Vibrational Raman Optical Activity as a Probe of Polyribonucleotide Solution Stereochemistry

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Abstract: The backscattered vibrational Raman optical activity (ROA) spectra of three single-stranded polyribonucleotides, poly(rA), poly(rC), and poly(rU), together with two double-stranded polyribonucleotides, poly(rA)·poly(rU) and poly(rG)·poly(rC), which both adopt A-type double helices, are reported in buffered H_2O and D_2O solutions between ∼650 and 1750 cm^{-1}. The ROA spectra are subdivided into three distinct regions that contain information on different stereochemical elements of nucleic acid structure. Between ∼1550 and 1750 cm^{-1}, ROA is generated through coupling of the vibrational coordinates on adjacent stacked bases that are chirally disposed. Large differences are observed between the spectra recorded in H_2O and D_2O in this region. Between ∼1200 and 1550 cm^{-1}, vibrational coordinates in the sugar and base rings couple in the normal modes to produce ROA signals that reflect the mutual orientation of these two rings and also the conformation of the sugar ring itself. Between ∼950 and 1150 cm^{-1}, a base-independent ROA triplet is observed that is characteristic of the particular sugar ring and sugar−phosphate backbone conformation present in A-type helices. These first results indicate that ROA is a powerful new probe of nucleic acid solution stereochemistry.

Introduction

Optical activity associated with the vibrations of chiral molecules may be measured by means of a small difference in the intensity of Raman scattering in right and left circularly polarized incident light.[1−5] Over the past few years, a series of instrumental advances[6−8] have paved the way for an increasing number of such vibrational Raman optical activity (ROA) studies on proteins and carbohydrates that have provided a fresh perspective on the solution conformation and dynamics of these two important classes of biopolymers (for a recent review see ref 9). We have recently extended the range of ROA studies to include pyrimidine nucleosides and demonstrated that ROA is sensitive to the type and conformation of the sugar ring, the mutual orientation of the sugar and base rings, and that no ROA is generated in the monomers by the normal modes localized in the planar base rings.[10] This work provides a springboard for the first ROA studies of polyribonucleotides reported here.

Another form of vibrational optical activity, complementary to ROA, is vibrational circular dichroism (VCD), the extension of UV CD into the infrared, which has also been used to study polynucleotides.[11,12] These VCD studies have identified two spectral windows for polynucleotides: 1550−1750 cm^{-1}, where the in-plane base stretching vibrations provide information on the interbase stereochemistry and stacking interactions, and 1000−1300 cm^{-1}, where the phosphate stretching vibrations probe the backbone stereochemistry. The present study shows that the useful spectral range for vibrational optical activity measurements of these biopolymers can be extended to 650−1750 cm^{-1} using ROA.

A large number of conventional Raman studies have been carried out on single- and double-stranded polyribonucleotides (for some reviews, see refs 13−17). These studies have produced assignments for several marker bands, the intensity and/or band position of which are correlated with changes in nucleic acid conformation and which have been used to monitor key structural elements such as the hydrogen bonding between bases, base stacking, and backbone conformation. ROA is also able to probe these features, but more incisively because it can cut through the complexity of conventional vibrational spectra by probing directly the crucial chiral elements of biomolecular structure.[9]

Having previously demonstrated that our current ROA instrument is able to provide high-quality spectra of polyribonucleotides,[9] we embarked on the present investigation to characterize the ROA spectra of single- and double-stranded polyribonucleotides adopting the A-type conformation with a

