vibrational spectra in terms of chromophore structure in other retinal pigments such as rhodopsin, halorhodopsin, and sensory rhodopsin.

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Supplementary Material Available: The Cartesian coordinates used in the calculations and a complete description of the force field will be found in Tables XIV and XV (7 pages). Ordering information is given on any current masthead page.

Vibrational Circular Dichroism of Poly(ribonucleic acids). A Comparative Study in Aqueous Solution

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Abstract: Vibrational circular dichroism data of several synthetic polyribonucleotides have been measured at neutral pH and room temperature in the 1750–1550-cm^{-1} region with sodium cacodylate buffer in D_2O as a solvent and are compared to similar data for monomers and dimers. Polynucleotides studied include homopolymers, some random copolymers, and two double stranded RNAs. The mononucleotides yield no significant VCD whereas, in most cases, the polymers have relatively larger, conservative bisignate VCD signals. The VCD magnitudes of the homodimers, ApA and CpC, are significantly smaller than those of the corresponding polymers but have the same sign pattern. This pattern is consistent with the result of coupled oscillator calculations for these two dimers. VCD of poly(C) has also been measured as a function of temperature and pD. Variation in VCD band shape and magnitude can be correlated to base stacking, base pairing, and degree of order.

Vibrational circular dichroism (VCD) has developed over the past decade from its initial status as an unusual physical phenomenon to one that can be routinely measured on a variety of compounds over a wide spectral range.[1-5] Stereochemists have long appreciated that the multiple, local chromophores accessible with VCD (or the complementary Raman circular intensity differential[2]) offer a potential source of new experimentally derived information about solution-phase molecular conformation. This promise is beginning to be realized via both theoretical and empirical analyses of the spectra.[6-9]

One field of seemingly useful application for VCD is that of biopolymer conformation. Several studies of polypeptide and oligopeptide VCD and the relationship of that data to secondary structure have appeared from our and other laboratories.[9] Until this paper, no parallel work on nucleic acids has appeared. Here we report the first VCD measurements of riboxy-dinucleoside monophosphates and polynucleotides which were made on synthetic samples studied in neutral aqueous solution in the base stretching (C=O, C=C, C=N) region, 1750–1550 cm^{-1}. Our results will be correlated to previous conformational studies on these species.

While extensive use of electronic CD has been made to interpret nucleic acid base stacking, conformational change, and duplex formation,[10] the parallel application of infrared (IR) spectroscopy has been less extensive.[11,12] This perhaps results from the small

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Table I. Absorption Frequency (ν_{max}), VCD Zero-Crossing Frequency (ν_0), Anisotropy Factor ($\Delta A/A$), and Experimental Parameters[a]

sample and source[b]	ν_{max} (cm^{-1})	ν_0 (cm^{-1})	$\Delta A/A^c \times 10^4$	concn (mg·mL^{-1}); pD; and absorbance
ApA, s	1625	1625	0.32	50; 7.7; 0.4
CpC, s	1653	1650	0.46	50; 7.1; 0.3
UpU, s	1657	1657	0.42	50; 7.3; 0.4
poly(A), s	1629	1630	2.6	20; 7.3; 0.1
poly(C), s	1655	1658	2.0	40; 7.6; 0.2
poly(I), s	1684	1686	5.7	30; 7.5; 0.2
poly(G), s	1684	1684	0.60	30; 7.6; 0.2
poly(U), p	1659	1663	0.56	50; 7.7; 0.4
poly(A,C), p	1650, 1625	1654	1.2	40; 7.7; 0.1
poly(C,U), p	1657	1657	1.1	50; 7.2; 0.2
poly(I,C), p	1691, 1650	1695	4.8	40; 7.3; 0.2
poly(A,U), p	1670, 1627	1671	4.7	35; 7.1; 0.1
poly(I)·poly(C), p	1694, 1647	1696	6.0	40; 7.4; 0.2
poly(A)·poly(U), p	1672, 1631	1671	7.2	40; 7.1; 0.2

[a] Resolution is 11 cm^{-1} for all experiments. [b] s–Sigma Chemical Company; p–P. L. Biochemicals, Inc. [c] Measured as peak-to-peak ΔA divided by the maximum A.

effects of stacking on IR accessible base stretching frequencies and on the difficulty of doing IR experiments on samples in aqueous solution. Our VCD results, on the other hand, show strong effects of base stacking and associated conformational changes. The bases can be thought of as achiral, planar aromatics coupled to a relatively distant chiral backbone. Stacking effects are due to a through-space coupling that is largely dipolar in nature, and as such, it has a large effect on electronic spectra but a relatively small one on IR spectra due to the relative size of the transition dipoles involved. Due to the base planarity, we expect to find only weak VCD in the C=O, C=N, and C=C stretching modes of the monomers. However, in dimers and polymers, the small coupling, that is difficult to establish in IR absorption, is expected to give rise to a significant bisignate VCD. We indeed find that this is true for many of the nucleic acids we have studied. To probe this source of VCD further, we have attempted to calculate the VCD with the coupled oscillator model[13] considering two adjacent residues from polymeric structure. In this paper, these experimental and calculational results are discussed in light of previously available data on the relevant nucleic acid conformations and vibrational modes.

Experimental Section

VCD measurements have been made for the following 5′-nucleoside monophosphates, AMP, CMP, UMP, and GMP; 3′→5′-dinucleoside monophosphates, ApA, CpC, and UpU; homopolynucleotides, poly(A), poly(C), poly(U), poly(G), and poly(I); random copolynucleotides, poly(A,C), poly(U,C), poly(A,U), and poly(I,C); and double-stranded polynucleotides, poly(A)·poly(U) and poly(I)·poly(C) (where the conventional abbreviations are used: A = adenine, C = cytosine, U = uracil, G = guanine, and I = inosine). All the polymers used have molecular weights >100 000. In the copolymers, the bases are stated to be in a nearly 1:1 ratio but occur in a random sequence. These samples were purchased from Sigma Chemical Co. and P. L. Biochemicals, Inc. and were used without further purification. The monomers and polymers used were either sodium or potassium salts, but the dimers were ammonium salts and were dissolved in D_2O and lyophilized before the solutions were prepared for VCD measurements.

