Infrared CD of Deoxy Oligonucleotides. Conformational Studies of 5′d(GCGC)3′, 5′d(CGCG)3′, 5′d(CCGG)3′, and 5′d(GGCC)3′ in Low and High Salt Aqueous Solution

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ABSTRACT Infrared (vibrational) circular dichroism (VCD) spectra have been obtained for the self-complementary tetranucleotides, 5′d(CGCG)3′, 5′d(GCGC)3′, 5′d(CCGG)3′, and 5′d(GGCC)3′. In buffered aqueous solution at low salt concentration, these tetramers exhibit spectra associated with right-handed polymers, although the spectra differ significantly for the four species. In high salt solution, a B → Z transition occurs in 5′d(CGCG)3′, while the other tetranucleotides appear only slightly altered. Temperature dependent studies of these oligonucleotides reveal a greater amount of thermal stability for the tetramers in low salt than for the high salt solutions. VCD intensities computed via the exciton formalism are compared with observed results.

INTRODUCTION

Infrared (vibrational) circular dichroism (VCD) has recently been used as a novel probe to monitor the solution conformation of biological molecules (Keiderling, 1990; Diem, 1993). VCD signals in the 1600–1750 cm^{-1} region of peptides and nucleic acids are due to the very polar and intense carbonyl stretching vibrational modes, which can couple with each other via a dipolar coupling mechanism. The interactions between these vibrations are strongly dependent on geometrical factors, and consequently, give rise to VCD signals which exhibit conformational sensitivity. The VCD spectra can be related computationally, using the exciton formalism (Tinoco, 1963; Gulotta et al., 1989), to structural parameters of the polymers under investigation. In the case of VCD spectra of DNA and RNA, information on the overall alignment of the carbonyl groups can be obtained, and thus, details on the position of the bases.

In this publication, we report experimental VCD spectra of four self-complementary tetramers, 5′d(CGCG)3′, 5′d(GCGC)3′, 5′d(CCGG)3′, and 5′d(GGCC)3′ (henceforth referred to simply as CGCG, GCGC, CCGG, and GGCC, respectively) in low and high ionic strength solutions. In addition, computed VCD results for these tetramers are reported. The tetramers are sufficiently simple to allow for VCD calculations for various trial structures, and search observed and calculated spectra for best agreements.

The motivation for this work is to demonstrate the sensitivity of VCD toward the different structural features of these four tetramers, and toward conformational changes brought on by variation of temperature and the ionic strengths of the solvent. Solid phase structural data, based on x-ray crystallography, and solution phase electronic CD, Raman, and NMR conformational data are available and permit correlations of VCD features and conformation to be carried out.

Most solution phase spectral results published to date for these tetramers were interpreted in terms of an overall B-type structure (Patel, 1976, 1977, 1979; Kastrup et al., 1978; Quadrifoglio et al., 1981; Thomas and Peticolas, 1984). Electronic CD spectra of GCGC and CGCG were found to be similar to those spectra observed for poly(dG-dC)·poly(dG-dC), whereas the CD data for the two nonalternating tetramers, GGCC and CCGG, were found to differ significantly from those of the polymer. The Raman spectra of the two alternating tetradeoxynucleotides indicated that at low NaCl concentrations, both tetranucleotides maintain a B form structure, while at high NaCl concentrations only CGCG assumes a double helical Z form. As the temperature was raised to 50°C, the B form DNA melted noncooperatively (Thomas and Peticolas, 1984). Temperature-dependent studies on CGCG inferred a thermally induced Z → B transition prior to melting (Thomas and Peticolas, 1989).

NMR data were interpreted in terms of right-handed helicity, but the exact form is not quite clear for these small oligonucleotides. A forms were eliminated as likely structures, based on the calculated chemical shifts of the nonexchangeable ring protons. The temperature dependence coefficients of certain chemical shifts and the observed hydrogen-deuterium exchange rates, indicate that significant end fraying occurs in some of these tetramers (Patel, 1976, 1977, 1979).

Our VCD results indicate an overall right-handed helicity of these samples in low salt solution, but the computational result indicate that the structures are less rigidly defined than the larger polymers studied earlier. In fact, VCD data based on A-type structures, which are less tightly wound and more open, generally fit the observed data better. Thus, the VCD results suggest that the tetramers may deviate significantly from a standard canonical B form conformation.

Received for publication 23 March 1993 and in final form 16 June 1993.
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0006-3495/93/09/1262/10 $2.00

MATERIALS AND METHODS

All absorption and VCD spectra were obtained on a dispersive VCD instrument, optimized in the 6-μm region, which was built in our laboratory (Lee and Diem, 1992). All VCD spectra shown are an average of 20–60 scans, depending on polymer concentration, obtained with a time constant of 1 s, and a scan speed of about 1 cm^{-1}/s. Baselines acquired for the buffer were subtracted from the sample spectra. Samples (with concentrations of 13 mg/ml) were contained between CaF_2 windows, at a path length of 50 μm. Some spectra were obtained at lower and higher concentrations as well to check for concentration-dependent effects.

The infrared absorption spectra were collected at sampling conditions identical to those employed for the VCD acquisitions. However, since the VCD spectrometer is a single beam instrument, two measurements needed to be carried out. First, the transmission spectrum of the sample cell, containing the solvent/buffer mixture was collected under conditions identical to those for the sample. Subsequently, the transmission spectrum of the nucleotide sample was measured. These spectra were ratioed and converted to absorbance scales. Infrared spectra as small as 10 milliabsorbance units can easily be measured reproducibly due to the enormous sensitivity of the HgCdTe detector used in the instrument and due to the phase-sensitive detection methods used to reduce effects of instrument drifts. The observed absorbance spectra were identical to those observed via commercial FT infrared spectrometers.

Temperature control was achieved by circulating thermostated water through the IR sample holder. Temperatures were calibrated by inserting a miniature thermocouple between the windows of a sample cell filled with water. We believe the reported temperatures to be accurate within ± 2°C.