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(1) Barron, L. D. *Molecular Light Scattering and Optical Activity*; Cambridge University Press: Cambridge, U.K., 1982.
(2) Nafie, L. A. *Appl. Spectrosc.* **1996**, *50*, 14A−26A.
(3) Hecht, L.; Barron, L. D. In *Modern Techniques in Raman Spectroscopy*; Laserna, J. J., Ed.; Wiley: Chichester, U.K., 1996; pp 265−304.
(4) Diem, M. *Modern Vibrational Spectroscopy*; Wiley: New York, 1993.
(5) Hoffmann, G. G. In *Infrared and Raman Spectroscopy*; Schrader, B., Ed.; VCH Publishers: Weinheim, Germany, 1995; pp 543−72.
(6) Hecht, L.; Barron, L. D.; Gargaro, A. R.; Wen, Z. Q.; Hug, W. *J. Raman Spectrosc.* **1992**, *23*, 401−11.
(7) Hecht, L.; Barron, L. D. *Faraday Discuss.* **1994**, *99*, 35−47.
(8) Hug, W. *Chimia* **1994**, *48*, 386−90.
(9) Barron, L. D.; Hecht, L.; Bell, A. F. *Appl. Spectrosc.* **1996**, *50*, 619−29.
(10) Bell, A. F.; Hecht, L.; Barron, L. D. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 553−62.
(11) Keiderling, T. A. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum: New York, 1996; pp 555−98.
(12) Ting, X.; Goss, G. D.; Diem, M. *Biophys. J.* **1993**, *65*, 1255−61.
(13) Hartman, K. A.; Lord, R. C.; Thomas, G. J. In *Physico-Chemical Properties of Nucleic Acids*; Duchesne, J., Ed.; Academic Press: London, 1973; pp 1−89.
(14) Tsuboi, M; Takahasi, S; Harada, I. In *Physico-Chemical Properties of Nucleic Acids*; Duchesne, J., Ed.; Academic Press: London, 1973; pp 92−145.
(15) Peticolas, W. L.; Kubasek, W. L.; Thomas, G. A., Tsuboi, M. In *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; Wiley: New York, 1987; Vol. 1, pp 81−134.
(16) Nishimura, Y; Tsuboi, M. In *Advances in Spectroscopy*; Clark, R. J. H., Hester, R. E., Eds.; Wiley: Chichester, U.K., 1986; Vol. 13, 177−232.
(17) Peticolas, W. L.; Evertsz, E. In *Methods in Enzymology*; Lilley, D. M. J., Dahlberg, J. E., Eds.; Academic: London, 1992; Vol. 211, pp 335−52.

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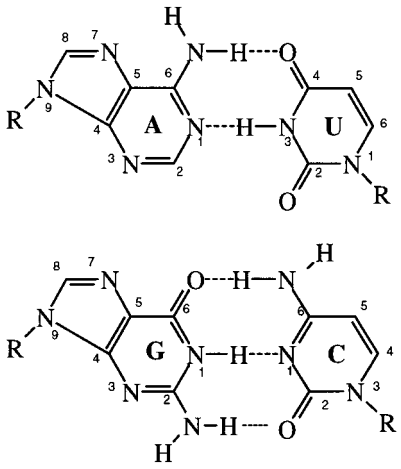

Figure 1. Structural formulas and atomic numbering schemes for the complementary Watson−Crick base pairs A·U and G·C. R represents the ribose−phosphate backbone.

view to identifying a series of marker bands for different conformational types and also to gain some insight into how ROA is generated in these molecules. In each case the individual strands are homopolymers containing one of the four common RNA bases which can form complementary Watson−Crick hydrogen-bonded base pairs (adenine with uracil and guanine with cytosine), the structural formulas and atomic numbering schemes of which are depicted in Figure 1.

Experimental Section

The homopolyribonucleotides polyadenylic acid (poly(rA)), polycytidylic acid (poly(rC)), and polyuridylic acid (poly(rU)) were supplied as K^+ salts and the complexes poly(rA)·poly(rU) and poly(rG)·poly(rC) as Na^+ salts by Sigma Chemical Corp. and were used without further purification. The samples for ROA measurements were prepared by dissolving the polyribonucleotide into between 100 and 150 μL of the appropriate buffer. These solutions were then passed through 0.45 μm Millipore membrane filters into quartz microfluorescence cells before being centrifuged for approximately 10 min. The amino and hydroxyl hydrogen atoms are readily exchangeable and were replaced by deuterium atoms by dissolving the polyribonucleotides in deuterated buffer and left to equilibrate for 3−4 days prior to ROA acquisition. For the conditions reported here we apparently have no significant deuteration of the C(8)H position of the purine bases as monitored by the relative intensities of bands at ∼1460 and 1480 cm^{-1} in the conventional Raman spectra.[18] We did not experience any particular problems with large fluorescence backgrounds, but all the samples were left for several hours in the laser beam to burn off residual fluorescence to provide a stable background for ROA measurements. The samples were found to be stable in the laser beam for several days with no significant change in either the Raman or ROA spectra.

The unpolarized backscattering Raman and ROA spectra of all the samples presented here were recorded on the ROA instrument GUROAS3. This is an upgraded version of the instrument GUROAS1 previously described in detail elsewhere.[6,7] The most important difference between the two is the introduction of a new *f*/1.4 stigmatic spectrograph based on a novel transmissive diffraction grating which, like the astigmatic single-grating *f*/4.1 spectrograph in GUROAS1, is equipped with a Peltier-cooled back-thinned charge coupled device (CCD) detector for a virtually shot noise limited detection of Raman scattered photons, plus a high optical density holographic notch filter (OD > 8) for an efficient suppression of the Rayleigh line. This upgrading has produced an approximately 5-fold increase in the speed of ROA measurements with slightly improved resolution. In addition, the aluminium-coated 45° mirror has been replaced with an enhanced silver-coated mirror (Balzers, Silflex) and a new temperature-stabilized electro-optic modulator (EOM) has been employed. These last two components have considerably improved the artifact suppression which is particularly important for ROA measurements on polyribonucleotides since they contain several intense strongly polarized Raman bands.

