Nucleic Acid Vibrational Circular Dichroism, Absorption, and Linear Dichroism Spectra. II. A DeVoe Theory Approach

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ABSTRACT The DeVoe polarizability theory is used to calculate vibrational circular dichroism (VCD) and infrared (IR) absorption spectra of four polyribonucleotides: poly(rA) · poly(rU), poly(rU) · poly(rA) · poly(rU), poly(rG) · poly(rC), and poly(rC$^+$) · poly(rI) · poly(rC). This is the first report on the use of the DeVoe theory to calculate VCD, oriented VCD, IR absorption, and IR linear dichroism (LD) spectra of double- and triple-stranded polyribonucleotides. Results are reported for DeVoe theory calculations—within the base-stretching 1750–1550 cm^{-1} spectral region—on several proposed multistranded polyribonucleotide geometries. The calculated spectra obtained from these proposed geometries are compared with previously reported measured and calculated VCD and IR spectral results. Base-base hydrogen-bonding effects on the frequencies and magnitudes of the base carbonyl stretching modes are explicitly considered. The good agreements found between calculated and measured spectra are proposed to be further evidence of the usefulness of the DeVoe theory in drawing three-dimensional structural conclusions from measured polyribonucleotide VCD and IR spectra.

INTRODUCTION

Over the past decade or so, vibrational circular dichroism (VCD) spectroscopy has become a powerful technique for differentiating between solution biopolymer geometries. During this period, the VCD and infrared (IR) absorption spectra for a number of synthetic and natural polynucleotides have been reported (cf. Annamalai and Keiderling, 1987; Zhong et al., 1990; Wang and Keiderling, 1992; Xiang et al., 1993; Birke et al., 1993; Yang and Keiderling, 1993; Wang et al., 1994). With continued improvements in experimental measurement techniques (cf. Keiderling, 1996), the number of available measured polynucleotide VCD and IR spectra should increase in the future. In this regard, it is imperative that improved theoretical methods be developed to structurally interpret these measured spectra. Toward this end, several previous attempts have been made at calculating, from proposed geometries, the VCD and IR spectra of a number of polynucleotides (e.g., Annamalai and Keiderling, 1987; Zhong et al., 1990; Wang and Keiderling, 1992; Xiang et al., 1993; Birke et al., 1993; Self and Moore, 1997).

Recently, the DeVoe polarizability theory (DeVoe, 1964, 1965, 1969, 1971)—previously shown to be one of the most useful theoretical methods for calculating ultraviolet CD and absorption spectra of polynucleotides—has been employed to calculate VCD, oriented VCD, IR absorption, and IR linear dichroism (LD) spectra of four, single-stranded, homopolyribonucleotides (Self and Moore, 1997). From the previous agreements found between calculated and measured spectra (for single-stranded homopolyribonucleotides), it was concluded (Self and Moore, 1997) that the DeVoe polarizability theory is a useful means of calculating the VCD and IR spectra of polynucleotides.

This paper reports the first application of the DeVoe theory to calculate VCD, oriented VCD, IR absorption, and IR LD spectra of double- and triple-stranded polyribonucleotides. Calculations are reported for the nucleic acid base-stretching region of the IR spectrum (1750–1550 cm^{-1}) for proposed geometries of poly(rA) · poly(rU), poly(rU) · poly(rA) · poly(rU), poly(rG) · poly(rC), and poly(rC$^+$) · poly(rI) · poly(rC).

THEORY

Isotropic VCD and IR

A thorough discussion of the use of the DeVoe polarizability theory in calculations of polynucleotide VCD, oriented VCD, IR absorption, and IR LD spectra has been presented previously (Self and Moore, 1997). The equations and calculational methods used in obtaining the isotropic VCD and IR results reported in this work are the same as described previously (Self and Moore, 1997).

Briefly, the equations for frequency (ν)-dependent isotropic IR extinction (ϵ_ν) and VCD [$\Delta\epsilon_\nu = (\epsilon_l - \epsilon_r)$] may be written

$$\epsilon_\nu = -(8\pi^2\nu N_o/6909)\Sigma_{ij}\,\mathrm{Im}(A_{ij})_\nu \mathbf{e_i}\cdot\mathbf{e_j} \qquad (1)$$

$$\Delta\epsilon_\nu = (24\pi^2\nu^2 N_o/3300)\Sigma_{ij}[(\mathbf{e_i}\times\mathbf{e_j})\cdot\mathbf{R_{ij}}]\mathrm{Im}(A_{ij})_\nu \qquad (2)$$

where

$$(A_{ij})_\nu = [\delta_{ij}/(\alpha_i)_\nu + G_{ij}]^{-1};$$

$$G_{ij} = [(\mathbf{e_i}\cdot\mathbf{e_j}/|\mathbf{R_{ij}}|^3 - 3(\mathbf{e_i}\cdot\mathbf{R_{ij}})\cdot(\mathbf{R_{ij}}\cdot\mathbf{e_j})/|\mathbf{R_{ij}}|^5)]/e_{diel} \quad \text{and} \quad \mathbf{R_{ij}} = \mathbf{R_j} - \mathbf{R_i}.$$

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The sums over **ij** (Σ_{ij}) are over all oscillators in the species of interest; $\mathbf{e}_i$ and $\mathbf{e}_j$ are unit vectors in the directions of oscillators **i** and **j**; $(\alpha_i)_\nu$ (in units of cm^{-3}) are frequency-dependent complex polarizabilities of oscillator **i** (Self and Moore, 1997); G_{ij} (in units of cm^{-3}) is a normalized dipole interaction energy, between oscillators **i** and **j**, obtained by dividing the quantum mechanical perturbation energy V_{ij} by the product of the absolute magnitudes of the electric dipole transition moments; $\mathbf{R}_{ij}$ (in units of cm^{-1}) is the vector distance between oscillators **i** and **j**; N_o is Avogadro's number; δ_{ij} is the Kronecker delta; and e_{diel} is an effective isotropic dielectric constant which, for biopolymers, commonly falls within the range of 1–4. In this work, as was done previously (Self and Moore, 1997), e_{diel} is set to a value of 2.7.

VCD and LD for oriented polynucleotides

With the helix axis oriented parallel to the z axis of a Cartesian coordinate frame, Eqs. 1 and 2 can be modified to obtain equations for the VCD and LD of oriented polynucleotides (Self and Moore, 1997). For radiation linearly polarized parallel to the helix axis, the extinction $(\epsilon_{para})_\nu$ as a function of frequency may be calculated by

$$(\epsilon_{para})_\nu = -(8\pi^2\nu N_o/6909)\Sigma_{ij}\,\mathrm{Im}(A_{ij})_\nu e_i^z e_j^z \qquad (3)$$

where the superscript z specifies the z component of the oscillator unit vectors. For radiation linearly polarized perpendicular to the helix axis, the extinction (ϵ_{perp}) may be calculated by

$$(\epsilon_{perp})_\nu = -(8\pi^2\nu N_o/6909)\Sigma_{ij}\,\mathrm{Im}(A_{ij})_\nu(e_i^x e_j^x + e_i^y e_j^y) \qquad (4)$$

where the superscripts x and y specify the respective components of the oscillator unit vectors. For z-incident circularly polarized radiation (propagation direction parallel to the helix axis), the oriented VCD $(\Delta\epsilon_z)_\nu$ as a function of frequency may be calculated by

$$(\Delta\epsilon_z)_\nu = (24\pi^2\nu^2 N_o/3300)\Sigma_{ij}[(e_i^x e_j^y - e_j^y e_j^x)R_{ij}^z]\,\mathrm{Im}(A_{ij})_\nu \qquad (5)$$

where the superscripts x, y, and z specify the contributing components of the respective oscillator unit vectors. For x-y incident circularly polarized radiation (propagation direction perpendicular to the helix axis), the oriented VCD, $(\Delta\epsilon_{xy})_\nu$ as a function of frequency, may be calculated by

$$(\Delta\epsilon_{xy})_\nu = (24\pi^2\nu^2 N_o/3300)\Sigma_{ij}[(e_i^y e_j^z - e_j^z e_j^y)R_{ij}^x - (e_i^z e_j e^x - e_j^x e_j^z)R_{ij}^y]\,\mathrm{Im}(A_{ij})_\nu \qquad (6)$$

