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

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ABSTRACT Infrared (IR) vibrational circular dichroism (VCD), absorption, and linear dichroism (LD) spectra of four homopolyribonucleotides, poly(rA), poly(rG), poly(rC), and poly(rU), have been calculated, in the 1750–1550 cm^{-1} spectral region, using the DeVoe polarizability theory. A newly derived algorithm, which approximates the Hilbert transform of imaginaries to reals, was used in the calculations to obtain real parts of oscillator polarizabilities associated with each normal mode. The calculated spectra of the polynucleotides were compared with previously measured solution spectra. The good agreement between calculated and measured polynucleotide spectra indicates, for the first time, that the DeVoe theory is a useful means of calculating the VCD and IR absorption spectra of polynucleotides. For the first time, calculated DeVoe theory VCD and IR absorption spectra of oriented polynucleotides are presented. The calculated VCD spectra for the oriented polynucleotides are used to predict the spectra for such measurements made in the future. The calculated IR spectra for the oriented polynucleotides are useful in interpreting the linear dichroism of the polynucleotides.

INTRODUCTION

A number of theoretical procedures have been employed for the calculation of the optical and chiroptical properties of nucleic acids (cf. DeVoe, 1964, 1965, 1969; Johnson and Tinoco, 1969; Rizzo and Schellman, 1984). Of these methods, the DeVoe polarizability theory (DeVoe, 1964, 1965) has been found to be one of the most useful. Although derived through a classical approach, the DeVoe theory has been shown (Rhodes and Chase, 1967) to be equivalent to the quantum mechanical, time-dependent Hartree theory, within the approximations common to all perturbation treatments of polymer optical and chiroptical properties. The DeVoe theory is all-order in the intercoulombic interactions between radiation-driven, frequency-dependent oscillators (transitions) in the species of interest. The theory has the advantage of using as input, unperturbed empirical frequency-dependent absorption data from which complex frequency-dependent polarizabilities are obtained. As output, the theory provides, directly, perturbed extinction (absorption) and circular dichroism (CD) spectra, instead of the dipole and rotational strengths commonly provided by other large molecule theoretical procedures. This spectral form of output provides the added advantage of allowing a direct comparison between calculated and measured spectra, even in spectral regions of overlapping transition bands.

Subsequent to its original application by DeVoe to calculate the UV absorption spectrum of uracil crystals and the hypochromism of DNA (DeVoe, 1969, 1971), the DeVoe theory was used with success in the calculation of UV CD and absorption spectra of polynucleotides and other species of interest (Hug et al., 1974; Cech et al., 1976a,b; Cech, 1975; Zandomeneghi et al., 1976; Cech and Tinoco, 1977; Greve et al., 1977; Applequist et al., 1979; Moore, 1980; Johnson et al., 1981; Williams and Moore, 1983; Moore and Williams, 1986). However, to date, no examples have been published showing the use of the theory to calculate vibrational CD (VCD), oriented VCD, infrared (IR) absorption, or IR linear dichroism (LD) spectra of any nucleic acid. This paper reports the first application of the DeVoe theory to calculations of the VCD, oriented VCD, IR absorption, and IR LD spectra of nucleic acids. In the following, calculated VCD and absorption spectra for four homopolyribonucleotides, poly(rA), poly(rU), poly(rC), and poly(rG), are compared with measured spectra. Oriented VCD and LD spectra are presented to predict the results to be expected when these properties of the homopolynucleotides are measured in the future.

THEORY

A complete discussion of the DeVoe polarizability theory has been published elsewhere (DeVoe, 1969, 1971). In using the DeVoe theory, it is assumed that the optical and chiroptical properties of the species of interest are due to interactions between oscillators—generated by absorption of electromagnetic radiation—located on polarizable groups within the species. The optical and chiroptical properties are then calculated by assuming that each oscillator, so generated, is dynamically coupled, to all-order, with all other oscillators within the species of interest. The equations for the resulting dynamically coupled electric and magnetic dipole oscillators are a function of frequency-dependent complex polarizabilities (Moore, 1980) of each radiation-absorbing, polarizable group. Optical and chiroptical spectra for the species of interest are then calculated by evaluating these equations at selected frequencies in the electromagnetic spectral region of interest.

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For the calculated VCD, IR absorption, and LD spectra presented herein, it was assumed that no substantial magnetically allowed oscillators contributed, because for each vibrational transition, the electrons (along with the atomic nuclei) follow linear paths. Furthermore, the effects of static fields were ignored, and each electric oscillator was assumed to be completely localized within the molecular subgroup to which it was assigned. Therefore, analogous to equations presented previously for DeVoe theory UV absorption (extinction) and UV CD (Cech, 1975), the equations for frequency (ν)-dependent IR extinction (ϵ_ν) and VCD ($\Delta\epsilon_\nu = \epsilon_l - \epsilon_r$) spectra may be written

$$\epsilon_\nu = -[(n_s^2 + 2)^2/9n_s](8\pi^2\nu N_o/6909)\Sigma_{ij}\,\mathrm{Im}(A_{ij})_\nu \mathbf{e}_i \cdot \mathbf{e}_j \qquad (1)$$

$$\Delta\epsilon_\nu = [(n_s^2 + 2)/9900]\Sigma_{ij}(C_{ij})_\nu\,\mathrm{Im}(A_{ij})_\nu \qquad (2)$$

where

$$(A_{ij})_\nu = [\delta_{ij}/(\alpha_i)_\nu + \mathbf{e}_i \cdot \mathbf{T}_{ij} \cdot \mathbf{e}_j]^{-1};$$

$$(C_{ij})_\nu = 24\pi^2\nu^2 N_o[(\mathbf{e}_i \times \mathbf{e}_j) \cdot \mathbf{R}_{ij}];$$

$$\mathbf{T}_{ij} = (\mathbf{e}_i \cdot \mathbf{e}_j/|\mathbf{R}_{ij}|^3 - 3\mathbf{e}_i \cdot \mathbf{R}_{ij} \cdot \mathbf{e}_j \cdot \mathbf{R}_{ij}/|\mathbf{R}_{ij}|^5)/e_{\mathrm{diel}}$$

and

$$\mathbf{R}_{ij} = \mathbf{R}_j - \mathbf{R}_i$$

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** (the real parts of these complex polarizabilities are more distinctly defined in the Methods section, below); $\mathbf{T}_{ij}$, in units of cm^{-3}, is the well-known point-dipole interaction tensor that is related to the quantum mechanical perturbation energy; $\mathbf{R}_{ij}$, 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; n_s (herein set equal to 1) is the frequency-dependent index of refraction of the solvent; and e_{diel} is an isotropic effective dielectric constant which, for biopolymers, commonly falls within the range of 1–4; herein, e_{diel} is set to 2.7.