ROA is measured by synchronizing the Raman spectral acquistion with the operation of the EOM switching between right and left circular polarization states in the incident laser beam at a suitable rate (i.e., the reciprocal exposure time) and by calculating and accumulating circular intensity differences (CIDs) of individual modulation cycles in a computer memory for a preset acquisition time. All spectra are displayed in analogue-to-digital converter (ADC) units as a function of the Stokes Raman wavenumber shift with respect to the exciting laser line. The raw experimental data from ∼650 to 1750 cm^{-1} are depicted as circular intensity sums (CISs) $I^R + I^L$, referring to the parent Raman spectrum, together with the associated circular intensity differences (CIDs) $I^R - I^L$, characterizing the ROA spectrum, where I represents the Raman scattered intensity and the superscripts R and L refer to right and left circular polarization states in the incident radiation, respectively. A useful dimensionless quantity for comparing ROA data collected on different instruments is the normalized Δ-value defined as $(I^R - I^L)/(I^R + I^L)$. In our figures, approximately 20% of the Raman background has been removed in each case to allow the bands to be seen more clearly.

The 514.5 nm argon ion laser line was used with an incident laser power at the sample of ∼700 mW. A 100 μm entrance slit corresponding to a spectral resolution of approximately 10 cm^{-1} at 550 nm was employed. The Raman and ROA spectra for individual samples were recorded at concentrations of between 40 and 50 mg/mL in sodium phosphate buffer (pH 7.0, 20 mM) with 100 mM added NaCl, or in deuterated sodium phosphate buffer (pD 7.4, 20 mM) with 100 mM added NaCl. No significant differences were found in the ROA for samples prepared in buffers containing 1 mM EDTA. Acquisition times were 8 h except for poly(rU) which was recorded over 16 and 24 h in H_2O and D_2O solutions, respectively.

Results and Discussion

The backscattered Raman and ROA spectra of poly(rA), poly(rU), poly(rC), poly(rA)·poly(rU) and poly(rG)·poly(rC) in buffered H_2O and D_2O solutions are presented in Figures 2−6, respectively. Both poly(rA)·poly(rU) and poly(rG)·poly(rC) adopt an A-type double-helical structure with Watson−Crick-type base pairing between the two homopolyribonucleotide strands, whereas poly(rA) and poly(rC) are predominantly single-stranded helices and poly(rU) is mainly a random coil under the conditions described here.[19,20] Because of its considerable propensity to form stable quadruple-stranded structures,[19−21] poly(rG) was omitted from this particular study. Using this set of spectra we can compare the ROA generated by each of the individual bases on going from a single- to a double-stranded structure. ROA spectra were also recorded in D_2O to assist in our assignments. Our investigation has revealed that the ROA spectra can be conveniently subdivided into the three distinct regions indicated at the bottom of Figure 2 that probe different aspects of nucleic acid stereochemistry and are discussed separately in the following sections.

Base Stacking Region (1550−1750 cm^{-1}). This region of the polyribonucleotide ROA spectra is found to exhibit characteristic sign patterns, listed in Table 1, which not only reflect which bases are present but also are specific for the particular base stacking arrangement. The normal modes responsible for generating the ROA in this region originate in the planar base rings and are generally assigned to C═O and skeletal ring stretching vibrations.[13−17] Since the base rings are achiral, these particular vibrations cannot generate any ROA unless there is

(18) Lamba, O. P.; Becka, R.; Thomas, G. J., Jr. *Biopolymers* **1990**, *29*, 1465−77.

(19) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(20) Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr. *Physical Chemistry of Nucleic Acids*; Harper and Row: New York; 1974.

(21) Pochon, F.; Michelson, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *53*, 1425−30.

