Circular Dichroism Spectra of DNA Oligomers Show That Short Interior Stretches of C·C$^+$ Base Pairs Do Not Form in Duplexes with A·T Base Pairs†

Eric L. Edwards,‡ Robert L. Ratliff,§ and Donald M. Gray*,‡

Program in Molecular and Cell Biology, Mail Stop FO 31, The University of Texas at Dallas, Box 830688, Richardson, Texas 75083-0688, and Genetics Group, Life Sciences Division, Mail Stop 886, Los Alamos National Laboratory, Box 1663, Los Alamos, New Mexico 87545

Received January 15, 1988

ABSTRACT: Circular dichroism (CD) experiments were carried out on a series of DNA oligomers to determine if short internal stretches of protonated cytosine–cytosine (C·C$^+$) base pairs could coexist with adenine–thymine (A·T) base pairs. (1) C·C$^+$ base pairs did form in the absence of A·T base pairs in the individual oligomers d(AACC)$_5$ and d(CCTT)$_5$, as indicated by the appearance of a long-wavelength CD band centered at 282–284 nm, when the pH was lowered to 6 or 5 at 0.5 M Na$^+$. A comparison of measured with calculated spectra showed that d(CCTT)$_5$ at pH 5, 0.5 M Na$^+$, 20 °C, likely adopted a structure with a central core of stacked C·C$^+$ base pairs and looped-out thymines. Under the same conditions, it appeared that C·C$^+$ base pairs also formed in d(AACC)$_5$, but with the adenines remaining intrahelical. Each of these oligomers showed a cooperative transition for formation of C·C$^+$ base pairs as the temperature was lowered, with C·C$^+$ base pairs forming at a higher temperature in d(CCTT)$_5$ than in d(AACC)$_5$. A·T base pairs formed in equimolar mixtures of d(AACC)$_5$ plus d(CCTT)$_5$ as monitored by an increase in the negative magnitude of the 250-nm CD band. However, a large increase did not appear at about 285 nm in CD spectra of the mixtures, showing that there were no stacked C·C$^+$ base pairs in the d(AACC)$_5$·d(CCTT)$_5$ duplex even though they formed under the same conditions in the individual strands. Thus, in this duplex, A·T base pairs prevented the formation of neighboring internal C·C$^+$ base pairs. (2) CD measurements were also made of d(A$_{10}$C$_4$T$_{10}$). At pH 5 there was evidence for formation of a duplex with A·T base pairs, but no C·C$^+$ base pairs were detected. (3) Finally, we found that the individual oligomers d(A$_6$C$_6$A$_6$) and d(T$_6$C$_6$T$_6$) were able to form C·C$^+$ base pairs, but only A·T base pairs were present in 1:1 mixtures of the two strands, even at pH values below the pK_a for C·C$^+$ base pair formation in the individual strands. Thus, short internal stretches of 2, 4, or 6 C·C$^+$ base pairs were incompatible with A·T base pairs in complexes of these oligomers under the conditions studied.

Protonated cytosine–cytosine base pairs (C·C$^+$ base pairs),[1] in which one proton is shared by two cytosines, can form in cytosine-containing DNA sequences at close to neutral pH (Inman, 1964; Gray et al., 1980, 1984, 1988; Brown et al., 1985). The formation of stacked C·C$^+$ base pairs in the acid self-complex of poly[d(C)] results in characteristic CD spectral changes, including greatly increased positive CD values at long wavelengths (>280 nm) where the absorption of cytosine increases upon protonation (Gray & Bollum, 1974; Marck et al., 1978; Gray et al., 1988). For poly[d(C)], C·C$^+$ base pairs form with a pK_a of 7.4 at 0.05 M Na$^+$ (Inman, 1964). CD experiments have shown that C·C$^+$ base pairs may also form upon protonation of mixed sequence polymers. CD and photochemical studies showed that poly[d(CT)] can form a self-complex with a pK_a of 6.2 at 0.05 M Na$^+$; this self-complex consists of a central core of stacked C·C$^+$ base pairs with the intervening thymines looped out into solution (Gray et al., 1980; Brown et al., 1985). In the case of poly[d(CG)], CD bands indicative of C·C$^+$ base pairs appear at pH values <3 (0.1 M Na$^+$) during formation of a structure in which the guanines may remain intrahelical (Antao et al., 1986).

Naturally occurring eukaryotic DNA sequences frequently have regions of high cytosine content that may play important structural or functional roles. Two yeast mitochondrial DNA replication origins contain the sequences d(CCCACCCCCTCCCCC) and d(CACCCACCCCCTCCCCC) (Baldacci et al., 1984). *Drosophila virilis* DNA contains a region 400 nucleotides long with the repeating sequence d(CT)$_{8-18}$ d(C)$_{4-5}$ (Tautz & Renz, 1984). There are 32 tandem repeats of the sequence d(CCTCT) that occur about 2000 times in chicken genomic DNA; these repeats are highly susceptible to cleavage by single-strand-specific nucleases, but only at pH values below 5.5 (Dybvig et al., 1983). An S1 hypersensitive site near the 5′ end of the chicken β-globin gene contains the sequence d(CTCCTCTTCCCC) that upon supercoiling adopts an unknown conformation that relieves torsional stress (Cantor & Efstratiadis, 1984). The 5′ end of the chicken pro-α2(I) collagen gene also contains a cytosine-rich sequence, d(TCCCTCCCTTCCTCCCTCCCT), that is endonuclease sensitive at pH 4.5 (Finer et al., 1984). A d(C)$_{22}$ tract closely neighbored by d(CCT)$_{15}$ and d(CGCAC)$_5$ sequences at its 5′ and 3′ ends, respectively, in Bermuda land crab DNA can adopt two different types of altered conformations under the influence of negative super-

† This work was performed E.L.E. in partial fulfillment of the requirements for the Ph.D. degree in the Program in Molecular and Cell Biology at The University of Texas at Dallas. Support was provided by NIH Research Grant GM 19060 and by Grant AT-503 from the Robert A. Welch Foundation. Work at the Los Alamos National Laboratory was supported in part by the U.S. Department of Energy.

‡ The University of Texas at Dallas.

§ Los Alamos National Laboratory.

[1] Abbreviations: C·C$^+$, base pair of two cytosines with a shared proton; A·T, Watson–Crick base pair of adenine and thymine; CD, circular dichroism; NMR, nuclear magnetic resonance.

0006-2960/88/0427-5166$01.50/0

helicity and decreased pH (Fowler & Skinner, 1986). Finally, d($C_4A_4C_4A_4C_4$) sequences at the ends of macronuclear DNA molecules of the protozoan *Oxytricha nova* are essential for coherence into an unusual structure (Oka & Thomas, 1987).

An important question is whether short stretches of C·C$^+$ base pairs can form in the interior of antiparallel DNA sequences in juxtaposition with normal Watson–Crick base pairs. If so, the possibility would exist that C·C$^+$ base pairs might form adjacent to Watson–Crick base pairs and help to stablize intrastrand loops. Also, C·C$^+$ base pairs might help to stabilize the pairing of sequences that are not strictly complementary. There is no direct evidence for the type of base pairing or the strand sense in the protonated conformation of poly[d(C)]. A centrosymmetric, parallel-strand pairing of cytosines with three hydrogen bonds has been found in crystal structures of the dinucleotides r(CpA) (Westhof & Sundaralingham, 1980) and d(CpG) (Cruse et al., 1983; Coll et al., 1987). NMR studies on the self-complex of d$(CT)_3$ at low pH showed that the cytosines adopted anti conformations about their glycosyl bonds, and it was suggested that C·C$^+$ base pairs were formed between parallel strands of this oligomer (Sarma et al., 1986). However, it would be possible to accommodate anti glycosyl bonds in cytidines paired between antiparallel strands if a type of C·C$^+$ base pair formed that has two hydrogen bonds (Gray et al., 1984). The only other indication of strand sense in a structure containing C·C$^+$ base pairs in solution was work that established that A·T and C·C$^+$ base pairs can form together in the oligomer d($C_4A_4T_4C_4$). This latter work suggested that, since A·T base pairs were between antiparallel strands, the neighboring C·C$^+$ base pairs were of the same strand orientation (Gray et al., 1984).