The tetranucleotides 5′d(CGCG)3′, 5′d(GCGC)3′, 5′d(CCGG)3′, and 5′d(GGCC)3′ were either purchased from Pharmacia Biotech Inc., Newark, NJ (lot numbers: 00013285, 00132496, 00132895 [5′d(CGCG)3′]; 00132497, 00132853, [5′d(GCGC)3′]; 00137916 [5′d(CCGG)3′] and 00132793, 00139711 [5′d(GGCC)3′]) or were synthesized at the Hunter College Sequencing and Synthesis facility and purified according to literature procedures (Applied BioSystems, Inc., 1987). The results were found to be identical for the commercial samples and those synthesized in-house.

Both the sodium cacodylate and the sodium chloride (Sigma Chemical Co., St. Louis, MO) were lyophilized from D_2O (Sigma) to remove a trace amount of H_2O, which reduces the sample transmission at 6 μm. Tetramers were then redissolved in D_2O, added to buffer/salt solutions such that the final buffer concentration was 10 mM cacodylate with NaCl concentrations either 0.096 or 2.5 M NaCl, and were equilibrated for about 30 min at 2°C. The pD of the solutions was 6.6. Spectra were obtained at 2, 25, and 40°C, and again at 2°C. All spectral data were found to be completely reversible with temperature.

Infrared VCD and absorption intensity calculations were carried out using the "degenerate extended coupled oscillator" (DECO) formalism (Xiang et al., 1993) and programs developed in our laboratory. Input parameters for these calculations are the Cartesian coordinates of the C=O oscillators, an unperturbed C=O stretching frequency, and the dipole transition moment observed for the nucleotide bases. These parameters were obtained as follows.

The C=O coordinates of all four tetramers, in both A and B forms, were created via the molecular graphics program MacroModel (Still, 1989) and transferred to our DECO program. The dipole transition moments for both C and G were obtained, by a suitable conversion, from the ϵ_{max} value (950 liters/mol·cm) observed for a GMP/CMP adduct (Zhong et al., 1990). A value of 1650 cm^{-1} for the unperturbed stretching frequency ν_o was used. Details of these calculations have been reported (Diem, 1993).

RESULTS

Results for the four tetramers will be discussed in the following section one at a time, starting with low ionic strengths and low temperature solutions. A comparison between calculated and observed spectra will be emphasized for all low salt solutions.

Studies at low temperature and low salt concentration

VCD of CGCG

Fig. 1 shows observed IR absorption and VCD spectra of CGCG in low ionic strength solution. Under these conditions, the nucleotide is thought to exist in a right-handed double stranded form, although the exact conformation is not well established (vide infra). The calculated spectra shown in Fig. 1 were obtained using A and B form geometries. For all calculations, we assumed that the infrared absorption and VCD features in the 1600 to 1750 cm^{-1} region are due to C=O stretching interactions only. We have recently reported the effects of including ring stretching vibrations into the calculated results (Xiang et al., 1993); however, these vibrations contribute mostly at the lower end of the spectral range mentioned above (approximately 1615 cm^{-1}). Thus, any VCD features observed below 1625 cm^{-1} will not be reproduced by the calculations.

The infrared absorption spectra of CGCG appears poorly resolved with two broad maxima at 1652 and 1685 cm^{-1} and a low frequency (1626 cm^{-1}) shoulder. The VCD spectrum exhibits a negative-positive couplet (1694/1679 cm^{-1}) which resembles that of B form poly(dG-dC)·poly(dG-dC), which has a negative-positive couplet at 1698/1677 cm^{-1}. In CGCG, this couplet is followed by a VCD minimum at 1658 and a positive peak at 1650 cm^{-1}, which do not appear in the polymer, and thus, indicate a deviation from the canonical B form.

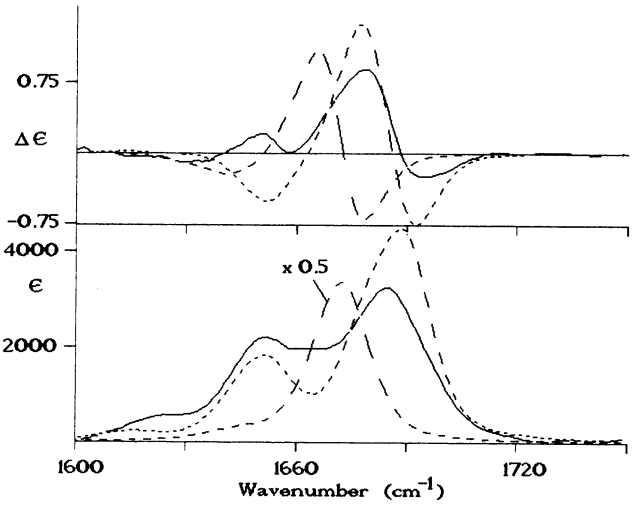

FIGURE 1 Observed (*solid traces*) and calculated (*dashed traces*) VCD (*top*) and infrared absorption (*bottom*) spectra of 5′d(CGCG)3′. Experimental conditions: tetramer concentration approximately 23 mg/ml in 0.096 M NaCl, 0.01 M sodium cacodylate in D_2O at 2°C. Computational results for A form (*short dashes*) and B form (*long dashes*)

The calculated VCD features for both A and B form geometries, are also shown in Fig. 1. For both calculations, identical values for ν_o (1650 cm^{-1}) were used. However, peak positions and relative intensities agree better for the A form structure, for which the frequency splitting in the absorption spectra is predicted to a much better degree (1616, 1652, and 1689 cm^{-1}). The observed VCD spectrum is more positively biased than either of the calculated traces. Thus, the minimum in the observed VCD spectrum at 1658 cm^{-1} is actually calculated as a negative peak at 1654 cm^{-1} in the A form geometry. Although the calculations based on the A form fit the observed data better, we do not necessarily imply that the tetramer exists in this conformation (vide infra).