METHODS

LD and VCD equations for oriented helices

By slight modification of Eqs. 1 and 2 above, the IR extinction and VCD spectra for oriented helices may be calculated. The following applies for helices oriented with their screw axes parallel to the z axis of a Cartesian reference frame.

The extinction for radiation polarized parallel to the helix axis, ϵ_{para}, is calculated (Eq. 3) from the z components of the oscillator unit vectors $\mathbf{e}_i$ and $\mathbf{e}_j$. Thus $\mathbf{e}_i \cdot \mathbf{e}_j$, in Eq. 1, may be replaced with $e_i^z \cdot e_j^z$, the product of the z components of unit vectors **i** and **j**. Similarly, the extinction for radiation polarized perpendicular to the helix axis, ϵ_{perp}, may be calculated from the x and y components of unit vectors **i** and **j**. To calculate ϵ_{perp}, $\mathbf{e}_i \cdot \mathbf{e}_j$ may be replaced (Eq. 1) with $e_i^x \cdot e_j^x + e_i^y \cdot e_j^y$. The LD as a function of frequency, $(\epsilon_{\mathrm{para}} - \epsilon_{\mathrm{perp}})_\mathrm{v}$, may then be calculated by

$$(\epsilon_{\mathrm{para}} - \epsilon_{\mathrm{perp}})_\nu = -[(n_s^2 + 2)^2/9n_s]$$
$$\cdot (8\pi^2\nu N_o/6909)\Sigma_{ij}\,\mathrm{Im}(A_{ij})_\nu[e_i^z \cdot e_j^z - (e_i^x \cdot e_j^x + e_i^y \cdot e_j^y)] \quad (3)$$

$\Delta\epsilon$ for radiation incident parallel to the helix axis may be calculated by noting that only the x and y components of $\mathbf{e}_i$ and $\mathbf{e}_j$ and the z component of $\mathbf{R}_{ij}$ contribute. Thus, for z-incident radiation, the VCD as a function of frequency may be calculated from Eq. 2, using

$$(C_{ij})_\nu = 24\pi^2\nu^2 N_o[(e_i^x \cdot e_j^y - e_i^y \cdot e_j^x)R_{ij}^z]$$

Similarly, $\Delta\epsilon$ for radiation incident perpendicular to the helix axis may be calculated from Eq. 2, using x-z, y-z components of $\mathbf{e}_i$ and $\mathbf{e}_j$ and x-y components of $\mathbf{R}_{ij}$, thus,

$$(C_{ij})_\nu = 24\pi^2\nu^2 \mathrm{N}_o[(e_i^y \cdot e_j^z - e_i^z \cdot e_j^y) \cdot R_{ij}^x - (e_i^z \cdot e_j^x - e_i^x \cdot e_j^z)R_{ij}^y]$$

It should be noted that, to date, polarized VCD measurements on nucleic acids have not been made, because of problems of dealing with an oriented sample and its intrinsic birefringence. These problems apply particularly for radiation incident perpendicular to the screw axes of oriented helices.

Polarizabilities

To calculate DeVoe theory VCD, absorption, and LD spectra, it is necessary to construct, for each oscillator, frequency-dependent complex polarizabilities from extinction bands resolved from measured absorption spectra of isolated groups (e.g., monomers, groups of bonds, bonds, or atoms) in the species of interest. Accordingly, for the calculations presented herein, IR absorption spectra (Tsuboi et al., 1973; Smith, 1974, 1979) of 5′-adenosine monophosphate (AMP), 5′-uridine monophosphate (UMP), 5′-guanosine monophosphate (GMP), and 5′-cytidine monophosphate (CMP) were resolved into Lorentzian band shapes with maximum frequencies previously assigned (Smith, 1974, 1979) to normal modes localized within particular subgroups (bonds or groups of bonds) on each mononucleotide. The direction for each oscillator, associated with a particular absorption band, was represented by a unit vector in the direction of the subgroup normal mode. Any vectors located at the same point in space were prohibited from interaction; i.e., for such dipoles, the interaction tensor was set to zero. The imaginary parts of the complex polarizabilities for each oscillator were obtained as described previously (DeVoe, 1969, 1971). Finally, for each oscillator, the real parts of the complex polarizabilities were obtained through an approximation to the Hilbert transform of imaginaries to reals.

The Hilbert (Kronig-Kramers) transform of imaginary to real polarizabilities as a function of frequency [$\mathrm{Im}(\alpha_\mathrm{v})$ to $\mathrm{Re}(\alpha_\mathrm{v})$] is a numerical procedure which, if accuracy is desired, consumes a large amount of computer time (DeVoe, 1969, 1971). The accuracy of the transform depends upon the number of frequency points selected within the spectral range of interest: the more frequency points selected, the more accurate the transform. However, the larger the number of frequency points considered, the longer the computer time required to achieve the transform. To save computer time and still achieve great accuracy, it would be advantageous to have an analytic solution for the transform. Toward this end, an empirically derived algorithm has been developed (Moore, D.S., unpublished work) that rapidly and accurately estimates the results of the Hilbert transform of imaginary polarizabilities to real polarizabilities, at frequency intervals of 0.01 wave number, in any spectral region of interest, i.e.,