As noted previously (Self and Moore, 1997), VCD measurements on oriented polynucleotides in the base-stretching region have not been made, to date, principally because of some technical problems of dealing with oriented samples and their intrinsic birefringence (particularly for x-y incident circularly polarized radiation).

METHODS

Polarizabilities

Complex frequency-dependent polarizabilities were calculated (Self and Moore, 1997) via extinction bands (ϵ_ν) resolved from measured IR absorption spectra of the 5′-ribonucleoside monophosphates: AMP, UMP, GMP, and CMP. In this work, the polarizabilities for the normal modes of non-base-paired 5′-inosine monophosphate (IMP) were taken to be identical to those of 5′-GMP, because their measured IR absorption spectra in the 1750–1550 cm^{-1} spectral region are essentially the same.

For each (j) extinction band $(\epsilon_j)_\nu$, the complex frequency-dependent polarizability $(\alpha_j)_\nu$ is determined by its frequency-dependent real $(\beta_j)_\nu$ and imaginary $(\chi_j)_\nu$ parts, i.e.,

$$(\alpha_j)_\nu = (\beta_j)_\nu + i(\chi_j)_\nu \qquad \text{where } i = (-1)^{1/2}$$

$(\chi_j)_\nu$ can be determined directly from $(\epsilon_j)_\nu$ and $(\beta_j)_\nu$ can be estimated from $(\chi_j)_\nu$ using numerical transforms, e.g., Hilbert or Kronig-Kramers (see Self and Moore, 1997).

Assignment of normal mode oscillations

Shown in Table 1 are normal mode (oscillator) Lorentzian band frequencies (ν_{max}), magnitudes (ϵ_{max}), and half-widths at half-height (Δ) assigned for the normal modes considered in this work. The values for ν_{max} and ϵ_{max} appearing in parentheses (Table 1) are those used when the associated carbonyl groups are involved in a base-paired, hydrogen bond(s) in the environment of a multihelical structure. The remaining parameters are the same as those assigned previously (Self and Moore, 1997) from the measured aqueous

TABLE 1 Input vibrational Lorentzian* band parameters§

Base#	Oscillator#	ν_{max} (cm^{-1})	(M^{-1} cm^{-1}) ϵ_{max} (cm^{-1})	Δ
(A) Adenine	I C5=C4–N3	1623	850	10
	II C8=N7	1576	200	10
(C) Cytosine	I C2=O(· · ·H)	1652 (1685)	950 (1000)	15
(C+)	I C2=O	1720	1000	15
	II C5=C6–N1	1617	470	15
	III C4=N3	1585	180	15
(G) Guanine	I N1–C6=O(· · ·H)	1675 (1690)	980 (1000)	15
(I) Inosine	I N1–C6=O(· · ·H)	(1685)	(1200)	15
(G/I)	II C4=C5	1650	225	15
(G/I)	III C8=N7	1577	225	15
(G/I)	IV H–N1–C2	1565	225	14
(U) Uracil	I C2=O(· · ·H)	1697 (1700)	640 (1300)	10
	II C4=O(· · ·H)	1655 (1670)	1210 (1250)	11
	III C5=C6	1617	240	12

*A Lorentzian band as a function of frequency, ν, is defined: $\epsilon_\nu = \epsilon_{max}\Delta^2/[(\nu - \nu_{max})^2 + \Delta]$, where ϵ_{max}, ν_{max}, and Δ are, respectively, the extinction coefficient at maximum absorption, the frequency at maximum absorption, and the band half-width at half-height. The dipole strength of such a band may be estimated: $D = 0.92 \times 10^{-38}\ \epsilon_{max}\ \pi\Delta/\nu_{max}$.

§The parameters in parentheses are those assigned for base-paired, hydrogen-bonded carbonyl group vibrations.

#See Fig. 1. (. . .H) indicates a hydrogen bond to another base.

solution IR absorption spectra of the 5′-ribonucleoside monophosphates: AMP, UMP, GMP, and CMP. The oscillator parameters for 5′-Inosine monophosphate (I) were taken to be identical to those of GMP, except for the N1-C6═O base-paired, hydrogen-bonded combination stretching vibration (Table 1).

Upon base pair formation (e.g., A:U or G:C) in the environment of multihelical polynucleotide structures, the electrostatic environment of each oscillating group in each monomer unit of each base pair is different from that of the oscillator on the monomer unit free in aqueous solution. For some IR absorbances, this environmental change is observed to result in a shift in vibrational absorption frequency maximum (ν_{max}) and/or shift in the intensity of extinction maximum (ϵ_{max}). The vibrations showing the largest shifts in ν_{max} (and/or ϵ_{max}) on base-pairing seem to be those localized, predominantly, on groups (e.g., carbonyls) involved in hydrogen bond formation between the monomers of the base pairs (Miles, 1964; Akhebat et al., 1992; Wang et al., 1994; Liquier and Taillandier, 1996). Thus, in assigning the normal modes for such groups in the base-paired environment of the double- and triple-stranded helices, considered herein, we have attempted to explicitly account for these ν_{max} and ϵ_{max} shifts.

Shown in Fig. 1 are examples of the base-pairing schemes (Watson-Crick, Hoogsteen, and reverse Hoogsteen) encountered in the polyribonucleotide geometries considered in the present calculations. The ν_{max} and ϵ_{max} values of the C2═O and C4═O carbonyls of U (Fig. 1, *A–C*) (each of which may or may not participate in base-paired hydrogen bond-