VCD of GCGC

Observed and calculated VCD spectra for this tetramer are shown in Fig. 2. The observed IR absorption spectrum contains two peaks at 1683 and 1651 cm^{-1}, with a shoulder at about 1620 cm^{-1}. The corresponding VCD spectrum exhibits a negative (1690)-positive (1676 and 1662)-negative (1647 cm^{-1}) couplet. The VCD zero crossings occur very near the infrared absorption maxima. In overall appearance, this spectrum is very similar to the one observed for B form poly(dA)·poly(dT) (Zhong et al., 1990).

VCD and absorption spectra calculated for the A form structure are shown in Fig. 2. Although this spectrum reproduces the observed traces reasonably well, we also show the VCD band shape obtained for a 50:50 mixture of A and B forms, which reproduces the observed spectrum even better. The question of whether there is an equilibrium between the two canonical forms, or whether the true form is static but in-between the two extreme forms, will be discussed later (vide infra).

VCD of CCGG

CCGG shows a broad, relatively weak negative (1690 cm^{-1}) positive (1667 and 1654 cm^{-1}) couplet in the VCD spectrum and two broad infrared absorptions with maxima at 1680 and 1649 cm^{-1} (cf. Fig. 3). Among the four tetramers studied, CCGG exhibits the simplest VCD spectrum, consisting basically of a simple, slightly positively biased couplet, with the possibility of two overlapping positive peaks. Although all other techniques agreed that CCGG is least B-like (vide infra), we find that the spectrum resembles the VCD signals observed for B family poly(dG-dC)·poly(dG-dC), shifted to lower wavenumber by about 10 cm^{-1}. Our calculated results, however, do not support a B form either, since the VCD spectra of the B form are much larger than the observed signals, although the sign pattern and the splitting are reproduced well. The A form spectra, on the other hand, produce VCD signals of the proper magnitude, and a better intensity distribution between the two absorptions calculated at 1702 and 1648 cm^{-1}.

VCD of GGCC

The most complex VCD patterns are observed for GGCC, shown in Fig. 4. The infrared absorption spectrum shows two broad peaks at 1677 and 1647 cm^{-1}, and a shoulder at 1620 cm^{-1}. The frequencies are somewhat lower than those observed for the other tetramers. The VCD spectrum exhibits a negative-positive couplet (1688/1672 cm^{-1}), which is followed by a minimum at 1665 cm^{-1} and a strong positive peak at 1650 cm^{-1}. The couplets are nearly conservative. The calculations using the B form geometry (not shown) predict a positive (1681 cm^{-1})-negative (1668 cm^{-1})-positive (1635 cm^{-1}) pattern, which is in total disagreement with the observed data. The A form-derived spectra fit the observed spectrum somewhat better (cf. Fig. 4), although the couplet

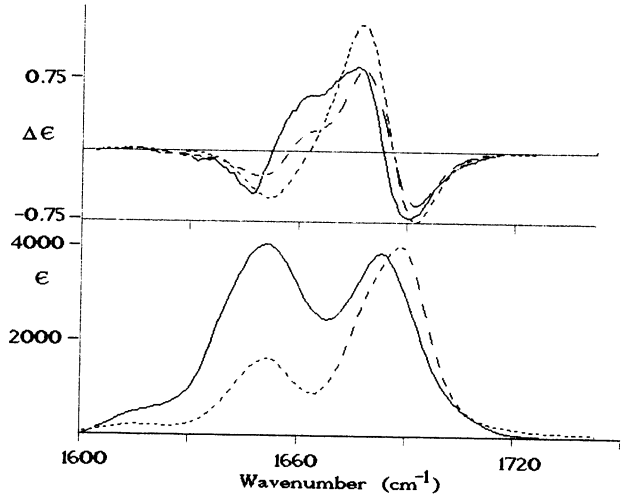

FIGURE 2 Observed (*solid traces*) and calculated (*dashed traces*) VCD (*top*) and infrared absorption (*bottom*) spectra of 5′d(GCGC)3′. Experimental conditions: tetramer concentration approximately 13 mg/ml in 0.096 M NaCl, 0.01 M sodium cacodylate in D_2O at 2°C. Computational results for A form geometry (*short dashes*) and 50:50 mixture of A and B form (*long dashes*)

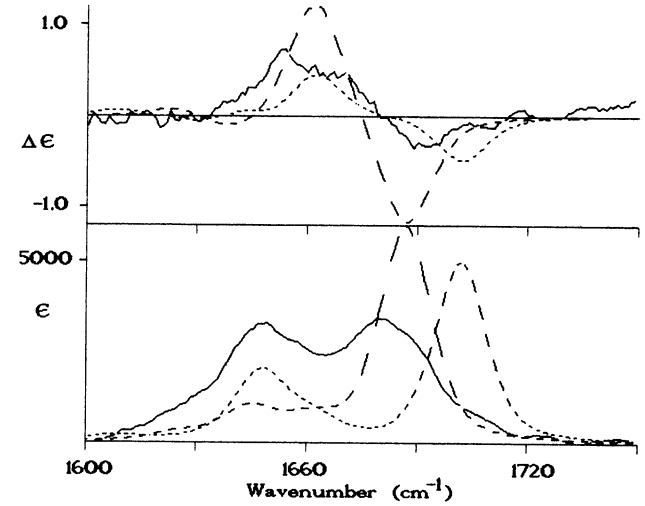

FIGURE 3 Observed (*solid traces*) and calculated (*dashed traces*) VCD (*top*) and infrared absorption (*bottom*) spectra of 5′d(CCGG)3′. Experimental conditions: tetramer concentration approximately 13 mg/ml in 0.096 M NaCl, 0.01 M sodium cacodylate in D_2O at 2°C. Computational results for A form geometry (*short dashed*) and B form (*long dashes*).