$$\mathrm{Re}(\alpha_\nu) = 9.96|\boldsymbol{\mu}|^2 f_\nu f_\eta/\nu_{\max}$$

where $\nu_{\max}$ is the wavenumber of absorption maximum ($\epsilon_{\max}$); ν is a wavenumber in the spectral region of interest; and $|\boldsymbol{\mu}|^2$ is the dipole strength associated with the absorption band;

$$f_\nu = 1 + \nu^2(\nu_{\max}^2 - \nu^2)/[(\nu_{\max}^2 - \nu^2)^2 + (2\Delta)^2\nu^2]$$

where Δ is the half-width (units of cm^{-1}) of the absorption band;

$$f_\eta = k(2A\eta - B\eta^2 - 2C\eta^5/3 + 1)$$

where $\eta = e^{-|d|^2}$, $d = (\nu_{\max} - \nu)/2\Delta$ and k is a function of d:

a. for $|d| > (20)^{1/2}$: $A = B = C = 0$ and $k = 1$;

b. for $1 < |d| \leq (20)^{1/2}$: $A = 1, B = C = 0$ and

$k = 0.9365$ when $d < 0$, $|d| \leq 1.15$;

$k = 1.040$ when $d < 0$, $|d| > 1.15$;

$k = 1.040$ when $d > 0$, $|d| > 1.25$;

$k = 0.9260$ when $d > 0$, $|d| \leq 1.25$

c. for $0.5 < |d| \leq 1$: $A = B = 1, C = 0$ and

$k = 0.9314$ when $d > 0$, $|d| \leq 0.60$;

$k = 0.9514$ when $d > 0$, $|d| \leq 0.65$

d. for $|d| \leq 0.5$: $A = B = C = 1$ and

$k = 1.063$ when $d < 0$, $|d| \leq 0.35$;

$k = 1.018$ when $d > 0$;

For the above Re(α_ν) equation, it was found necessary to introduce corrections (via f_η) so that the real parts of the polarizabilities produced would be within about ±2% of the values calculated from the Hilbert transform. As $\nu \to \nu_{\max}$ (or $d \to 0$), different f_η corrections—calculated from a, b, c, and d, above—were necessary. For example, it was found that no corrections were needed ($f_\eta = k$; see a above) for $|d|$ greater than ~4.47 (or $|d| > (20)^{1/2}$).

Assignment of normal mode oscillations

The normal mode frequencies, widths at half-height (Δ), Lorentzian band magnitudes ($\epsilon_{\max}$) used in the present calculations are shown in Table 1. The locations and directions of the oscillators along with the base [adenine (A), uracil (U), guanine (G), and cytosine (C)] numbering systems are shown in Fig. 1. The measured IR spectrum of 5′-AMP (Tsuboi et al., 1973; Smith, 1974, 1979) shows two major absorptions in the 1750–1550 cm^{-1} region: a relatively strong absorption at 1623 cm^{-1} and a relatively weak one at 1576 cm^{-1} (see Table 1 and Fig. 1). The absorption at 1623 cm^{-1} was assigned as a predominantly coupled (65%) C5═C4:(35%) C4-N3 vibration, and that at 1576 cm^{-1} as a predominant C8═N7 stretch. The measured IR spectrum of 5′-UMP shows three major peaks in the 1750–1550 cm^{-1} region: two relatively strong absorptions at 1697 cm^{-1} and 1655 cm^{-1}, and a relatively weak absorption at 1617 cm^{-1}. The absorption at 1697 cm^{-1} is assigned to a coupled C2═O/C2-N1 vibration (85% C2═O:15% C2-N1). The absorption at 1655 cm^{-1} is assigned to a coupled C4═O/C4-N3 vibration (95% C4═O:5% C4-N3). The absorption at 1617 cm^{-1} is assigned to a C5═C6 stretch mode. The measured IR spectra of 5′-CMP show three major absorptions in the 1750–1550 cm^{-1} region: 1652 cm^{-1} (predominantly C2═O stretch); 1617 cm^{-1} [assigned as predominantly 70% C5═C6:30% C6-N1 stretches] and 1585 cm^{-1} (predominantly C4═N3 stretch) (see Table 1 and Fig. 1). The measured IR spectrum of 5′-GMP shows four major absorptions (Table 1 and Fig. 1) in the 1750–1550 cm^{-1} spectral region: 1675 cm^{-1} [predominantly, 80% C6═O stretch:20% C6-N1 stretch (Miyazawa et al., 1958)]; 1650 cm^{-1} (predominantly C4═C5 stretch); 1577 cm^{-1} (predominantly C7═N8 stretch); and 1565 cm^{-1} [predominantly, 60% in-plane N1-H bend:40% C2-N1 stretch (Miyazawa et al., 1958)]. The modes as assigned, above, imply relatively localized motions such as would occur in isolated, vibrational model systems. In these assignments we assume that the point oscillator dipole of interest is localized at the geometric center of the bonds (or bond) described, with the major contribution as indicated. For each oscillator, the approximate net dipole oscillator direction is shown in Fig. 1, and its approximate location (**R**$_i$) is assumed to be at the geometric center of the contributing bond(s).

TABLE 1 DeVoe theory Lorentzian* input band parameters

Base#	—Oscillator—		$\nu_{\max}$ (cm^{-1})	$\epsilon_{\max}$ (M^{-1} cm^{-1})	Δ (cm^{-1})
A	I	C5═C4—N3	1623	850	10
	II	C8═N7	1576	200	10
C	I	C2═O	1652	1000	14
	II	C5═C6—N1	1617	470	12
	III	C4═N3	1585	180	15
G	I	N1—C6═O	1675	980	15
	II	C4═C5	1650	225	15
	III	C8═N7	1577	225	15
	IV	C2—N1—H	1565	225	14
U	I	N1—C2═O	1697	640	13
	II	N3—C4═O	1655	1210	13
	III	C5═C6	1617	240	12

*A Lorentzian band as a function of frequency, ν, is defined: $\varepsilon_\nu = \varepsilon_{\max}\Delta^2/[\nu - \nu_{\max}]^2 + \Delta]$, where $\varepsilon_{\max}$, $\nu_{\max}$, 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: $D = 0.92 \times 10^{-38}\ \varepsilon_{\max}\pi\Delta/\nu_{\max}$.

