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Conformational transitions of synthetic DNA sequences with inserted bases, related to the dodecamer d(CGCGAATTCGCG)

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ABSTRACT

Conformational transitions for a series of imperfect palindromes related to the dodecamer d(CGCGAATTCGCG) have been investigated. These sequences are: two isomeric 13-mers - d(CGCAGAATTCGCG) (13-merI) and d(CGCGAATTACGCG) (13-merII), 17-mer d(CGCGCGAATTACGCGCG) and 15-mer d(CGCGAAATTTACGCG). Insertion of a single adenine nucleotide prevents these sequences from being self-complementary. Analysis of thermodynamic parameters derived from the melting profiles together with other data at higher concentrations (NMR and calorimetry) indicates that the insertion of the additional nucleotide which lacks a complement in the opposite strand does not change the enthalpy of the duplex formation, but does alter the number of stable nucleation configurations. The relative position of the insertion within the self-complementary sequence determines the equilibrium between the duplex form and the single-stranded hairpin loop. C-G segments separated by the insertion from the rest of the molecule can undergo an independent conformational transition at high salt concentration, probably to the Z form.

INTRODUCTION

Sequences with inverted repeat symmetry (palindromes), which are either perfect, or have central, non-symmetric regions, occur commonly in natural DNA molecules and appear to have an important role in specific protein - DNA interactions. Representative examples that have been extensively studied are the binding site for the lac operator and CAP (1), and the cleavage sites for restriction endonucleases (2). Quasipalindromes are often found in sites of frequent mutations (3). Since such sequences are complementary, or almost complementary within one strand, the possibility of hairpin formation giving rise to a cruciform structure (3,4,5) is an attractive hypothesis for explaining their unique recognition properties and their role in DNA expression.

Several studies on sequence-dependent conformational flexibility, utilizing model synthetic DNA oligomers have been reported. It was shown that even for the perfectly self-complementary dodecamer d(CGCGAATTCGCG) (6) and its sequence isomer d(CGCGTATACGCG) (7), two forms - a duplex and a single

stranded hairpin loop - can coexist in solution. This is also true for the 11-mer d(CGCGATTCGCG) (8), which forms a duplex with a mismatched base pair. The equilibrium between the two forms is shifted toward the duplex at higher ionic strength and oligomer concentration. At a given set of conditions, the thermal stability of the duplex depends on the sequence. It was shown by NMR spectroscopy (9) that the 13-merI d(CGCAGAATTCGCG), derived from the dodecamer (10) by inserting an adenosine in the fourth position from the 5'-end, exists in solution as an improper duplex with an unpaired adenine residue intercalated into the double helix. A preliminary x-ray study (11) is in agreement with the NMR results. We report here that at optical concentrations (up to 100 μM) 13-merII d(CGCGAATTACGCG) adopts exclusively the hairpin configuration.

Conformational preferences were subsequently studied for two other homologous sequences: 17-mer d(CGCGCGAATTACGCGCG) and 15-mer d(CGCGAAATTTACGCG). In the first case the CG segments of 13-merII were elongated on both ends by adding two CG nucleotides, in the second case the central tetranucleotide was substituted by hexanucleotide. In parallel studies 13-merII and 17-mer were investigated by NMR spectroscopy (12) at concentrations at least a hundred times higher. In this paper the thermodynamic parameters of the conformational transitions of these sequences over a wide range of concentrations are compared and discussed in order to better distinguish and characterize duplex to hairpin transitions postulated for natural DNA.

MATERIALS AND METHODS

Sample preparation. DNA oligomers were synthetized by the phosphoramidite solid phase method, using a Vega Coder 300 automated synthetizer, and were purified by reverse-phase HPLC, followed by precipitation with ethanol. The concentrations of the oligomers were determined spectrophotometrically at 90°C. Extinction coefficients at 90°C were calculated assuming ϵ of 7000 per pyrimidine and 14000 per purine. Oligomers were dissolved in 10 mM sodium phosphate buffer at pH 7. In the case of spermine, 10 mM sodium cacodylate was used.

UV - spectroscopy. UV - absorbance spectra and temperature profiles were recorded using a thermoelectrically controlled Perkin-Elmer Lambda 4B spectrophotometer. The temperature was increased continuously at a rate of 0.5 °C/min.