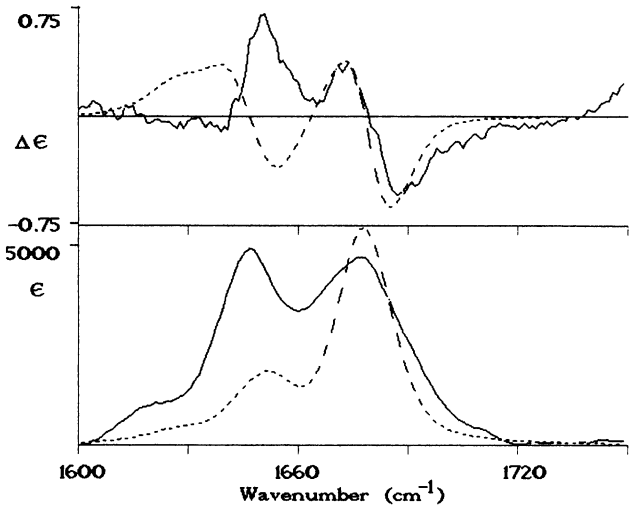

FIGURE 4 Observed (*solid traces*) and calculated (*dashed trace*) VCD (*top*) and infrared absorption (*bottom*) spectra of 5′d(GGCC)3′. Experimental conditions: tetramer concentration approximately 13 mg/ml in 0.096 M NaCl, 0.01 M sodium cacodylate in D_2O at 2°C. Computational results for A form geometry.

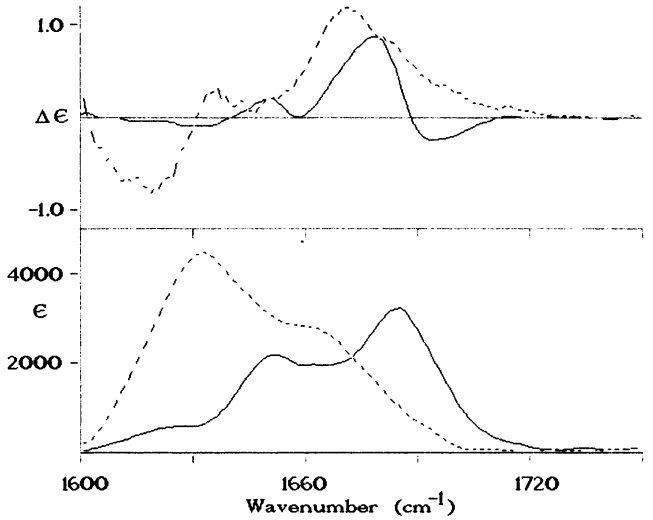

FIGURE 5 Observed infrared VCD (*top*) and absorption (*bottom*) spectra of 5′d(CGCG)3′ in low ionic strength (c = 0.096 M NaCl, solid line) and in high ionic strength solution (c = 2.5 M NaCl, *dashed trace*).

at low frequency is predicted with the improper sign pattern. Of all tetramers studied, GGCC has the least overall agreement between observed and any of the calculated spectra.

According to previous studies, GGCC was found to be in a conformation similar to the B form, whereas CCGG was found to least B-like. Our studies disagree on this point, since CCGG showed VCD of a normal right-handed form, whereas GGCC shows very unusual spectral features. These differences between VCD and other techniques will be elaborated upon later in the discussion.

Studies at low temperature and high salt concentration

Under high salt condition and at 2°C, CGCG exhibits spectral features (Fig. 5) resembling the Z form of poly(dG-dC)·poly(dG-dC) (Gulotta et al., 1989). The high frequency negative VCD contribution of the low salt, right-handed form has completely vanished, and is replaced by a broad, positive peak with a shoulder at 1683 and a maximum at 1672 cm^{-1}. Toward lower frequencies, there is a minimum at 1646 cm^{-1}, a small positive peak at 1637 cm^{-1}, and a broad negative peak at 1619 cm^{-1}. In the polymer, the minimum at 1646 cm^{-1} is observed as a negative peak. The IR absorptions are shifted toward lower wavenumber by approximately 20 cm^{-1} compared to the B form, and occur at 1639 and 1633 cm^{-1}.

For GCGC, the spectral band shapes in high concentration (2.5 M NaCl) salt solution are preserved, as compared to the low salt solution, indicating that GCGC does not undergo a B → Z transition. However, depending on the tetramer concentration, we do observe slight changes in the VCD spectra between low and high ionic strength solutions. These changes are more pronounced in at low (~7 mg/ml) concentrations of the tetramer, indicating that at high concentrations, end-to-end interactions occur between the tetramer units, which make them less susceptible to the effects of high ionic strength.

In the case of GGCC and CCGG, little spectral change is observed at high ionic strengths. The CCGG VCD spectrum is slightly more positively biased and exhibits less high frequency negative intensity. For GGCC, the signal amplitude decreases in high ionic strength solution.

Temperature effects

All tetramers exhibit similar thermal behavior: as the temperature is raised to 25°C, the amplitude of the VCD signals diminishes, and at 40°C, only very weak VCD features remain (Fig. 6). This behavior is more prominent at low con-

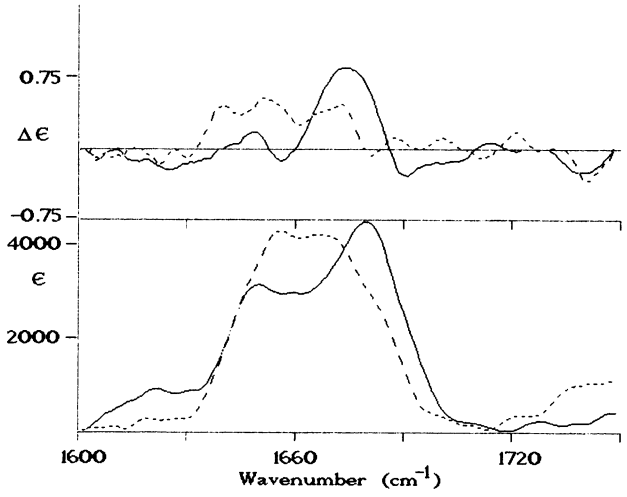

FIGURE 6 Observed infrared VCD (*top*) and absorption (*bottom*) spectra of 5′d(CGCG)3′ in low ionic strength solution at 2°C (*solid line*) and 40°C (*dashed trace*). Tetramer concentration 7 mg/ml.

centrations of the tetramers, since end-to-end stacking occurs at higher concentrations. Furthermore, the two IR absorption peaks coalesce into one broad peak with increasing temperature.