Typical IR-compatible solvents such as $CHCl_3$, CCl_4, etc., are poor solvents for nucleic acids and hence aqueous solutions must be used. Since D_2O is more transparent than H_2O for the 1750–1550-cm^{-1} region, sodium cacodylate buffer, prepared with 0.05 M cacodylate and 0.1 M NaCl in D_2O, was used as the solvent. Nucleic acid solutions of about 100 μL were prepared in the buffer with 30–50 mg/mL concentration. The pD of each solution was then obtained with use of a micro-combination electrode (Ingold) and a Corning 145 pH meter. The solutions were titrated with NaOD or DCl solutions in D_2O to bring the pH meter reading within the 6.7–7.3 range corresponding to pD ~ 7.1–7.7 [pD = pH(meter) + 0.4].[14,15] (For neutral D_2O, the pD is estimated to be 7.4.[15]) Solutions of some polymers are very viscous and, sometimes, they even appear to gel at the concentrations used. Furthermore poly(G) and GMP are known to form aggregates in solution.[16] Both the gelled and aggregated samples described above could not be significantly diluted because of the requirements of small path length for VCD measurement (see below). Poly(I) was difficult to dissolve and had to be warmed to obtain a homogeneous solution. When the solution was cooled no precipitation was found.

The absorption and VCD spectra were measured with use of the previously detailed UIC dispersive VCD instrument[1] and a Presslok IR sample cell (Barnes Engineering Co.) composed of CaF_2 windows separated by a Teflon spacer. We have found that a 15–25 μm path length is optimal for VCD measurements in the 1750–1550-cm^{-1} region with these D_2O-based solutions. Need for such a small path length for VCD has prohibited us from studying more dilute samples, although path lengths as long as 50 μm have been used in previous IR absorption measurements.[11,12] To obtain higher resolution IR spectra and to estimate molar extinction coefficients of CMP and AMP for our coupled oscillator calculations, we used a Digilab FTS-60 FTIR spectrometer.

To prepare the sample, a small amount of solution (~20 μL) is first placed on one of the windows, and then a Teflon spacer and the other window are carefully placed over the sample and the windows are pressed together in the sample holder. Care was taken so that no air bubbles were trapped in the viscous sample. The actual path length was not known accurately, as we believe that it depended on how the windows are pressed together. Hence, the data presented here are in terms of absorbance (A and ΔA). Extinction coefficients for many of these nucleic acids are available.[17] Peak absorbances of the polymers measured (except poly(U)) were in the range of 0.1–0.2 absorbance units whereas those for the dimers and poly(U) were between 0.3 and 0.4 to enhance the signal. Absorbances specific to the data presented as well as concentrations used are given in Table I. Small values were used because of path length and concentration limitations and to minimize absorption artifacts. However, for ease of comparison of VCD spectra, we have normalized all the figures presented to a peak absorbance of 1.0 which, for that band, allows the VCD to be directly read off in terms of $\Delta A/A$. For the purpose of obtaining VCD base lines, identical scans of the solvent buffer have been used in the case of the polymers. However, in the case of dimers, and as a check for some of the homopolymers, the VCD base lines were obtained with use of monomer scans. This latter method directly subtracts the monomeric contribution from the VCD. For the polymers with large VCD (see results) little difference was found with use of monomers as base lines.

Temperature-dependent VCD spectra of poly(C) and poly(I) were measured at a variety of temperatures between 10 and 70 °C. For these experiments, a variable-temperature cell, designed in our laboratory, was used which has demountable CaF_2 windows; and temperature was controlled by circulating water from a Neslab thermostated bath. Additionally, pD-dependent spectra were measured for poly(C) in solution adjusted to various pD values between 6.1 and 7.3. For this experiment,

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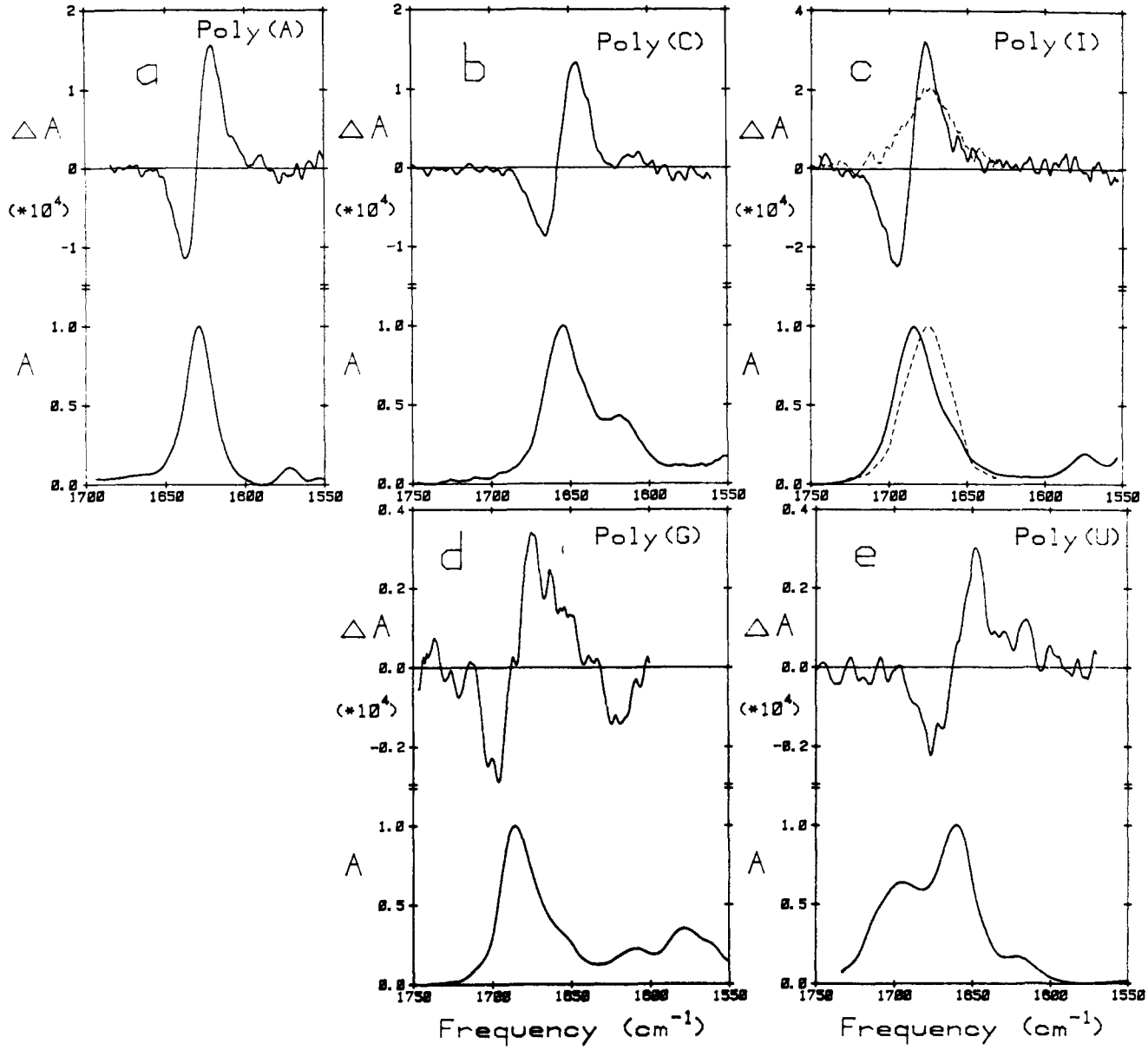