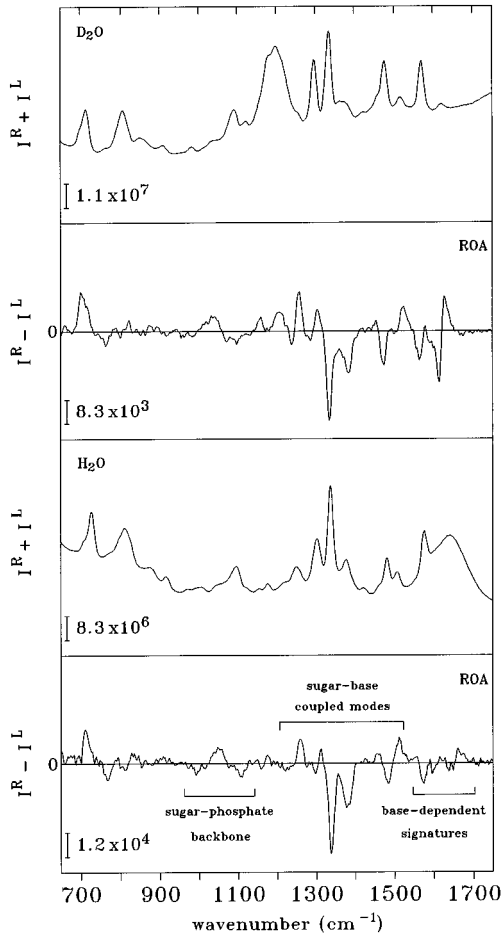

Figure 2. Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of poly(rA) in H_2O (bottom) and D_2O (top).

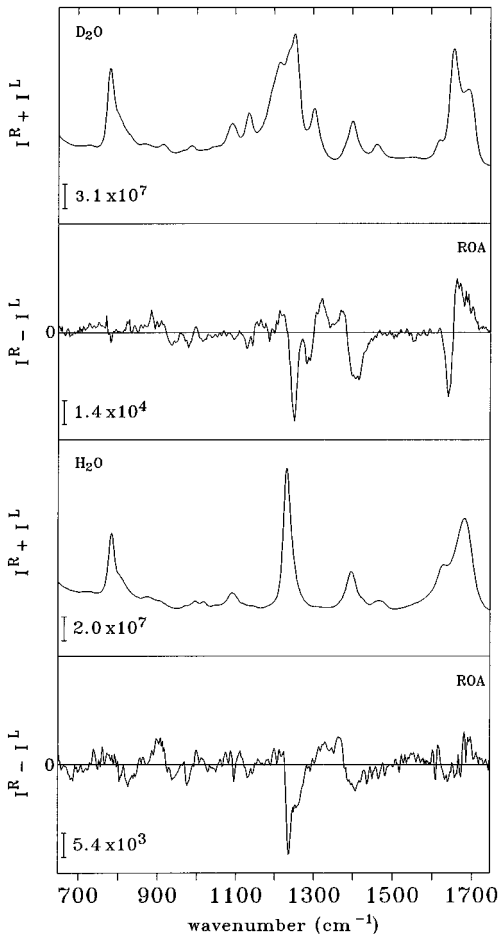

Figure 3. Backscattered Raman and ROA spectra of poly(rU) in H_2O (bottom) and D_2O (top).

some coupling between adjacent bases that are chirally disposed (for the monomers, no ROA is observed in this region[10]). There are two distinct possibilities: either the bases interact *inter*molecularly through the hydrogen bonding that can occur between adjacent polyribonucleotide strands or *intra*molecularly through the base stacking that is known to occur within a single polyribonucleotide strand. The latter must be the case for the single-stranded polyribonucleotides (Figures 2−4) since there are no *inter*strand hydrogen bonds present. However, it is nonetheless possible that the coupling of the vibrational coordinates through *inter*strand hydrogen bonds may also contribute to the generation of the ROA in the double-stranded polyribonucleotides (Figures 5 and 6). As we shall see, the similarities in the ROA spectra of the single- and double-stranded polyribonucleotides studied here imply that the same mechanism for generation of ROA may be common to both. If *intra*strand coupling of the bases is responsible for generating ROA, and assuming that a single vibrational coordinate such as the C(4)═O stretch in poly(rU) or the C(4)═C(5) stretch in poly(rA) dominates a particular normal mode, then the associated normal coordinates will describe a helix running along the strand. This would provide the chirality necessary for generating ROA signals in this region.

This region of the ROA spectra exhibits dramatic changes upon deuteration (Figures 2−6): not only do we observe the characteristic wavenumber shifts that are also evident in the conventional Raman spectra,[13−17] but specific ROA signals experience dramatic intensity increases. The wavenumber shifts may be accounted for by the hydrogen−deuterium exchange of the labile NH and NH_2 groups of the bases. In H_2O, NH and NH_2 deformations are known to couple with the C═O and skeletal ring stretching coordinates in this region.[13−17] Upon deuteration, the NH and NH_2 deformations are replaced by ND and ND_2 deformations which couple very little with the base stretching coordinates,[13,14] the result being the observed wavenumber shifts. However, we are unable at present to explain the observed ROA intensity increases. Having discussed some of the general features of the ROA spectra in this region, we now concentrate on the interpretation and assignment of the individual polyribonucleotides.

