Circular dichroism spectra show that repeating dinucleotide DNAs may form helices in which every other base is looped out

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ABSTRACT

We have found that the CD spectrum of poly[d(C-T)] undergoes a marked change upon protonation, taking on the unmistakable features of the spectrum of poly[d(C)·d(C⁺)]. The acid poly[d(C-T)] structure appears to be one in which each of the T residues is individually looped out of a helix formed by stacked C·C⁺ base pairs. We also present data for poly[d(C-A)] and review data for the other four possible repeating DNA dinucleotide sequences. These data show that self-complexes in which up to 50% of the residues are extra-helical may be unexceptional.

INTRODUCTION

Synthetic DNA polymers of various sequences have been shown to undergo acid-induced structural rearrangements with concomitant changes in their circular dichroism (CD) spectra.¹⁻⁶ One of the most interesting CD changes that can occur upon acidification of C-containing polymers is an increase in the positive CD at wavelengths as long as 300-310 nm, where the absorption is dominated by cytosine. In the case of poly[d(G)·d(C)], this change has been related by two groups of workers to the formation of a poly[d(C)] self-complex, probably poly[d(C)·d(C⁺)].²⁻⁴

Increases in the long-wavelength CD signal have been shown to occur at low pH for the C-containing polymers, poly[d(G-C)·d(G-C)], poly[d(A-G)·d(C-T)], and poly[d(A-C)·d(G-T)] by Guschlbauer and his coworkers¹ and for poly[d(A-G-G)·d(C-C-T)] and poly[d(A-C-C)·d(G-G-T)] by Gray *et al.*⁵ The long wavelength CD increase occurs upon direct addition of acid to solutions of these polymers. When poly[d(A-G)·d(C-T)] and poly[d(A-G-G)·d(C-C-T)] are dialyzed into a low pH buffer containing a moderate concentration of salt, other CD changes occur, and Lee *et al.*⁶ have shown that under such conditions these poly[d(purine)·d(pyrimidine)] polymers rearrange to form a poly[d(pyrimidine)·d(purine)·d(pyrimidine)] triplex and a free poly[d(purine)] strand.

The long-wavelength CD change seen after direct addition of acid to DNA

polymers was suggested by Gray *et al.*⁵ to be related to the formation of $C \cdot C^+$ base pairs. To investigate this possibility further, we have isolated free poly[d(C-T)] and have found a remarkable similarity between the spectrum of the structure that this polymer forms at acid pH and the spectrum of poly-[d(C)·d(C⁺)]. We interpret this similarly to mean that $C \cdot C^+$ base pairs are stacked in acid poly[d(C-T)], with alternating thymidylic acid residues looped out. In this paper we present CD data for poly[d(C-T)] and poly-[d(A-C)] and review CD data for other single-stranded polymers with an alternating base sequence. We conclude that it may not be unusual for DNAs to form a structure in which opposing non-complementary bases are looped out. Although stable helical structures with non-complementary bases looped out in one strand have been reported for synthetic RNAs (reviewed by Lomant and Fresco⁷), ours is the first report that DNAs may form helical structures in which every other base is looped out in both strands.

MATERIALS AND METHODS

Poly[d(C-T)] was prepared by preferential acid hydrolysis of the polypurine strand in poly[d(A-G)·d(C-T)]. Poly[d(A-G)·d(C-T)] synthesis was performed by procedures analogous to those previously described for poly[d(A-C)·d(G-T)] synthesis.⁸ The poly[d(A-G)] strand was degraded by heating, to 100°C for 35 min, a 1 ml solution containing 600 µg of poly[d(A-G)·d(C-T)] in 0.01 M Na⁺ (phosphate buffer, pH 7.0), to which 20 µl of 5 M H₂SO₄ were added. (This procedure was taken from Ref. 9.) The purine digestion products were removed by dialysis of the sample into 0.01 M Na⁺ (phosphate buffer, pH 7.0). Concentrated NaCl was added to raise the Na⁺ concentration to 0.08 M. The extinction coefficient of single-stranded poly[d(C-T)] was taken to be 7500 liter/(mol·cm) at 260 nm as reported by Lee *et al.*⁶