Figure 1. Absorption and VCD spectra of homopolyribonucleotides in D_2O buffer (0.05 M NaCacodylate + 0.1 M NaCl) at 11 cm^{-1} resolution; the plots are for (a) poly(A), VCD with 3 s time constant and AMP base line; (b) poly(C), VCD with 3 s time constant and D_2O buffer base line; (c) poly(I) at 25 °C (—) and 60 °C (---), VCD with 3 s time constant and D_2O buffer base line; (d) poly(G), VCD with 10 s time constant and GMP base line; and (e) poly(U), VCD with 10 s time constant and UMP base line. All VCD measurements were averaged for 4 scans. An offset of ~10% peak-to-peak ΔA has been used in making these plots. For further experimental parameters, see Table I. Note that poly(I) is plotted with twice and poly(U) and poly(G) one-fifth the ΔA scale of poly(A) and poly(C).

we could not add DCl directly to a stock solution to reduce the pD because this results in precipitation at these high concentrations. Alternatively, we added various amounts of DCl to the solvent buffer itself and then prepared fresh sample solutions with these pD-altered solvents by slowly adding poly(C). The pD of the resulting solution was then recorded and VCD was measured. For example, the buffer solvent whose pD was altered to 2.4 by DCl addition yielded a solution of poly(C) with pD 6.1. In fact, no measurements were possible below this value because the solution gels.

Results

Spectra. The main absorption features in the 1750–1550-cm^{-1} region arise from base modes; for each case considered, the maximum occurs at (A) 1625, (U) 1657, (C) 1652, (G) 1666, and (I) 1672 cm^{-1}.[11,17,18] These bands have been assigned to have a large contribution from C=O stretch vibrations for U, G, I, and C and from C=C stretches for A. In the case of U, the other C=O stretching band is less intense and occurs at 1691 cm^{-1}. The monomers AMP, CMP, UMP, and GMP yield no measurable VCD to the noise and base line artifact limits ($\Delta A/A < 2 \times 10^{-5}$) of these aqueous solution experiments. Thus we will not discuss the monomer results further.

Spectra for poly(A), poly(C), poly(I), poly(G), and poly(U) are shown in Figure 1. All these polymers give VCD having a bisignate pattern with negative to higher and positive to lower energy (which can be termed a positive couplet[19]). Of these systems, poly(I) clearly gives the largest VCD being about 2–3 times larger than poly(A) or poly(C) which are comparable ($\Delta A/A \sim 2 \times 10^{-4}$, peak-to-peak). Poly(U) and poly(G) both had weak VCD in this region with comparable magnitudes ($\Delta A/A \sim 5 \times 10^{-5}$), and it was necessary to measure their VCD against the respective monophosphates to suppress artifacts. Since GMP and poly(G) differ in absorbance at the lower energy portion of this region (~1620 cm^{-1}), there may be a residual artifact in that band. The large difference in the poly(G) and poly(I) VCD magnitudes suggests that, though they have very similar base structures and IR absorption spectra, their conformations must

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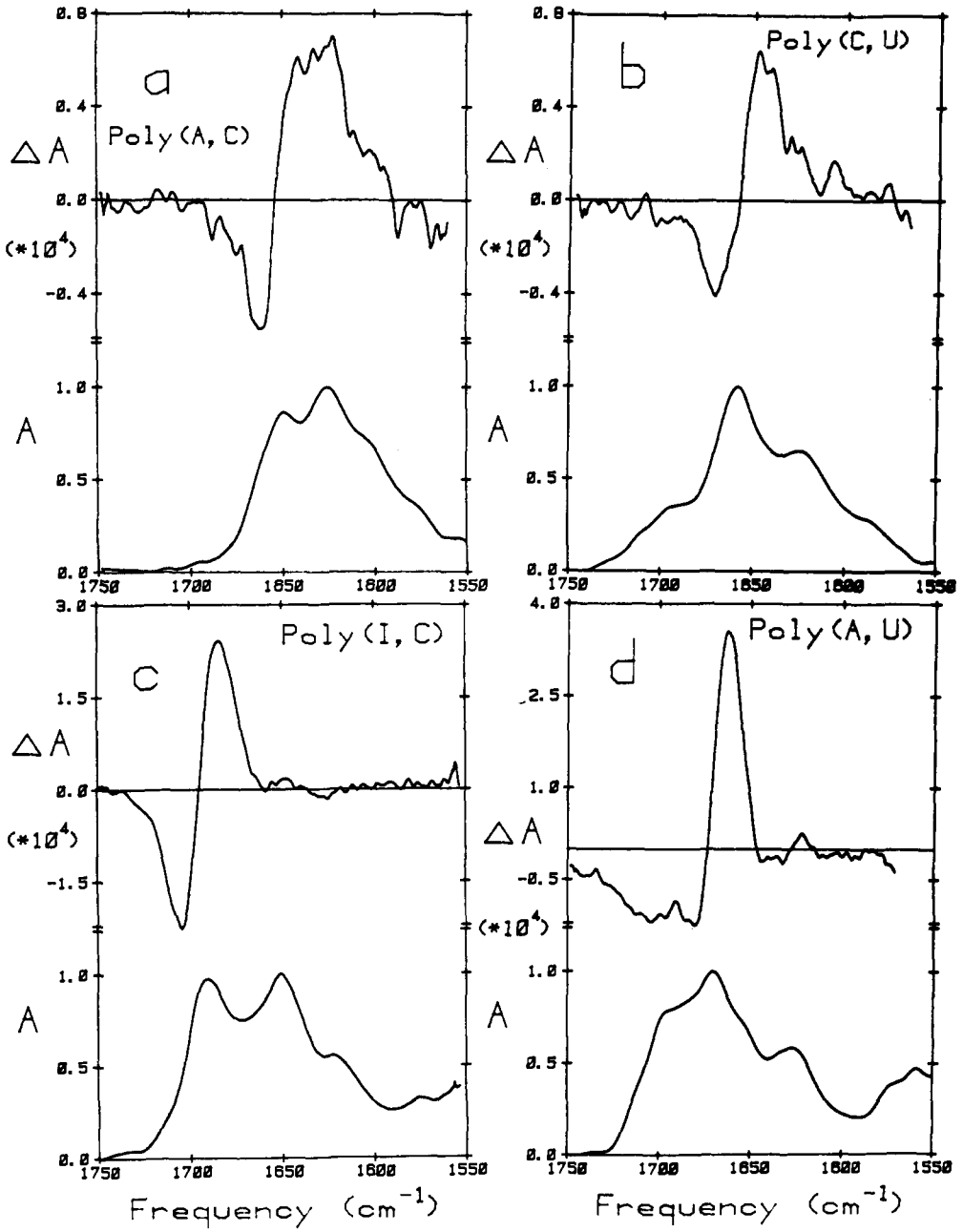