#See Fig. 1.

Measured spectra

Measured ϵ and $\Delta\epsilon$ spectra for poly(rA) and poly(rU) were taken from Yang and Keiderling (1993). Measured ϵ and $\Delta\epsilon$ spectra for poly(rG) and poly(rC) were computed from the measured A and ΔA spectra published by

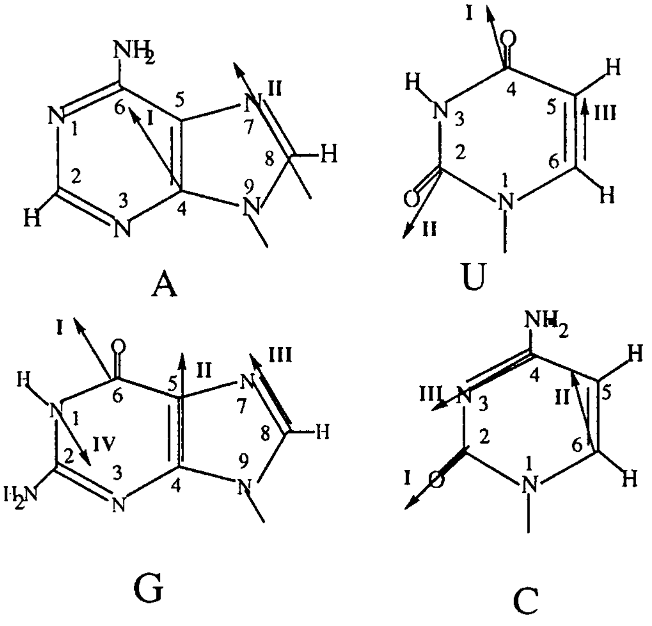

FIGURE 1 Structures and numbering systems for the nucleic acid bases. The directions and magnitudes of the oscillator vectors are not to scale and their directions are drawn approximately.

Annamalai and Keiderling (1987) (using these workers' estimates of sample pathlengths and concentrations). As pointed out by Annamalai and Keiderling (1987), concentrations and pathlengths used for the A and ΔA measurements were not precisely known; thus the calculated ϵ and $\Delta\epsilon$ spectra are only approximations to the actual spectra.

RESULTS AND DISCUSSION

Poly(rA) spectra

Measured VCD and absorption spectra for poly(rA) have been reported previously (Annamalai and Keiderling, 1987; Tsuboi et al., 1973; Xiang et al., 1993; Yang and Keiderling, 1993). Fig. 2 shows DeVoe theory calculated VCD (*top*) and absorption (*bottom*) spectra for A-form poly(rA) geometry (Arnott et al., 1972) compared with measured spectra of Yang and Keiderling (1993). Overall it is observed that the measured and calculated VCD and absorption spectra (Fig. 2) are in good agreement. However, the measured IR absorption spectrum of Yang and Keiderling (1993) (Fig. 2, *bottom*) shows a peak, near 1665 cm^{-1}, which does not appear in the measured poly(rA) absorption spectra reported elsewhere (Tsuboi et al., 1973; Xiang et al., 1993; Annamalai and Keiderling, 1987). Because no adenine normal mode assignments have been made previously for this 1665 cm^{-1} region, no attempts have been made, in the present calculations, to include such a band. Aside from this 1665 cm^{-1} region, the remainders of the measured spectra (Fig. 2) are virtually the same as reported elsewhere (Tsuboi et al., 1973; Xiang et al., 1993; Annamalai and Keiderling, 1987). Additionally, the poly(rA) spectra measured by Yang and Keiderling (1993) are reported in extinction units (ϵ and $\Delta\epsilon$) and are of better quality than those reported elsewhere. Thus we choose to compare our calculated spectra with the measured spectra of Yang and Keiderling (1993).

Xiang et al. (1993) have calculated rotational and dipole strengths, in the C$=$C stretching region, for poly(rA) in A-form RNA geometry (Arnott et al., 1972), using an extension of the coupled oscillator theoretical principles developed by Tinoco (1962, 1963, 1964). The resulting calculated rotational and dipole strengths from this so-called degenerate extended coupled oscillator (DECO) theory were fit to assumed band shapes and then compared with measured spectra in the 1660–1600 cm^{-1} region (Xiang et al., 1993). These calculated A-form poly(rA) results of Xiang et al. (1993) agree qualitatively with the DeVoe theory poly(rA) spectra shown in Fig. 2. Additionally, previous DeVoe theory UV CD calculations on poly(rA) (Moore and Williams, 1986) are in agreement with a single-stranded A-RNA geometry for poly(rA) in mild aqueous media.

Shown in Fig. 3 are calculated VCD and absorption spectra for oriented poly(rA) in single-stranded A-RNA conformation. The calculated VCD and absorption spectra (Fig. 3) show that the VCD (compare Figs. 2 and 3, *top*) of poly(rA) in the 1700–1550 cm^{-1} spectral region is due, predominantly, to light propagating parallel to the helix axis, whereas the absorption spectrum (compare Figs. 2 and 3, *bottom*) is due predominantly to light polarized perpendicular to the helix axis. The linear dichroism (Eq. 3) for the oriented helix can be easily calculated (from the spectra shown in Fig. 3, *bottom*) by subtracting the spectrum for light polarized perpendicular (ϵ_{perp}) to the helix axis from the absorbance for light polarized parallel (ϵ_{para}) to the helix axis. Thus the linear dichroism for poly(rA) is predicted to