To determine if short stretches of cytosines could form C·C$^+$ base pairs in the interior of an antiparallel duplex, we used CD measurements to study (1) mixtures of d$(AACC)_5$ with d$(CCTT)_5$, (2) the oligomer d($A_{10}C_4T_{10}$), which has self-complementary A_{10} and T_{10} blocks, and (3) mixtures of d($A_6C_6A_6$) with d($T_6C_6T_6$). When base-paired, these oligomers potentially could form antiparallel DNA duplexes having interior blocks of 2, 4, or 6 C·C$^+$ base pairs. Using CD measurements as in previous work, we were able to separately distinguish the formation of A·T and C·C$^+$ base pairs in these oligomers at a variety of pH values and Na$^+$ concentrations. We found that C·C$^+$ base pairs were not formed in the interior of any of these DNA duplexes. In the course of this study, we found that C·C$^+$ base pairs could form in the individual strands of d$(AACC)_5$, d$(CCTT)_5$, d($A_6C_6A_6$), and d($T_6C_6T_6$) but that this type of base pairing was disallowed when A·T base pairs formed in d$(AACC)_5$·d$(CCTT)_5$ and d($A_6C_6A_6$)·d($T_6C_6T_6$) duplexes. Consequently, for the oligomers studied, A·T base pair formation precluded the formation of adjacent, short stretches of C·C$^+$ base pairs.

Materials and Methods

Preparation of DNA Oligomers. Oligomers d$(AACC)_5$, d$(CCTT)_5$, and d($A_{10}C_4T_{10}$) were synthesized by solid-phase phosphite triester methods on a Beckman System 1 DNA synthesizer. Oligomers d($A_6C_6A_6$) and d($T_6C_6T_6$) were synthesized manually by using a DNA synthesis kit from New England Biolabs, Inc. All of the synthesized oligomers lacked 3′- and 5′-terminal phosphates. The DNA oligomers were subsequently purified by electrophoresis on a 20% (w/v) polyacrylamide gel in 7 M urea (Frank & Koster, 1979). Standards of d$(T)_{12-18}$ and d$(A)_{20}$, obtained from P-L Biochemicals, Inc., were used to provide length markers during electrophoresis. Under the gel conditions employed, no sequence-dependent effects on electrophoretic mobility were noted. Bands containing the oligomers were visualized by their quenching of ultraviolet light after the gels were laid on a fluor-containing thin-layer chromatography plate. Oligomers of the correct length were electroeluted from gel slices in an ISCO Model 1750 sample concentrator. The oligomers were precipitated by the addition of 4 volumes of ethanol to a solution adjusted to 0.1 M Na$^+$, which was then placed at −20 °C for 1 h. After the precipitates were centrifuged for 30 min at 15600*g* in an Eppendorf microcentrifuge, the oligomer pellets were finally resuspended in 0.01 M Na$^+$ (phosphate) buffer, pH 7, 6, or 5. Na$^+$ concentrations of the samples were increased to 0.1 or 0.5 M by adding aliquots of 4 M NaCl. Oligomer concentrations were corrected for the dilution, which was at most 10%, due to the addition of NaCl.

Extinction Coefficients. Extinction coefficients for the single-stranded oligomers were calculated from first-neighbor equations by using known extinction coefficients of dimers and monomers (Cantor et al., 1970). The calculated extinction coefficients at 260 nm were 9800, 7650, 8760, 9300, and 7830 L·mol^{-1}·cm^{-1} for d$(AACC)_5$, d$(CCTT)_5$, d($A_{10}C_4T_{10}$), d($A_6C_6A_6$), and d($T_6C_6T_6$), respectively. In the calculations of the extinction coefficients for d($A_{10}C_4T_{10}$) and d($A_6C_6A_6$), the absorbance components representing the d$(A)_{10}$ and d$(A)_6$ portions of the sequences were corrected by a factor of 0.8 to take into account the significant second-neighbor effects that contribute to the absorbance of poly[d(A)] (0.8 is the ratio of the measured to the calculated extinction coefficient of poly[d(A)]). Oligomer concentrations were determined by using absorbance measurements along with the calculated extinction coefficients.

Mixing Experiments. Mixtures of d$(AACC)_5$ with d$(CCTT)_5$ and of d($A_6C_6A_6$) with d($T_6C_6T_6$) were made with various molar proportions of the two single strands. The mixtures were heated to 60 °C, and spectra were recorded at decreasing temperatures, allowing 30 min to equilibrate at each temperature. No spectral changes were noted after 30 min at a given temperature, and annealing profiles obtained with this procedure were reproducible.

Absorption and CD Spectra. Absorption spectra were taken with a Cary-Varian Model 118 spectrophotometer. CD spectra were taken with a Jasco Model J-500A circular dichrometer. The circular dichrometer was calibrated by using *d*-10-camphorsulfonic acid (Aldrich Chemical Co.) to give a value within 2% of 0.336-deg ellipiticity at 290.5 nm for a 0.1% (w/v) solution (Chen & Yang, 1977). The ratio of the magnitudes of the peaks at 192.5 and 290.5 nm was −2.05 ± 0.3. Measurements were taken with a spectral bandwidth of 1 nm, a time constant of 1 s, and a scan speed of 10 nm/min. Digitized data were collected every 0.1 nm on a Jasco DP-500N data processor and smoothed twice with a 7-point third-order polynomial. Every 10th point was transferred to a Hewlett-Packard 9816S computer. The data were then smoothed with a 13-point quadratic-cubic function (Savitzky & Golay, 1964). CD values are reported as $\epsilon_L - \epsilon_R$ in units of L·mol^{-1}·cm^{-1}. During spectral measurements, samples were maintained at constant temperature with an accuracy of ±0.5 °C.

CD spectra of polymers used in calculations were similar to those previously published for two forms of poly[d(C)] (the single-stranded form at pH 8, 0.01 M Na$^+$, 20 °C, and the acid self-complex at pH 7, 0.01 M Na$^+$, 20 °C; Gray et al., 1988), for poly[d(T)] and poly[d(A)] (both at pH 7, 0.1 M Na$^+$, 20 °C; Steely et al., 1986), and for poly[d(CT)] and poly[d(AC)] (both at pH 7, 0.01 M Na$^+$, 20 °C; Gray et al., 1980).

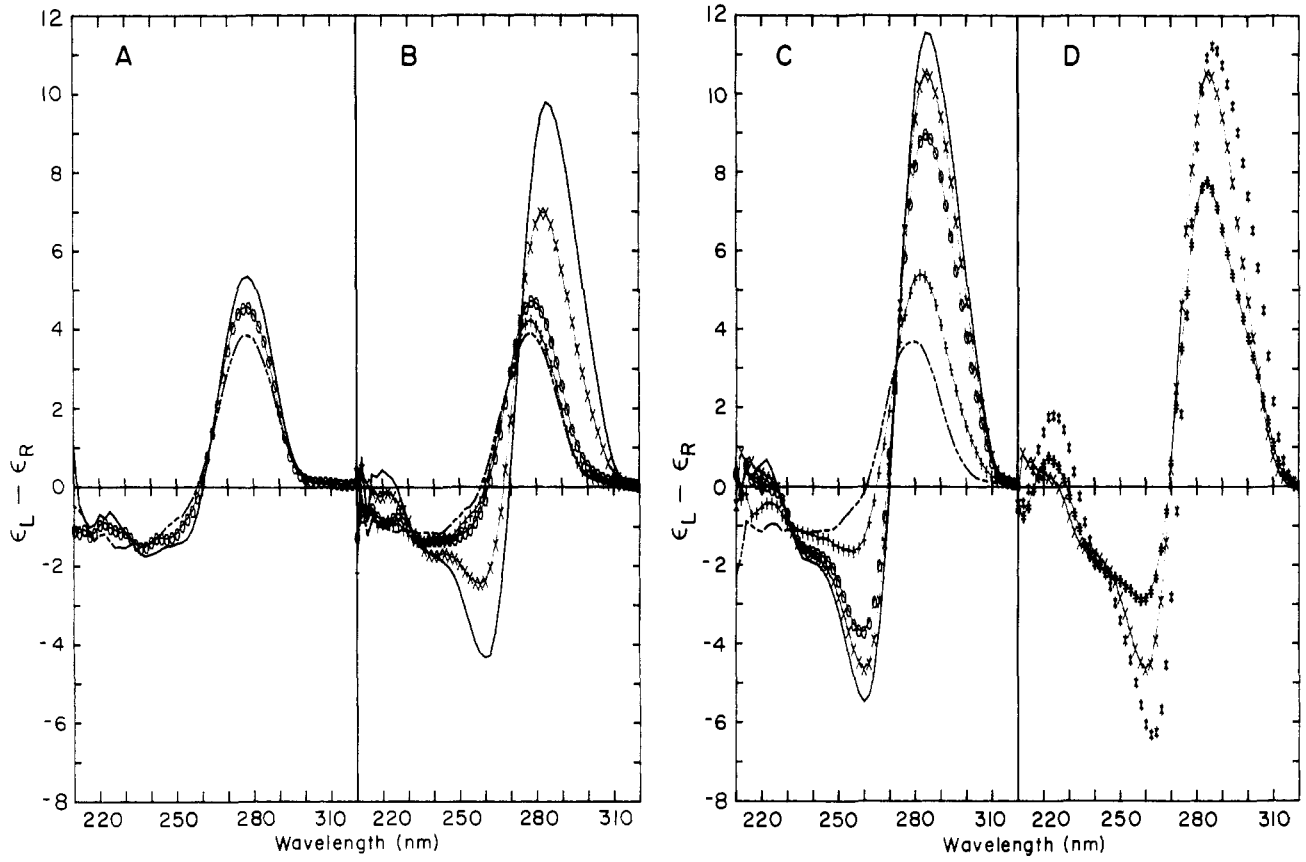
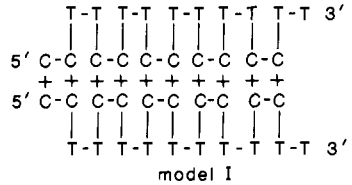