Figure 2. Plots of absorption and VCD for random copolyribonucleotides in D_2O buffer (as in Figure 1) at 11-cm^{-1} resolution: (a) poly(A,C), (b) poly(C,U), (c) poly(I,C), and (d) poly(A,U). VCD data collected against D_2O buffer (base line) at 3 s time constant were averaged for 4 scans. Offset in ΔA used in these plots is $<1.5 \times 10^{-5}$. Note the ΔA scale change in plots c and d. Further experimental details are in Table I.

significantly differ in some respect (see Discussion).

The random copolymers studied (Figure 2) give different patterns and are much less systematic. Poly(C,U) and poly(I,C) give rise to nearly conservative couplets but of quite different magnitudes. The poly(I,C) VCD has a strong positive couplet at 1695 cm^{-1} which is somewhat smaller in magnitude but almost identical in shape to that of poly(I). Hence the poly(I,C) VCD correlates well to the inosine absorption band at ~1691 cm^{-1} but not at all to the cytosine band at ~1650 cm^{-1}. This makes it appear that the cytosine mode is partially quenched in the poly(I,C) VCD based on an analysis of what one would qualitatively expect from summing the poly(I) and poly(C) spectra. By comparison, poly(C,U) has a VCD band shape and frequency reflecting the positive couplet part of the poly(C) VCD but having only about half the magnitude. While still a bisignate VCD, the poly(A,C) couplet is spread over both the C and A vibrations. The positive feature is much broader than the negative one and again the $\Delta A/A$ value of poly(A,C) is only about half the value of either poly(C) or poly(A). Poly(A,U) gives a very intense positively biased VCD at 1665 cm^{-1} with only a hint of a positive couplet. The main VCD feature correlates most strongly to the absorption maximum of U.

For comparison to the polynucleotide results above, we also measured VCD of the dimers: ApA, CpC, and UpU which gave rise to positive couplets (Figure 3) reflecting those found in the homopolynucleotides. However, the magnitudes in terms of $\Delta A/A$ for ApA and CpC were at least four times smaller than those found for the corresponding polymers. The UpU VCD was also smaller than that of poly(U), but the result is not quantitatively reliable considering the small magnitudes of both and our noise/artifact limitations.

Since the poly(A,U) and poly(I,C) VCD signals were much larger than those of the other copolymers and since these polymers both involve complementary bases, samples of poly(I)·poly(C) and poly(A)·poly(U) were purchased and their VCD measured. The results for these double-stranded RNA's are shown in Figure 4. In both cases, the signals are more intense than had been seen for the single-stranded polynucleotides that make them up and for the corresponding copolymers. The poly(A)·poly(U) spectrum has a 3-peak (−+−) VCD which is dominated by the strong

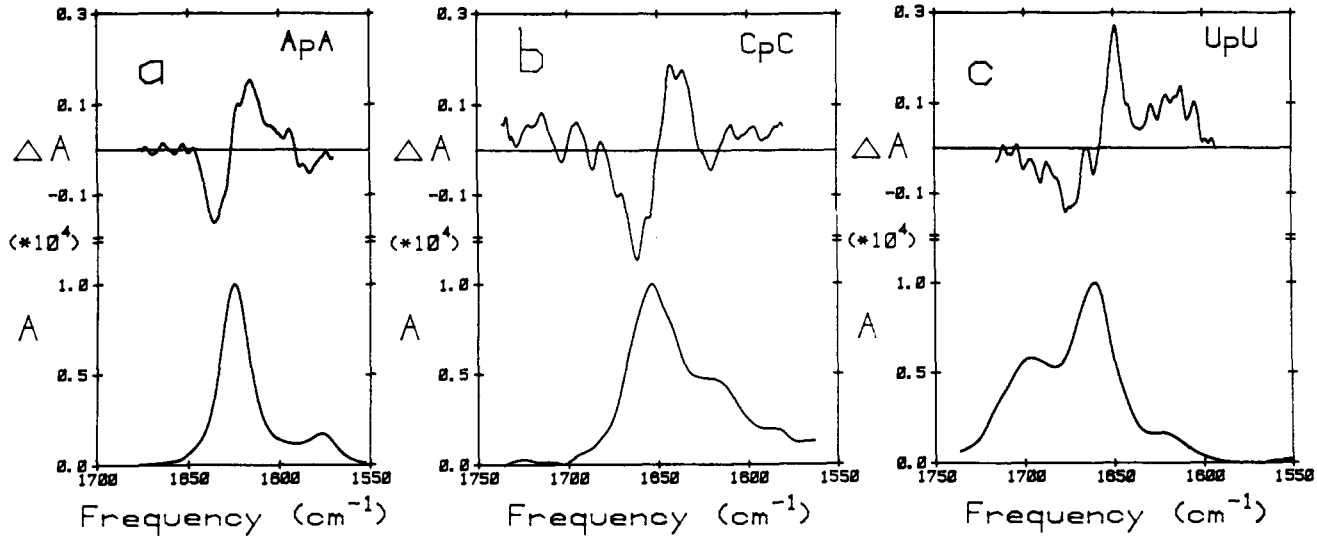

Figure 3. Absorption and VCD curves of riboxy-dinucleoside monophosphates in D_2O buffer (as in Figure 1) at 11 cm^{-1} resolution. The plots are for (a) ApA, (b) CpC, and (c) UpU. For VCD measurements, (a) 6, (b) 6, and (c) 4 scans were averaged at 10 s time constant against base line runs of (a) AMP, (b) CMP, and (c) UMP. In these plots ΔA has been offset by $<1 \times 10^{-5}$. See Table I for more experimental details.