Analysis of the melting profiles. The van't Hoff transitions enthalpies were obtained from a nonlinear least squares analysis of the temperature de-

pendence of the optical density using a two-state model (13). The two-state expression for the dependence of the optical density, O.D., on temperature is :

$$O.D. = \frac{\epsilon_A c_t}{1 + k} + \frac{\epsilon_B c_t}{1 + 1/k}$$

for concentration independent transitions, and

$$O.D. = \epsilon_A c_t/2 + (\epsilon_B - \epsilon_A/2)\cdot(k/4)\cdot[(1+8c_t/k)^{1/2} - 1]$$

for bimolecular reactions.

In these expressions, ϵ_A and ϵ_B are the absorption coefficients per mole of the low temperature and high temperature species respectively, c_t is the total strand concentration, and k is the equilibrium constant for the process. The temperature dependence of the optical density is determined in part by the temperature dependence of k which can be expressed as :

$$k = k_m \exp\{-\Delta H(T-T_m)/RT_mT\}$$

where k_m is the equilibrium coefficient at $T=T_m$, the midpoint of the transition where the numbers of strands in both states are equal and ΔH is the van't Hoff enthalpy at T_m expressed per mole of low temperature species. In addition to the temperature dependence of k, a linear dependence of the absorption coefficients ϵ_A and ϵ_B has been observed in this and other studies (13). When an extended asymptote was observed in either the pre- or post-transition region, the corresponding absorption coefficient was allowed to vary linearly with temperature in the overall non-linear least squares fit. If the initiation or completion of the phase transition extended beyond the available temperature range of our instrumentation or if a linear region could not be discerned between two neighboring transitions, the temperature dependence of the corresponding absorption coefficient was not included in the least squares fit. On the basis of the analyses conducted in the present study as well as those reported in the literature, the errors introduced into the reported thermodynamic quantities by ignoring the temperature dependence of the absorption coefficients are less than 10% for ΔH and less than 2 °C for T_m and do not affect the conclusions of this study. When two distinct sigmoidal regions were observed, they were analyzed in two ways, individually and jointly as a sum of the above expressions, with essentially indistinguishable results. In some situations, a seemingly single profile, when fit to a two-state model, could be seen to deviate significantly from the model by appearance of a characteristic pattern in the residuals. In these instances we presumed that the profile was the sum of overlapping

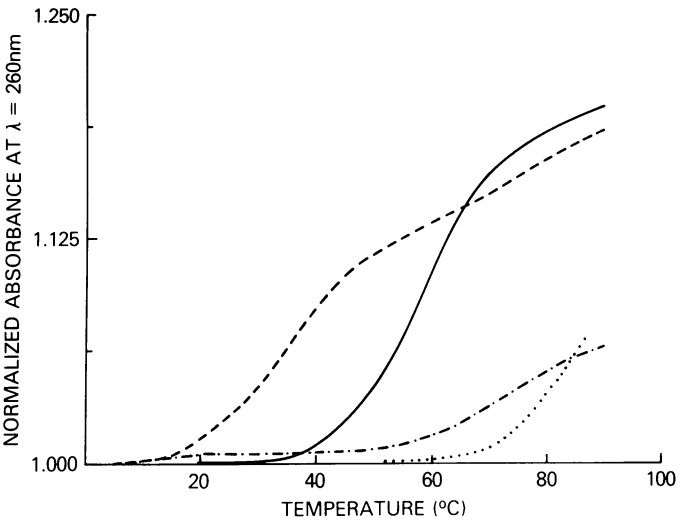

Figure 1: Temperature transition profiles in 0.1 M NaCl of: dodecamer (——), 13-merI (- - -), 13-merII (-•-•) and 15-mer (••••). Optical densities of solutions were about 1.0 at λ=260 nm at 5 °C.

steps, which prevented the calculation of reliable values of ΔH and T_m.

CD Spectroscopy. CD spectra were recorded on a JASCO 500 A spectrophotometer equipped with a thermal block allowing measurements at controlled temperatures.

Calorimetry. The thermal scans were performed with a Hart Scientific DSC 7707 differential scanning calorimeter which has been described elsewhere (14). The volume of the sample was 0.9 ml and scans from 15 to 95 °C were made at a rate of 15 °C/hr. The calorimetric enthalpy was determined as an average of three successive thermal scans after subtracting a baseline tangent to the pre-transition and post-transition baselines.