FIGURE 1: Measured and calculated CD spectra of $d(CCTT)_5$. Measured spectra were taken as the temperature was lowered from 60 to 5 °C: (- - -) 60 °C; (+) 40 °C; (o) 30 °C; (×) 20 °C; (—) 5 °C. Measured spectra are shown at (A) pH 7, (B) pH 6, and (C) pH 5, all at 0.5 M Na^+. (D) The measured CD spectrum at pH 5, 20 °C (×), is compared with two calculated spectra. One CD spectrum (*) was calculated for a structure (model I) having stacked $C{\cdot}C^+$ base pairs and looped-out T's; the calculation was 0.5 × [CD spectrum of single-stranded poly[d(T)]] + 0.5 × [CD spectrum of the acid self-complex of poly[d(C)]]. A second CD spectrum (#) was calculated for a structure (model II) having stacked $C{\cdot}C^+$ base pairs and intrahelical T's; the calculation was 0.25 × [CD spectrum of single-stranded poly[d(T)]] + 0.5 × [CD spectrum of single-stranded poly[d(CT)]] + 0.25 × [CD spectrum of the acid self-complex of poly[d(C)]].

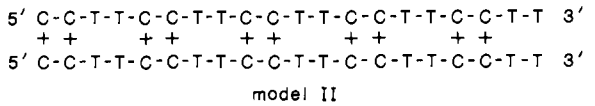
Results and Discussion

$d(CCTT)_5$ and $d(AACC)_5$. The individual $d(CCTT)_5$ and $d(AACC)_5$ oligomers were studied to determine under what conditions $C{\cdot}C^+$ base pairs would form within each of these sequences. CD spectra of $d(CCTT)_5$ at pH 7, 6, and 5 (at a Na^+ concentration of 0.5 M) are shown in Figure 1, panels A–C, respectively. At each pH, representative spectra are shown at several decreasing temperatures. Only small changes in band magnitudes occurred at pH 7 as the temperature of $d(CCTT)_5$ was lowered. Small increases in band magnitudes (such as seen at 277 nm in Figure 1A) are typical of increased base stacking in a single-stranded oligomer. However, as shown in Figure 1B, when the oligomer was at a reduced pH of 6, a decrease in temperature resulted in a dramatic increase in the magnitude of the positive band, a red shift of the positive band from 278 to 284 nm, and the appearance of a negative band at 261 nm. These CD changes were comparable to the ones seen during formation of the protonated self-complex of poly[d(C)] (Gray & Bollum, 1974) and during the formation of a loop-out structure for poly[d(CT)] (Brown et al., 1985), both of which are stabilized by stacked $C{\cdot}C^+$ base pairs. As the temperature of $d(CCTT)_5$ at pH 6 was decreased, the CD band magnitudes increased over a narrow range of temperatures between 30 and 5 °C, indicating a cooperative structural transition that involved the formation of $C{\cdot}C^+$ base pairs. When the pH was lowered to 5, these CD changes commenced at even higher temperatures, as is shown in Figure 1C. Also, at pH 5, CD band magnitudes were greater than at pH 6, indicating that $C{\cdot}C^+$ base pair formation was more complete at the lower pH.

The measured CD spectrum of $d(CCTT)_5$ at pH 5, 0.5 M Na^+, 20 °C, is compared in Figure 1D with spectra calculated for two different model structures having $C{\cdot}C^+$ base pairs. Model I is a helix with a continuous stack of 10 $C{\cdot}C^+$ base



model I



model II

pairs as in the acid self-complex of poly[d(C)], with extrahelical thymines stacked as in single-stranded poly[d(T)]. Model II is a structure in which the opposing thymines remain intrahelical and in which stacked d(TC) and d(CT) neighbors contribute to the CD. In this latter case the duplex has five separate regions of two stacked $C{\cdot}C^+$ base pairs each. Strand polarity is arbitrarily shown as parallel.

The CD spectrum calculated for model I was the average of the CD spectrum of single-stranded poly[d(T)] and the CD spectrum of the acid self-complex of poly[d(C)]. No CD contributions of d(CT) or d(TC) stacks were included. The CD spectrum calculated for model II was one-fourth of the CD spectrum of single-stranded poly[d(T)], one-fourth of the CD spectrum of the self-complex of poly[d(C)], plus half of the CD spectrum of single-stranded poly[d(CT)]. This second

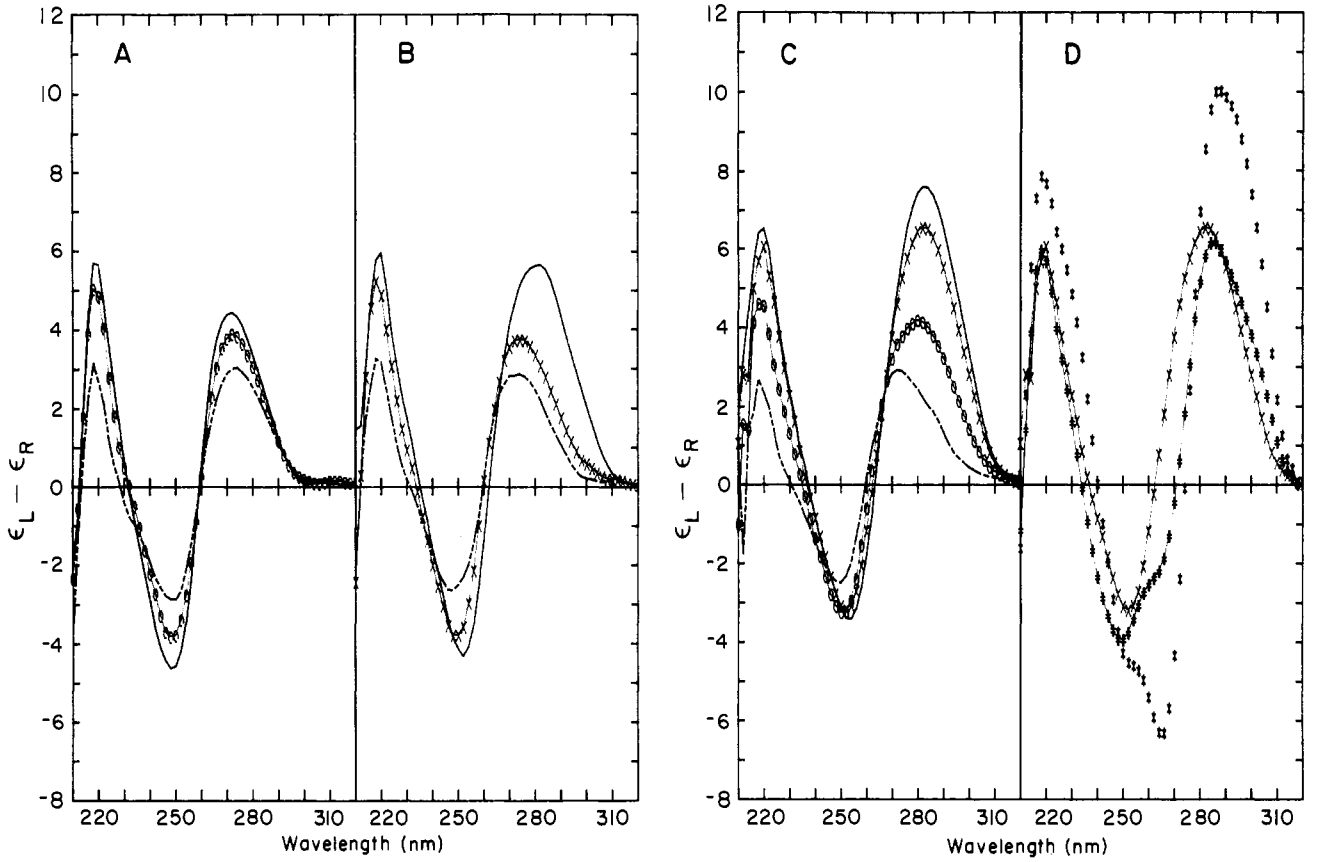


FIGURE 2: Measured and calculated CD spectra of d(AACC)$_5$. Measured spectra were taken as the temperature was lowered from 60 to 5 °C: (- - -) 60 °C; (○) 30 °C; (×) 20 °C; (—) 5 °C. Measured spectra are shown at (A) pH 7, (B) pH 6, and (C) pH 5, all at 0.5 M Na$^+$. (D) The measured CD spectrum at pH 5, 20 °C (×), is compared with two calculated spectra. One CD spectrum (*) was calculated for a structure (model I′) having stacked C·C$^+$ base pairs and looped-out A's; the calculation was 0.5 × [CD spectrum of single-stranded poly[d(A)]] + 0.5 × [CD spectrum of the acid self-complex of poly[d(C)]]. A second CD spectrum (#) was calculated for a structure (model II′) having stacked C·C$^+$ base pairs with intrahelical A's; the calculation was 0.25 × [CD spectrum of single-stranded poly[d(A)]] + 0.5 × [CD spectrum of single-stranded poly[d(AC)]] + 0.25 × [CD spectrum of the acid self-complex of poly[d(C)]].

calculation included CD contributions from neighboring cytosines and thymines.