Poly(rA). In aqueous solution at neutral pH, poly(rA) adopts a single-stranded right-handed helical conformation with an appreciable degree of base stacking.[19,20] The conventional Raman spectrum in H_2O consists of a band at ∼1576 cm^{-1} and another broad band at ∼1640 cm^{-1} that is assigned to the solvent (Figure 2). In D_2O, small wavenumber shifts are evident and an additional weak Raman band at ∼1621 cm^{-1} is revealed by the removal of the solvent scattering. The conventional Raman band observed at ∼1576 cm^{-1} in H_2O and at ∼1570 cm^{-1} in D_2O is assigned to a skeletal stretching mode of the adenine base.[13−17] This band generates weak negative ROA signals at ∼1573 and 1564 cm^{-1} in H_2O and D_2O, respectively. The most interesting observation in this region for poly(rA) is the ROA couplet in the D_2O spectrum centered at ∼1620 cm^{-1}, positive at higher and negative at lower wavenumber, and associated with the conventional Raman band at ∼1621 cm^{-1}. This band is assigned to a skeletal stretching vibration of the base with a major contribution coming from the C4═C5 stretching coordinate.[14] No similar signal occurs at this wavenumber in H_2O; instead, a very weak couplet at higher wavenumber (centered at ∼1655 cm^{-1}) is observed.

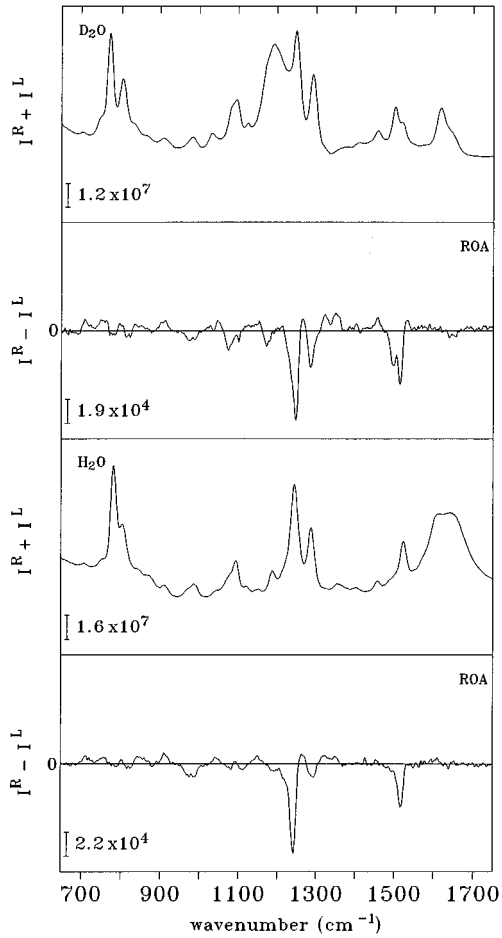

Figure 4. Backscattered Raman and ROA spectra of poly(rC) in H_2O (bottom) and D_2O (top).

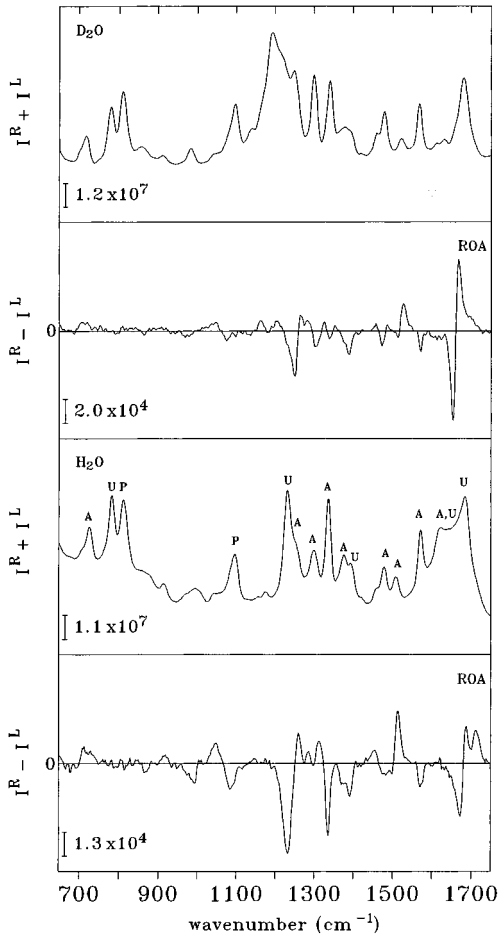

Figure 5. Backscattered Raman and ROA spectra of poly(rA)·poly(rU) in H_2O (bottom) and D_2O (top). The more intense conventional Raman bands are marked A and U, depending on whether they originate predominantly in the adenylic acid strand or the uridylic acid strand, respectively, and the bands that involve vibrations of the phosphate group are marked P. The remaining weaker Raman bands can mainly be attributed to the sugar ring.