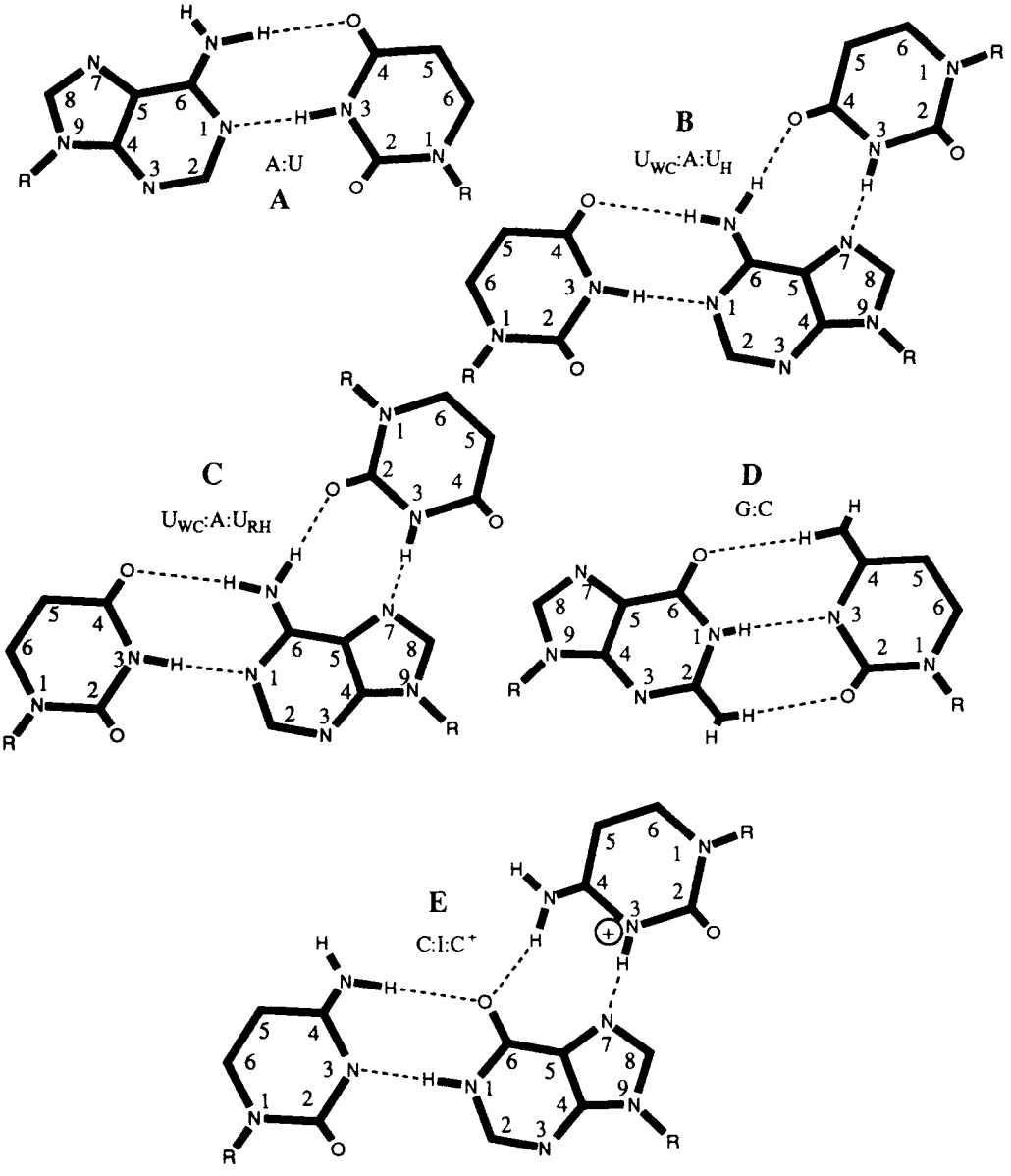

FIGURE 1 Base-pairing for the duplex and triplex polynucleotide structures considered in this paper. The subscripts WC, H, and RH on U represent, respectively, Watson-Crick, Hoogsteen, and reverse Hoogsteen base pairing of U to the A.

ing, depending on the polymer geometry) can be greatly affected by the presence or absence of H bonds with other bases. In a Watson-Crick ($U_{WC} \cdot A$) base pair (Fig. 1, *A–C*), the C4═O carbonyl of U is involved in a hydrogen bond with the NH_2 group of A, whereas the C2═O of U is not. In a Hoogsteen ($A \cdot U_H$) base pair (Fig. 1 *B*), the C4═O carbonyl of U is involved in a base-paired hydrogen bond with A, whereas the C2═O is not. In a reverse Hoogsteen ($A \cdot U_{RH}$) base pair (Fig. 1 *C*), the C2═O carbonyl is involved in a hydrogen bond with NH_2 of A, whereas the C4═O is not. We have assigned ν_{max} and ϵ_{max} parameters for the base-paired hydrogen-bonded carbonyls (shown in parentheses, Table 1) of U (relative to those of U free in solution) consistent with measured values found in the IR absorption spectra of polyribonucleotides. Those C2═O and C4═O parameters of U (Table 1) not involved in base-paired hydrogen bonds have been assigned previously (Self and Moore, 1997). The Watson-Crick, base-paired, hydrogen-bonded parameters for C4═O of U (parentheses, Table 1) were assigned in accordance with measured IR spectra of poly(rA) · poly(rU) (Yang and Keiderling, 1993; Xiang et al., 1993), where all evidence suggests Watson-Crick base-pairing between A and U. These assigned base-paired, hydrogen-bonding parameters of C4═O on U are shown (in parentheses) in Table 1. Attempts were made at assigning base-paired, hydrogen-bonded parameters for C2═O by considering the measured IR spectrum of poly(rU) · poly(rA) · poly(rU_{RH}), which, if it were to exist in solution, would contain a base-paired, hydrogen-bonded C2═O in a reverse Hoogsteen ($A \cdot U_{RH}$) base pair; no satisfactory results were obtained—perhaps future IR spectral measurements on crystals known to contain $A \cdot U_{RH}$ base pairs (Hoogsteen, 1963) could shed some light on the correct parameters to assign in this case.

The measured IR absorbances for the combination N1-C6═O of G and the C2═O of C in poly(rG) · poly(rC) (hydrogen-bonded in Watson-Crick base-pairs; Fig. 1 *D*) show shifted ν_{max} values (Xiang et al., 1993) relative to those measured for these groups on G and C free in solution. The ν_{max} shift of these hydrogen-bonded carbonyls can be as great as 15–30 cm^{-1}, depending on the type of change in environment experienced by the monomers (Miles, 1964; Akhebat et al., 1992; Wang et al., 1994; Liquier and Taillandier, 1996). Free in mild aqueous media, the N1-C6═O combination stretch on G has a ν_{max} of 1675 cm^{-1}, whereas in poly(rG) · poly(rC), it appears to shift to the blue by 15 cm^{-1} to 1690 cm^{-1} (Liquier and Taillandier, 1996). Similarly, the C2═O carbonyl on C (free in solution) has a ν_{max} of 1652 cm^{-1}, which appears in the base-paired hydrogen-bonded case to shift by ~33 cm^{-1} to 1685 cm^{-1} (Liquier and Taillandier, 1996). Accordingly, we have assigned the base-paired, hydrogen-bonded ν_{max} parameters for the N1-C6═O combination stretch of G and the C2═O stretch of C to 1690 cm^{-1} and 1685 cm^{-1}, respectively (see values in parentheses, Table 1). Furthermore, the ϵ_{max} values of the base-paired, hydrogen-bonded carbonyls on both G and C appear to be slightly larger in magnitude than those on the free (in solution) carbonyls. We have tentatively assigned a base-paired hydrogen-bonded magnitude (ϵ_{max}) of ~1000 M^{-1} cm^{-1} for the N1-C6═O combination stretch vibration of the G and C2═O stretch vibration of C (see values in parentheses, Table 1).

