.21 mM (phosphate), as a function of temperature.

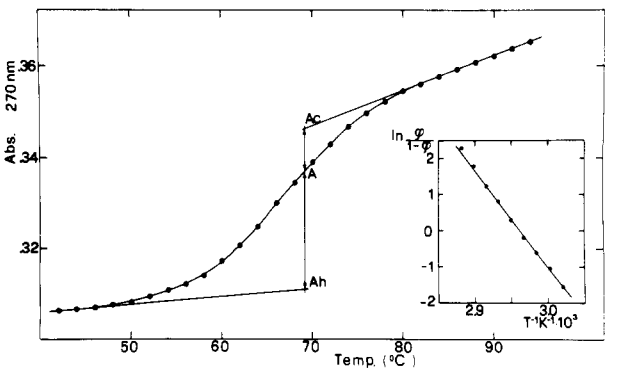

FIGURE 5: Absorbance at 270 nm versus temperature profile of d(CGCGCGAAAAACGCGCG) in 4.6 M $NaClO_4$, 0.1 M Tris·HCl (pH 7.4), and a nucleotide concentration of 0.1 mM (phosphate); the insert shows the correspondent van't Hoff plot.

formations, respectively. The $\Delta\epsilon$'s, relative to the limit forms, have been estimated from the CD spectra at 0 °C and 1 M $NaClO_4$ for $\Delta\epsilon_B$, 0 °C and 4.6 M $NaClO_4$ for $\Delta\epsilon_Z$, and 90 °C and 4.6 M $NaClO_4$ for $\Delta\epsilon_C$. They have been considered constant with the temperature and salt concentration, even though a small, but not relevant, dependence was observed. The coil fractions f_C at each temperature were determined by a subsequent UV melting experiment (of the same solution) following the absorbance at the B–Z isobestic point (270 nm). Therefore, the system of eq 1 and 2, relative to each CD spectrum at a given temperature, was solved to afford estimates of f_Z and f_B. The variation of the fractions in B, Z, and coil conformation in 4.6 M $NaClO_4$ (buffer A) is displayed in Figure 4. It can be noted that in this buffer slightly more than 20% of the Z population reverts into the B conformation during the denaturation process. The same experiment was repeated in buffer B, where the Z conformation was expected to be more stable. In fact the fraction f_B in this case was found to be very low (below 5%). In the case of the two 17-mers **A5** and **T5** they did not show any significant thermally induced shifts of the Z conformation in favor of the B one at 4.6 M (or higher) $NaClO_4$, a behavior already seen for the hairpin structure d(CGCGCGCGCGTTTTCGCGCGCGCG) in 5 M NaCl (Germann et al., 1985).

Denaturation Experiments. The two 17-mers **A5** and **T5** were melted in buffer A at concentrations in the range 0.03–0.3 mM (phosphate). Melting profiles were recorded at 270 nm and resulted to be monophasic. Upon a 10-fold increase of the nucleotide concentration no effect on the midpoint temperature, T_M, was observed. This behavior suggests that the monophasic transition is due to the denaturation of the hairpin structure whose stem is in the left-handed conformation as shown by the negligible B-type contribution in the CD spectrum. A typical melting profile is reported in Figure 5 for **A5**. On melting of the tetradecamers **TA** and **AT** in buffer A, the thermally induced reversion of molecules from the Z to the B conformation is added to the main unstacking reaction, as shown by CD measurements (see Figures 3 and 4). Therefore, we recorded melting profiles at the B–Z isobestic point, 270 nm, in order to separate the two effects and to measure only an order–disorder process. The melting profiles of both **TA** and **AT** were revealed to be monophasic and the apparent T_M's shown to be independent of the oligomer concentration. This finding indicates that, in buffer A and in the concentration range explored, also the 14-mers exist as monomolecular hairpin structures. Because of the partial Z to B reversion in buffer A, we followed the denaturation of the 14-mers **TA** and **AT** in buffer B too, since its higher $NaClO_4$ content (6 M) assured a negligible presence of thermally induced B-hairpins during the melting experiments. This is shown by the strict similarity of the absorption versus temperature profiles at 270 nm (B–Z isosbestic point) and at 247 nm (where the Z to B transition exhibits a significant hyperchromic effect).