Poly(rU). At room temperature, poly(rU) adopts a predominantly random structure with only a small degree of base stacking.[19,20] This is reflected throughout the entire ROA spectra of poly(rU) in H_2O and D_2O (Figure 3), which contain signals that are between three and four times weaker than the other single-stranded polyribonucleotides studied here. In H_2O, a weak broad ROA couplet centered at ~1680 cm^{-1}, negative at lower and positive at higher wavenumber, is observed for poly(rU). Upon deuteration, this couplet is shifted down to ~1656 cm^{-1} and there is a dramatic increase in intensity relative to the remainder of the spectrum. This couplet is associated with the conventional Raman band at ~1655 cm^{-1} in D_2O that is usually assigned to a normal mode having a large contribution from the C(4)═O stretching coordinate of the uracil base.[14] However, for uracil bases (in which there are two carbonyl groups present) extensive mixing of the carbonyl vibrations may be expected.[22] The fact that we still observe some ROA signal, albeit weak, means that there must still be some small degree of base stacking present in poly(rU).

Poly(rC). Relating the ROA sign pattern and intensity between 1550 and 1750 cm^{-1} to base stacking can be used to interpret the ROA spectra of poly(rA) and poly(rU). However, poly(rC) in H_2O only exhibits a very weak ROA couplet centered at ~1620 cm^{-1} which has the opposite sign relative to the ROA couplets observed in the other polyribonucleotides studied here as shown in Figure 7. Conventionally, poly(rC) is thought to adopt a right-handed helical structure with a moderate degree of base stacking.[19,20] However, from a 2D-NMR study on poly(rC) in aqueous solution, Broido and Kearns proposed a novel single-stranded left-handed helical structure with the sugar−phosphate backbone inside the helix and the bases on the outside.[23] This structure is stabilized by *intra*molecular hydrogen bonds between the carbonyl group of one base and the amino group of an adjacent base. The observed hypochromism of poly(rC) in UV absorption and conventional Raman measurements was explained in terms of some interaction between the bases involving this *intra*molecular hydrogen bonding. One characteristic of this unusual structure is that the bases are not stacked but instead are tilted at an angle of ~45° to the helix axis and are not arranged parallel to one another. This unstacked structure is not unique, as recent studies on $d(A^+-G)_{10}$ have shown that this particular oligonucleotide can exist in a single-stranded, unstacked ordered structure.[24,25]

Since the intensities in the remainder of the ROA spectrum are consistent with an ordered structure for poly(rC) in neutral aqueous solution, it seems then that the weak ROA couplet intensity observed for poly(rC) in this region might be interpreted in terms of this left-handed helical structure. The fact that the bases are tilted by 45° relative to the helix axis, and are not parallel to one another, means that the distances between

(22) Bandekar, J.; Zundel, G. *Biopolymers* **1984**, *23*, 2623−8.

(23) Broido, M. S.; Kearns, D. R. *J. Am. Chem. Soc.* **1982**, *104*, 5207−16.

(24) Shiber, M. C.; Lavelle, L.: Fossella, J. A.; Fresco, J. R. *Biochemistry* **1995**, *34*, 14293−99.

(25) Mukerji, I.; Shiber, M. C.; Spiro, T. G.; Fresco, J. R. *Biochemistry* **1995**, *34*, 14300−3.