RESULTS

13-mer II : d(CGCGAATTACGCG). Absorbance versus temperature profiles registered at λ = 260 nm in 0.1 M NaCl are shown for the 13-mers I and II and for the dodecamer (see also (6)) in fig. 1. In contrast to the dodecamer and to sequence I, II did not exhibit a pronounced hyperchromic effect. This behavior did not change either upon 10-fold increase of the DNA concentration, or in the presence of $MgCl_2$, tetramethylammonium chloride or spermine (data not shown). The enthalpy change accompanying this transition was investigated using a differential scanning micro-calorimeter. The

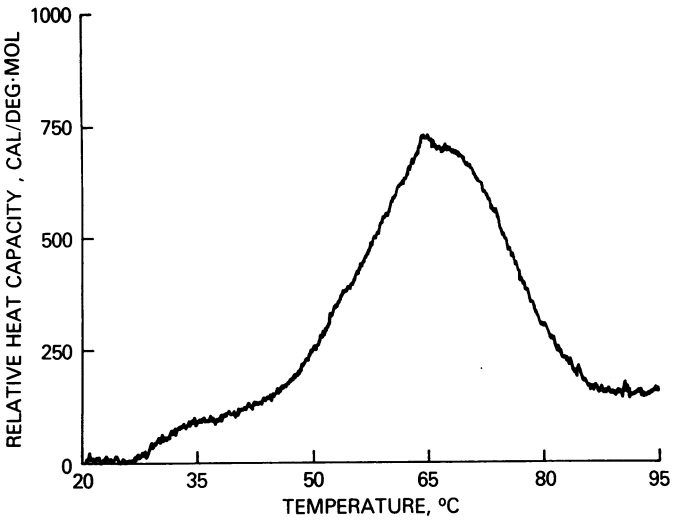

Figure 2: Calorimetric heat capacity versus temperature for 13-merII in 0.1M NaCl. The oligomer concentration is 1.3 mM in single strands.

relative heat capacity versus temperature for 13-mer II is shown in fig. 2. From the area under the heat capacity curve, a transition enthalpy of 22 kcal/mol of single strand was calculated. This value can be compared with

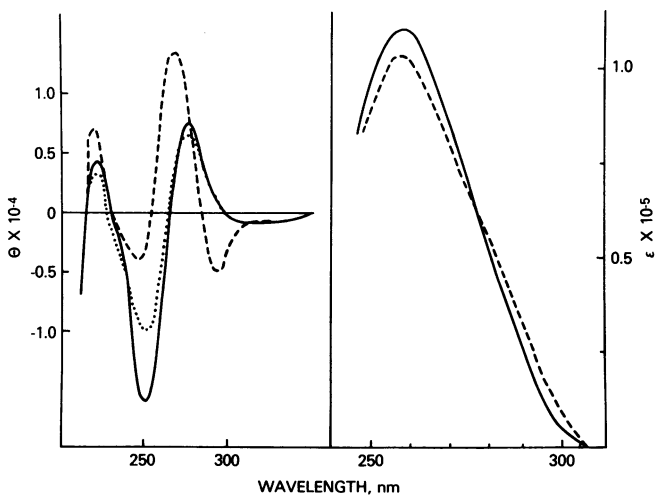

Figure 3: Circular dichroic spectra (left) and UV absorbance spectra (right) of 13-merII in 1M NaCl (——) and 5 M NaCl (- - -) at 5 °C. Also shown (left) (••••) is circular dichroic spectrum in 5M NaCl at 60 °C. Units for the molar ellipticity [θ] are degree × cm^2/dmol.