Poly[d(A-C)] was prepared by separation of the strands of poly[d(A-C)·d(G-T)] in an alkaline CsCl gradient.¹⁰ The poly[d(A-C)] sample consisted of long oligomers with an average chain length of 134 nucleotides. This was one of the longer chain length fractions resulting from a partial DNase I digestion and fractionation as reported by Hayes *et al.*¹⁰ The dried, salt-free fraction was dissolved in 0.01 M Na⁺ (phosphate buffer, pH 7.0), and concentrated NaCl was added to raise the concentration of Na⁺ to 0.1 M. The extinction coefficient was taken to be 9800 liter/(mol·cm) at 260 nm. This value is a first-neighbor estimate from the extinction coefficients of d(CpAp), d(ApC), and mononucleotides dC and dA as reported by Cantor *et al.*¹¹

Digitized CD spectra of the mononucleotides as reported in Ref. 11 were kindly provided by the authors.

Spectral measurements were obtained on a Varian Cary Model 61 circular dichrometer calibrated with d-10-camphorsulfonic acid (Aldrich Chemical Co.) to give a value within 2% of 0.336 degrees ellipticity at 290.5 nm for a 0.1% solution, as recommended by Chen and Yang.¹² Data taken from earlier work have been scaled to this new calibration value. Data collected by a Varian Spectroscopy 100 with an on-line Varian 620/i computer are plotted at nanometer intervals to directly show the random error due to noise in each spectrum. CD spectra per mole of monomer are plotted as $\epsilon_L - \epsilon_R$ in units of liter/(mol·cm); $\epsilon_L - \epsilon_R = \theta / (32.98lc)$ where θ is the measured ellipticity in degrees, l is the pathlength in cm, and c is the molar concentration of monomer subunits.

RESULTS

Poly[d(C-T)]. The measured CD spectrum of poly[d(C-T)] at pH 7.0 and a Na^+ concentration of 0.08 M is shown in Figure 1. This spectrum (shown as xxx) is that of single-stranded poly[d(C-T)] as indicated by a reasonably close agreement with the spectrum (shown as - - -) calculated from the CD spectra of d(CpT) and d(TpC) dinucleotide phosphates and dC and dT monomers by the first-neighbor formula:

$$\Delta\epsilon[\text{calc for ss}] = \Delta\epsilon[\text{d(CpT)}] + \Delta\epsilon[\text{d(TpC)}] - (\Delta\epsilon[\text{dC}] + \Delta\epsilon[\text{dT}])/2. \quad (1)$$

The agreement between the measured and calculated spectra confirms that the sample does contain C-T and T-C neighbors. For example, if only C-C neighbors or C monomer residues were present, the crossover would be near 240 nm¹¹ instead of at 258 nm as observed and as predicted by Equation (1).

Upon lowering of the pH to 5 by adding 1/10 volume of dilute H_3PO_4 , the CD spectrum of poly[d(C-T)] undergoes dramatic changes, including the appearance of a large positive band at long wavelengths (spectrum shown as ooo in Figure 1). This spectrum shows all the features found for the acid self-complex of poly[d(C)] in 0.02 M Na^+ at pH 7.0.² It should be noted that the "acid form" of poly[d(C)], which occurs upon protonation of one-half of the bases, exists at pH 7.0.¹³ We denote this self-complex of poly[d(C)] as poly[d(C)·d(C⁺)]. The spectrum measured for poly[d(C-T)] at pH 5 is rather well accounted for by a spectrum (shown as — — —) calculated as the simple average of poly[d(C)·d(C⁺)]² and dT spectra,¹¹ both at pH 7.0. (Changes in the CD spectra of poly[d(C)·d(C⁺)] and dT between pH 7.0 and pH 5.0 are quite minor.^{3,14}) The poly[d(C)·d(C⁺)] spectrum dominates this calculated spectrum, which accordingly has approximately one-half the molar magnitude of the spectrum previously measured for poly[d(C)·d(C⁺)]. This is especially true for the major positive band at about 288 nm in the calculated spectrum.

FIGURE 1. Circular dichroism spectra of poly[d(C-T)] at 20°C: (xxx spectrum) at pH 7.0, 0.08 M Na⁺; (ooo) spectrum at pH 5, 0.07 M Na⁺; (---) first-neighbor calculation of the CD spectrum of single-stranded poly[d(C-T)]; (— — —) average of the CD spectra of poly[d(C)·d(C⁺)] and thymidine. Inset: the melting profile of the pH 5 form of poly[d(C-T)] monitored by the circular dichroism at 285 nm.