Table I: Thermodynamic Data for Z-Hairpin Denaturation in Buffer A (4.6 M $NaClO_4$ and 0.5 mM Tris, pH 7.4) at 25 °C

sample	ΔH^a (kcal/mol)	ΔS^b (eu)	T_M^c (°C)
d(CGCGCGTTTTTCGCGCG)	52	154	65
d(CGCGCGAAAAACGCGCG)	52	153	67
d(CGCGCGTACGCGCG)	38[d]	118	50[e]
d(CGCGCGATCGCGCG)	36[d]	112	49[e]
d(CGCGCG)	49		

[a] Estimated error ±2 kcal/mol. [b] Estimated error ±6 eu. [c] Semitransition temperature; estimated error ± 1 °C. [d] The same values, within the experimental error, have been found also in buffer B. [e] Temperature at which $f_Z = f_C$ (see Figure 4).

Analysis of the Hairpin–Coil Transition. The conformational equilibrium which reflects the one-step melting profile exhibited by the four oligomers in buffer A or B has been analyzed in term of a two-state model (Borer et al., 1974). The enthalpy changes for the Z–coil transition (in buffer A for the 17-mers and in buffer B for the 14-mers) were determined by shape analysis of the melting profiles at 270 nm, the T_M of this intramolecular equilibrium being independent of the nucleotide concentration. The ΔH values, reported in Table I, are averages of three methods: linear plots of ln K versus $1/T$ (Albergo et al., 1981); slope at T_M of a plot reporting the fraction of coil as a function of T (Breslauer et al., 1975); and best fit of the melting curves according to the procedure of Freier et al. (1983). The error estimate on ΔH and ΔS is at most ±5%, while the T_M's were determined with an uncertainty of ±1 °C (see preceding paper in this issue for further details).

The denaturation enthalpies of **TA** and **AT** Z-hairpins in buffer A were determined by plotting ln (f_C/f_Z) versus $1/T$ (Figure 6), where f_C and f_Z were determined as described above. These values were almost coincident with those obtained in buffer B: even if ΔH could be salt concentration dependent, the difference between 4.6 and 6 M $NaClO_4$ is expected to be very small (Manzini et al., 1987). The simple application of the two-state model computations to the 14-mers denaturation profiles in buffer A led to values of ΔH of about 34 kcal/mol: it is apparent that the difference between these values and those reported in Table I arises from the inadequacy of applying the two-state model in this case, where the third species B-hairpin is present. Analogously, the apparent T_M's (i.e., the temperatures at which $f_C = 0.5, f_Z + f_B = 0.5$) are

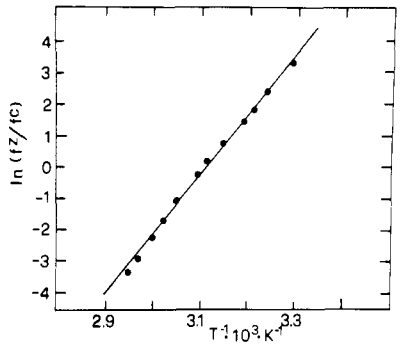

FIGURE 6: van't Hoff plot for the coil to Z-hairpin conformational transition of d(CGCGCGTACGCGCG) in buffer A. Fractions f_Z and f_C have been obtained by a combination of CD and UV experiments.

some degrees higher than those pertaining to the Z–coil equilibrium ($f_C = f_Z, f_B \neq 0$). The coincidence of the ΔH values (38 kcal/mol) for **TA** obtained in buffer B by analyzing the melting curves recorded at 247 nm (where the difference in absorbance between B- and Z-hairpins is significant) and at 270 nm (B–Z isosbestic point) further supports the nearly total absence of B-hairpins in this buffer.