TABLE I. Midpoints of the transitions of 13-merII recorded at λ=274 nm

concentration of NaCl [M]	10μM of single strand	100μM of single strand
0.1	68.2	67.6
1.0	67.7	67.0
2.0	65.9	-
3.0	64.3	-
4.0	62.8	-
5.0	61.5	61.7

the van't Hoff enthalpies obtained from the optical melting profiles (see discussion). As shown in fig. 3, increasing the NaCl concentration caused significant changes in both the CD and UV spectra of sequence II. The changes are similar to those reported (15) for the B to Z transition of poly(d(G-C)), but are much less pronounced: in 5 M NaCl, the negative band at 295 nm has only 37% the intensity of the positive band at 268 nm and a residual, short-wavelength negative band is still present. Only minimal change in the CD spectrum was noticed in the presence of 2.5 M MgCl and in 0.1M $Co(NH_3)_6^{3+}$ (not shown) . With increasing temperature above 25 °C the high salt CD spectrum reverts to the B type. UV spectra at low and high salt concentrations exhibit hyperchromicity at 285 nm and an isosbestic

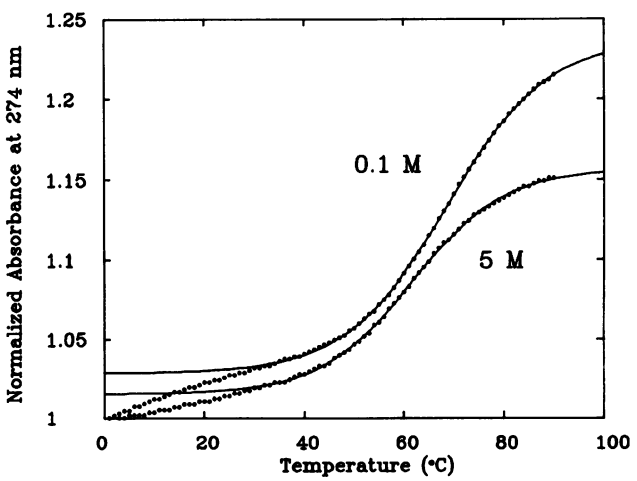

Figure 4: Temperature transition profiles of 13-merII recorded at λ=274 nm in NaCl concentrations as indicated. Solid line shows fitting to two-state model with flat lower baseline.

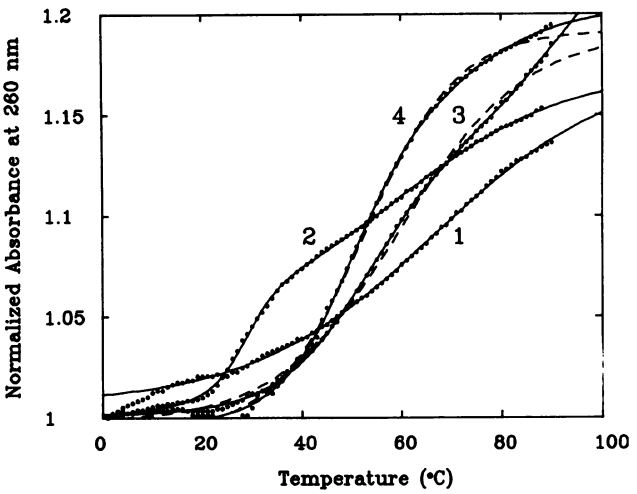

Figure 5: Temperature transition profiles at λ=260 nm of 15-mer (••••). Curve 1 in 0.1M NaCl, 7μM of single strand; curve 2 in 0.1M NaCl, 70μM single strand; curve 3 in 1M NaCl, 70 μM single strand; curve 4 in 1 molar equivalent of spermine, 70 μM single strand.

point at 274 nm. Melting profiles at DNA strand concentrations of 10 μM and 100 μM were recorded at this isosbestic point to separate the order-disorder effect from the thermally induced Z to B transition. Values of T_m are shown in Table I. The profiles at 0.1 M and 5 M NaCl, presented in fig. 4, both show only a one-step transition in the same temperature range as the profile recorded at λ = 260 nm and shown in fig. 1. Notice the higher hyperchromicity and lower T_m at λ = 274 nm as compared with values estimated from profile at λ = 260 nm. T_m decreases monotonically with increasing salt concentration from 0.1 M to 5 M NaCl. There was no increase in T_m upon 10 fold increase of DNA concentration.

17-mer d(CGCGCGAATTACGCGCG). 17-mer exhibits melting behavior qualitatively similar to 13-mer II. The observed transition is concentration independent but, as shown in fig. 1, is shifted to higher temperatures and is not yet complete at 95 °C. The CD spectrum in 5 M NaCl shows analogous but more pronounced changes than observed for 13-mer II. The negative short wavelength band disappears completely and the ratio of ellipticity at λ = 295 and 268 nm is 0.4 (not shown).