($\epsilon_L - \epsilon_R$ values are expressed per mole of nucleotide or nucleoside monomer.)

The close agreement of this calculated spectrum with the measured acid poly[d(C-T)] spectrum, not only in shape but also in molar magnitude, is strong evidence that all of the cytosine bases in acid poly[d(C-T)] are interacting in a fashion very similar to that of the bases in poly[d(C)·d(C⁺)].

The acid poly[d(C-T)] structure in 0.07 M Na⁺ melts with a T_m of about 45°C, as shown in the inset in Figure 1. The cooperative loss of the CD band at 285 nm with increasing temperature serves to confirm that the acid poly[d(C-T)] structure at 20°C contains C·C⁺ pairs that are stacked. Upon lowering the temperature to 20°C, the CD spectrum readily returns to that of the original structure.

Poly[d(C)·d(C⁺)] melts with a T_m of about 96°C in 0.1 M Na⁺, pH 5.¹³ In 0.07 M Na⁺, pH 5, the T_m of poly[d(C)·d(C⁺)] would be even higher, since the effect of salt concentration on the T_m of this polymer is opposite to that found for natural DNAs.¹³ Thus, the acid poly[d(C-T)] structure is more thermolabile by over 50°C than is poly[d(C)·d(C⁺)] under similar conditions.

The possibility that the CD spectrum of acid poly[d(C-T)] arises from alternately stacked T-T and C·C⁺ base pairs (i.e. from C⁺-T and T-C⁺ stacked neighbors) and is fortuitously similar to the CD of poly[d(C)·d(C⁺)] is very unlikely. Direct evidence that C⁺-T and T-C⁺ stacked neighbors do not give rise to an enhanced positive CD at 290 nm comes from the spectrum reported by Lee *et al.*⁶ for the triplex poly[d(C-T)·d(A-G)·d(T-C⁺)]. This triplex contains C⁺-T and T-C⁺ stacked neighbors, but has no enhanced positive CD contribution at long wavelengths.

We infer that the thymidylic acid residues must be individually looped out in the acid poly[d(C-T)] structure. The spectrum calculated for acid poly[d(C-T)] is not greatly different if the spectrum of poly[d(T)] is substituted for the spectrum of dT monomer. This is because both the poly[d(T)] and dT monomer spectra are minor compared with that of poly[d(C)·d(C⁺)]. If some interaction exists among the looped-out thymidylic acid residues, it does not give rise to an unusually large optical activity.

Confidence in the interpretation of the CD spectrum of acid poly[d(C-T)] is justified by the characteristic and large CD of adjacent C·C⁺ base pairs. Once this type of structure with alternate bases looped out was recognized, we proceeded to investigate and review the CD spectra of other polymer sequences, to see if they could be similarly interpreted.

Poly[d(A-C)]. We have measured the CD spectra of a sample of d(A-C) _{$\bar{n}$} of average length 134 nucleotides ($\bar{n} = 67$). The measured CD spectrum of this sample of "poly[d(A-C)]" at neutral pH has the bands predicted from a first-neighbor calculation for the single-stranded polymer using an equation analogous to Equation (1), although the calculated and measured positive bands differ in magnitude. The measured (xxx) and calculated (- - -) curves are shown in Figure 2. Upon addition of dilute H₃PO₄ to reduce the pH to 4.0,

FIGURE 2. Circular dichroism spectra of poly[d(A-C)] at 20°C: (xxx) spectrum at pH 7.0, 0.10 M Na⁺; (ooo) spectrum at pH 4, 0.07 M Na⁺; (---) first-neighbor calculation of the CD spectrum of single-stranded poly[d(A-C)]; (- · -) average of the CD spectra of poly[d(C)·d(C⁺)] and deoxyadenosine. Inset: the melting profile of the low pH form of poly[d(A-C)] monitored by the circular dichroism at 285 nm.

the measured CD spectrum shows an enhanced positive band shifted to longer wavelengths (maximum near 285 nm; spectrum shown as ooo). This again suggests the formation of stacked C·C⁺ base pairs. The agreement of the acid poly[d(A-C)] spectrum with a simple average of poly[d(C)·d(C⁺)] and dA monomer spectra at pH 7 (calculation shown as - · - in Figure 2) is not as

close as in the case of acid poly[d(C-T)], especially in the 255-280 nm region. However, this could be due to the optical activity of the looped-out deoxyadenylic acid residues, for which we have no adequate estimate. (The dA monomer itself does not have a very different optical activity in neutral and acid solutions.¹⁴) The acid poly[d(A-C)] structure in 0.07 M Na⁺ melts cooperatively with a T_m of about 30°C, as shown in the inset in Figure 2.