The measured spectrum of d(CCTT)$_5$ at pH 5, 0.5 M Na$^+$, 20 °C, more closely matched the calculated spectrum for model I (Figure 1D). We concluded that a majority of the d(CCTT)$_5$ oligomer was in a loop-out structure at pH 5, similar to that previously found for the acid self-complex of poly[d(CT)] (Brown et al., 1985), with adjacent thymines being looped out two at a time in the case of d(CCTT)$_5$. It should be noted that the looped-out thymines of d(CCTT)$_5$ could be involved in intrastrand stacks of only two at a time along the same strand, although they could also be involved in interstrand stacks with thymines displaced a half helical turn in the opposite strand.

CD spectra were measured for d(AACC)$_5$ under the same pH and Na$^+$ concentrations as those used for d(CCTT)$_5$. Panels A–C of Figure 2 show representative CD spectra at decreasing temperatures for d(AACC)$_5$ at pH 7, 6, and 5, respectively (all at 0.5 M Na$^+$). As shown in Figure 2A, relatively small increases in the CD bands were observed when the temperature was lowered at pH 7, consistent with the oligomer remaining single stranded, but with increased base stacking, as the temperature was lowered at this pH. Figure 2B shows that when the pH was lowered to 6, the CD spectrum qualitatively changed at the lowest temperature; most notably there was a red shift of the long-wavelength band from about 275 to 282 nm and an increase in its magnitude. When the pH was lowered to 5 (Figure 2C), the 282-nm band increased still further in magnitude at the lowest temperatures. Apparently d(AACC)$_5$ also formed a self-complex, although it required lower pH and/or temperature than did d(CCTT)$_5$ (compare spectra in Figures 1 and 2).

A priori, it was possible that, at low pH, d(AACC)$_5$ adopted various types of base pairs including C·C$^+$, A$^+$·A$^+$, and A·C$^+$ pairs. However, the T_m of the self-complex of d(AACC)$_5$ was lower than that of the self-complex of d(CCTT)$_5$ by about 9 °C at pH 5, 0.5 M Na$^+$, suggesting that d(AACC)$_5$ did not form both C·C$^+$ and A$^+$·A$^+$ base pairs or all A·C$^+$ base pairs. Furthermore, the pK_a for A$^+$·A$^+$ base pair formation in poly[d(A)] is 4.4 (Adler et al., 1969), significantly below the pH where spectral changes for the d(AACC)$_5$ transition are seen. Finally, the spectral changes that occurred during the transitions of d(CCTT)$_5$ and d(AACC)$_5$ were compared. Spectra of the compounds at high pH were subtracted from their respective spectra at low pH (at 5 °C, where base pairing was maximal at the low pH). The resulting difference CD spectra shown in Figure 3A were similar in that both had positive and negative bands centered at about 290 and 263 nm, suggesting that the same type of base pair was present in each self-complex. The self-complex of d(CCTT)$_5$ may have had more stacked C·C$^+$ base pairs than did the self-complex of d(AACC)$_5$, since it had a difference CD spectrum closer in magnitude to that of the self-complex of poly[d(C)], which is also shown in Figure 3A on a comparable scale. We concluded that the acid self-complexed forms of both d(CCTT)$_5$ and d(AACC)$_5$ probably contained C·C$^+$ base pairs, although the possibility of A·C$^+$ base pairs in the d(AACC)$_5$ self-complex could not be rigorously excluded.

Figure 2D shows the spectrum of d(AACC)$_5$ at pH 5, 0.5 M Na$^+$, 20 °C, together with calculated spectra for two dif-

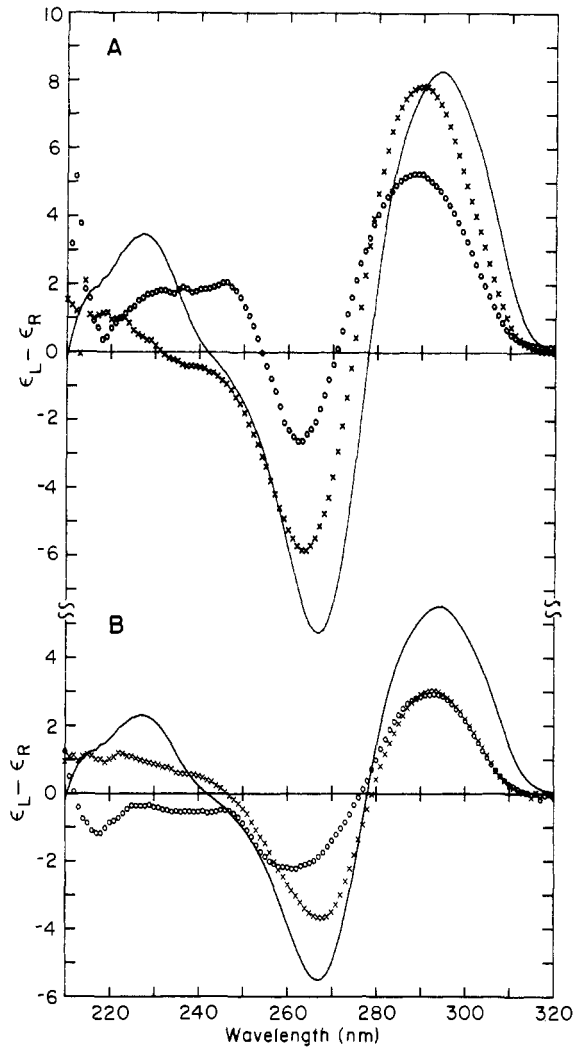
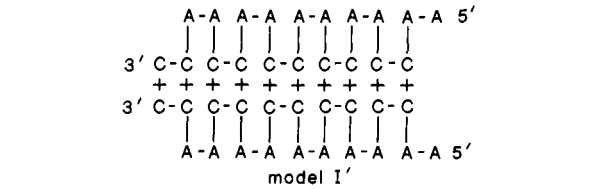
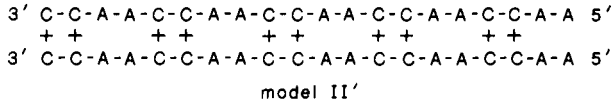


FIGURE 3: CD difference spectra (low pH minus high pH): (A) (×) d(CCTT)$_5$, (○) d(AACC)$_5$, and (—) poly[d(C)]; (B) (×) d($T_6C_6T_6$), (○) d($A_6C_6A_6$), and (—) poly[d(C)]. Spectra were calculated by subtracting the CD spectra of the single-stranded forms at pH 7, 0.5 M Na^+, 5 °C from the CD spectra of the double-stranded forms at pH 5, 0.5 M Na^+, 5 °C, except for poly[d(C)] for which the spectrum of the single-stranded form at pH 8 was subtracted from the CD spectrum of the self-complexed form at pH 7 (both at 0.01 M Na^+, 20 °C). The difference spectra for poly[d(C)] have been reduced by half in panel A and to one-third in panel B for comparison with the difference spectra of oligomers having these fractions of cytosine.

ferent model structures having C·C$^+$ base pairs, analogous to those presented above for d(CCTT)$_5$. Model I′ is a helix with



model I′



model II′

a continuous stack of 10 C·C$^+$ base pairs as in the self-complex of poly[d(C)], with extrahelical adenines stacked as in single-stranded poly[d(A)]. Model II′ is a structure in which the opposing adenines remain intrahelical and in which stacked d(AC) and d(CA) neighbors contribute to the CD. The schematic drawings of these two models are again shown with an arbitrary parallel strand orientation.