15-mer d(CGCGAAATTTACGCG). UV versus temperature profiles at λ = 260 nm under various conditions are shown in fig. 5. At a strand concentration of 7 μm in 0.1 M NaCl, the broad melting profile is similar to the profile of

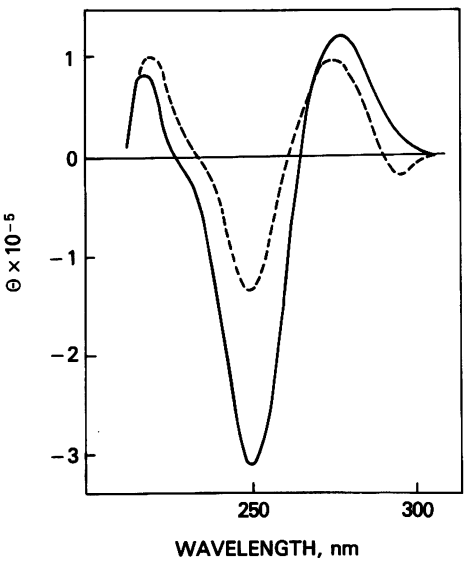

Figure 6: Circular dichroic spectra of 15-mer, 70 μM strand concentration in 0.1M NaCl (——) and 5M NaCl (- - -). Units as in fig. 3.

13-mer II (fig. 1). Upon 10 fold increase in the DNA concentration, a "sharp" transition appeared, followed by a stepwise increase in the absorption up to 90 °C. In the presence of 1 molar equivalent of spermine, a one step cooperative transition is present. CD spectra of a 70 μM solution of 15-mer in 0.1 M and 5 M NaCl are shown in fig. 6. There is a significant change in the magnitude of the Cotton effect and shape of the spectrum in high NaCl concentration.

DISCUSSION

Analysis of the melting profiles. Previous studies (6,7) have already shown that two ordered forms of self-complementary oligonucleotides - a duplex and a single stranded hairpin loop - can coexist in solution. Theoretically possible structures for the oligomers investigated here are depicted in fig. 7. The 13-merI has been shown by NMR (9) and X-ray diffraction (11) to form an improper duplex with the unpaired adenosines intercalated into the double helix. Also, a calorimetric enthalpy of 104 kcal/mol and T_m = 52°C have been reported for the 13-merI duplex to random coil transition in 0.1 M NaCl at a strand concentration of 0.7 mM (9).

In the optical studies we report here, the assignment to mono or

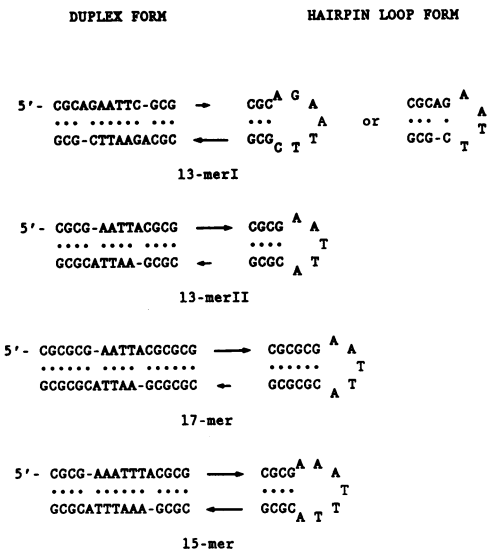

Figure 7: Theoretically possible conformations of investigated polynucleotides. (Although the possibility of forming double helices with mismatched base pairs by above sequences cannot be exluded, such structures would require five mismatched base pairs in case of 13-merI and three in case of 13-merII, making them energetically less favorable).

bimolecular transition of an observed temperature profile can be made on the basis of the concentration dependence of the midpoint of the transition. Upon assignment, the van't Hoff enthalpy can be calculated from the profile. We have observed for the 13-merI at a strand concentration of 10 μM a cooperative transition with Tm = 35 °C, and corresponding ΔH = 37 kcal/mol of duplex, followed by a further stepwise increase in the optical density. We interpret this result as duplex to hairpin loop transition, followed by hairpin melting. The van't Hoff enthalpy of the hairpin to coil transition is 25 kcal/mol of single strand which, when combined with the value for the duplex to hairpin transition gives a value of 87 kcal/mol of duplex for overall duplex to coil enthalpy change. This value is in good agreement with the NMR measurements (7) of 90 kcal/mol for disruption of the duplex formed by the sequence d(CGCGTATACGCG). Although the Van't Hoff enthalpy change for duplex to coil transition of the dodecamer d(CGCGAATTCGCG) in 0.1 M NaCl was calculated (6) from the melting profile (shown also in fig. 1 for comparison) to be 42 kcal/mol, it is clear from the profiles shown in ref. (6) that the melting of the duplex overlaps with the melting