There are three types of base pairs other than C·C⁺ base pairs that could exist in an acid poly[d(A-C)] structure: (1) A⁺·A⁺ base pairs could form as in acid poly[d(A)].¹⁵ However, under conditions comparable to those needed to form the acid poly[d(A-C)] self-complex, we have not found a self-complex to be formed for poly[d(A-T)]. At a NaCl concentration of 0.1 M and a pH of 3, the CD spectrum of poly[d(A-T)] is that of the single-stranded form. Therefore we do not believe the acid poly[d(A-C)] structure is being stabilized by A⁺·A⁺ base pairs. (2) A⁺·C wobble-type base pairs as drawn by Topal and Fresco¹⁶ could lead to a uniform helical structure with antiparallel strands. (3) A·C⁺ base pairs could possibly form with the added hydrogen shared between the N3 of cytosine and the N1 of adenine, with a second hydrogen bond being formed between the O2 of cytosine and the amino group of adenine, again leading to a uniform structure, but with parallel strands. In either of these latter two cases, we would expect the acid poly[d(A-C)] structure to be considerably more stable than that of the acid poly[d(C-T)] structure, which contains looped-out bases. However, as seen by comparing the insets of Figures 1 and 2, under comparable conditions the poly[d(A-C)] self-complex melts with a T_m 15°C lower than that of the poly[d(C-T)] self-complex. Therefore, we believe it is unlikely that the poly[d(A-C)] structure consists of A⁺·C or A·C⁺ base pairs.

Although the interpretation of the CD of the acid poly[d(A-C)] structure is not as clear as in the case of acid poly[d(C-T)], this is the first evidence for a structure formed by this sequence under acidic conditions. The CD data are not inconsistent with a structure containing stacked C·C⁺ pairs with looped-out deoxyadenylic acid residues.

Poly[d(G-A)]. Poly[d(G-A)] and poly[d(G-G-A)] both form self-complexes as described by Lee *et al.*⁶ Both melt cooperatively, with the T_m of the poly[d(G-G-A)] self-complex being 10° higher than that of the poly[d(G-A)] self-complex under comparable conditions. The T_m of the poly[d(G-A)] self-complex is unaffected by pH over the range 5 to 8, so protonated bases are not necessary to form this structure. Lee *et al.*⁶ propose a structure with hydrogen bonding between G and G and between A and A. However, if the gua-

nines are hydrogen-bonded to each other as in the four-stranded structure found for self-complexed poly[r(G)]¹⁷, with two hydrogen bonds between the guanines of neighboring strands, there would only be one hydrogen bond between similarly positioned adenines. The CD spectrum reported by Lee *et al.*⁶ for the poly[d(G-A)] structure has a strong positive peak at 260 nm, much as is seen for a poly[d(G)] self-complex,² suggesting that the guanines do indeed interact with each other. We asked whether the CD spectrum would also be consistent with looped-out deoxyadenylic acid residues.

Figure 3 shows a comparison of the measured CD of the poly[d(G-A)] self-

FIGURE 3. Circular dichroism spectra of poly[d(G-A)]. (—) measured spectrum as reported by Lee *et al.*⁶ for poly[d(G-A)] at 25°C in 0.2 M NaCl, 50 mM Na acetate, pH 5; (---) calculated spectrum that is an average of poly[d(G)] self-complex and deoxyadenosine spectra, both at pH 7.

complex and a simple average of poly[d(G)] self-complex and dA monomer spectra. The agreement is fairly good, not only in shape but also in molar magnitude of the major positive peak. The latter deserves attention, since the large magnitude of the 285 nm band of the self-complexed poly[d(G)] spectrum, averaged together with the dA monomer spectrum, would not be approached on a molar scale by the poly[d(G-A)] spectrum unless all of the G residues in the poly[d(G-A)] structure were stacked in a manner similar to that in the poly[d(G)] self-complex, with looping out of the interspersed A residues.