The IR spectral properties for triplex poly(rC^+) · poly(rI) · poly(rC) have been described in detail (Akhebat et al., 1992; Wang et al., 1994). The C6═O of I is presumed to be hydrogen-bonded to an amino hydrogen of C and an amino hydrogen of C^+ (Fig. 1 *E*). The ν_{max} for the N1-C6═0 combination stretch vibration of I appears to be slightly less shifted (Akhebat et al, 1992; Wang et al., 1994) than the base-paired, hydrogen-bonded C6═O of G. We have tentatively assigned a ν_{max} of 1685 cm^{-1} and an ϵ_{max} of 1200 M^{-1} cm^{-1} for the N1-C6═O base-paired, hydrogen-bonded combination vibration of I (parentheses, Table 1). The remaining oscillator parameters for I were assumed to be the same as those assigned for G. In poly(rC^+) · poly(rI) · poly(rC), neither the C2═O carbonyl on C nor the C2═O carbonyl on C^+ (Fig. 1 *E*) is hydrogen-bonded, but they are not in equivalent electrostatic environments. The C2═O carbonyl absorption on free C has been assigned previously (Self and Moore, 1997) at a ν_{max} of 1652 cm^{-1} (Table 1). Such a relatively low ν_{max} for a carbonyl group occurs when the group is conjugated with an aromatic system (as is the case for all of the nucleotide carbonyl groups). The conjugation with the ring electrons apparently results in significant accumulation of electron density on the carbonyl oxygen of C, giving this carbonyl bond substantial single-bond character. Consequently, this single-bond character should result in a smaller C2═O ν_{max}, as is observed, relative to an isolated, nonconjugated C═O bond. Placing a positive charge (e.g., at the N3 position of C; Fig. 1 *E*) on this aromatic ring would draw electron density away from the carbonyl oxygen toward the carbonyl carbon, and thereby make the carbonyl group less like a nonconjugated one (i.e., more double-bond character would be induced in the C═O bond) and, hence, a shift of the carbonyl stretching vibration ν_{max} to a larger value. Thus the non-base-paired C2═O absorption of poly(rC^+) (Fig. 1 *E*) has been assigned a ν_{max} of 1720 cm^{-1} (Table 1), in line with the frequency shift expected for a carbonyl with a predominant double bond character (Akhebat et al., 1992). Additionally, the ϵ_{max} value for this 1720 cm^{-1} absorption appears to be slightly larger than that for a non-base-paired C2═O absorption; a tentative value of 1000 M^{-1} cm^{-1} has been assigned (parentheses, Table 1).

RESULTS AND DISCUSSION

Poly(rA) · poly(rU) spectra

It is clear that poly(rA) · poly(rU) at room temperature and in mild aqueous media does exist in the A-RNA duplex conformation. Previously Moore and Williams (1986) obtained excellent results in comparisons of calculated (for

A-RNA geometry) ultraviolet CD spectra with measured spectra.

VCD and IR absorption spectra have been measured, in the 1750–1600 cm^{-1} region, for poly(rA) · poly(rU), by Xiang et al. (1993) and Yang and Keiderling (1993) at 20°C and 25°C, respectively, in mild aqueous media. These measured spectra are compared (Fig. 2) with spectra calculated for poly(rA) · poly(rU) in A-form RNA geometry (Arnott and Dover, 1972, 1973). It can be seen (Fig. 2, *light solid line*) that the magnitudes of the measured VCD (*top*) and measured IR absorption spectra (*bottom*) reported by Yang and Keiderling (1993) are much larger than those reported (*bold solid line*) by Xiang et al. (1993). It is unlikely that these spectral differences are due to the small 5°C temperature difference in the measurement conditions, but more likely are due to mistakes in computing the reported ϵ and $\Delta\epsilon$ values. The dissymmetry ratio, $\Delta\epsilon/\epsilon$, at ~1675 cm^{-1} from the Yang and Keiderling (1993) measurements (*light solid line*) is $\sim 5 \times 10^{-4}$, whereas that from the Xiang et al. (1993) measurements (*bold solid line*) is $\sim 2 \times 10^{-4}$. Thus, with pathlengths and concentrations omitted as sources of error, the Yang and Keiderling (1993) and the Xiang et al. (1993) measurements still differ in magnitude by a factor of ~2.5. Otherwise, the two measured VCD spectra (Fig. 2, *top*) show among themselves similar spectral features, as do the two measured IR spectra (Fig. 2, *bottom*). The calculated VCD spectrum (Fig. 2, *top*, *dashed line*) for poly(rA) · poly(rU) in A-RNA geometry is observed to be consignant with the measured VCD spectrum (*light solid line*) reported by Yang and Keiderling (1993) in the 1700–1600 cm^{-1} spectral region. However, this measured spectrum (*light solid line*) of Yang and Keiderling (1993) is seen to have a negative VCD band at ~1720 cm^{-1} not found in the calculated (*dashed line*) spectrum. The highest frequency absorption band assigned for the calculated spectra (Fig. 2) is that of the non-hydrogen-bonded C2═O of U, with a ν_{max} of 1697 cm^{-1} (Table 1). The calculated 1697 cm^{-1} absorption band is observed (Fig. 2, *bottom*) to overlap with the calculated hydrogen-bonded C4═O absorption band of U (ν_{max} 1670 cm^{-1}), reproducing the double-humped IR absorption band feature seen in the 1700–1650 cm^{-1} range of the measured IR absorption spectrum. In the spectral region of the C5═C4-N3 combination stretch of A (~1625 cm^{-1}), the calculated VCD spectrum (Fig. 2, *top*) and the calculated IR absorption spectrum (Fig. 2, *bottom*) are seen to be in excellent agreement with the measured VCD and IR absorption spectra. The dissymmetry ratio, $\Delta\epsilon/\epsilon$, at ~1675 cm^{-1} for the calculated spectra ($\sim 1 \times 10^{-4}$) is on the same order of magnitude as the $\Delta\epsilon/\epsilon$ from both measured spectra (the calculated $\Delta\epsilon/\epsilon$ are 1/5 and 1/2 the magnitudes, respectively, of the measured (*light solid line*) Yang and Keiderling (1993) and measured (*bold solid line*) Xiang et al. (1993) spectra).

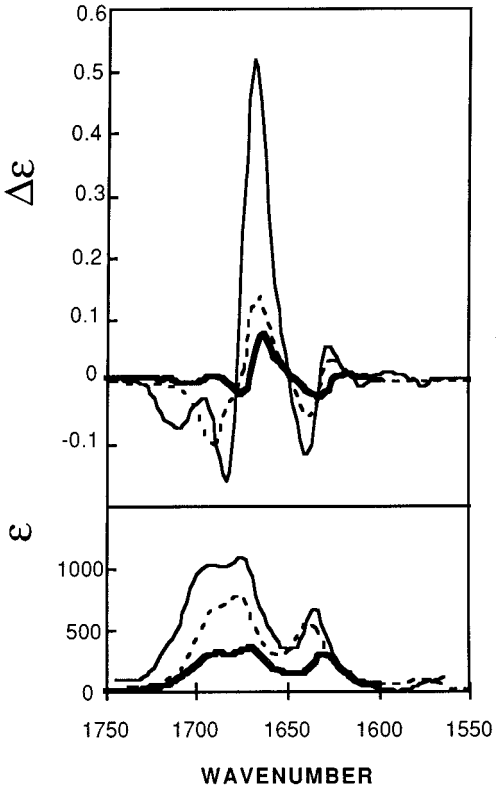

FIGURE 2 Measured and calculated poly(rA) · poly(rU) VCD (*top*) and IR absorption (*bottom*) spectra per nucleotide (total 15 bp). Measured spectra (*light solid line*) are from Yang and Keiderling (1993) and (*bold solid line*) Xiang et al. (1993). Calculated spectra (*dashed line*) for A-form RNA (Arnott and Dover, 1972; Arnott et al., 1976) geometry.