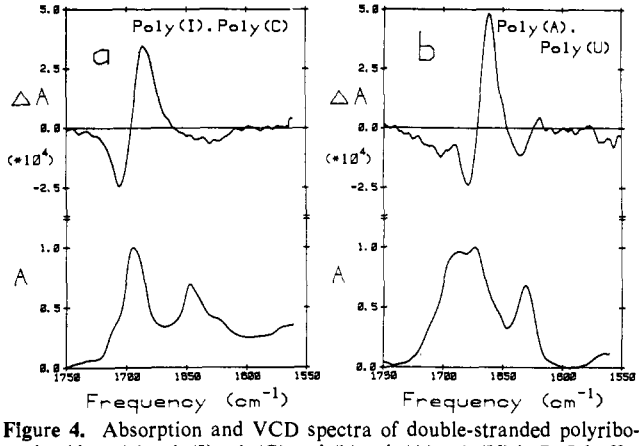

Figure 4. Absorption and VCD spectra of double-stranded polyribonucleotides: (a) poly(I)·poly(C) and (b) poly(A)·poly(U) in D_2O buffer (see Figure 1) at 11-cm^{-1} resolution. Two scans of VCD at 3 s time constant against D_2O buffer (baseline) were averaged. Offset in ΔA for these plots is $\sim 6 \times 10^{-5}$. Table I contains further experimental details.

positive central band at $\sim$1665 cm^{-1} closely resembling the poly(A,U) result. The poly(I)·poly(C) spectrum is dominated by a strong positive couplet at 1695 cm^{-1} which closely resembles that seen in poly(I,C) also at 1695 cm^{-1} and in poly(I) at 1685 cm^{-1}.

The values of $\Delta A/A$ (peak-to-peak values of the bisignate features) and band frequencies measured for the various compounds discussed above are summarized in Table I. In order to develop some understanding of the environmental dependence and stability of these VCD spectra, we have investigated both the temperature and pD dependence of the poly(C) results. Poly(C) was chosen for this test because its conformation is known from previous work to be pD and temperature dependent.[14,20] Over the range from 10 to 50 °C the poly(C) VCD steadily decreased in magnitude by a factor of 2 but kept the same band shape. With pD change, the signal stayed constant between pD values of 7.4 and 6.2 and then dropped sharply at pD 6.1 to less than half of its former value. Both these results, a slow change with temperature and a sharp one with pD, are consistent with earlier observations with electronic CD, Raman, IR, and UV spectroscopies to study poly(C) temperature and pD dependencies.[14,20]

The anisotropy factor ($\Delta A/A$ ratio) for poly(I) is substantially larger than that of the other single-stranded homopolynucleic acids. To gain further insight into this result we have also done a preliminary VCD melting experiment for poly(I) between 30 and 70 °C. We indeed found a sharp transition at about 52 °C. The large bisignate VCD found at low temperatures suddenly changes into a monosignate (+) VCD of smaller magnitude above this temperature. This is shown in Figure 1c as the dashed spectrum. The high-temperature form has a much broader and slightly lower frequency ($\sim$10 cm^{-1}) absorption band. We have also measured the spectra after cooling the sample back to 35 °C (total elapsed time $\sim$1 h). Both the VCD and absorption bands returned to their original shapes, but the $\Delta\epsilon/\epsilon$ value was only $\sim$80% of its original value. No attempt was made to study the time course of this reconstitution.

Calculations. To aid in understanding the VCD of the ApA and CpC dimers as well as the poly(A) and poly(C) VCD, we used coupled oscillator theory[13] to calculate the VCD expected from the dipolar coupling of the most intense base modes in a model dimer system. The methods used for this analysis have been described in detail previously.[21] In brief, we have assumed that the dipoles of interest are localized on the bond with major contribution to the normal mode in each case; that the loss in degeneracy of the symmetric (+) and antisymmetric (−) contributions is due to dipole–dipole coupling; that the unperturbed dipole magnitude can be obtained by integrating the absorption spectrum of the monomer (as measured by us); and that the line shapes can be approximated by two overlapping Gaussians of the same sign in absorption but opposite signs in VCD.

In order to evaluate $R_{\pm}$, the rotational strengths of the two bands, the relative orientations of the two dipoles are needed. For this, we assumed that the bases in the dimers have the same geometry as has been postulated for them in the polymers. For poly(C) and poly(A), right-handed helical structures have been proposed by Arnott et al.[22] and Saenger et al.,[23] respectively. Additionally, Broido and Kearns[24] have, from 2-D NMR results, proposed an alternate, non-base-stacked, left-handed structure for poly(C). Accordingly we have done coupled-oscillator calculations with all three of the above proposed geometries centering the dipoles on $C_2{=}O_2$ for CpC and $C_5{=}C_6$ for ApA.[12] In *N*-deuteriated compounds these bonds were found to have the maximum contribution to the stretching vibrations of interest in this work.[12]

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Table II. Results of DCO Calculations

structure	ref	ν_{max} (cm^{-1})	ϵ_m[a] ($L \cdot M^{-1} \cdot cm^{-1}$)	$\mu^b \times 10^{19}$ (esu·cm)	$(2V/h)^b$ (cm^{-1})	$R_+{}^b \times 10^{42}$ $(esu \cdot cm)^2$	$(\Delta A/A)_d \times 10^4$ calcd[c]	$(\Delta A/A)_d \times 10^4$ obsd	$(\Delta A/A)_p \times 10^4$ calcd[c]	$(\Delta A/A)_p \times 10^4$ obsd
right helix "CpC"	22	1651	980	4.21	12.2	−12.4	1.9	0.46	3.8	2.0
left helix "CpC"	24	1651	980	4.21	−12.3	6.2	0.95	0.46	1.9	2.0
right helix "ApA"	23	1625	1000	3.72	25.0	−6.9	2.8	0.32	5.6	2.6

[a] Molar extinction coefficient at 8 cm^{-1} resolution for monomers. [b] Defined in ref 21. [c] $(\Delta A/A)_p = 2(\Delta A/A)_d$, based on eq 1; the subscripts p and d refer to polymer and dimer, respectively. Gaussian half-widths used: 16 cm^{-1} for CpC and 18 cm^{-1} for ApA.