A calculation (not shown) which is the average of self-complexed poly[d(G)] and single-stranded poly[d(A)] spectra is similar to the calculation shown, at wavelengths above 240 nm. Below 240 nm, however, the calculation which includes the poly[d(A)] spectrum is more positive, with a large peak at 215 nm, for which there is no evidence in the measured spectrum.

The major discrepancy between the spectra shown in Figure 3 is between 260 and 285 nm. The magnitude of the measured spectrum is greater in this 260-285 nm region than is that of the calculated spectrum. Qualitatively this difference is like the major difference between the calculated and measured spectra shown in Figure 2 for acid poly[d(A-C)]. This would be consistent with both structures containing looped-out A residues which contribute to a positive CD between 255 and 285 nm.

We conclude that the CD spectrum of the poly[d(G-A)] self-complex can be best interpreted as arising from a structure of stacked, paired G residues, with looped-out A residues.

Poly[d(G-T)]. We have earlier reported¹⁸ that at 0°C poly[d(G-T)] exists in two different hypochromic self-complexed forms, with different CD spectra. One form dominates at moderate salt concentrations (e.g. 0.1 M Na⁺) and another dominates at high salt concentrations (e.g. 1.0 M Na⁺).¹⁸ The form seen at lower ionic strength has been interpreted as containing only G-T base pairs.^{18,19} However, we now note that there is a striking resemblance of the CD change upon formation of the self-complex of poly[d(G-T)] at 0.1 M Na⁺ to the CD change upon formation of a poly[d(G)] self-complex, both self-complexed forms gaining a positive CD band near 258 nm and a negative band at 240 nm.^{2,18} The form of poly[d(G-T)] at 0.1 M Na⁺ has an additional positive band at 275-280 nm not found in self-complexed poly[d(G)]. This latter band is the one most sensitive to salt concentration, being decreased in a continuous fashion as the salt concentration is raised to 1 M Na⁺. Simultaneous with this CD change, the poly[d(G-T)] self-complex becomes very much more thermostable. (The T_m is below 5°C in 0.1 M Na⁺ but is about 30°C in 1.0 M

Na^+).¹⁸ A possible interpretation of these results is that poly[d(G-T)] can exist in a form with paired G residues and looped-out T residues as well as in a form containing G-T base pairs. We believe that a mixture of forms occurs even at 0.1 M Na^+ and that G-T pairs (to which we assign the positive band at 275-280 nm) are disfavored at higher salt concentrations. Additional experiments will be necessary to explore this possibility.

Recent NMR data show that G-T base pairing can exist in poly[d(G-T)].²⁰ These experiments were done at a polymer concentration of approximately 30 mg/ml and a salt concentration of about 0.3 M NaCl and 10 mM cacodylate. It is not certain to which self-complexed form of poly[d(G-T)], as characterized by the CD technique, these data would correspond.

Poly[d(G-C)]. Poly[d(G-C)] exists in a number of conformations characterized by very different CD spectra.^{1,21,22} Of particular interest in the present context is an acid-induced conformational change described by Thiele et al.¹ The conformation assumed by poly[d(G-C)] at pH 2.8 in 0.15 M NaCl has an enhanced positive band near 288 nm which extends above 300 nm and a large negative band with a maximum near 265 nm. This structure is reversed upon back titration to neutral pH to give the CD spectrum generally thought to be that of the B conformation. The dominant CD characteristics of the acid form are almost certainly those of poly[d(C)·d(C⁺)] and strongly suggest that poly[d(G-C)] may form a structure with looped-out guanylic acid residues.

This observation adds to the findings by NMR²³ and X-ray diffraction^{24,25} that guanylic acid and cytidylic acid may have very different conformations in a poly[d(G-C)] sequence. The conformational variability of this polymer may be even greater than that needed to form left-handed helices in fibers.²⁵ (It has been proposed that the left-handed Z-DNA conformation in crystals and fibers corresponds to a salt-induced solution conformation of poly[d(G-C)] that has a CD spectrum very different from the spectrum of the acid-induced conformation.)^{24,25}

Poly[d(A-T)]. In preliminary experiments, we have found the CD spectrum of poly[d(A-T)] in acid solution (down to pH 3.0) to be either that of the single-stranded polymer or of the poly[d(A-T)·d(A-T)] double helix. No unusual CD spectral features were observed.