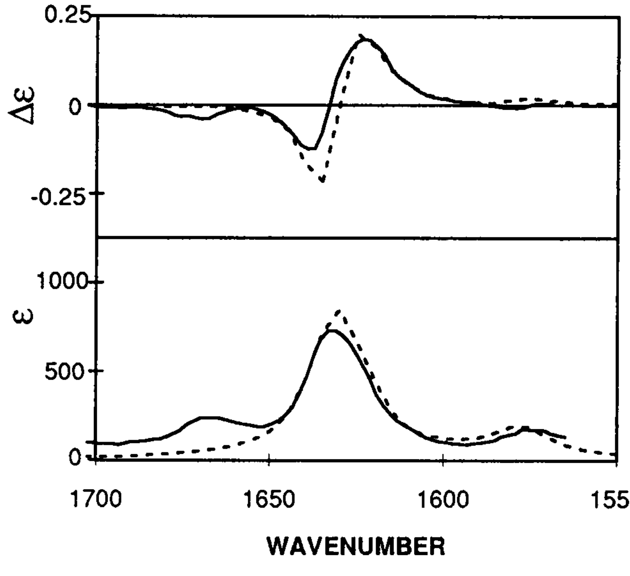

FIGURE 2 Measured (——) and calculated (– – –) poly(rA) VCD (*top*) and absorption (*bottom*) spectra per nucleotide. Calculated spectra are in single-stranded A-RNA geometry (Arnott et al., 1972); measured spectra are from Yang and Keiderling (1993).

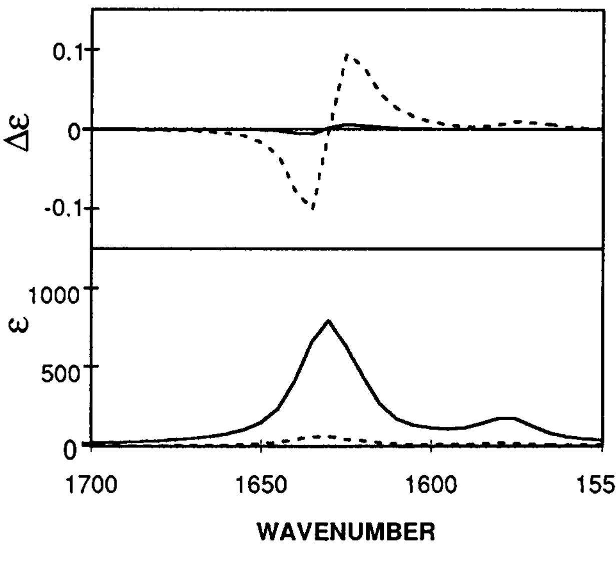

FIGURE 3 Calculated oriented poly(rA) VCD and absorption spectra for single-stranded A-RNA geometry (Arnott et al., 1972). VCD (*top*): Light incident parallel (– – –) and perpendicular (——) to the helix axis; absorption (*bottom*): light polarized parallel (– – –) and perpendicular (——) to the helix axis.

be negative over the entire 1700–1550 cm^{-1} spectral region. No measured LD spectra have been reported for poly(rA) in the 1700–1550 cm^{-1} spectral region.

Poly(rC) spectra

There is some controversy over the geometry of poly(rC) in mild aqueous media. Based on 2D proton-NMR results, Broido and Kearns (1982) have suggested a single-stranded, left-handed helical geometry for poly(rC), whereas, based on DeVoe theory UV CD calculations, Williams and Moore (1983) have suggested a single-stranded, right-handed helical geometry similar to the x-ray diffraction-determined geometry for poly(rC) (Arnott et al., 1976). Previous simple coupled-oscillator VCD rotational strength calculations—using only C=O stretches—by Annamalai and Keiderling (1987) for dimeric (CpC) structures were not able to distinguish between the proposed left- and right-handed geometries. Using DeVoe theory, VCD spectral calculations, and only C=O stretches, we have obtained results similar (not shown) to those of Annamalai and Keiderling (1987): the calculated VCD spectra for the left- and right-handed models [CpC and poly(rC)] were found to be consignant (same sign and shape); the A-form models produced higher intensity VCD spectra. Xiang et al. (1993), using their DECO approach, calculated (VCD) rotational strengths and (absorbance) dipole strengths for poly(rC) in A-form RNA geometry. Their calculations were made using C=O and C=C stretches, with the (somewhat artificial) assumption that the C=O and C=C transitions do not interact. The separate C=O and C=C calculations were then added together (Xiang et al., 1993) to give fitted VCD of poly(rC) with correct band signs, but with essentially no overlap—contrary to measurement—between the C=O and C=C absorption bands.

Shown in Fig. 4 are DeVoe theory calculated VCD (*top*) and absorption (*bottom*) spectra compared with the measured poly(rC) spectra of Annamalai and Keiderling (1987). Calculations were made for the proposed single-stranded, left-handed geometry (Broido and Kearns, 1982; Fig. 4, *long dashes*) and the right-handed geometry (Arnott et al., 1976; Fig. 4, *short dashes*) of poly(rC). The major absorbances—C=O, C=C, and C=N stretches—appearing in the 1750–1550 cm^{-1} region were included in the calculations with no restrictions on interactions between them. It is noted (Fig. 4) that the DeVoe theory calculated VCD spectra–with C=O, C=C, and C=N stretches included—do distinguish between the proposed left-handed (Broido and Kearns, 1982) and right-handed (Arnott et al., 1976) poly(rC) geometries. Thus, including contributions from the nearby (in frequency) C=C (and, to a minor extent, the C=N) stretches is important for these calculations. The calculated VCD spectrum—with C=O, C=C, and C=N stretches included—for the single-stranded, right-handed A-RNA conformation (Arnott et al. (1976), is seen (Fig. 4, *top*) to be in good agreement with the measured VCD spectrum of poly(rC), whereas that for the Broido and Kearns (1982) left-handed geometry is not. Furthermore, the calculated absorption spectra (differing only in intensity; Fig. 4, *bottom*) for both the left-handed and right-handed geometries are seen to reproduce the overlapping transitions observed in the measured poly(rC) absorbance spectrum (Fig. 4, *bottom*), contrary to the DECO calculations of Xiang et al. (1993). The relative intensity differences between the calculated VCD spectra (Fig. 4, *top*) of the two poly(rC) models can be easily explained by the calculated spectra of the oriented poly(rC) models (Figs. 5 and 6).