Considering only the interaction of adjacent bases with dipoles oriented according to either the poly(A) or the two poly(C) structures, the coupled oscillator calculations predict a positive couplet in all three cases. The results are summarized in Table II. The agreement in sign of this result with that found experimentally is quite gratifying.

Both the right-[22] and left-handed[24] poly(C) structures are predicted to have the same sign pattern but the former is calculated to have twice the magnitude of the latter. The splittings between ν_+ and ν_- turn out to be essentially the same in both cases. With a 16 cm^{-1} half-width (at 1/e) the $\Delta\epsilon/\epsilon$ ratio (equivalent to $\Delta A/A$) is predicted to be 1×10^{-4} and 2×10^{-4}, respectively, for the left- and right-handed CpC structures. These values are 2–4 times larger than those found experimentally. In the case of ApA, the calculated splitting is larger than the experimental absorption spectrum half-width and, thus, must be in error. Using instead a half-width of 18 cm^{-1}, which would be compatible with the experimental absorption, we obtain a broad calculated absorption band and a $\Delta\epsilon/\epsilon$ value of $\sim 3 \times 10^{-4}$, still substantially larger than the value seen experimentally.

Discussion

First it must be remarked that these are the first VCD data to be presented for nucleic acids and that our experiments demonstrate the measurability of such VCD and its sensitivity to nucleic acid conformation. All the data presented are for in-plane base modes, C=O and C=C stretches, as we wished to concentrate on that aspect of the structure. However, it should be noted that other modes such as the PO_2^- stretches yield measurable VCD[18] and could provide a different, complementary source of structural information.

Second, we note from the results in Table I that the $\Delta A/A$ values vary over a large range for the systems we have studied. Since, in these molecules, ϵ varies by only a small amount on a per subunit basis, this variation must be attributable to the VCD itself. That effect, in turn, appears to be primarily due to base–base coupling because the monophosphates do not give measureable VCD in this region, as might be expected from the vibrations of a planar purine or pyrimidine which are only weakly coupled to those of the chiral ribose entity. While the dimers give weak, measureable VCD signals, they appear to be qualitatively related to those found for the corresponding homopolymers but are much smaller, particularly in the cases of ApA and CpC. The UpU and poly(U) VCD magnitudes are both near the limit of our ability to measure ΔA quantitatively. The homopolymer VCD results fall in three distinct classes: weak (U and G), moderate (A and C), and high intensity (I).

On the other hand, the two examples we have presented of double-stranded RNAs exhibit significantly more intense VCD than do the single-strand examples. In short, there is a general progression in magnitude that appears to correlate with the expected stability of the helical secondary structure since the double-strand RNA should be more stable than the single strand which itself is more stable than the dimer.

It must be clear that these molecules are not expected to exist in a single, uniform conformation. In fact, the dimers are probably quite fluctional and the polymers are similarly thought to be composed of mixed helix and random components.[25] It has been suggested by Johnson and co-workers[26] that quantitative CD studies of polynucleotides should be undertaken at the equivalence point of this conformational equilibrium so that the structural composition of the sample would be well-defined. Unfortunately, given our current experimental constraints, this was not possible for the VCD measurements presented above. In that respect, our data must be viewed from a somewhat less quantitative perspective.

From comparison of monomer and dimer results, it is apparent that coupling-induced VCD is measured even at the dimer level in these systems. If a substantial portion of the sample were randomly configured, it might be expected to have VCD like the monomer while the stacked conformer should give VCD indicative of coupling. Since both conformers would contribute to the absorption, the resultant $\Delta A/A$ magnitude in the dimer spectrum would be much smaller than the calculated coupled oscillator results; but the calculated sign pattern would still be appropriate for comparison to experiment. In that light, it is very gratifying that by using geometrical parameters derived from poly(C) and from poly(A) one can calculate coupled oscillator VCD band shapes (Table II) that agree in sign with the experiments. However, it should be noted that the quantitative agreement is much better for CpC than ApA. Calculationally, this lack of agreement for ApA centers chiefly in the dipolar splitting, 2 V/h. The source of this must be in a poor representation for the location and orientation of the dipoles. Since the in-plane, C=C vibrations are undoubtably highly mixed, choosing a particular C=C bond for the dipole location and orientation is probably too simple. In addition, the poly(A) structure from which we obtained coordinates was postulated from an ApApA crystal structure. Which of these is the chief source of the calculational problem is, at present, unclear. By comparison, for CpC the somewhat more unique C=O bond was used with substantially better results.

Homopolymers and Homodimers. For electronic CD, the results of polymer (CD_p) and dimer (CD_d) spectra have been related by assuming only nearest neighbor interactions and correcting for monomer (CD_m) contribution as follows:[26,27]

$$CD_p = 2*CD_d - CD_m \quad (1)$$

Using analogous logic and normalizing to absorbance rather than residue concentration, due to our experimental limitations, we would expect $(\Delta A/A)_p$ to be twice $(\Delta A/A)_d$ since the monomer contribution is negligible. However, we see a much bigger change (4–8 times) from dimer to polymer in the A and C cases and much less for U. As noted above, eq 1 is dependent on the assumption that the sample is in a well-defined conformation which almost certainly is not the case here. Thus the fact that our homopolymer VCD is of greater magnitude than dimer VCD can be viewed as being consistent with the coupled oscillator type expectations and with the assumption that the polymer has a more stable stacked structure than the dimer. Quantitatively, this fails for U which may be due to the low values in these cases. Clearly, detailed temperature-dependent studies of these results are needed before more definitive conclusions can be reached. It is interesting to note that by applying eq 1 to the calculated dimer, $(\Delta A/A)_d$, results in Table II, we find agreement between the calculated $(\Delta A/A)_p$ values and the experimental homopolymer results to a factor of 2. Since the polymer structure is expected to be more stable than that of the dimer, this is consistent with dipolar

(25) See, for example: Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; Freeman: San Francisco, 1980; Vol. III, Chapter 22.

(26) Causley, G. C.; Staskus, P. W.; Johnson, W. C., Jr. *Biopolymers* **1983**, *22*, 945–967.

(27) Cantor, C. R.; Tinoco, I., Jr. *J. Mol. Biol.* **1965**, *13*, 65–77.

coupling as being the origin of the observed VCD.