The thermodynamic data suggest that (a) on hairpin formation, the 17-mer fragments are accompanied, with respect to the 14-mer ones, by a more favorable enthalpy of stacking (−52 versus −38 kcal/mol), and by a larger loss of entropy (−152 versus about −115 eu), and (b) both **A5** and **T5** exhibit a higher thermal stability (ΔT about 16 °C) with respect to **TA** and **AT**, which is enthalpic in origin.

Discussion

The data reported in this paper, based on optical and chirooptical measurements, offer a first thermodynamic picture of hairpin formation in oligodeoxynucleotides with the stem in the left-handed conformation. Previous studies have already shown that, analogously to poly(dG-dC) (Pohl & Jovin, 1972) and to oligo(dC-dG) (Quadrifoglio et al., 1981), the stem part of hairpin loop structures can undergo a B to Z conformational transition in high-salt aqueous solution. A complete B to Z transformation was reported for d-(CGCGCGCGCGTTTTCGCGCGCGCG) (Germann et al., 1985) and for **T5** (Xodo et al., 1986), while for the 13-mer d(CGCGAATTACGCG) (Millar et al., 1987) both B and Z hairpins appear to coexist in 5 M NaCl. We found that, in both 17-mers **T5** and **A5**, $NaClO_4$ induces a cooperative B to Z transition with a midpoint at 1.8 M salt, as occurring for poly(dG-dC) (Pohl & Jovin, 1972). Hence it appears that a four- or five-residue loop does not impose any relevant restriction to the conformational capacity of a $(CG)_n$ stem to adopt a left-handed conformation. in studying the conformational transition properties of the 17-mer **A5**, which differs from **T5** by having a central stretch of purines instead of pyrimidines, we intended to investigate the influence of the loop composition on the B- to Z-hairpin transition, as compared to the one occurring in **T5**. The presence of the bulkier adenines in the loop did not cause any significant variation either in the cooperativity or in the extent of dehydrating conditions required to obtain a complete transition. However, when we considered the effect of the loop size on the B–Z transition, by studying the behavior of hairpins **TA** and **AT**, which possess only a two-residue central segment, more severe conditions were necessary in order to have the B–Z transition of the stem reach completion. Indeed, the concentration of $NaClO_4$ at the semitransition point is in these cases about 3 and 3.5 M, respectively.

The data in Table I show that the presence of the loop in **A5** and **T5** increases very slightly the enthalpy change on denaturation of the hairpin with respect to the unconstrained stem, namely, the duplex of the hexamer d(CGCGCG), by a few kcal/mol. This enthalpic stabilization was also observed (in both heptadecamers) at low salt concentration (preceding paper) and attributed, on the basis of model building studies of closely related hairpin structures (Haasnoot et al., 1986; Hare & Reid, 1986), to the stacking interactions between thymidine residues in the loop and the flanking C·G base pair at the top of the stem. Thus, it seems reasonable to expect also in hairpins with a left-handed stem a similar propagation of the stacking interactions from the stem into the first bases of the loop. Moreover, Table I shows that, in high-salt conditions, the overall hairpin stability depends strongly on the size of the central residue stretch: in fact, in buffer A, both hairpins **TA** and **AT** exhibit a significantly lower stability (T_M = ca. 50 °C) with respect to both **A5** and **T5** (T_M = ca. 66 °C).

This thermal behavior is exactly the opposite of that shown by the same hairpins at low ionic strength, where both **TA** and **AT** possess a greater stability (ΔT about 15 °C) with respect to **A5** and **T5** (preceding paper), which was found to be entropic in origin. By contrast, the lower thermodynamic stability in buffer A of **TA** and **AT** is due essentially to enthalpic reasons. This can be inferred by inspection of Table I: the less favorable denaturation entropy of the 14-mers is more than counterbalanced by their markedly lower denaturation enthalpy. The denaturation enthalpy change of about 37 kcal/mol for **TA** and **AT**, as compared to the ΔH relative to the denaturation of the duplex of d(CGCGCG) in the same buffer A (49 ± 2 kcal/mol), suggests that the stem part of the hairpins **TA** and **AT** includes five Watson–Crick base pairs. This intimation is consistent with the lower thermal stability of both **TA** and **AT** with respect to **A5** and **T5** (ΔT about 16 °C). Thus it appears that in high-salt aqueous solution, contrary to what has been observed at low ionic strength, where the possibility of a two-base loop was evidenced (Orbons et al., 1986; preceding paper), the dC·dG base pair closing the two-base loop is sacrificed in favor of a four-residue loop.