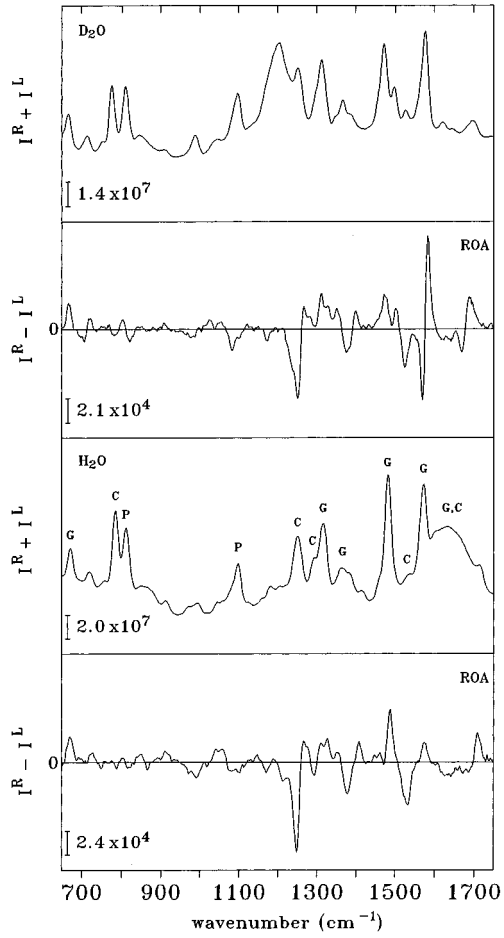

Figure 6. Backscattered Raman and ROA spectra of poly(rG)·poly(rC) in H_2O (bottom) and D_2O (top). The more intense conventional Raman bands are marked G and C, depending on whether they originate predominantly in the guanylic acid strand or the cytidylic acid strand, respectively, and the bands that involve vibrations of the phosphate group are marked P. The remaining weaker bands can mainly be attributed to the sugar ring.

Table 1. Position and Sign of Distinctive ROA Signals in the Base Stacking Region (1550−1750 cm^{-1})

polyribonucleotide	solvent	signal position (cm^{-1}) and sign[a,b]		
poly(rA)	H_2O	1573(−)w	1655(−,+)w	
	D_2O	1564(−)w	1620(−,+)s	
poly(rC)	H_2O		1620(+,−)w	
	D_2O		1638(−)w	
poly(rU)	H_2O			1680(−,+)m
	D_2O			1656(−,+)s
poly(rA)·poly(rU)	H_2O	1572(−)w		1682(−,+)m
	D_2O	1572(−)w		1660(−,+)s
poly(rG)·poly(rC)	H_2O	1577(+)m		
	D_2O	1577(−,+)s		1680(−,+)m

[a] (−,+) denotes a couplet negative at lower and positive at higher wavenumber. [b] w, m, and s denote weak, medium, and strong relative ROA intensities.

the same groups on adjacent bases will increase, which will weaken the interaction between them, and so could explain the weak ROA couplet strength. Furthermore, a left-handed structure could account for the observed sign inversion. However, we cannot rule out the conventional base stacking of a right-handed helical structure of poly(rC). Within a simple two-group model of ROA,[1,26] certain alignments of the bases in right-handed helical structures may also theoretically generate

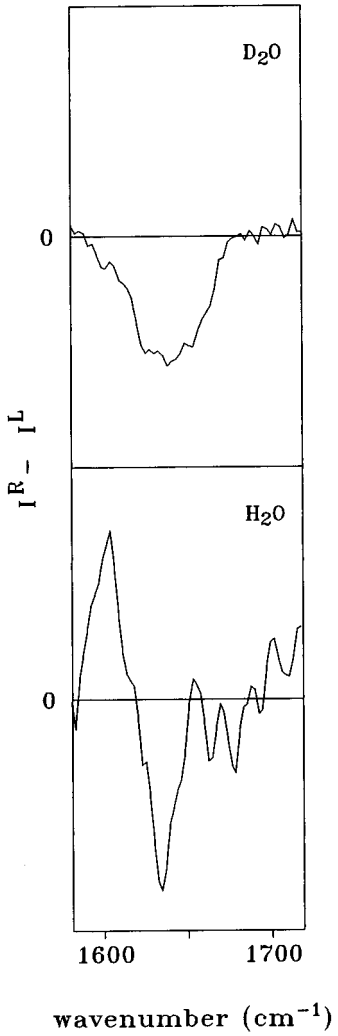

Figure 7. Backscattered Raman and ROA spectra of poly(rC) between 1580 and 1720 cm^{-1}. Same conditions as in Figure 4 except ROA was acquired over 50 h.

an ROA couplet positive at lower and negative at higher wavenumber. Nonetheless, it is clear that there is some structural difference between poly(rC) and the other polyribonucleotides studied here.