In high salt solutions, the decrease in VCD intensities occurs at lower temperatures, and most VCD features have disappeared at 25°C. This is in line with experimental results from other techniques which state that the thermal stability is lower for the high salt conformations, since high concentrations of ions tend to induce less stable conformations, such as a more open A form in GCGC, or the Z form in CGCG.

We observed, for all cases, an increase in the infrared absorption intensity, which resembles the hypochromic effect observed in the UV absorption spectra, and a shift toward lower wavenumber with increasing temperature. All temperature effects are completely reversible, and, upon cooling samples to the initial low temperatures, the peaks observed originally are restored.

DISCUSSION

VCD is a very young spectroscopic technique, which was first applied to nucleic acids in 1987 (Annamalai and Keiderling, 1987). However, VCD instrumentation has advanced to such a level of sophistication that spectra of nucleotides can be collected on less than 1 mg of sample in a few hours to yield a signal-to-noise ratio of better than 100:1 (cf. Fig. 1). The quality and reproducibility of these data is such that a quantitative comparison of the VCD structural results with those obtained via other spectroscopic techniques is now possible.

Due to the fact that the VCD features reported here originate from the dipolar coupling of C=O stretching vibration transition moments, there exists an empirical model to compute VCD intensities from structural data. We have used degenerate and nondegenerate coupled oscillator calculations in an attempt to establish a quantitative interpretation of VCD results (Gulotta et al., 1989; Zhong et al., 1990; Xiang et al., 1993), and to correlate observed VCD signals to known structural features in a number of model DNA systems. In view of the approximate nature of the computational model, the agreement between observed and calculated spectral features has often been astounding, and has increased our willingness to accept VCD as a new conformational probe.

The four self-complementary tetradeoxynucleotides reported here have been studied before using crystallographic, CD, vibrational, and NMR techniques. However, there seem to exist somewhat diverging interpretations of these data, and the next part of the discussion seeks to consolidate the previously obtained spectral information into one picture.

Conformational results from other techniques

The original studies on 5′d(CGCG)3′ revealed that the tetramer crystallizes as a left-handed double helix depending on the salt concentration (Drew et al., 1978; Crawford et al., 1980). The base stacking is similar to that of the crystalline hexamer, although the cytosine has a slightly different orientation (Wang et al., 1979; Crawford et al., 1980). More than one structure exists for the crystallized tetramer and hexamer, which indicates that a family of left-handed DNA molecules prevails (Crawford et al., 1980).

CD in the 220–280-nm range indicates that, at low ionic strengths, CGCG and GCGC exist in right-handed structures (Kastrup et al., 1978) very similar to that of poly(dG-dC)·poly(dG-dC) (Pohl and Jovin, 1972), whereas CCGG and GGCC exhibit totally different CD features. The latter of these two, GGCC, showed CD which was consistent with model calculations, using the nearest neighbor approach (NNA, Gray and Tinoco (1970)), and it was concluded that this structure was not unusual, although the observed CD did not appear to be a standard B form. The CD reported by Kastrup et al. (1978) for CCGG at low temperature and relatively high concentrations (50 μM), however, exhibited all features which are now associated with Z form DNA (vide infra).

Quadrifoglio et al. (1981) reported the CD spectra of CGCGCG at low and high salt concentration. The low ionic strength data agree well with those reported for CGCG, but at the low concentration used it was not possible to convert CGCG to the Z form, even at very high salt concentrations. However, CGCGCG was found to adopt a Z form readily, and the Z form features reported are similar to the anomalous CD observed by Kastrup et al. (1978) for CCGG.

Raman data on all four tetramers were reported by Thomas and Peticolas in 1984, and rectified some of the misconceptions which arose from the earlier CD studies. In this Raman study, the structures of the tetramers were elucidated by a comparison of their Raman spectra to reference spectra of poly(dG-dC)·poly(dG-dC) in the standard B and Z forms. Marker bands, which were used for the identification of the B conformation, include the band at 830 cm^{-1}, which is attributed to the C2′-endo furanose ring conformation. Furthermore, a band which occurs at 682 cm^{-1} in the B-from due to the guanine markedly decreases and a new band appears at 627 cm^{-1} in the Z form. Other bands in the base vibrational region between 1200 to 1600 cm^{-1} also exhibit conformational sensitivity. The C=O stretching region, in which all the infrared absorption and VCD spectra were observed, is not discussed at all in the Raman studies, presumably because of the relative weakness of these bands in Raman scattering and solvent interference.

The Raman results indicate that CGCG, GCGC, and GGCC are in a right-handed conformation similar to that of poly(dG-dC)·poly(dG-dC), and that only CCGG is markedly different from a standard B form. CCGG, however, did not show Raman marker bands typical of the A form (805 and 814 cm^{-1}).

The Raman studies indicated that CGCG does change conformation under high salt conditions. The inability to observe the salt dependence in the earlier CD study was attributed to absence of duplex formation since a low concentration of the tetramer was used in the CD study (Thomas and Peticolas, 1984).

NMR data in general agreed with a right-handed conformation of the tetramers in low ionic strength solution, but did not clearly favor a B form geometry. In addition, end fraying was postulated from hydrogen-deuterium exchange rate studies (Patel, 1976, 1977, 1979).

All of the previously discussed studies agree that the four tetramers exist in a right-handed conformation in low ionic strength solutions, although the exact nature of the conformation is not clear. The Raman data (except for CCGG) are consistent with a canonical B form structure. CD data suggest that the alternating tetramers are in a B-type conformation and that the GGCC exists in a similar form, although its CD spectrum is unusual.

VCD and its origin in the self-complementary tetranucleotides

The VCD data on all four tetramers are sufficiently different from those of $d(CG)_5$ or poly(dG-dC)·poly(dG-dC) to suggest a significant perturbation of the canonical B form conformation. In addition, the VCD spectra of the four tetramers differ markedly among themselves, whereas the CD spectra were similar within the alternating and the nonalternating series.