Xiang et al. (1993) have also calculated rotational and dipole strengths for poly(rA) · poly(rU) in the 1700–1600 cm^{-1} spectral region, using their degenerate extended coupled oscillator (DECO) method, in which coupling is allowed only between degenerate oscillators. They included three vibrations in their calculations: the C═C-N combination stretch of A (which they located at 1625 cm^{-1}) and the C2═O and C4═O stretches of U (which they located at 1687 and 1654 cm^{-1}, respectively). Using their DECO method (in which three separate coupled oscillator calculations were made (one calculation per oscillator), and the three results summed then fitted to band shapes), they calculated a VCD spectrum for poly(rA) · poly(rU) that agreed qualitatively with the measured spectrum in terms of band signs. However, their calculated IR absorption spectrum did not show good agreement with the measured IR spectrum in terms of band locations, because the observed ν_{max} shifts (and ϵ_{max} changes) of the base-paired, hydrogen-bonded C4═O (of U) were not explicitly considered in their work, as done in the calculations reported in this work. Herein, the ν_{max} values (Table 1) of C4═O and C2═O of the (Watson-Crick) base-paired U are closer to each other than those for U free in solution or in single-stranded poly(rU) (Self and Moore, 1997).

Shown in Fig. 3 are calculated VCD and IR absorption spectra for oriented models of poly(rA) · poly(rU) in A-RNA geometry (Arnott and Dover, 1972). The highly stacked A-RNA geometry yields a DeVoe theory oriented VCD (Fig. 3, *top*) dominated by light propagating parallel

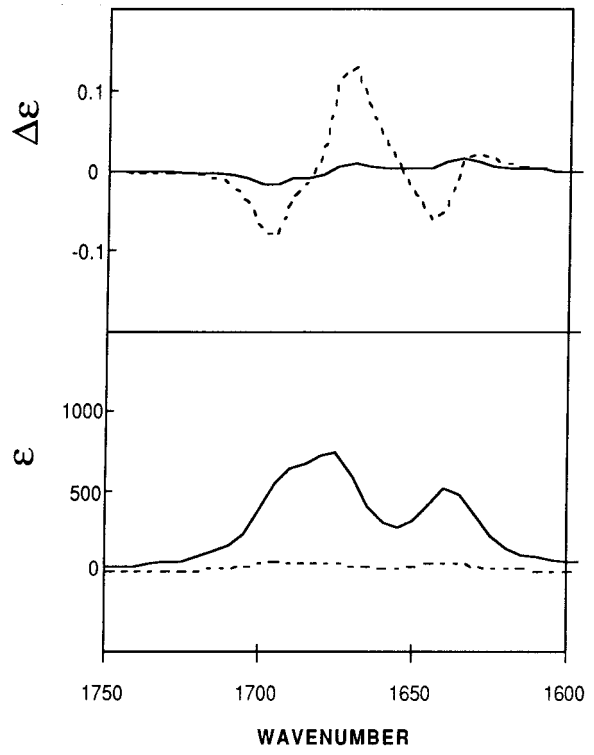

FIGURE 3 Calculated VCD (*top*) and IR absorption (*bottom*) spectra for oriented poly(rA) · poly(rU) in A-form RNA geometry (Arnott and Dover, 1972; Arnott et al., 1976). VCD (*top*): circularly polarized light propagating along (– – –) and perpendicular to (——) the helix axis; IR absorption (bottom): light polarized parallel (– – –) and perpendicular (——) to the helix axis.

(*dashed line*) to the helix axis. The absorption spectrum is seen (Fig. 3, *bottom*) to be dominated by light polarized perpendicular (*light solid line*) to the helix axis, indicating that the poly(rA) · poly(rU) vibrations used in the calculations are all predominantly polarized perpendicular to the helix axis.

Poly(rU) · poly(rA) · poly(rU) spectra

Recently Wang et al. (1994) characterized triplex solution structures of pyrimidine-purine-pyrimidine nucleic acids via VCD measurements in the base stretching (1750–1550 cm^{-1}) spectral region. These workers point out that all triplex structures studied showed a consistent five-peak (from larger to smaller wavenumber) −, +, +, −, + VCD sign pattern, suggesting (along with evidence from other techniques) that the triplexes have nearly the same base-stacking patterns (notwithstanding small variations in the geometry of the ribose-phosphate backbone—the vibrations of which appear below 1500 cm^{-1}). Furthermore, using the U_{WC}:A:U_H triplex geometry (where U_{WC} and U_H indicate Watson-Crick and Hoogsteen pairing, respectively; see Fig. 1 *B*), these workers attempted, without success, to calculate via their previously described DECO method, the typical −, +, +, −, + VCD spectral sign pattern measured for triplex polyribonucleotides. Another variation of the ECO method was then tried by these workers, but good agreement with the measured sign pattern was still missing. Subsequently, these workers then ignored the C4═C5 oscillator of A in the triplex calculations, considering only the C2═O and C4═O oscillators of U. (In effect, this neglect of the A oscillators results in a calculation of the VCD of duplex poly(rU) · poly(rU_H) separated by an optically and chiroptically inactive A-form poly(rA).) These ECO calculations of the presumed poly(rU_{WC}) · poly(rA) · poly(U_H) VCD were also found to be inconsistent with the measured triplex VCD sign pattern, but the calculated IR absorption spectrum was found to be reasonably consistent with that measured. Finally, these workers switched the C2═O and C4═O oscillator frequencies and changed the triplex structure from poly(rU_{WC}) · poly(rA) · poly(U_H) (Fig. 1 *B*) to poly(rU_{WC}) · poly(rA) · poly(U_{RH}) (Fig. 1 *C*), i.e., the triplex was assumed to have a reverse Hoogsteen rather than a Hoogsteen A:U base-pair. Their calculation for poly(rU_{WC}) · poly(rA) · poly(U_{RH}) (ignoring the C4═C5 vibration of rA) was found to give the characteristic five-band −, +, +, −, + VCD sign pattern measured for poly(rU) · poly(rA) · poly(U). Thus it was suggested from these results that the predominant geometry for poly(rU) · poly(rA) · poly(U) in solution might be that of poly(rU_{WC}) · poly(rA) · poly(U_{RH}).