TABLE II. Van't Hoff enthalpies evaluated from melting profiles at λ=260 nm

sequence	concentration [μM]	duplex → hairpin T_m [°C]	duplex → hairpin ΔH [kcal/mol of duplex]	hairpin → coil T_m [°C]	hairpin → coil ΔH [kcal/mol of strand]	duplex → coil (*) ΔH [kcal/mol of strand]
13-merI	10	35	37	75	25	87
13-merII	10-100	not observed		73	29	88
	$4.5 \cdot 10^3$ (**)	6	30	not measured		
15-mer	7	not observed		71	14	
	70	28	74	71	16	106

footnote: (*) calculated as sum of two terms.
(**) from ref (12), NMR data.

of the hairpin and the resulting profile is a sum of these two transitions. (See also the discussion in ref.(7), and below for the case of 15-mer.) The calorimetric value for the dodecamer was 102 kcal/mol of duplex (16), identical to the 13-merI (9).

The lack of a concentration dependence on the structural transition and the value of ΔH obtained from the differential scanning calorimeter, considerably lower than the value expected for the duplex to coil transition, indicates that only the single stranded hairpin loop was formed under the reported here conditions for 13-merII (see fig. 7). A more pronounced hyperchromicity at 274 nm, as compared to the hyperchromicity at 260 nm also indicates that that the transition involved primarily C-G pairs (17). The van't Hoff enthalpy, expressed as kcal/mole of single strand estimated from the profile at 260 nm is 29, and from the profile at 274 nm is 25, or 23 if fitted with the flat base line as shown in fig. 4. These minor differences arise from the dispersion of the hyperchromic effect (18). The value of 23 agrees very well with the value obtained from differential scanning calorimetry. An independent NMR study (12) of 13-merII, performed at much higher DNA concentration, showed the presence of some fraction of the duplex at low temperature. At a total strand concentration of 4.5 mM, the midpoint

of the duplex to hairpin transition was found to be 6 °C, which is at least 50 °C lower than the transition for 13-merI at a comparable concentration.

The structural transition for 17-mer is not yet complete at 95 °C and evaluation of its characteristics is not possible. On the basis of the NMR study (12) it forms an identical loop as the 13-merII, but with a stem two G-C pairs longer.

Similarly to the 13-merII, the 15-mer shows only the hairpin melting at 7 μM oligomer concentration. However, a biphasic melting profile, was observed at a strand concentration of 70 μM. When fit to two transitions this profile gave a ΔH value of 75 kcal/mol of duplex for duplex to hairpin transition and 15 kcal/mol of single strand for hairpin to coil transition. The latter value was identical with the value which we obtained from the profile at 7 μM strand concentration. Although curves 3 and 4 in fig. 5 appear to exhibit a one-step temperature profile, simulation with two overlapping transitions gave a better fit to the observed profile. However, reliable estimates of ΔH for the two transitions could not be obtained from the analysis. The addition of spermine, or an increase in the salt concentration shifted the first transition to higher temperature, where it overlapped with the hairpin melting. Thus, what appeared to be one transition was in fact composed of two unresolvable transitions. This example shows, that care is required while evaluating numerical values from melting profiles, and additional information about the system is often necessary for a proper analysis.