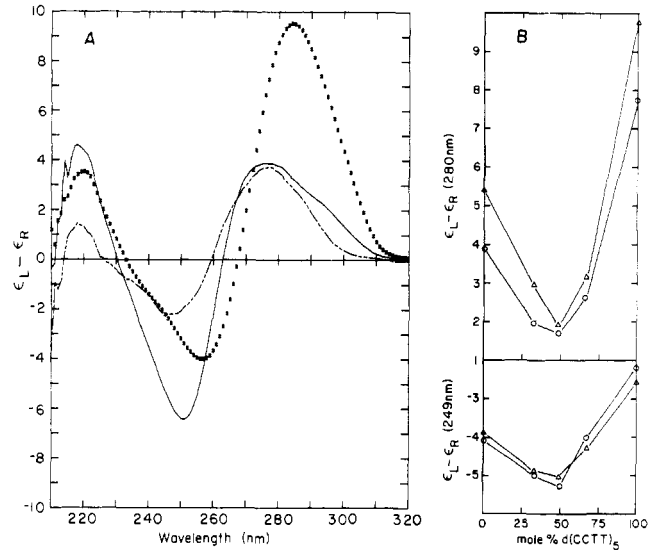


FIGURE 4: (A) Measured CD spectra of the 1:1 mix of d(AACC)$_5$ with d(CCTT)$_5$ at pH 5, 0.5 M Na^+, compared with the average of the CD spectra of the separated strands under the same conditions at a temperature where the individual strands were in self-complexes. Measured spectra were at 60 (- - -) and 5 °C (—). Average spectrum is for the separated strands at 5 °C (*). (B) Mixing curves of d(AACC)$_5$ with d(CCTT)$_5$ at pH 6, 0.1 M (○) and 0.5 M (△) Na^+, 5 °C, showing CD changes at two wavelengths for increasing mole percentage of d(CCTT)$_5$.

Spectra were calculated for these two structures in a fashion parallel to that used above to calculate spectra for the models of the d(CCTT)$_5$ self-complex. The calculated spectra are shown in Figure 2D, together with the measured spectrum of d(AACC)$_5$ at pH 5, 0.5 M Na^+, 20 °C. Given the simple nature of the calculations, the agreement seemed to be quite good between the measured spectrum for the self-complex of this oligomer and the calculated spectrum for model II′. This indicated not only that the adenines remained intrahelical in the d(AACC)$_5$ self-complex but also that C·C$^+$ base pairs were probably formed in the self-complex to the exclusion of A$^+$·A$^+$ and A·C$^+$ base pairs. The dissimilar band magnitudes of the difference spectra for d(AACC)$_5$ and d(CCTT)$_5$, shown in Figure 3A, may have been due to the fact that a continuous stack of 10 C·C$^+$ base pairs was present only in the d(CCTT)$_5$ self-complex.

Mixtures of d(CCTT)$_5$ and d(AACC)$_5$. In previous work we found that the pK_a for C·C$^+$ base pair formation in a self-complex of d($C_4A_4T_4C_4$) that had both A·T and C·C$^+$ base pairs was higher than that in the self-complex of d(C)$_8$ (Gray et al., 1984). That is, A·T base pairs appeared to stabilize adjacent C·C$^+$ base pairs in the self-complex of d($C_4A_4T_4C_4$). Consequently, we expected that a duplex of d(AACC)$_5$·d(CCTT)$_5$ might form that would have stable base pairs of both types and in which the pK_a for the formation of C·C$^+$ base pairs might even be raised.

As discussed above, C·C$^+$ base pairs formed in the individual d(AACC)$_5$ and d(CCTT)$_5$ oligomers at pH 6 or 5, resulting in an increased positive CD band at 282–284 nm. However, 1:1 mixtures of the oligomers at pH 6 and 5 showed either a decrease or little change in the magnitude of this CD band. Figure 4A shows measured CD spectra at 60 and 5 °C of a 1:1 mixture of d(AACC)$_5$ with d(CCTT)$_5$ at pH 5, 0.5 M Na^+. Two important CD characteristics were noted as the temperature was dropped below the T_m of 35–40 °C. First, a band close to 250 nm tripled in negative magnitude as the temperature was lowered (Figure 4A). Simultaneously, the absorbance at 264 nm decreased by 10% (not shown). Previous CD work has shown that there is an increase in the negative

magnitude of a 250-nm band when A·T base pairs are formed in poly[d(A)]·poly[d(T)] and poly[d(AT)·d(AT)] duplexes (Greve et al., 1977). Consequently, we concluded that A·T base pairs formed in the 1:1 mixture of $d(AACC)_5$ with $d(CCTT)_5$ at pH 5.0, 5 °C. Second, there was no positive band at long wavelengths (with a peak near 285 nm) that would have indicated the presence of C·C$^+$ base pairs in the mixture. This was not due to a compensating negative CD from the A·T base pairs; there is a small decrease in the CD between 265 and 280 nm during pairing of poly[d(A)] with poly[d(T)] (Steely et al., 1986), but it is much smaller than the increase in CD at these wavelengths when C·C$^+$ base pairs form. Thus, the CD changes that occurred when A·T base pairs formed in $d(AACC)_5$·$d(CCTT)_5$ did not mask the changes that would have been seen if C·C$^+$ base pairs had formed concomitantly. Also shown in Figure 4A is the average of the CD spectra of the $d(AACC)_5$ and $d(CCTT)_5$ self-complexes at pH 5, 0.5 M Na^+, 5 °C, to demonstrate the magnitude of the long-wavelength CD band that might have been measured if C·C$^+$ base pairs had been present in the mixture.

Similar results were obtained when mixtures of the oligomers were made at pH 6. Figure 4B shows changes in the CD bands that monitor either the formation of A·T base pairs (249 nm) or C·C$^+$ base pairs (280 nm) for mixtures of differing oligomer stoichiometries. The lower panel shows that A·T base pairing was maximal at a strand ratio of 1:1, while the upper panel shows that C·C$^+$ base pairing reached a minimum at a 1:1 strand ratio. Thus, A·T base pairs not only did not raise the pK_a of the adjacent cytosines to facilitate C·C$^+$ base pair formation but actually prevented the formation of adjacent C·C$^+$ base pairs in the $d(AACC)_5$·$d(CCTT)_5$ duplexes at pH values sufficiently low to allow such pairing in the individual strands.

$d(A_{10}C_4T_{10})$. The oligomer $d(A_{10}C_4T_{10})$, which has self-complementary A_{10} and T_{10} regions, was studied to see if a sequence of four cytosines might pair in the interior of a DNA duplex. This oligomer can potentially form a hairpin as well as a duplex, and the annealing profiles were indeed biphasic at 0.1 M Na^+, as shown in the inset to Figure 5. It appeared that the transition to form a duplex was at the lower temperature, since the annealing temperature of this transition was increased as the nucleotide concentration increased from 1.07×10^{-5} to 1.24×10^{-4} M (Figure 5, inset). Others have also found that the duplex form of a self-complementary DNA oligomer can be the stable form at low temperatures (Schleffler et al., 1968; Marky et al., 1983).

CD spectra of $d(A_{10}C_4T_{10})$ are shown in Figure 5 at 60 and 5 °C. The increase in the negative magnitude of the 249-nm band indicated the pairing of A and T bases. At the same time, the CD above 280 nm, used to monitor C·C$^+$ base pair formation, did not increase in magnitude as the temperature was lowered from 60 to 5 °C. The pairing of cytosines could have resulted in an increase at long wavelengths that was one-fourth to one-third as large as that shown in Figure 3A for $d(AACC)_5$ or $d(CCTT)_5$. The measured spectrum of $d(A_{10}C_4T_{10})$ at a low temperature was close to a calculated spectrum based on the assumption that none of the cytosines were paired while 60% of the possible A·T pairs were formed (Figure 5). Thus, there was no CD evidence for C·C$^+$ base pair formation in this oligomer at pH 5, 5 °C, nor was there spectral evidence for cytosine base pairs at other, higher temperatures.

$d(A_6C_6A_6)$ and $d(T_6C_6T_6)$. Finally, we investigated the possibility that a set of oligomers having blocks of six cytosines might pair and simultaneously form A·T and C·C$^+$ base pairs.