Thus, one needs to discuss both the differences observed among the four tetramers, and interpret the overall VCD features of the tetramers in terms of those of known structures. The VCD spectra of B form polymers, such as $d(CG)_5$ or poly(dG-dC)· poly(dG-dC), is characterized by a negative peak at 1699 cm^{-1} and a positive peak at 1682 cm^{-1}, with the zero crossing (1692 cm^{-1}) nearly exactly under the main infrared absorption feature (1690 cm^{-1}). Other, weaker absorption peaks are observed at 1655 and 1625 cm^{-1}. A deflection or a slightly negative peak is observed at 1660 cm^{-1} in the VCD spectrum. These main features are relatively constant between poly(dG-dC)·poly(dG-dC) and $d(CG)_5$ and have been reproduced computationally for a number of right-handed structures (Gulotta et al., 1989; Zhong et al., 1990). Thus, these features are considered prototypical for the B form. However, VCD spectra for A form polynucleotides have recently been reported (Wang and Keiderling, 1992; Xiang et al., 1993). These spectra are similar, yet distinguishable from those of B form polymers, and some of the spectral features of the tetramers are best described as mixtures of A and B forms (vide infra).

In all tetramers, the highest frequency VCD signal is negative, at slightly lower wavenumber than in the polymer. This negative peak is followed by a positive feature, which occurs at about 1678 cm^{-1} in the alternating and about 1655 cm^{-1} in the nonalternating tetramers. In addition, three of the four tetramers have one lower intensity couplet which varies strongly in frequency, and therefore, is superimposed onto different parts of the VCD peaks among the four tetramers.

The absorption spectrum of CGCG resembles that of the polymeric form, and one may conclude that CGCG exists in a right-handed conformation similar to the B form. However, structural perturbations do occur, most likely at the terminal base pairs, since the VCD calculated for a B form conformation does not fit the observed data as well as those calculated for the much more open A form structure.

The VCD of GCGC is still similar to that of CGCG, but has a distinct low frequency negative peak, which is reproduced in the calculations using the A form geometry. Also, the lower frequency infrared absorption at 1651 cm^{-1} is the dominant peak. Its VCD and absorption spectra very much resemble that of B form poly(dA)·poly(dT). A mixture of A and B form geometries reproduces the observed VCD band shapes best, indicating that the true structure might correspond to a right-handed conformation which has unwound significantly from the tight B form conformation.

CCGG appears to be right-handed as well with a nearly conservative couplet. Yet, the splitting between the positive and negative members of the couplet is largest in CCGG, and this splitting is best reproduced computationally by an A form structure.

The VCD of GGCC do not resemble any other features observed for G and C containing deoxynucleotides, although the sign pattern at higher wavenumber, a negative-positive couplet, mimics that of a right-handed helix.

The differences between the VCD spectra of the four tetramers can be explained relatively easily. The most dominant interactions giving rise to VCD intensities are those between adjacent base pairs in a double strand. Within a given base pair, the coupling of the carbonyl groups produces minimal VCD intensities because of the near exact antiparallel alignment of the C=O groups (cf. Fig. 7).

In CGCG, the orientation of the four carbonyl groups in two subsequent base pairs is either such that all four carbonyl groups are nearly parallel as shown in Fig. 7 *a*, or are twisted by nearly 90° with respect to the previous layer, as shown in Fig. 7 *b*. These two cases will be referred to as the "parallel interaction" and the "90° interaction," respectively. Due to the handedness of the twist in the second case, a large optical activity is induced by the dipolar coupling of the transitions. Thus, the VCD in CGCG is determined by two "parallel interactions" and one "90° interaction," whereas it is determined by two "90° interactions" and one "parallel interaction" in GCGC. Thus, it is not surprising that the observed and computed VCD of 5′d(CGCG)3′ and 5d(GCGC)3′ are different. Similar arguments can be made for the nonalternating tetramers.

Fig. 8 shows the carbonyl group coordinates for A form conformations. It can be seen from these graphs that the C=O groups are displaced further from the helix axes, and the structure is much more open, and less tightly wound. At this point, we wish to emphasize that we do not imply that the tetramers do exist in an A form structure, but that the VCD is better reproduced for less tightly wound geometry encountered in the canonical B form. These changes in structure could be static or dynamic: VCD cannot differentiate whether or not there is a fast dynamic equilibration between A, B, or any other geometries or whether the true geometry is static and corresponds to structural parameters in between those of the extreme A and B form geometries.