DISCUSSION AND CONCLUSION

CD spectra are very sensitive to the sequence of neighboring bases in polynucleotides. The characteristic CD spectrum of double-stranded poly-

$[d(C) \cdot d(C^+)]^{1,2}$ is caused by the stacking of adjacent $C \cdot C^+$ base pairs. The formation of isolated planar $C \cdot C^+$ base pairs alone would not be expected to give rise to the large CD bands observed for $\text{poly}[d(C) \cdot d(C^+)]$. The positive band of this unique CD spectrum extends to longer wavelengths than most other CD bands found in polynucleotides. Thus, the existence of this characteristic spectrum in acid $\text{poly}[d(C-T)]$ and $\text{poly}[d(G-C)]$ structures, and to a lesser extent in an acid $\text{poly}[d(A-C)]$ structure, strongly implies that these repeating dinucleotide polymers can form a helix of stacked $C \cdot C^+$ base pairs, with T, G, or A residues looped out. Similarly, a pair of CD bands at 258 and 240 nm that are characteristic of self-complexed $\text{poly}[d(G)]$ are found in $\text{poly}[d(G-A)]$ and in one of two $\text{poly}[d(G-T)]$ self-complexes. Therefore, these polymers may contain stacked G·G base pairs with extrahelical T or A bases. It would be interesting if conditions could be found in which $\text{poly}[d(G-C)]$ had stacked G·G base pairs with extrahelical C bases. The only repeating dinucleotide polymer for which we have no CD evidence for a structure with extrahelical bases is $\text{poly}[d(A-T)]$.

Stacked $C \cdot C^+$ base pairs probably also occur in repeating trinucleotide polymers. $\text{Poly}[d(A-G-G) \cdot d(C-C-T)]$ undergoes a rearrangement upon direct addition of acid to give a quite definite long-wavelength CD band like that of $\text{poly}[d(C) \cdot d(C^+)]^5$, a change that is probably caused by self-complexing of the $\text{poly}[d(C-C-T)]$ strands. A more modest but similar shift occurs in the spectrum of acid $\text{poly}[d(A-C-C) \cdot d(G-G-T)]^5$. In these cases one-third of the nearest-neighbors are C-C in the sequence of one strand, so the CD of the acid-induced structures of the isolated strands will have to be quantitated to determine whether the number of stacked $C \cdot C^+$ base pairs is larger than one-third and whether the T residues (or A residues) do indeed loop out. As mentioned earlier, a cooperative melting transition has been reported for isolated $\text{poly}[d(G-G-A)]^6$, for which one explanation is that this repeating trinucleotide forms a self-complex involving long stretches of stacked G·G pairs, with the A residues looped out.

The existence of looped-out bases in polynucleotide sequences is not a new finding. As reviewed by Lomant and Fresco,⁷ non-complementary bases in one of two polyribonucleotide strands of a double helix (with Watson-Crick pairs) always prefer to loop out and adopt extrahelical conformations. A space-filling model made by these authors showed that a single residue could be looped out with minimum distortion of a double helix. We have confirmed that a space-filling model can be built, with conformational angles falling in the ranges usually observed for nucleic acids,²⁶ of an acid $\text{poly}[d(C-T)]$

structure that consists of every other T residue looped out of a helix composed of stacked $C \cdot C^+$ base pairs. Extrahelical bases arranged at the outer radius of such a structure could not be extensively stacked and would not be expected to contribute very much to the CD spectrum, consistent with the data shown in Figure 1.

Whether such structures with extrahelical bases are of biological importance remains to be determined. If $\text{poly}[d(C) \cdot d(C^+)]$ contains $C \cdot C^+$ base pairs as proposed by Langridge and Rich²⁷ for $\text{poly}[r(C) \cdot r(C^+)]$, the strands must be parallel, unless the base-sugar conformation is syn in one strand and anti in the other. Likewise, if $\text{poly}[d(G)]$ forms a self-complex with pairing between bases in a multiple-stranded structure like that described for $\text{poly}[r(G)]$,¹⁷ it, too, must consist of parallel strands. Thus, helical structures made of $C \cdot C$ or $G \cdot G$ base pairs would not be expected to form by local back-folding in a strand of natural DNA. However, parallel pairing could occur between a strand of natural DNA and a separate C-rich or G-rich control sequence, or between regions far apart on the same strand, with concomitant looping out of a recognizable pattern of T and A residues, perhaps to signal the start of some enzymatic process.

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