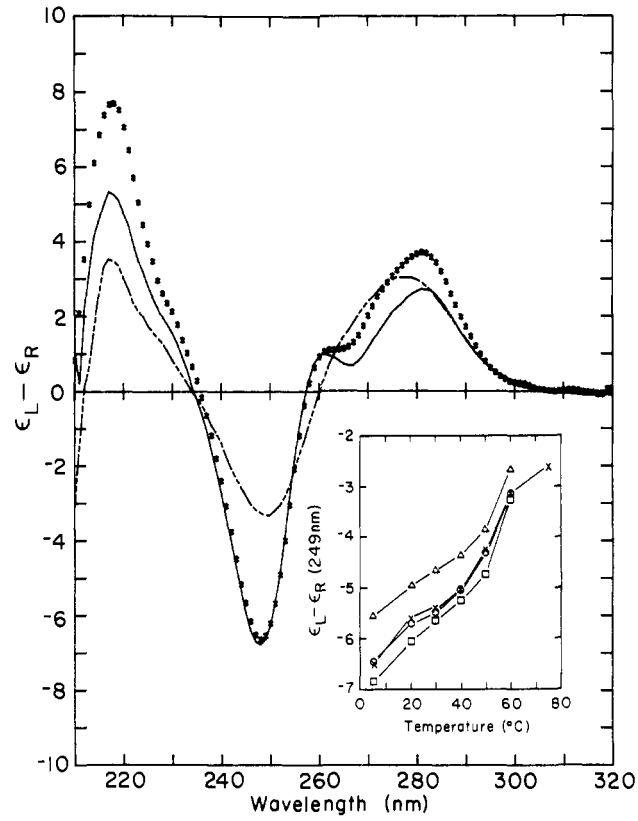


FIGURE 5: Measured and calculated CD spectra of $d(A_{10}C_4T_{10})$. The measured spectra were taken at pH 5, 0.1 M Na^+, first at 60 °C (- - -) and then at 5 °C (—). Also shown is a spectrum calculated for $d(A_{10}C_4T_{10})$ with 0% C·C$^+$ base pairs and 60% A·T base pairs (*). The calculation was $(^1/_6) \times$ [CD spectrum of single-stranded poly[d(C)]] + $(0.6 \times {}^5/_6) \times$ [CD spectrum of double-stranded d-(A_{10})·$d(T_{10})$] + $(0.4 \times {}^5/_6) \times$ [CD spectrum of single-stranded $d(A_{10})$ + $d(T_{10})$]. (Inset) Annealing profiles of $d(A_{10}C_4T_{10})$ at the following nucleotide concentrations and pH values (all at 0.1 M Na^+): (○) 1.04×10^{-4} M, pH 7; (×) 1.24×10^{-4} M, pH 6; (□) 1.24×10^{-4}, pH 5; (△) 1.07×10^{-5} M, pH 5.

CD spectra of the individual oligomers $d(A_6C_6A_6)$ and $d(T_6C_6T_6)$ are shown in Figures 6 and 7, respectively. For both oligomers at pH 7, lowering the temperature resulted in slight increases in most CD band magnitudes, indicating that the oligomers remained single stranded but with increased base stacking (Figures 6A and 7A). Once the pH was dropped to 5, however, an increase in the CD at long wavelengths provided evidence for the formation of self-complexes by both oligomers at sufficiently low temperatures (Figures 6B and 7B). The difference CD (spectra at pH 5 minus spectra at pH 7, 5 °C) for these two oligomers are compared in Figure 3B with the difference spectrum of poly[d(C)] on a comparable scale. The similarities among these difference spectra, especially in the band at about 292 nm, leaves little doubt that a stack of C·C$^+$ base pairs could be formed in the separate $d(A_6C_6A_6)$ and $d(T_6C_6T_6)$ oligomers.

Mixtures of $d(A_6C_6A_6)$ with $d(T_6C_6T_6)$. Mixtures were made of $d(A_6C_6A_6)$ with $d(T_6C_6T_6)$ at pH 7, 6, 5, and 4. Mixing curves of $d(A_6C_6A_6)$ with $d(T_6C_6T_6)$ were made at pH 6 to determine the strand stoichiometry in the resulting complex. As before with mixtures of $d(AACC)_5$ with $d(CCTT)_5$, there were increases and decreases in the CD bands at 250 and 280 nm, respectively, that were maximal at a 1:1 oligomer ratio (data not shown). There was no CD evidence for pairing of cytosines, even at pH 4 (data not shown), which is below the pK_a of the cytosine N3. CD spectra of the 1:1 mixture of $d(A_6C_6A_6)$ and $d(T_6C_6T_6)$ at a few temperatures at pH 5 are shown in Figure 8. As the temperature was

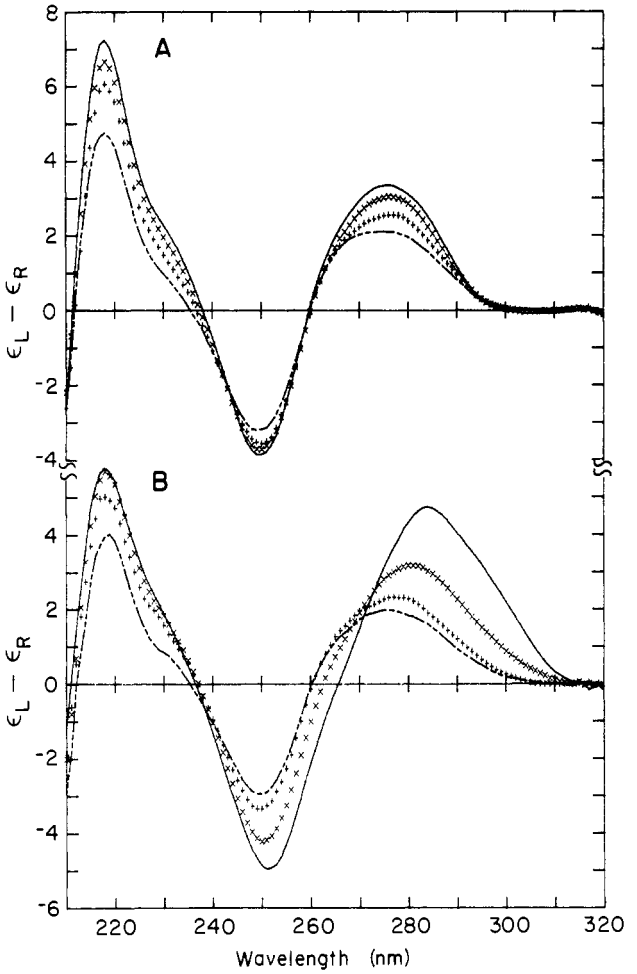


FIGURE 6: Measured CD spectra of $d(A_6C_6A_6)$ taken as the temperature was lowered from 60 to 5 °C: (- - -) 60 °C; (+) 40 °C; (×) 20 °C; (—) 5 °C. Spectra are shown at (A) pH 7, 0.5 M Na^+, and (B) pH 5, 0.5 M Na^+.

lowered from 60 to 5 °C the negative band at 249 nm increased in magnitude, indicative of A·T base pair formation. However, there was no long-wavelength positive band with a peak above 280 nm in spectra of the mixture at low temperatures, and thus there was no evidence for $C{\cdot}C^+$ base pairs. The average spectrum of the individual oligomers is included in Figure 8 to show the magnitude of the CD band above 280 nm that might have been present if $C{\cdot}C^+$ base pairs had formed. The paired oligomers obviously did not contain both types of base pair.

CONCLUSIONS

This work demonstrated the ability of the individual oligomers $d(AACC)_5$, $d(CCTT)_5$, $d(A_6C_6A_6)$, and $d(T_6C_6T_6)$ to form $C{\cdot}C^+$ base pairs when the pH was lowered to 6 or 5, as monitored by an increase in the magnitude of the long-wavelength CD band above 280 nm. This transition was facilitated by lowering the pH or the temperature. Also, raising the Na^+ concentration from 0.1 to 0.5 M helped stabilize $C{\cdot}C^+$ base pairs in these complexes (data not shown). This was in contrast to the case with poly[d(C)], which is more stable at lower Na^+ concentrations (Inman, 1964). Thus, in the case of these oligomer complexes, which have only one-third to half of the bases as cytosines, the repulsion of the phosphates was stronger than the attraction between the hemiprotonated cytosine bases and the negatively charged phosphates, which dominates in the case of poly[d(C)] (Cantor & Schimmel, 1980). The individual oligomers $d(CCTT)_5$,