Using the assigned A and U base parameters (Table 1) along with the DeVoe theory, and including all base stretching vibrations, we have calculated the VCD and IR absorption spectra for poly(rU_{WC}) · poly(rA) · poly(U_{RH}) and poly(rU_{WC}) · poly(rA) · poly(U_H). Shown in Fig. 4 are the resulting DeVoe theory calculated VCD (*top*, *dashed line*) and IR absorption (*bottom*, *dashed line*) for poly(rU_{WC}) · poly(rA) · poly(U_{RH}) (*left*) and poly(rU_{WC}) · poly(rA) · poly(U_H) (*right*) compared with the measured (*light solid line*) poly(rU) · poly(rA) · poly(U) spectra (Yang and Keiderling, 1993). For these calculations, the U_{WC}:A base pairs of the triplex structures were stacked in duplex A-form RNA geometry (Arnott and Dover, 1972), and the U_{RH} or U_H of each base trio was generated in the major groove of the duplex by overlaying the A:T_{RH} or A:T_H (where T = U) base pair (Hoogsteen, 1963) on the A of the duplex U_{WC}:A base pairs. Both the VCD and IR absorption spectra calculated (*dashed line*) for poly(rU_{WC}) · poly(rA) · poly(U_{RH}) (Fig. 4, *left*) and poly(rU_{WC}) · poly(rA) · poly(U_H) (Fig. 4, *right*) from the DeVoe theory are seen to be in good agreement with the measured spectra. However, in considering the detailed shapes of the calculated VCD spectra, it can be observed that the calculated VCD for poly(rU_{WC}) · poly(rA) · poly(U_H) (Fig. 4, *top right, dashed line*) is in much better agreement with the measured VCD spectrum (Fig. 4, *top right, solid line*) than is the calculated spectrum for poly(rU_{WC}) · poly(rA) · poly(U_{RH}) (Fig. 4, *top left, dashed line*). The calculated −, +, +, −, + VCD band sign pattern in the calculated poly(rU_{WC}) · poly(rA) · poly(U_H) VCD spectrum (Fig. 4, *top right, dashed line*) appears at the

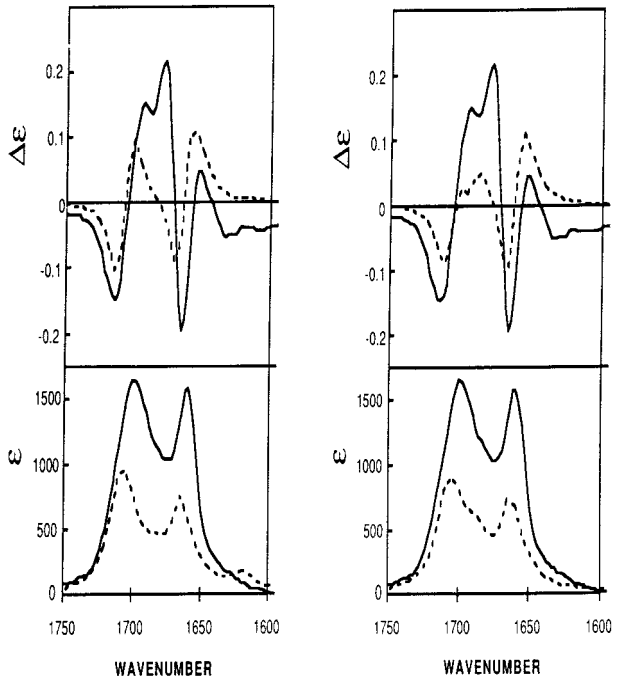

FIGURE 4 Measured (——) and calculated (– – –) poly(rU) · poly(rA) · poly(rU) VCD (*top*) and IR absorption (*bottom*) spectra per nucleotide (total 10 base trios). (*Left*) Calculated spectra (– – –) for triplex A-RNA (Arnott et al., 1976) geometry [poly(rU_{WC}) · poly(rA) · poly(rU_{RH})] with reverse Hoogsteen A:U base-pairing. (*Right*) Calculated spectra (– – –) for triplex A-RNA (Arnott et al., 1976) geometry [poly(rU_{WC}) · poly(rA) · poly(rU_H)] with Hoogsteen A:U base-pairing. Measured spectra from Yang and Keiderling (1993).

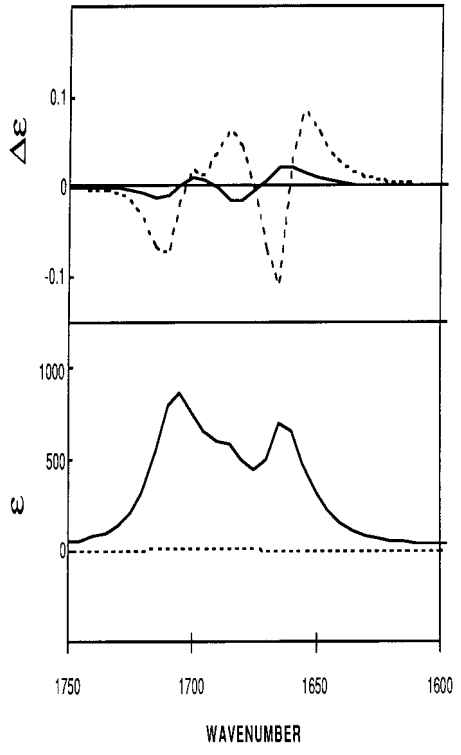

FIGURE 5 Calculated VCD and IR absorption spectra for oriented poly(rU_{WC}) · poly(rA) · poly(rU_H) in triplex A-RNA (Arnott et al., 1976) geometry. (*Top*) VCD spectra: light propagating along (– – –) and perpendicular to (——) the helix axis. (*Bottom*) IR absorption spectra: light polarized parallel (– – –) and perpendicular (——) to the helix axis.

same frequencies as those in the measured (*solid line*) VCD spectrum. Moreover, the calculated DeVoe theory IR absorption spectrum (Fig. 4, *bottom, dashed line*) for poly(rU_{WC}) · poly(rA) · poly(U_H), although of reduced magnitude, is virtually congruent with the measured (*solid line*) IR absorption spectrum. Furthermore, at ~1675 cm^{-1} (Fig. 4, *right*), the dissymmetry ration, $\Delta\epsilon/\epsilon$, for the DeVoe theory calculated poly(rU_{WC}) · poly(rA) · poly(U_H) spectra is found to be virtually identical to that obtained from the measured poly(rU) · poly(rA) · poly(U) spectra.

Shown in Fig. 5 are DeVoe theory calculated VCD (*top*) and IR absorption (*bottom*) for oriented triplex poly(rU_{WC}) · poly(rA) · poly(rU_H). As in the case of duplex poly(rU) · poly(rA) in A-form RNA geometry, the VCD for light propagating parallel to the helix axis of the oriented triplex (Fig. 5, *top*, *dashed line*) is much larger than the VCD for light propagating perpendicular to the helix axis (Fig. 5, *top*, *solid line*) and, therefore, is the primary source for the calculated isotropic VCD (Fig. 5, *top right, dashed line*). The calculated isotropic IR absorption spectrum of poly(rU_{WC}) · poly(rA) · poly(rU_H) (Fig. 4, *bottom right, dashed line*) is seen to be due to light polarized perpendicular to the triplex helix axis (Fig. 5, *bottom, solid line*); the contribution for light polarized parallel to the triplex helical axis (Fig. 5, *bottom, dashed line*) is seen to be negligible.