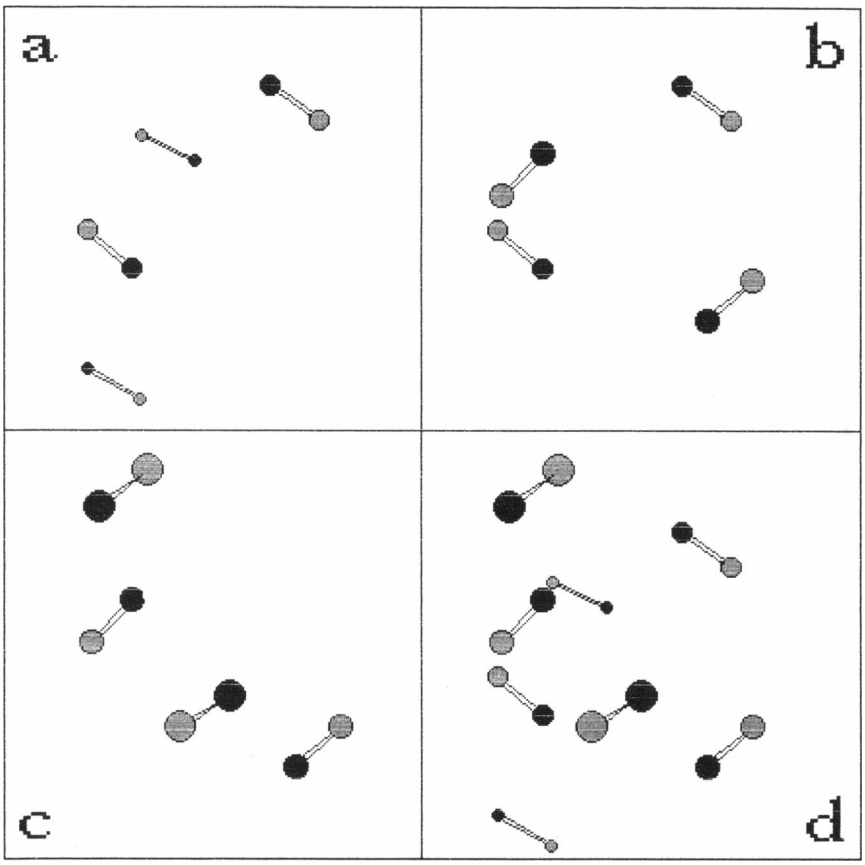

FIGURE 7 Positions of the carbonyl groups participating in the dipolar coupling in 5′d(CGCG)3′, viewed along the *Z* axis. Coordinates were calculated from canonical B form poly-(dG-dC)·poly(dG-dC). The black circles represent carbon atoms, and the shaded ones represent oxygen atoms. Larger atoms are closer to the viewer (with more positive *Z* coordinates). (*a*) 0.3 and 3.7 Å plane; (*b*) 3.7 and 7.0 Å plane; (*c*) 7.0 and 10.4 Å plane; (*d*) all planes.

Comparison of VCD conformational results with those obtained from other spectroscopic techniques

The differences in the structural conclusions obtained for the four tetramers via the various spectroscopic techniques are much harder to explain and need to be discussed in detail.

There has been a growing amount of spectroscopic evidence (Lee et al., 1989; Gounarides, 1993) which suggest that VCD detects different solution conformation than does NMR spectroscopy. This evidence has been collected so far for small peptides, for which NMR often is not able to detect *any* solution structure. The differences in these results are due to the different time scales of the experiments: for flexible molecules, such as a small linear peptide, conformational transitions occur faster than the NMR time scale, but slower than VCD time scale, which is less than a picosecond. Thus, different averaging rules will apply to both techniques.

In the nucleotides discussed here, the difference in time scales between NMR and VCD are less important, since the tetramers are thought to exist as relatively stable, hydrogen-bonded double helices. Dynamic and static end fraying occurs most likely in all the tetramers to a varying degree, but these effects yielded similar results in both NMR and VCD, namely that significant deviation from canonical B form structure exists.

VCD data on 5′d(CGCG)3′ (Fig. 1) are more similar to the VCD results on poly(dG-dC)·poly(dG-dC). NMR data suggest that end fraying is less prevalent in CGCG (Patel, 1976, 1979) than in GCGC, and that it retains more of a base-paired and base-stacked structure. Fraying will mainly alter the conformation of the external base pairs and reduces their dipolar coupling, and will leave the central (GC) duplex in the case of the 5′d(CGCG)3′ and a central (CG) duplex in 5′d-(GCGC)3′. In the latter case, with strong end-fraying effects, the spectrum should be primarily determined by the central (CG) pair, for which VCD data have been calculated (Zhong et al., 1990). The sign pattern of the calculated data agrees with the observed spectrum of 5′d(GCGC)3′. In 5′d-(CGCG)3′, on the other hand, significant coupling between the center and the end pairs occurs, and the spectra appear more like those of the polymer.

The discrepancy between Raman and VCD derived conformations must be sought in terms of the different regions of the molecules sampled by the two techniques, rather than their time scales. Most Raman interpretations are based on the vibrations of the ribose and phosphate moieties of the oligomer or polymer. When a polymer undergoes a B → Z transition, every other (deoxy-) ribose group will change from the C2′-endo furanose to the exo conformation. However, the presence of the marker band due to the C2′-endo furanose in the tetramers does not imply necessarily that the *overall* conformation is unchanged. It merely implies that the furanose conformation is unchanged and that a slight unwinding or opening of the duplex could have happened undetected.