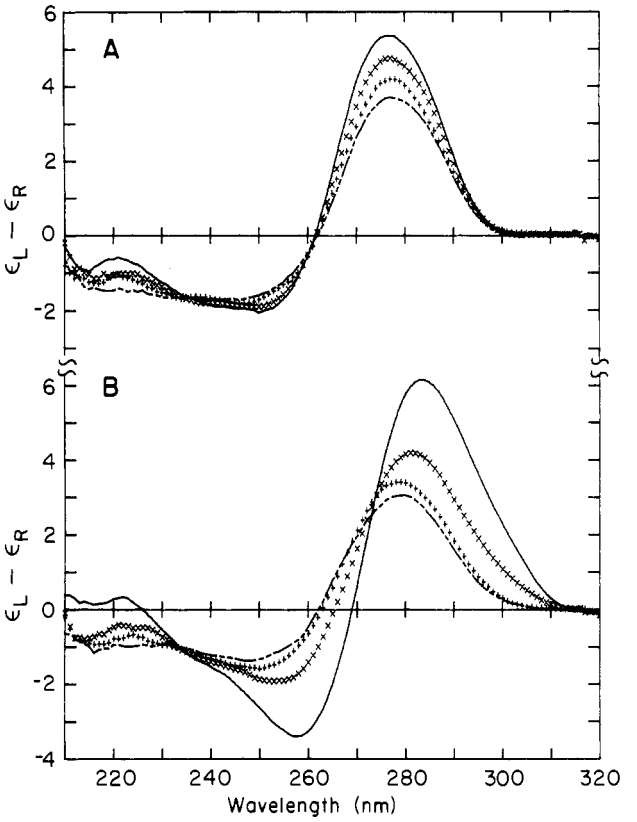


FIGURE 7: Measured CD spectra of $d(T_6C_6T_6)$ taken as the temperature was lowered from 60 to 5 °C: (- - -) 60 °C; (+) 40 °C; (×) 20 °C; (—) 5 °C. Spectra are shown at (A) pH 7, 0.5 M Na^+, and (B) pH 5, 0.5 M Na^+.

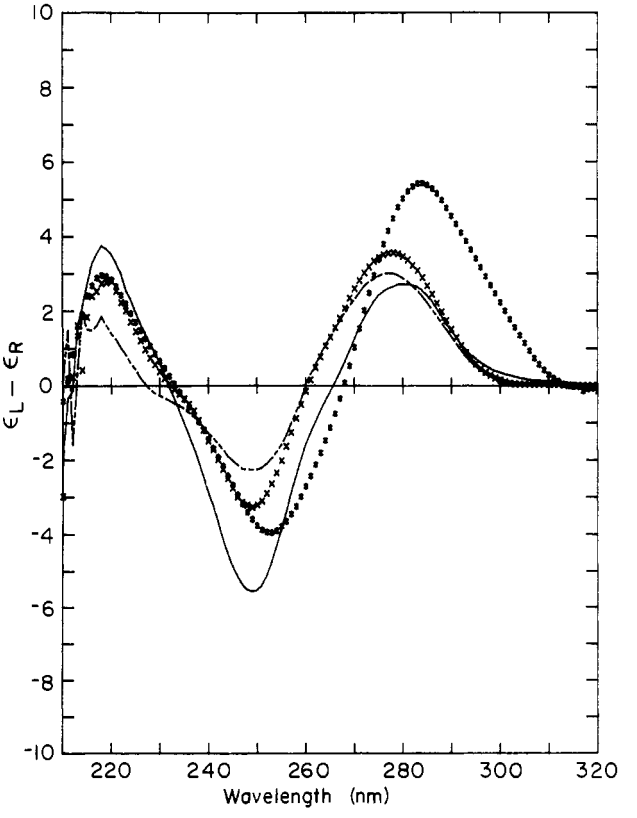


FIGURE 8: Measured CD spectra of the 1:1 mix of $d(A_6C_6A_6)$ with $d(T_6C_6T_6)$ at pH 5, 0.5 M Na^+ compared with the average of the CD spectra of the separated strands under the same conditions at a temperature where the individual strands were in self-complexes. Measured spectra were at (- - -) 60 °C; (×) 20 °C, and (—) 5 °C. The average spectrum (*) is for the individual strands at 5 °C.

d($A_6C_6A_6$), and d($T_6C_6T_6$) each formed a complex having a stretch of 6 or 10 stacked C·C$^+$ base pairs, the internal thymines being looped out in the case of d(CCTT)$_5$ (see the comparison of measured and calculated CD spectra in Figure 1D). In the case of d(AACC)$_5$, 2 C·C$^+$ base pairs apparently alternated with two intrahelical adenines (see Figure 2D).

The T_m of the d(CCTT)$_5$ self-complex varied from about 15 °C (at pH 6, 0.1 M Na$^+$) to 35 °C (at pH 5, 0.5 M Na$^+$) and that of the d(AACC)$_5$ self-complex varied from <10 °C (at pH 6, 0.1 M Na$^+$) to 27 °C (at pH 5, 0.5 M Na$^+$) (data not shown). The fact that the T_m of the d(CCTT)$_5$ oligomeric loop-out structure was less than we previously found for the poly[d(CT)] loop-out structure (with a T_m of about 42 °C at pH 5.5, 0.09 M Na$^+$) was at least partially a consequence of the short length of the oligomer. It may also be more destabilizing to have two adjacent thymines looped out as in the oligomeric self-complex. More work will be needed to access the influence of the lengths of C and T blocks on the stability of such loop-out structures.

An increase in the negative magnitude of the 250-nm CD band provided evidence for the formation of A·T base pairs in d($A_{10}C_4T_{10}$) (0.1 M Na$^+$), mixtures of d(AACC)$_5$ with d(CCTT)$_5$ (0.1 or 0.5 M Na$^+$), and mixtures of d($A_6C_6A_6$) with d($T_6C_6T_6$) (0.1 or 0.5 M Na$^+$) at low temperatures, pH 5 or 6 (including data not shown). The absence of a long-wavelength CD band with a peak above 280 nm showed that C·C$^+$ base pairs did not concomitantly form in any of these complexes, although such base pairs could readily form at pH 5, 0.5 M Na$^+$, within the four individual strands that were used to make the mixtures. Mixtures made at low pH of d(AACC)$_5$ with d(CCTT)$_5$ (Figure 4B) and of d($A_6C_6A_6$) with d($T_6C_6T_6$) showed that maximal A·T base pairing and minimal C·C$^+$ base pairing occurred at a strand ratio of 1:1. Thus, a major conclusion of this work was that short internal stretches of 2, 4, or 6 C·C$^+$ base pairs could not form next to neighboring A·T base pairs in these oligomer duplexes. Our results with the mixtures of d(CCTT)$_5$ and d(AACC)$_5$ showed that A·T base pairs were more stable than an equal number of C·C$^+$ base pairs in self-complexes of the individual strands (at 0.1 or 0.5 M Na$^+$, pH 5 or 6). Aboul-ela et al., (1985) previously showed that a single internal cytosine–cytosine mismatch does not add to the stability of an antiparallel DNA duplex.

Why were C·C$^+$ base pairs unable to form in the d(AACC)$_5$·d(CCTT)$_5$, d($A_{10}C_4T_{10}$)·d($A_{10}C_4T_{10}$), and d($A_6C_6A_6$)·d($T_6C_6T_6$) duplexes under conditions where A·T base pairs formed? One possibility was that sequences of 2, 4, or 6 adjacent C·C$^+$ base pairs were insufficiently long to be stable in the oligomeric complexes. The results for the individual strands of d($A_6C_6A_6$) and d($T_6C_6T_6$) argued against this restriction for six adjacent cytosines. In this case, six contiguous cytosines were long enough for each of these two oligomers, by themselves, to form C·C$^+$ base pairs when no other constraints (such as an additional type of base pairing) were imposed on the complex. Also, five sets of two adjacent cytosine base pairs were sufficient to stabilize the d(AACC)$_5$ self-complex (Figure 2D).

A second possible reason for the absence of C·C$^+$ base pairs in any of the complexes that contained A·T base pairs was that the interior cytosine regions may have been constrained from pairing by the presence of junctions with A·T base pairs. The oligomer d($C_4A_4T_4C_4$) studied by Gray et al. (1984), which showed simultaneous formation of both C·C$^+$ and A·T base pairs, had blocks of cytosines at the ends of the sequence and may have had minimal junction constraints. However, known junction effects between two types of conformation are relatively nondisruptive. The junction between a left-handed Z-DNA sequence and a right-handed B-DNA sequence is about 3 base pairs and does not affect the ability of bases to pair on either side of this region (Wells et al., 1983). A similar case was noted for a junction between A-DNA and B-DNA in the oligomer dG_n·(rC_{11}dC_{16}). The dG·dC porton of the sequence adopts the B conformation, while the dG·rC sequence assumes the A conformation. The junction between these two regions is localized to 1 base pair, and there is complete base pairing and base stacking on both sides of the junction (Selsing & Wells, 1979). On the other hand, the difference in the C1′–C1′ distance for a C·C$^+$ base pair (8.7 Å) and for a Watson–Crick base pair (10.8 Å) may be sufficiently large to result in a more significant junction effect.