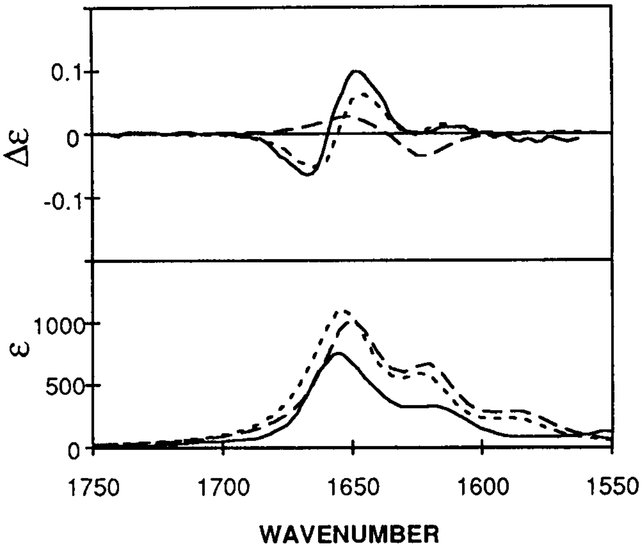

FIGURE 4 Measured and calculated poly(rC) VCD (*top*) and absorption (*bottom*) spectra per nucleotide: measured (———) ϵ and $\Delta\epsilon$ spectra converted from measured A and ΔA spectra of Annamalai and Keiderling (1987); calculated spectra in single-stranded right-handed (- - -) poly(rC) geometry (Arnott et al., 1976) and single-stranded left-handed (– – –) poly(rC) geometry (Broido and Kearns, 1982).

Shown in Figs. 5 and 6 are calculated VCD and absorption spectra for the oriented right-handed and left-handed poly(rC) models, respectively. In the 1750–1550 cm^{-1} spectral region, the highly stacked A-RNA conformation for poly(rC) yields a DeVoe theory-oriented VCD (Fig. 5, *top*) due, predominantly, to light propagating parallel to the helix axis. Because of the highly stacked nature of the A-RNA conformation, the absorption spectrum is seen (Fig. 5, *bottom*) to be dominated by light polarized perpendicular to the helix axis. In contrast, the bases in the left-handed Broido and Kearns (1982) geometry are very much less stacked (i.e., the bases are located on the outside of the helix) and are oriented so that the low intensity VCD calculated for light propagating perpendicular to the helix axis predominates (Fig. 6, *top, solid line*). Hence summation of these oriented VCD spectra leads to the low-intensity isotropic VCD spectrum (Fig. 4, *top, long dashes*) for left-handed poly(rC), which is similar to the spectrum for light polarized perpendicular to the helix axis. Thus—from Figs. 5 and 6 (*bottom*)—the linear dichroism ($\epsilon_{\text{para}} - \epsilon_{\text{perp}}$) for the A-RNA conformation can be predicted (Fig. 5, *bottom*) to be of a large negative magnitude, over the entire spectrum, whereas the linear dichroism for the left-handed conforma-

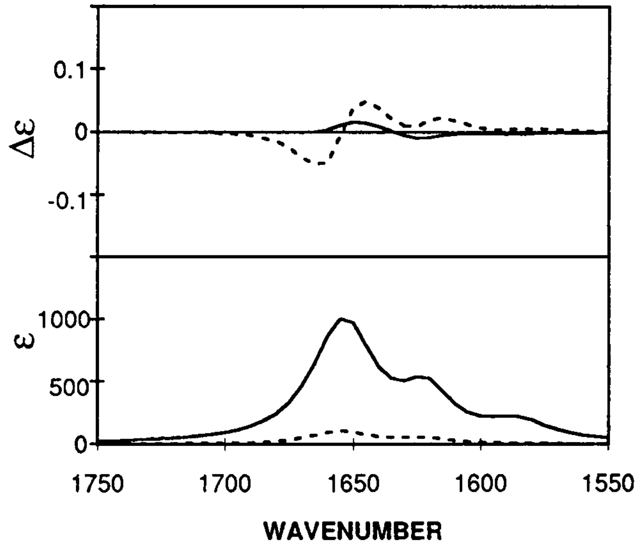

FIGURE 5 Calculated VCD and absorption spectra per nucleotide for oriented poly(rC) in right-handed single-stranded poly(rC) (Arnott et al., 1976) geometry. VCD (*top*): Light incident parallel (– – –) and perpendicular (——) to the helix axis; absorption (*bottom*): light polarized parallel (– – –) and perpendicular (——) to the helix axis.

tion is predicted (Fig. 6, *bottom*) to be of a relatively small positive magnitude in the region of the C═O vibration and a relatively large negative magnitude over the C═C and C═N vibrations.

Poly(rG) spectra

Guanine residues have been reported to be particularly prone to self-aggregration, even in short oligonucleotides. Gray and Bollum (1974) have concluded from UV CD studies on oligo and poly(dG) that these molecules exist in two forms in mild aqueous media: a single-stranded and an ordered, self-complexed, multistranded form. In an immunological study of poly(rG), Souleil and Panijel (1968) found evidence for the simultaneous existence of two forms of this polymer in aqueous solution: a single-stranded and a four-stranded form. DeVoe theory UV CD calculations by Moore and Williams (1986) on weighted averages of specific ratios of single-stranded, right-handed A-form poly(rG) and left-handed, four-stranded, poly-(rG) produced a calculated weighted-average UV CD spectra similar to the measured UV CD spectrum for poly-rG.