Poly(rG) · poly(rC) spectra

Shown in Fig. 6 are DeVoe theory calculated (*dashed line*) VCD (*top*) and IR absorption (*bottom*) spectra for poly(rG) · poly(rC) in duplex A-form RNA geometry (Arnott and Dover, 1972, 1973), compared with measured (*solid line*) spectra by Xiang et al. (1993). The calculated IR absorption spectrum (Fig. 6, *bottom*) is seen to be in excellent agreement in shape and magnitude with the measured IR spectrum. Both measured and calculated IR spectra are seen to consist of three absorption bands with ν_{max} values of ~1690, 1655, and 1620 cm^{-1}. The calculated (*dashed line*) and measured (*solid line*) VCD spectra (Fig. 6, *top*) are seen to be in good agreement with each other, in terms of spectral shapes; however, the magnitude of the measured VCD spectrum (Fig. 6, *top*) has been scaled by a factor of 1/4. The $\Delta\epsilon/\epsilon$ values at ~1675 cm^{-1} for the measured and calculated spectra are on the same order of magnitude, at $\sim 5.8 \times 10^{-4}$ and $\sim 1.4 \times 10^{-4}$, respectively. Xiang et al. (1993) have reported calculated A-form RNA poly(rG) · poly(rC) rotational and dipole strengths, in the 1750–1600 cm^{-1} spectral region, using their ECO VCD calculational methods. Their results agree qualitatively with the results presented here, except that their calculated band frequencies were different from those presented here; they did not explicitly consider the frequency shifts of the carbonyl vibrations on forming base-paired hydrogen bonds.

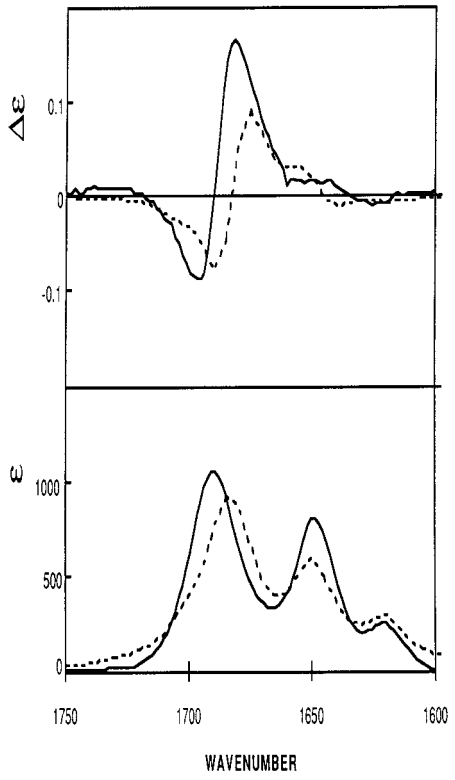

FIGURE 6 Measured (——) and calculated (– – –) poly(rG) · poly(rC) VCD (*top*) and IR absorption (*bottom*) per nucleotide (total 15 bp). Calculated spectra for duplex in A-RNA (Arnott and Dover, 1972; Arnott et al., 1976) geometry. Measured spectra are from Xiang et al. (1993).

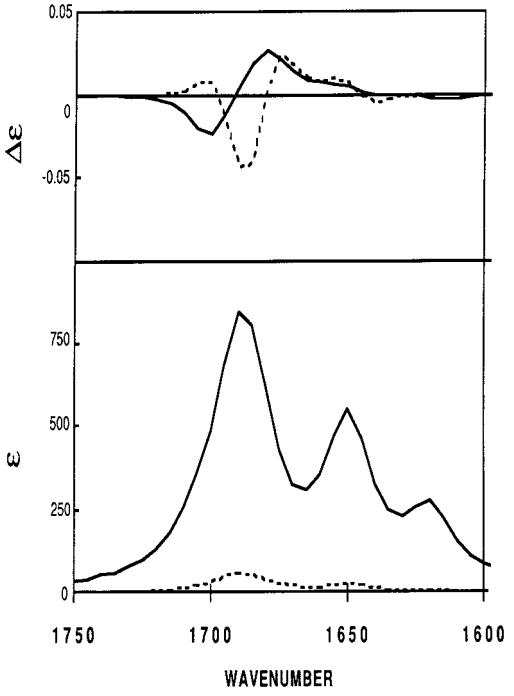

FIGURE 7 Calculated VCD and IR absorption spectra for oriented poly(rG) · poly(rC) in A-RNA geometry (Arnott and Dover, 1972; Arnott et al., 1976). VCD (*top*): Light propagating along (– – –) and perpendicular to (——) the helix axis. IR absorption (*bottom)*: Light polarized parallel (– – –) and perpendicular (——) to the helix axis.

Shown in Fig. 7 are calculated VCD and IR absorption spectra for oriented duplex A-form poly(rG) · poly(rC). The VCDs (Fig. 7, *top*) for light propagating parallel (*solid line*) and perpendicular (*dashed line*) to the helix axis are both seen to make significant contributions to the calculated isotropic VCD spectrum (Fig. 6, *dashed line*). The calculated IR absorption spectrum is seen (Fig. 7, *bottom*) to be dominated by light polarized perpendicularly (*solid line*) to the helix axis.

Poly(rC) · poly(rI) · poly(rC$^+$) spectra

Measured IR absorption spectra for poly(rC) · poly(rI) · poly(rC$^+$) have been reported by Akhebat et al. (1992) and Wang et al. (1994) have reported measured VCD and IR spectra for this triplex. Shown in Fig. 8 are measured (*solid line*) VCD (*top*) and IR absorption (*bottom*) for poly(rC) · poly(rI) · poly(rC$^+$) from Wang et al. (1994), compared with DeVoe theory calculated (*dashed line*) VCD and IR absorption spectra for the triplex A-form RNA geometry (Arnott et al., 1976). The base trio used for the model structure is shown in Fig. 1 *E*. The measured VCD and IR absorption spectra, as reported by Wang et al. (1994), are in ΔA and A, respectively, where $X = \Delta A = 9.1 \times 10^{-5}$ for the measured VCD spectrum (Fig. 8, *top*, *solid line*), and $Y = A = 0.75$ for the measured IR spectrum (Fig. 8, *bottom*, *solid line*). For the calculated VCD spectrum (Fig. 8, *top*, *dashed line*), $X = \Delta\epsilon = 0.075\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$ and for the calculated IR (Fig. 8, *bottom*, *dashed line*), $Y = \epsilon = 500\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$. Thus the dissymmetry ratio computed from the measured poly(rC) · poly(rI) · poly(rC$^+$) spectra ($\Delta A/A = 1.2 \times 10^{-4}$) is seen to be essentially the same as

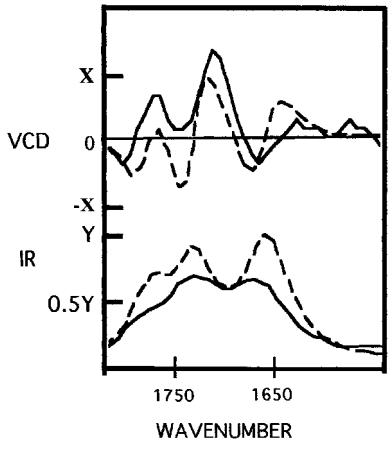

FIGURE 8 Measured and calculated poly(rC) · poly(rI) · poly(rC$^+$) VCD and IR absorption spectra per nucleotide (total 10 base trios). Measured spectra (——) [VCD (*top*): $X = \Delta A = 9.1 \times 10^{-5}$; IR (*bottom*): $Y = A = 0.75$] are from Wang et al. (1994). Calculated spectra (– – –) [VCD (*top*): $X = \Delta\epsilon = 0.075\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$; IR (*bottom*): $Y = \epsilon = 500\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$] for triple-stranded A-RNA (Arnott et al., 1976) geometry.

that computed from the calculated spectra ($\Delta\epsilon/\epsilon = 1.5 \times 10^{-4}$).