A third possibility is that C·C$^+$ base pairs only form between parallel DNA strands and thus cannot exist adjacent to A·T base pairs that are in an antiparallel strand orientation. When C·C$^+$ base pairs formed in the individual d(AACC)$_5$, d(CCTT)$_5$, d($A_6C_6A_6$), and d($T_6C_6T_6$) oligomers, the strands may have been oriented parallel to each other. Previous work on d($C_4A_4T_4C_4$) (Gray et al., 1984) that showed concomitant formation of C·C$^+$ and A·T base pairs would have to be explained by postulating the formation of a multistranded structure that contained DNA strands with both parallel and antiparallel orientations (Gray et al., 1988).

References

Aboul-ela, E., Koh, D., & Tinoco, I., Jr. (1985) *Nucleic Acids Res. 13*, 4811–4824.

Adler, A. J., Grossman, C., & Fasman, G. D. (1969) *Biochemistry 8*, 3846–3858.

Antao, V. P., Gray, C. W., Gray, D. M., & Ratliff, R. L. (1986) *Nucleic Acids Res. 14*, 10091–10113.

Baldacci, G., Cherif-Zahar, B., & Bernardi, G. (1984) *EMBO J. 3*, 2115–2120.

Brown, D. M., Gray, D. M., Patrick, M. H., & Ratliff, R. L. (1985) *Biochemistry 24*, 1676–1683.

Cantor, C. R., & Schimmel, P. R. (1980) *Biophysical Chemistry, Part III*, pp 1146–1147, W. H. Freeman, San Francisco.

Cantor, C. R., & Efstratiadis, A. (1984) *Nucleic Acids Res. 12*, 8059–8072.

Cantor, C. R., Warshaw, M. M., & Shapiro, H. (1970) *Biopolymers 9*, 1059–1077.

Chen, G. C., & Yang, J. T. (1977) *Anal. Lett. 10*, 1195–1207.

Coll, M., Solans, X., Font-Altaba, M., & Subirana, J. A. (1987) *J. Biomol. Struct. Dyn. 4*, 797–811.

Cruse, W. B. T., Egert, E., Kennard, O., Sala, G. B., Salibury, S. A., & Viswamitra, M. A. (1983) *Biochemistry 22*, 1833–1839.

Dybvig, K., Clark, C. D., Aliperti, G., & Schlesinger, M. J. (1983) *Nucleic Acids Res. 11*, 8495–8508.

Finer, M. H., Fodor, E. J. B., Boedtker, H., & Doty, P. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1659–1663.

Fowler, R. F., & Skinner, D. M. (1986) *J. Biol. Chem. 261*, 8994–9001.

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

Gray, D. M., & Bollum, F. J. (1974) *Biopolymers 13*, 2087–2102.

Gray, D. M., Vaughan, M., Ratliff, R. L., & Hayes, F. N. (1980) *Nucleic Acids Res. 8*, 3695–3707.

Gray, D. M., Cui, T., & Ratliff, R. L. (1984) *Nucleic Acids Res. 12*, 7565–7580.

Gray, D. M., Ratliff, R. L., Antao, V. P., & Gray, C. W. (1988) in *DNA and Its Drug Complexes, Proceedings of*

the Fifth Conversation in Biomolecular Stereodynamics (Sarma, R. H., & Sarma, M. H., Eds.) Vol. 2, Adenine, Schenectady, NY.
Greve, J., Maestre, M. F., & Levin, A. (1977) *Biopolymers 16*, 1489-1504.
Inman, R. B. (1964) *J. Mol. Biol. 9*, 624-637.
Marck, C., Thiele, D., Schneider, C., & Guschlbauer, W. (1978) *Nucleic Acids Res. 5*, 1979-1996.
Marky, L. A., Blumenfeld, K. S., Kozlowski, S., & Breslauer, K. J. (1983) *Biopolymers 22*, 1247-1257.
Oka, Y., & Thomas, C. A., Jr. (1987) *Nucleic Acids Res. 15*, 8877-8898.
Sarma, M. H., Gupta, G., & Sarma, R. H. (1986) *FEBS Lett. 205*, 223-229.
Savitzky, A., & Golay, M. J. E. (1964) *Anal. Chem. 36*, 1627-1639.
Scheffler, I. E., Elson, E. L., & Baldwin, R. L. (1968) *J. Mol. Biol. 36*, 291-304.
Selsing, E., & Wells, R. D. (1979) *J. Biol. Chem. 254*, 5410-5416.
Steely, H. T., Jr., Gray, D. M., & Ratliff, R. L. (1986) *Nucleic Acids Res. 14*, 10071-10091.
Tautz, D., & Renz, M. (1984) *J. Mol. Biol. 172*, 229-235.
Wells, R. D., Brennan, R., Chapman, K. A., Goodman, T. C., Hart, P. A., Hillen, W., Kellogg, D. R., Kilpatrick, M. W., Klein, R. D., Klysik, J., Lambert, P. F., Larson, J. E., Miglietta, J. J., Neuendorf, S. K., O'Connor, T. R., Singleton, C. K., Stirdivant, S. M., Veneziale, C. N., Wartell, R. M., & Zacharias, W. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 77-84.
Westhof, E., & Sundaralingham, M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1852-1856.

Base-Catalyzed Reversal of a Psoralen–DNA Cross-Link†

Yun-bo Shi,‡ H. Peter Spielmann, and John E. Hearst*
Department of Chemistry, University of California, Berkeley, California 94720
Received December 1, 1987; Revised Manuscript Received February 26, 1988

ABSTRACT: Base-catalyzed reversal of a psoralen–DNA cross-link has been observed under denaturing alkaline conditions at elevated temperatures. The cross-link was formed between 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen and the two thymidine residues (T) on opposite strands of the double-stranded DNA formed from the self-complementary oligonucleotide 5′-GGGTACCC-3′. In contrast to the photoreversal of the cross-link, which yields mostly the furan-side monoadducted oligonucleotide [Cimino, G. D., Shi, Y., & Hearst, J. E. (1986) *Biochemistry 25*, 3013-3020], base-catalyzed reversal of the cross-link yields only pyrone-side monoadducted oligonucleotides as identified on the basis of their mobilities on a 20% polyacrylamide–7 M urea gel and their chemical and photochemical properties. A mechanism has been proposed to explain the base-catalyzed reversal reaction. This observation suggests a way to make pyrone-side monoadducted DNA. It also suggests that caution must be taken when psoralen-adducted DNA is treated under denaturing alkaline conditions.

The widespread use of psoralen in the medical and biological fields (Song & Tapley, 1979; Parson, 1980; Fitzpatrick et al., 1982; Parrish et al., 1982; Cimino et al., 1985) has promoted detailed investigation of the photochemistry between psoralen and nucleic acids. Psoralens can intercalate between base pairs of double-stranded nucleic acids. Upon near-UV (320–380-nm) irradiation, the intercalated psoralens form a set of well-characterized adducts with pyrimidine bases (Straub et al., 1981; Kanne et al., 1982a,b; Peckler et al., 1982). The first step of the photoreaction yields either a furan-side monoadduct or a pyrone-side monoadduct, depending upon whether the 4′,5′-double bond of the furan ring or the 3,4-double bond of the pyrone ring of the intercalated psoralen photoreacts with the 5,6-double bond of a pyrimidine residue. The furan-side monoadduct can be driven into an interstrand diadduct upon absorption of a second photon if there is an adjacent pyrimidine residue located on the other strand available for photoreaction. Under this irradiation condition, the pyrone-side monoadduct cannot be converted into a diadduct since it does not absorb light in the 320–380-nm wavelength region.

We have previously reported the wavelength dependencies for the photoreactions of the diadduct and the monoadducts formed between the psoralen derivative (HMT)[1] 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen and thymidine (T) and these adducts in deoxyoligonucleotides (Cimino et al., 1986; Shi & Hearst, 1987a,b). We found that photoreversal of the cross-link in a double-stranded oligonucleotide yields mostly the furan-side monoadducted oligonucleotide. The pyrone-side monoadducted oligonucleotide is a minor product of the photoreaction. The pyrone-side monoadducted oligonucleotide exists as either the pyrone ring opened form or the pyrone ring closed form as revealed by analysis of the products on a denaturing polyacrylamide gel. While investigating the pH

† This work has been supported by NIH Grant GM 11180.
* To whom correspondence should be addressed.
‡ Present address: Department of Embryology, Carnegie Institution of Washington, 115 W. University Parkway, Baltimore, MD 21210.

[1] Abbreviations: HMT, 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; XL, HMT–DNA cross-link; T–HMT–T, thymidine–HMT–thymidine diadduct; C-M_{Py}, pyrone ring closed form of the pyrone-side monoadducted DNA; O-M_{Py}, pyrone ring opened form of the pyrone-side monoadducted DNA; DNase, deoxyribonuclease; HPLC, high-performance liquid chromatography.

0006-2960/88/0427-5174$01.50/0