Shown in Fig. 7 are DeVoe theory calculated VCD (*top*) and absorption (*bottom*) spectra compared with measured spectra of Annamalai and Keiderling (1987). The geometries used for the calculated poly(rG) spectra, shown in Fig. 7, are right-handed, four-stranded (*long dashes*) (Arnott et al., 1974), single-stranded A-RNA (*short bold dashes*) (Arnott et al., 1972), and left-handed four-stranded (Zimmerman et al., 1975) (*short dashes*). There is very little difference between the calculated VCD absorption spectra (Fig. 7, *bottom*) for the three different poly(rG) geometries, and they compare favorably with the measured absorption spectrum. In the case of the calculated VCD spectra, it can be seen (Fig. 7, *top*) that the right-handed, single-stranded and right-handed, four-stranded geometries yield spectra with the same signs of VCD as the measured VCD spectrum over most of the 1700–1550 cm^{-1} spectral region. (The small negative VCD band measured near 1610 cm^{-1} does not appear in the calculated spectra shown in Fig. 7. This small

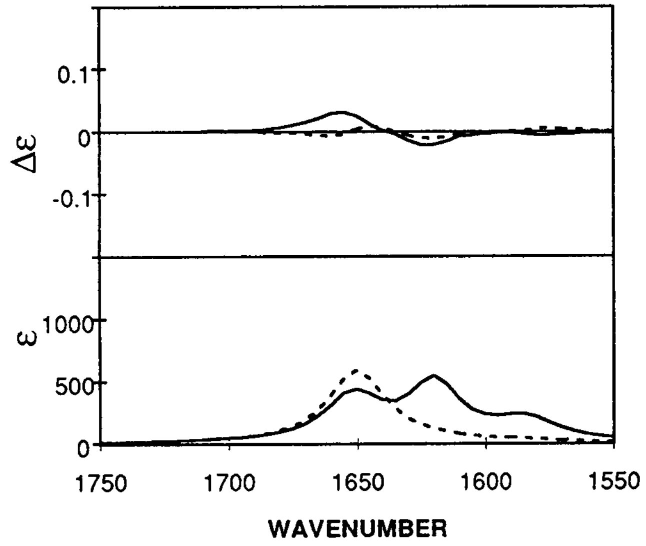

FIGURE 6 Calculated VCD and absorption spectra per nucleotide for oriented poly(rC) in left-handed, single-stranded poly(rC) (Broido and Kearns, 1982) geometry. VCD (*top*): light incident parallel (– – –) and perpendicular (——) to the helix axis; absorption (*bottom*): light polarized parallel (– – –) and perpendicular (——) to the helix axis.

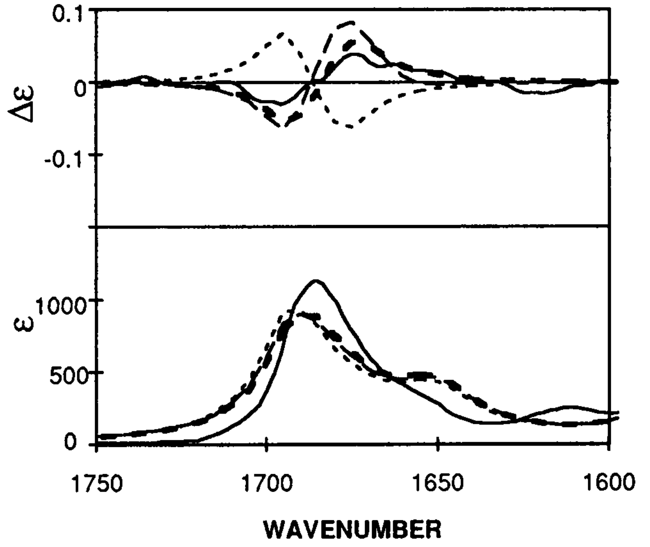

FIGURE 7 Measured and calculated poly(rG) VCD (*top*) and absorption (*bottom*) spectra per nucleotide. Measured (——) ϵ and $\Delta\epsilon$ spectra converted from measured A and ΔA spectra of Annamalai and Keiderling (1987); calculated spectra in right-handed, four-stranded (*long dashes*) poly(rG) geometry (Arnott et al., 1974); right-handed, single-stranded A-RNA (*bold dashes*) geometry (Arnott et al., 1972); and left-handed, four-stranded (*short dashes*) poly(rG) geometry (Zimmerman et al., 1975).

negative band may be due to an overtone or combination band not considered in the current calculations. Thus, in the following, this band is ignored in making spectral comparisons.) Any weighted-average VCD spectrum constructed from the right-handed single and four-stranded spectra would always have the same band signs but greater intensity than the measured VCD spectrum. The VCD band signs and magnitudes of the calculated poly(rG) spectrum could be brought into closer agreement with the measured VCD spectrum, if a weighted-average VCD spectrum were calculated from the calculated VCD spectra of the right-handed single-stranded and left-handed four-stranded geometries, as in previous UV CD calculations by Williams and Moore (1983). An example of such a weighted-average spectrum (80:20, single-strand: four-stranded) showing good agreement with the measured VCD spectrum is displayed in Fig. 8. Previous calculations by the DECO theory (Xiang et al., 1993) showed complete disagreement between the calculated and the measured (mild aqueous media) poly(rG) spectrum.

The oriented VCD and linear dichroism spectra (not shown) for these well-stacked poly(rG) helices are similar to those of the well-stacked helices considered previously: the oriented VCD spectra for light propagating parallel to the helix axis is much greater than that for light propagating perpendicular to the helix axis, and the LD ($\epsilon_{para} - \epsilon_{perp}$) is of a large negative magnitude over the entire 1700–1550 cm^{-1} spectral region.