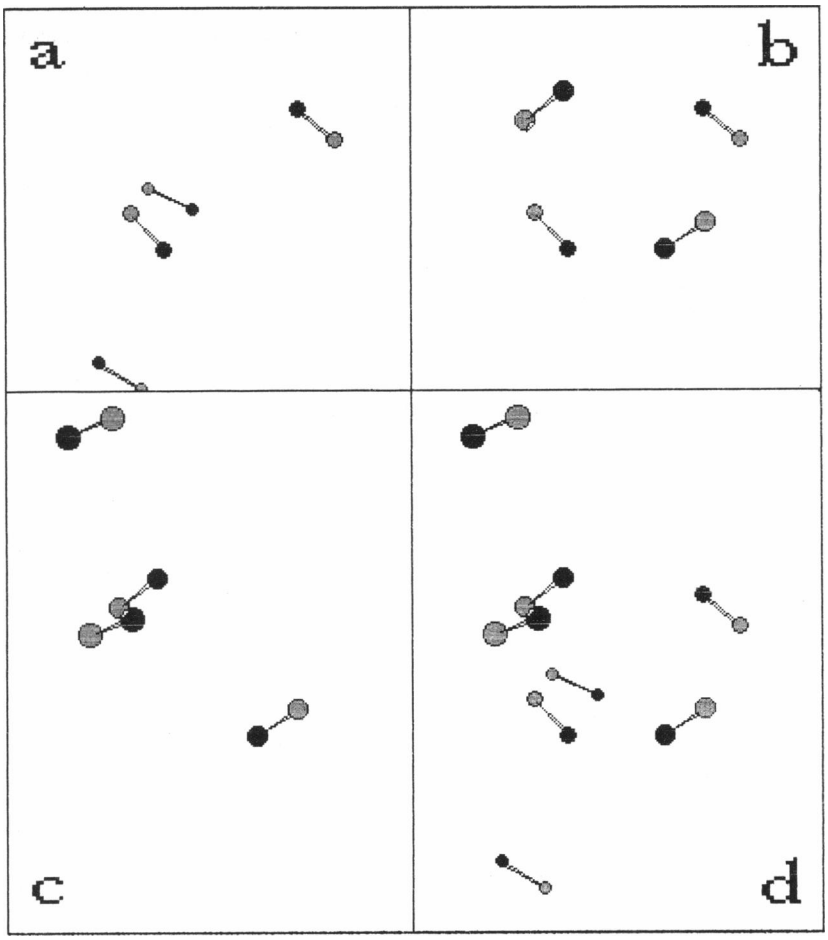

FIGURE 8 Positions of the carbonyl groups participating in the dipolar coupling in 5′d(CGCG)3′, viewed along the *Z* axis. Coordinates were calculated from canonical A form poly(dG-dC)·poly(dG-dC). The black circles represent carbon atoms, and the shaded ones represent oxygen atoms. Larger atoms are closer to the viewer (with more positive *Z* coordinates). (*a*) 0.7 and 2.7 Å plane; (*b*) 2.7 and 5.5 Å plane; (*c*) 5.5 and 9.0 Å plane; (*d*) all planes.

Chiroptical techniques, such as CD and VCD, are less sensitive than Raman spectroscopy to the conformation of the ribose and phosphate groups, but much more sensitive to overall alignment of the transitions which give rise to CD or VCD. Thus, the results from Raman and VCD data are not necessarily in contradiction, but rather supplementing each other. The Raman data suggest that the tetramers, with the exception of CCGG, have many local features consistent with B form conformation. The VCD data reported here are due to vibrations in the planar ring systems which couple over a number of neighboring rings. Thus, VCD is more sensitive to the relative orientation of the rings, and VCD results suggest that some unwinding, opening, and end-fraying has distorted the canonical structures.

In terms of this discussion, the disagreement between CD and VCD results for the nonalternating tetramers needs to be investigated further. As pointed out before by Thomas and Peticolas (1984), the CD data may be hampered by the fact that significant amounts of single stranded tetramers may exist at the low concentrations used for electronic CD. Nevertheless, for the alternating tetramers, the overall agreement between CD, VCD, and Raman spectral results is good. However, contradictions occur in CCGG and GGCC between CD and VCD results: the CD technique claims CCGG to be an exception, and GGCC to have an unusual spectrum which nevertheless can be interpreted in terms of a standard B form using the nearest neighbor approach (NNA).

Conceivably, the NNA is misleading in this case. In the VCD results presented here, the transition moments of the C═O stretching vibrations couple over more than just the nearest neighbors, although the transition moments are much smaller than those encountered in the electronic transitions of the bases. Thus, it appears that the NNA could give erroneous results in cases where long range effects are important.

The VCD results indicate that at low salt concentration and low temperature, the four tetramers exist in a right-handed conformation which is more unwound than the canonical B form, and may approach distances and dihedral angles between the C═O transition similar to those found in the A form. However, Raman data suggest that the backbone conformations still resembles most closely that of the B form. In high ionic strength solution, only CGCG assumes a left-handed conformation according to the VCD results, which is in agreement with previous studies. The left-handedness of CGCG is indicated in the VCD by a reversal of the couplet, in analogy to the results obtained for poly(dG-dC)· poly(dG-dC) and its methylated analogue (Gulotta, 1993). At increasing temperature, all tetramers melt reversibly, which is indicated in the VCD spectra by a

loss of detail until only a broad feature is left, and an increase in the integrated infrared absorption intensity. The denaturation point is reached at 40°C for low and about 25°C for high ionic strength solution.

We have carried out structure optimization on all tetramers, using the AMBER program energy minimization and considering solvent and counter ion effects. The results of these efforts will be reported at a later date, but preliminary indications of these calculations indicate that a opened conformation, similar to the A form, is most likely.

The general trend in thermal denaturation of the tetramers

Generally, there is a shift to lower wavenumbers in both IR and VCD spectra upon heating from 2 to 40°C (cf. Fig. 5). This decrease in the vibrational frequency in the carbonyl stretching region with increasing temperature has been observed previously (Bandekar and Zundel, 1984), and has been attributed to differences in solvation (Howard et al., 1969; Kölkenbeck and Zundel, 1975; Herbeck and Zundel, 1976) and also to differences in the dipole-dipole coupling of the C=O transition moments. In most cases, we have also observed an increase in the IR absorption intensity, as the temperature was raised to 40°C. This increase resembles the hypochromic effect observed in electronic transitions, and the origin of this effect in vibrational transitions is not understood at this time.

In low salt solution, the VCD spectra of the tetramers retain most of the initial spectral features as the temperature is raised to 25°C. At 40°C, the VCD spectra experience a further loss of intensity as well as a shift to lower frequencies. Raman data suggest that melting to single stranded conformation is complete at 50°C; thus, we believe that the remaining VCD signals are due to some residual order of melted single strands.

In high ionic strength solution, Raman spectroscopic studies suggest that a Z → B transition can be induced thermally for 5′d(CGCG)3′. Our VCD and IR spectra do not agree with these Raman findings, and a temperature-induced Z → B transition is not observed for CGCG by the use of VCD. Instead, as the temperature is increased from 2 to 25°C, there is a weakening of the VCD signal, and at 40°C, only broad, positive VCD feature remains in all tetramers. Thus, the high salt forms appear to be less thermally stable than their low salt counterparts. This can be due to a disruption in both the base-pairing and base-stacking interactions, both of which would lead to a loss of VCD signals.

CONCLUSIONS

VCD data on four guanosine and cytosine containing tetramers have been collected and interpreted in terms of right-handed structures which deviate from the canonical B form. The VCD-derived results are not necessarily in complete agreement with those obtained from other techniques. However, the differences can be reconciled considering the specific sensitivities for structural features of the spectroscopic techniques used.

Support of this research by the National Institute of General Medical Sciences (GM 28619) is gratefully acknowledged. The construction of the VCD instruments was supported by grants from the National Science Foundation (CHE 86-07934), several City University of New York faculty research awards, and from Instruments, SA, Metuchen, NJ. We also wish to acknowledge a stipend (to M. Gulotta) from a Biophysics Training grant (5T32 GM 08399).

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