Generally base-stacking interactions are thought to be a driving force for formation of helical structures in single-strand nucleic acids. Base stacking has also been observed in several dimers.[28,29] As noted above, the electronic CD spectra of specific homodimers and homopolymers are similar, but they are different in shape from those of the respective monomers.[30] The electronic CD magnitude per residue has been found to increase progressively with respect to the chain length until it reaches a maximum.[20,31] As noted, our data for CpC and ApA appear to fit this pattern, being 4–8 times weaker than for poly(C) and poly(A), respectively, yet of the same general band shape. By contrast, it is interesting to note that our polypeptide VCD studies indicate that the signals reach a stable level fairly early in helix formation as compared to electronic CD,[32] indicative of VCD and electronic CD having different distance dependences. The nucleic acid data may be less technique dependent from implications at this early stage in our study.

Previous CD, IR, and other measurements have determined several characteristics of the various polynucleotides studied here with which our VCD data can be correlated. In particular, in aqueous solution, at room temperature and neutral pH, poly(C), poly(U), and poly(A) are thought to exist as single strands[20,31] whereas poly(G) is multistranded.[16,33] Poly(I) is believed to exist as a single strand at low salt concentrations analogous to those used in this study.[34] Furthermore, numerous experimental studies indicate that the single-stranded poly(A) and poly(C) have a significant fraction of ordered helical structure whereas poly(U) has a primarily random structure at room temperature.[20,31]

In line with this latter result, we find that both poly(A) and poly(C) give rise to a moderate bisignate VCD of roughly the same magnitude, while the poly(U) VCD is 4–5 times smaller in magnitude but of similar shape. On the other hand, comparison of poly(I) and poly(G) VCD does not lead to any consistent interpretation. While the poly(G) VCD is very weak, that of poly(I) is an order of magnitude larger. The poly(I) positive couplet is also 2–3 times larger than that found for poly(A) and poly(C) and melts sharply to a less intense positive VCD band at ~52 °C. (The electronic CD spectral magnitudes for poly(G)[30] and poly(I)[35] do not have such a large difference, that of poly(I) being, in fact, smaller in magnitude.) The transition dipoles corresponding to the VCD-active modes in both G and I can be assumed to lie along their respective C=O bonds. Since these have the same relative geometry with respect to the backbone in each case, dipole coupling should give rise to very similar VCD if both had the same conformation. Hence, to the extent that this mechanism explains the VCD, we can conclude that the VCD indicates that poly(G) and poly(I) must assume substantially different conformations in solution, in spite of the similarity in their base structures. Small differences in the low-energy tails of the electronic CD might also suggest this.[30,35] The sharp melting and high VCD magnitude suggest stabilization of the poly(I) conformation by multistrand formation. This is known to occur in dilute solutions under high salt conditions where poly(I) sharply melts at 42 °C.[34] Perhaps the high concentrations we use for VCD studies also favor multistrand formation at a lower salt level. If so, the sharp melting transition we have noted at 52 °C might be concentration dependent. If poly(I) is multistranded and has such large VCD, it seems surprising that poly(G), known to have a stable, multistranded conformation, has such small VCD. It is conceivable that differences in helical parameters, relative orientation of bases, and number of strands for the two polymers, I and G, could lead to a substantial difference in VCD magnitude.

It should also be noted here that a large shift in IR frequency (>10 cm^{-1}) on going from monomer to polymer has been observed in this study for only G and I and not for the other bases: A, C, and U. These frequency changes are GMP 1667 cm^{-1}, poly(G) 1684 cm^{-1} and IMP 1672 cm^{-1}, poly(I) 1684 cm^{-1}. In addition, the absorption frequency of the high-temperature (melted) form of poly(I) drops down close to that of IMP. Further analysis of the multistranded structure of poly(I) in relation to poly(G) awaits more detailed studies underway in our laboratories.

The VCD temperature and pD dependences observed for poly(C) fit the previously proposed model of single-strand helical structure.[14,20] As temperature is increased, the $\Delta A/A$ value gradually decreases which is consistent with a decrease in the relative fraction of polynucleotide in the helical form due to a shift in the equilibrium between helix and coil. The remainder of the structure should be randomly oriented and might be expected to yield VCD something like that of the monomer and hence make little contribution to the observed signal. This assumes that the random coil form does not have significant local order. The pD dependence, on the other hand, shows a sharp change at pD ~6.1. Hartman and Rich observed this transition at pD 5.7 using infrared and ultraviolet absorption spectra on more dilute samples and attributed it to C·C base pairing facilitated by partial protonation of the cytosine bases.[14] The drop in VCD magnitude seen with decreasing pD then would correlate to a change in conformation from the single- to double-strand form.

Copolymers and Double-Stranded Polymers. The random copolymers (Figure 2) give rise to a somewhat different situation from the homopolymer results. Nevertheless, one can attempt a comparison of the VCD to the homopolymer result to seek any patterns useful for interpretation. The simplest case is that of poly(C,U) where the VCD is again the usual positive couplet centered over the absorption maximum at 1657 cm^{-1}. At this frequency the C=O stretching modes of C and U overlap spectrally. The poly(C,U) VCD appears to be just about the same shape as was found for poly(C) with a magnitude ($\Delta A/A$) that is roughly the average of that found for poly(C) and poly(U). For poly(A,C) a similar situation can be proposed except that both A and C have their absorption maxima separated leading to partially nonoverlapped contributions to the poly(A,C) observed VCD. Here a negative band occurs at ~1665 cm^{-1} and a weak, broad positive one is spread over 1650–1600 cm^{-1}. The poly(A) and poly(C) VCDs are both positive couplets of comparable magnitudes, but that of poly(A) is shifted down in energy by about 25 cm^{-1} from the poly(C) result. Addition of these two homopolymer spectra results in some cancellation between the positive lobe of C and negative lobe of A leading to a VCD spectral bandshape somewhat like that found for poly(A,C). In addition, one might expect an A–C interaction that could lead to a significantly different VCD pattern which would be superimposed on that generated by the A–A or C–C interactions. Poly(A,C) VCD magnitudes are significantly lower than the homopolymer results. This may be due to the dilution of A–A and C–C pairs in the random copolymer and/or to a decrease in stacking propensity brought on by heterogeneity in the chain.

This summing approach of independent spectra fails for poly(A,U) which has a nearly monosignate positive VCD at a frequency close to that of the U absorption maximum and is more than four times stronger than the VCD of the above discussed copolymers, poly(A,C) and poly(C,U). Paralleling the above discussed poly(C,U) and poly(A,C) results, we might have expected poly(A,U) to give a somewhat weak positive couplet. Clearly some A–U interaction enhances the signal and makes the poly(A,U) spectrum more complex than that of the first two examples.