Poly(rU) spectra

Brahms (1963) has measured the UV CD of poly(rU), in mild aqueous media, as a function of temperature. At room temperature, the UV CD of poly(rU) was found to be similar to the spectrum of its monomer, indicating an absence of substantial long-range poly(rU) secondary structure. UV CD calculations (Moore and Williams, 1986) on double-stranded and single-stranded polymer structures of poly(rU), as well as monomer structures of rU, indicate—in agreement with measurements—that a single-stranded A-RNA type solution geometry predominates for poly(rU) only at temperatures near 3°C. As the temperature is increased from 3°C toward room temperature, the strong-intensity UV CD, characteristic of long-range poly(rU) secondary structure, gradually diminishes toward a weaker intensity spectrum that is consignant with the 3°C spectrum. This indicates that an equilibrium exists between constantly fluctuating regions of helically stacked U residues (due to base-base hydrophobic interactions) and regions of randomly coiled U residues. In such a situation, it is expected that the UV CD (as well as the VCD) of randomly coiled poly(rU) regions will be small (because of random orientations) relative to the CD and VCD from the helically stacked regions. Therefore, the observed room temperature solution UV CD and VCD spectra for poly(rU) should be, basically, measures of the amount of helically stacked, room-temperature poly(rU) structure.

Shown in Fig. 9 are DeVoe theory calculated VCD (*top*) and absorption (*bottom*) spectra compared with measured spectra of Annamalai and Keiderling (1987). The geometry used for the calculated poly(rU) spectra is single-stranded, A-form RNA (Arnott et al., 1972). The relatively large-intensity calculated VCD (Fig. 9, *top*) for poly(rU) is observed to be approximately consignant with the weaker intensity measured VCD spectrum. The relatively weak intensity of the measured poly(rU) VCD spectrum indicates, consistent with previously mentioned UV CD calculations and measurements, that little long-range secondary structure

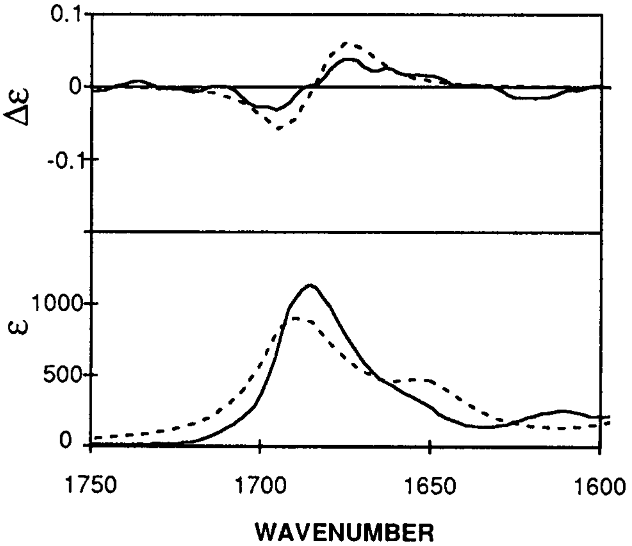

FIGURE 8 Calculated poly(rG) weighted-average (80:20, right-handed, single-stranded:left-handed, four-stranded) VCD spectrum (– – –) compared with measured spectrum (——) from Annamalai and Keiderling (1987).

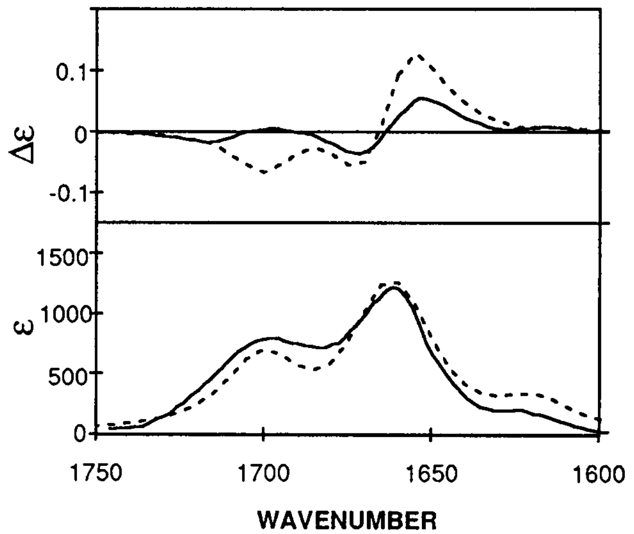

FIGURE 9 Measured (——) and calculated (– – –) poly(rU) VCD (*top*) and absorption (*bottom*) spectra per nucleotide. Calculated spectra are in single-stranded A-RNA geometry (Arnott et al., 1972); measured spectra are from Yang and Keiderling (1993).

exists for poly(rU) at room temperature. However, the consignancy of the calculated and measured VCD spectra indicates that whatever short-range order secondary structure does exist is stacked in a manner similar to that of the uracil residues in A-RNA geometry. The calculated absorption spectrum for single-stranded poly(rU) in A-RNA geometry is seen (Fig. 9, *bottom*) to be in good agreement with the measured spectrum, being approximately isochromic with the measured spectrum.

CONCLUSION

For structural models indicated to exist, in aqueous solution, by other techniques of measurement, the DeVoe theory calculated VCD and IR extinction spectra for the homopolyribonucleotides poly(rA), poly(rC), poly(rG), and poly(rU) have been found to be in good agreement (in the 1700–1550 cm^{-1} spectral region) with the VCD and IR extinction spectra measured in mild aqueous media. The calculations reported herein point out the necessity of employing correct model structure analyses when trying to correlate calculated VCD (or, in general, CD) spectra with measured VCD (or CD) spectra, especially when the VCD (or CD) basis set of parameters is not well established. At the very least, in assigning a particular structure (or structures) as the source of a particular VCD (or CD) spectrum, one must show that there is also good agreement between the measured and calculated extinction (absorption) spectrum. Overall, the agreements found between the measured homopolyribonucleotide VCD and extinction spectra and those calculated by the DeVoe theory (for model structures shown to exist under the conditions of measurement by other techniques) are found to be much better than agreements inferred previously from calculation.

As reported herein, the calculated oriented VCD and LD spectra are important for showing the dominant structural orientations contributing to the isotropic VCD and extinction spectra. In future calculations, e.g., for heteropolynucleotides, particularly in the spectral regions for phosphate group vibrations, these types of calculations—particularly calculated LD spectra—will be of great importance in obtaining additional structural information on nucleic acid geometries in solution.

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