Conclusion

The data reported in this paper, added to the recent developments on oligonucleotide loop formation (Orbons et al., 1985; Hare et al., 1986; Summers et al., 1985; preceding paper), suggest that both the base sequence and the conformation of the stem play an important role in determining the optimal loop size for hairpin stability. As for the influence of the stem helix geometry, it is not trivially connected with the interstrand distance between the phosphates of two paired bases. As anticipated in the preceding paper, this parameter is indeed similar for A and B helices (ca. 17.5 Å) (Wang et al., 1979), which appear to prefer different loop sizes, and it is lower for a Z helix (not more than ca. 15 Å) (Wang et al., 1979), which seems to require loops of length closer to those found for A-RNA than for B-DNA. In conclusion, the evidence presented in this paper shows that (a) the ability of a $d(CG)_n$ stem to assume the left-handed conformation in a hairpin upon exposure to increasing salt concentration is not abolished by the presence of the loop and depends on its size; (b) a loop of five residues, as that possessed by either **A5** or **T5**, slightly stabilizes enthalpically the hairpin with respect to the unconstrained duplex of d(CGCGCG), at high ionic strength, just as at low ionic strength (Xodo et al., 1986; Ikuta et al., 1986; Haasnot et al., 1986); (c) contrary to the behavior at low salt concentration, where a two-base loop was evidenced

for both **TA** and **AT** (preceding paper), at high salt concentration the enthalpic data as well as the T_M values suggest that a loop of four residues is more favored in the 14-mer fragments.

Registry No. **A5**, 115185-40-5; **T5**, 105417-61-6; **AT**, 115185-78-9; **TA**, 115185-38-1; d(CGCGCG), 58927-26-7.

REFERENCES

Albergo, D. D. P., Marky, L. A., Breslauer, K. J., & Turner, D. H. (1981) *Biochemistry 20*, 1409–1418.

Behe, M. J., Felsenfeld, G., Szu, S. C., & Charney, E. (1985) *Biopolymers 24*, 289–300.

Bevington, P. R. (1969) *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York.

Borer, P. N., Dengler, B., Tinoco, I., Jr., & Uhlenbeck, O. C. (1974) *J. Mol. Biol. 86*, 845–853.

Breslauer, K., Sturtevant, J., & Tinoco, I., Jr. (1975) *J. Mol. Biol. 99*, 549–565.

Frank, R., & Koster, H. (1979) *Nucleic Acids Res. 6*, 2069–2087.

Freier, S. M., Albergo, D. D., & Turner, D. H. (1983) *Biopolymers 22*, 1107–1131.

Germann, M. W., Schoenwälder, K.-H., & van de Sande, J. H. (1985) *Biochemistry 24*, 5698–5702.

Gralla, J., & Crothers, D. (1973) *J. Mol. Biol. 73*, 497–511.

Haasnoot, C. A. G., Hilbers, C. W., van der Marel, G. A., van Boom, J. H., Singh, U. C., Pattabiraman, N., & Kollman, P. A. (1986) *J. Biol. Struct. Dyn. 3*, 843–857.

Hare, D. R., & Reid, B. R. (1986) *Biochemistry 25*, 5341–5350.

Hilbers, C. W., Haasnoot, C. A. G., de Bruin, S. H., Joordens, J. J. M., van der Marel, G. A., & van Boom, J. H. (1985) *Biochimie 67*, 685–695.