CONCLUSION

Polynucleotides have been studied via infrared spectroscopy for over 40 years. Many of these studies have yielded valuable structural data on polynucleotides in solution. Naturally, during this time, some controversies have developed over the interpretation of various measurements. For example, poly(rU) · poly(rA) · poly(rU) was studied via IR spectroscopy by Miles (1964) and, based on model construction and infrared band frequency shifts, it was concluded that the triplex contained the base-pairing pattern shown in Fig. 1 *C*, i.e., poly(rU_{WC}) · poly(rA) · poly(rU_{RH}). In poly(rU_{WC}) · poly(rA) · poly(rU_{RH}), the U_{WC} base has a hydrogen-bonded C4═O and a non-hydrogen-bonded C2═O, and the U_{RH} has a hydrogen-bonded C2═O and a non-hydrogen-bonded C4═O. Thus, there should be four nonequivalent carbonyls, which should yield four different carbonyl IR absorption frequencies in the base stretching spectral region. The band assignments by Miles (1964) only accounted for three carbonyl bands; the non-hydrogen-bonded C2═O on U_{WC} and hydrogen-bonded C2═O on U_{RH} were taken to be equivalent. Thus, for poly(rU_{WC}) · poly(rA) · poly(rU_{RH}), there should be a total of four prominent bands in the base stretching region (three C═O bands for the Us and one C═C band for A). The conclusions of Miles (1964) were challenged by Arnott and Bond (1973). Based on x-ray crystallographic and molecular modeling studies, they concluded that this triplex did not contain reverse Hoogsteen A:U_{RH} pairs, but instead contained Hoogsteen only A:U_H, pairs, i.e., poly(rU_{WC}) · poly(rA) · poly(rU_H) (see Fig. 1 *B*). In the poly(rU_{WC}) · poly(rA) · poly(rU_H) triplex, the C4═O on both U_{WC} and U_H is hydrogen-bonded to A, whereas the C2═O on both U_{WC} and U_H is not hydrogen bonded to another base. Thus, poly(rU_{WC}) · poly(rA) · poly(rU_H) should have only two different types of C═O groups. In the VCD calculations presented in this work, we have shown that the Arnott and Bond (1973) proposed model, poly(rU_{WC}) · poly(rA) · poly(rU_H), produces calculated VCD and IR absorption spectra that compare more favorably with the measured spectra than the spectra calculated for the poly(rU_{WC}) · poly(rA) · poly(rU_{RH}) model. For both structures, we have calculated acceptable IR absorption spectra (Fig. 4). However, when compared with the measured spectrum, the VCD spectrum calculated from the poly(rU_{WC}) · poly(rA) · poly(rU_H) model is better than the VCD spectrum calculated from the poly(rU_{WC}) · poly(rA) · poly(rU_{RH}) model. This type of result has also been reported previously for VCD calculations on other polynucleotide structures. Self and Moore (1997) found that acceptable IR absorption spectra for poly(rC) (as well as poly(rG)) could be calculated from very different helical geometries. However, it was the calculated VCD spectra in comparison with the measured VCD spectra that allowed choices to be made between the competing geometries. Therefore, in trying to draw structural conclusions about polynucleotides in solution, it is best that acceptable (compared to measurements) IR absorption and VCD spectra be calculated for the geometries under consideration. Geometries proposed based solely on a good (compared to measurement) VCD spectrum, without a correspondingly good IR absorption calculation, should be suspect. Thus it is concluded that VCD and IR absorption spectral measurements can provide powerful information for elucidating polynucleotide solution structures when coupled with good VCD and IR spectral calculations on proposed polynucleotide geometries. The DeVoe theory, when parameterized appropriately, is capable of simulating the measured VCD and IR absorption spectra of double- and triple-stranded polynucleotide structures.

Furthermore, it is concluded that comparisons of calculated $\Delta\epsilon/\epsilon$ for proposed geometries with measured $\Delta\epsilon/\epsilon$ (or $\Delta A/A$) greatly facilitate interpretations when there are different measured $\Delta\epsilon$ and ϵ (or ΔA and A) spectra reported in the literature (e.g., as in the case herein for poly(rA) · poly(rU), poly(rG) · poly(rC), and poly(rC) · poly(rI) · poly(rC^+)). These types of comparisons between measured and calculated spectra for poly(rA) · poly(rU) and poly(rC) · poly(rI) · poly(rC^+) have been considered in the previous section. In the case of poly(rG) · poly(rC), the difference between the measured and calculated magnitudes (a factor of ~4) for the $\Delta\epsilon/\epsilon$ of this polymer may be a reflection of evidence presented by Thiele and Guschlbauer (1971) that this molecule, in aqueous solution, does not exist in pure duplex form at neutral pH and low ionic strength (the conditions used to obtain the measured VCD and IR spectra shown in Fig. 6). Furthermore, these workers (Thiele and Guschlbauer, 1971) have shown that both poly(rG) and poly(rC) are capable of forming, in such mild aqueous media, self-aggregated, multistranded structures with stabilities similar to that of duplex poly(rG) · poly(rC). Thus the difference between the measured dissymmetry ratio and that calculated for poly(rG) · poly(rC), in duplex A-RNA geometry, may be due to measured spectral contributions from these other possible multistranded structures. A similar conclusion was drawn from magnitude differences found, previously, between calculated (duplex A-RNA geometry) and measured ultraviolet CD spectra of poly(rG) · poly(rC) (Williams and Moore, 1983).

Finally, in comparing the calculated and measured VCD spectra of triplex poly(rC) · poly(rI) · poly(rC^+) (Fig. 8) with those of triplex poly(rU) · poly(rA) · poly(U) (Fig. 4, *left*), it is observed that both measured spectra show the −, +, +, −, + (larger to smaller wavenumber in the 1750–1600 cm^{-1} region) five-band sign pattern that Wang et al. (1994) generalized as being characteristic of triplex structures. However, it is noted that the measured VCD spectrum for poly(rC) · poly(rI) · poly(rC^+) (Fig. 8) contains a much smaller positive minimum between the two (second and third) adjacent positive bands (Fig. 8) than does the measured spectrum of poly(rU) · poly(rA) · poly(U) (Fig. 4,

left), indicating a much larger negative (and/or smaller positive) VCD contribution in this spectral region for poly(rC) · poly(rI) · poly(rC^+). In the case of the calculated spectrum for poly(rC) · poly(rI) · poly(rC^+) (Fig. 8), this minimum between the second and third adjacent positive bands is actually negative. This calculated spectrum thus shows a six-band −, +, −, +, −, + VCD sign pattern in which each of the three −, + pairs corresponds to overlapping VCD couplets for each of the three major IR absorption bands in this spectral region. Thus it is concluded that a measured spectrum of a triplex may be better characterized—by neglecting band signs—as containing (from larger to smaller wavenumber in the 1750–1600 cm^{-1} region) six alternating extrema: minimum, maximum, minimum, maximum, minimum, maximum (or, in short, simply, 3(minimax)).

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