The VCD of the last random copolymer studied, poly(I,C), looks virtually identical with that of poly(I) but is shifted up in frequency by ~10 cm^{-1}. The VCD associated with the C modes appears

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to be quenched, leaving only that of the poly(I)-type contribution. But due to the copolymer formation, one might have expected some decrease in $\Delta A/A$ in the I band unless stacking were enhanced in poly(I,C) or the I–C coupling gave a strong, constructive VCD contribution. The latter is extremely unlikely considering the difference in frequency of the absorbance maxima in poly(I,C) correlating to the two bases ($\sim$40 cm^{-1}) while the VCD exhibits a normal couplet centered slightly above the higher energy absorbance band of I. The resemblance of poly(I) and poly(I,C) VCD spectra is suggestive of some similarity being found in their structures. Thus if the poly(I) VCD is not characteristic of a single strand, then neither is the poly(I,C) VCD. Further discussion of this point follows in conjunction with the double-strand poly(I)·poly(C) results.

These comparably high $\Delta A/A$ magnitudes for poly(A,U) and poly(I,C) suggest that some alternate interaction between bases is operative in addition to stacking. Since both (A,U) and (I,C) are complementary Watson–Crick base pairs, it is natural to think that some base pairing, either intra- or interchain, occurs in each of these copolymers. To gain some insight into this possibility, we obtained VCD for double-stranded poly(I)·poly(C) and poly(A)·poly(U). From those results (Figure 4), it is clear that the same VCD sign pattern and bandshape emerges as seen in poly(I,C) and poly(A,U), respectively. However, the magnitudes are $\sim$50% higher. This result is consistent with partial double-strand formation in poly(A,U) as being the source of its increased VCD intensity and predominantly monosignate line shape centered on the U absorption maximum. In addition, for poly(A,U), the absorption envelop appears to reflect both the shifted U maxima seen in poly(A)·poly(U) as well as that found for the single strand. Such a profile is consistent with a mixture of hydrogen-bonded and non-hydrogen-bonded bases in a partially double-stranded conformation. It should be noted that double-strand formation in poly(A,U) has previously been invoked to help explain electronic CD results.[36]

Given the convincing evidence for poly(A,U), there is no reason to believe why such a partial double strand should not be present for poly(I,C). Paralleling the poly(I,C) result, the VCD band of poly(I)·poly(C) also moves up in frequency as compared to poly(I). Furthermore, the bisignate VCD seen in poly(C) is again absent in poly(I)·poly(C), and only a very weak monosignate (–) VCD is noticeable over the poly(C) band. Again, the poly(I,C) absorption envelop, being broader than that in the duplex, could encompass the bands seen for the hydrogen-bonded bases as well as those of the single-stranded form. Finally, it is worth noting that, of the four copolymers studied, poly(A,U) and poly(I,C) solutions were found to be the most viscous.

That said, it remains quite interesting that the poly(I,C) and the poly(I)·poly(C) spectra so closely resemble that of poly(I) *if* the latter is strongly influenced by multiple-strand formation. Following such an interpretation for poly(I), these results imply that the poly(I) multistrand structure must have a helical twist similar to that of the double-strand structure.

VCD and Electronic CD Comparisons. The differences in the relative magnitudes found for poly(A) + poly(U), poly(A,U), and poly(A)·poly(U) in VCD as compared to electronic CD[36] point up the difference in origin of the two effects. While, for example, the vibrational modes of A contributing to the VCD (C=C stretch) are not strongly affected by the hydrogen bonding present in base pairing, the π-electronic structure of the bases will be more significantly affected. Hence coupling and dipolar orientations in electronic CD will be significantly different for single-strand and double-strand (A,U) systems. However, the VCD coupling should be about the same having its primary change being due to orientational effects. Thus we feel that the VCD data, once a substantial base of survey results is built up, will serve as a useful complement to the more widely used electronic CD.

A striking example of this difference between electronic CD and VCD has been observed in this work. Both poly(A) and poly(C) have conservative bisignate VCD, but conservative electronic CD has been observed only for poly(A) in the near UV ($\sim$260 nm) while poly(C) has mainly monosignate (+) CD.[30] Again the VCD of CpC and ApA and the electronic CD of ApA are bisignate and conservative whereas the electronic CD of CpC is monosignate (+). The CD spectrum of ApA and in turn that of poly(A) has successfully been explained[37] by using a nearest neighbor interaction (exciton) model which is directly analogous to the coupled oscillator model we have used above for VCD. Such a model is obviously inadequate to explain the nonconservative CD of CpC and poly(C) which is considered to be due to the interaction with far-UV transitions[31] whereas, in VCD, interactions between different vibrations appear to be somewhat simpler.

Broido and Kearns have postulated that the poly(C) secondary structure is not one of a right-handed RNA helix[22] with conventional base stacking but is instead that of a left-handed helix with the bases unstacked yet hydrogen bonded on the outside.[24] This change in conformation should have significant effects on the electronic structure yet be still applicable for modelling with coupled-oscillator VCD. In fact, as noted above, calculations using both structures yield the same signed VCD for the C=O stretching mode differing only by a factor of 2 in magnitude. Hence, we cannot tell these two proposed poly(C) structures apart using only base modes. However, when we extend our measurements to the vibrations of the phosphate groups, we should be able to resolve the handedness of the helix *independent* of the orientation of the bases. Our preliminary calculations for these modes indicate that the sign of their VCD will depend on the handedness of the helix.[18] Again, the multiple chromophore aspects of VCD have important, complementary aspects for structural study.

Conclusions

Generally it is found that VCD magnitude corresponding to the base vibrations between 1600 and 1700 cm^{-1} increases as the degree of order increases. This is further enhanced by pairing between complementary bases. There is substantial evidence for partial double-strand formation in the random copolymer poly(A,U), but that is less clear in poly(I,C). Additionally, poly(I) might exist as a multistrand even at low salt conditions at the concentrations needed for VCD. Finally, the DCO model successfully predicts the observed VCD sign in poly(A) and poly(C).

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Registry No. ApA, 2391-46-0; CpC, 2536-99-4; UpU, 2415-43-2; poly(A), 24937-83-5; poly(C), 30811-80-4; poly(I), 30918-54-8; poly(G), 25191-14-4; poly(U), 27416-86-0; poly(A,C), 26182-06-9; poly(C,U), 26427-29-2; poly(I,C), 26301-44-0; poly(A,U), 25249-19-8; poly(I)·poly(C), 24939-03-5; poly(A)·poly(U), 24936-38-7.

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