Holak, T. A., Borer, P. N., Levy, G. C., van Boom, J. H., & Wang, A. H. J. (1984) *Nucleic Acids Res. 12*, 4625–4635.

Lilley, D. M. J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6468–6472.

Lilley, D. M. J. (1981) *Nucleic Acids Res. 9*, 1271–1289.

Manzini, G., Xodo, L. E., Quadrifoglio, F., van Boom, J. H., & van der Marel, G. A. (1987) *J. Biomol. Struct. Dyn. 4*, 651–662.

Marky, L. A., Blumenfeld, K. S., Kozlowski, S., & Breslauer, K. J. (1983) *Biopolymers 22*, 1247–1257.

Millar, M., Kirchhoff, W., Sussman, J. L., Appella, E., Chiu, Y. H., Cohen, J., & Sussmann, J. L. (1987) *Nucleic Acids Res. 15*, 3877–3890.

Muller, U. R., & Fitch, W. M. (1982) *Nature (London) 289*, 582–585.

Orbons, L. P. M., van der Marel, G. A., van Boom, J. H., & Altona, C. (1986) *Nucleic Acids Res. 14*, 4187–4195.

Panayotatos, N., & Wells, R. D. (1981) *Nature (London) 289*, 466–470.

Pohl, F. M., & Jovin, T. M. (1972) *J. Mol. Biol. 67*, 375–396.

Quadrifoglio, F., Manzini, G., Vasser, M., Dinkelspiel, K., & Crea, R. (1981) *Nucleic Acids Res. 12*, 2195–2206.

Rosenberg, M., & Court, D. (1979) *Annu. Rev. Genet. 13*, 319–351.

Roy, S., Weinstein, S., Borah, B., Nickol, J., Appella, E., Sussman, J. L., Miller, M., Shindo, H., & Cohen, J. S. (1986) *Biochemistry, 25*, 7417–7423.

Scheffler, I. E., Elson, E. L., & Baldwin, R. L. (1968) *J. Mol. Biol. 36*, 291–304.

Summers, M. F., Byrd, R. A., Gallo, K. A., Samson, C. J., Zon, G., & Egan, W. (1985) *Nucleic Acids Res. 13*, 6375–6386.

Tran-Dinh, S., Taboury, J., Neumann, J. M., Tam, H. D., Genissel, B., Langlois d'Estaintot, B., & Igolen, J. (1984) *Biochemistry 23*, 1362–1371.

van Boom, J. H., van der Marel, G. A., van Boeckel, C. A. A., Willie, G., & Hoyng, C. F. (1982) *Chemical and Enzymatic Synthesis of Gene Fragments* (Gassen, H. G., & Lang, A., Eds.) pp 53–70, Verlag Chemie, Weinheim.

van der Marel, G. A., van Boeckel, C. A. A., Wille, G., & van Boom, J. H. (1981) *Tetrahedron Lett. 22*, 3887–3890.

Vologodskii, A. V., Lukashin, A. V., Anshelevich, V. V., & Frank-Kamenetskii, M. D. (1979) *Nucleic Acids Res. 6*, 967–982.

Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. A., & Rich, A. (1979) *Nature (London) 282*, 680–686.

Wells, R. D., Goodman, T. C., Hillen, W., Horn, G. T., Klein, R. D., Larson, J. E., Muller, U. R., Neuendorf, S. K., Panayotatos, N., & Stirdivant, S. M. (1980) *Prog. Nucleic Acids Res. Mol. Biol. 25*, 167–267.

Wemmer, D. E., Hare, D. R., & Reid, B. R. (1985) *Nucleic Acids Res. 13*, 3755–3771.

Xodo, L. E., Manzini, G., Quadrifoglio, F., van der Marel, G. A., & van Boom, J. H. (1986) *Nucleic Acids Res. 14*, 5389–5398.

Xodo, L. E., Manzini, G., Quadrifoglio, F., van der Marel, G. A., & van Boom, J. H. (1988) *Biochemistry* (preceding paper in this issue).