Thermodynamics of the transitions. Van't Hoff enthalpies evaluated from profiles at λ = 260 nm in 0.1 M NaCl are compared in table II. ΔH for the duplex to coil transition were calculated as a sum of two terms. In the case of the 13-merII the value from the NMR study (12) had to be used, since it forms a stable duplex above 0 °C only in very high (millimolar) concentrations. Estimated values of ΔH for the duplex disruption to random coil are the same for the isomeric 13-mers. The difference of 7 kcal/mol of duplex in ΔH for the duplex to hairpin transition is a result of difference in ΔH for hairpin formation (from the coil). These differences are in the range of experimental error, but if real, are too small to account for the difference of 50 °C in the midpoint of duplex to hairpin transition. Thus the difference in duplex stability for 13-mers I and II is mostly entropic in origin. Since the ΔH and T_m for the hairpin to coil transition in both cases are similar, the difference in entropy must arise mainly from the configurational parameters of the duplex form and implies that there is a larger number of available stable states for the 13-merI duplex. This can

be explained by inspection of the two structures depicted on fig. 7. Both dimers have the same number of base pairs and only a small difference in stacking energy, resulting from inserting adenine residues in different positions, can be expected. On the other hand, the unpaired nucleotides are six base pairs apart in 13-merI duplex and only four base pairs apart in 13-merII. Current ideas on the structural stability of double helices suggest that a single base pair can dissociate and re-associate, and the helix remains stable if a critical nucleation length remains intact (19, 20). Insertion of a base lacking its complement in the opposite strand introduces a break in the zipper model of the double helix. (The term "zipper" is used here in the same manner as in (21), eg. it refers to the equilibrium distribution of species and does not make any assumption about the mechanism by which one species is transformed to another). On the basis of the kinetic properties of RNA oligomers, it has been shown that a stable nucleus consists of three adjacent A-U base pairs (19) or one to two G-C pairs (20). The probability of formation of a stable nucleus by six A-T base pairs is twice as great as for four A-T base pairs, and much higher if these are flanked by C-G pairs as in 13-merI. A greater number of stable nucleation configurations will result in a greater number of available states for the duplex. Thus the distance of the insertion from the "center of symmetry" of the palindrome determines the duplex - hairpin equilibrium.

This explanation is also consistent with the observed behavior of the 17-mer and 15-mer. Formation of the 17-mer by elongation of C-G segments at both ends of the 13-merII sequence shifted the equilibrium further toward the hairpin structure (12), in spite of increasing the number of base pairs in the possible duplex, while extension of the A-T central core of the 13-merII to form 15-mer resulted in the stabilization of the duplex form. An increase in the enthalpy change for an overall duplex to unstacked coil transition of 15-mer in comparison with both 13-mers is in agreement with the expected value for disruption of a duplex two A-T base pairs longer. Estimated values for the hairpin to coil enthalpy change revealed a decrease in enthalpy with elongation of a loop from five nucleotides (13-merII) to seven nucleotides (15-mer), while both hairpins melted in the same temperature range. Decrease in Tm, without change of enthalpy, has been reported elsewhere (22) for a different series of hairpins. This discrepancy may be due to the difference in the loop sequence (23).

Transitions in high salt concentrations. In 13-merII, 17-mer and 15-mer inserted adenosines separates C-G sections of the sequences from A-T sections. This provides a unique possibility to investigate independent

conformational transitions within segments of a single molecule. The lack of concentration dependence of the structural transition in high salt concentrations, indicates that 13-merII exists in a hairpin form up to 5 M NaCl concentration. Thus the stem of this loop undergoes a B to Z transition as visualized by the change in the CD spectrum. The possibility of adopting a left-handed conformation by the alternating C-G sequence in the stem of the hairpin loop has already been reported for the $d(C\text{-}G)_5T_4(C\text{-}G)_5$ hairpin (24) so the occurrence of such a transition does not appear to depend on the specific sequence of the loop. In the case of the 15-mer, CD spectra were recorded at a oligonucleotide concentration for which the duplex is present at room temperature. Although one should be very careful in drawing conclusions on the basis of the CD spectra alone, it is very tempting to interpret the spectrum in 5 M NaCl (fig. 6) as the sum of Cotton effects corresponding to helices with opposite handedness adopted by A-T and G-C segments of the duplex. We have recently crystallized this sequence (25), and x-ray diffraction studies are in progress.

CONCLUSIONS

1. The enthalpy part of the free energy for a duplex with insertions does not change significantly in comparison with the parent sequence and does not depend on the relative position of the insertion within the sequence.
2. The distance of the insertion from the "center of symmetry" of the palindrome is related to the probability of formation of a stable nucleus by the central core and thereby determines the duplex - hairpin equilibrium.
3. Segments separated by the insertions have to form independent nucleation centers, giving rise to the possibility of formation of distinct structural domains.

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