Upon deuteration, the ROA couplet centered at ∼1620 cm^{-1} is replaced by a negative ROA signal at ∼1638 cm^{-1}. This suggests that the normal mode responsible for the ROA couplet observed in H_2O involves a contribution from NH_2 deformations. The conventional Raman band in cytosine at 1617 cm^{-1} in D_2O has an important contribution from the C5═C6 stretching coordinate.[13,14] In H_2O, this mode couples with NH_2 scissoring motions and two bands are observed in the IR spectrum at 1603 and 1670 cm^{-1}.[14] In our conventional Raman spectra of poly(rC), bands appear at 1606 and 1618 cm^{-1} in H_2O and D_2O, respectively. These results also indicate that the NH_2 deformations play an important role in generating the ROA couplet observed in H_2O. It is interesting to note that NH_2 groups are proposed to be involved in *intra*molecular hydrogen bonding to the carbonyl group in the left-handed structure of poly(rC).[23]

Other chiroptical techniques have been applied to the study of poly(rC) in solution. VCD calculations predict that all polyribonucleotides that have a positive couplet in the base stretching region, as observed for poly(rC), are right-handed helices.[11,12,27] However, these calculations consider only the

(26) Barron, L. D.; Buckingham, A. D. *J. Am. Chem. Soc.* **1974**, *96*, 4769−73.

(27) Annamalai, A; Keiderling, T. A. *J. Am. Chem. Soc.* **1987**, *109*, 3125−32.

case where the bases are stacked parallel to one another and are normal to the helix axis. The near-UV CD spectrum of poly(rC) has a positive CD which was attributed to base tilting, leading to a contribution from far-UV transitions to the CD; whereas poly(rA) shows an almost conservative CD couplet arising mainly from degenerate exciton coupling.[28]

Poly(rA)·poly(rU). The two double-stranded polyribonucleotides studied here have different characteristic sign patterns in this region. For poly(rA)·poly(rU), which adopts an A-type double-helical conformation in neutral aqueous solution, there are four conventional Raman bands at ∼1572, 1624, 1684, and 1712 cm^{-1} in H_2O and five bands at ∼1568, 1613, 1631, 1659 and 1680 cm^{-1} in D_2O (Figure 5). The band at ∼1572 cm^{-1} in H_2O is associated with a weak negative ROA signal and is shifted only slightly by deuteration, which is also the case for a band at approximately the same wavenumber in single-stranded poly(rA). Thus, we can say that this band originates in the poly(rA) strand of the double helix and can be given a similar assignment to the ROA signal at ∼1573 cm^{-1} in the single-stranded poly(rA) discussed above. The two bands at ∼1613 and 1631 cm^{-1} in the deuterated form and at ∼1624 cm^{-1} in the nondeuterated form do not yield any associated ROA signals. This is somewhat surprising as it may have been expected that the moderately intense couplet observed at approximately this wavenumber in the ROA spectrum of poly(rA) in D_2O would also be present in the double-stranded form. It is unclear to us at present why this ROA signal should be quenched in the duplex structure. An ROA couplet centered at ∼1682 cm^{-1}, negative at lower and positive at higher wavenumber, is observed in the spectrum of poly(rA)·poly(rU) in H_2O which upon deuteration shifts to ∼1660 cm^{-1} and approximately doubles in intensity. The same sign pattern and approximately the same signal positions (Table 1) are observed for poly(rU) in H_2O and D_2O, although of much weaker intensity. This suggests that the same type of base stacking of the uracil bases is prevalent in single-stranded poly(rU) and the poly(rU) strand in double-stranded poly(rA)·poly(rU) and that only the degree of base stacking is different. In addition, the formation of *inter*molecular hydrogen bonds appears to have only a slight effect on the signal positions but not on the observed sign pattern.

Poly(rG)·poly(rC). For poly(rG)·poly(rC), which also adopts an A-type double helix in neutral aqueous solution, conventional Raman bands appear at ∼1573, 1640, and 1713 cm^{-1} in H_2O and at ∼1577, 1621, 1644, and 1695 cm^{-1} in D_2O (Figure 6). The conventional Raman band at ∼1573 cm^{-1} in H_2O generates a moderately intense positive ROA signal that becomes an intense couplet, negative at lower and positive at higher wavenumber, upon deuteration. The corresponding conventional Raman band is assigned mainly to a C4═C5 stretching vibration.[14] The broad Raman band at ∼1640 cm^{-1} is again assigned to the solvent and no substantial ROA signals are observed between ∼1600 and 1650 cm^{-1} in either H_2O or D_2O. However, in D_2O, an ROA couplet centered at ∼1680 cm^{-1}, negative at lower and positive at higher wavenumber, is observed which is not present in the H_2O spectrum. The corresponding conventional Raman band appears as a weak shoulder on the ∼1695 cm^{-1} band. The conventional Raman spectra of single-stranded poly(rC) in H_2O and D_2O exhibit no bands above ∼1650 cm^{-1}. On this basis, we may assign this ROA couplet to the guanine base C(6)═O stretching mode.[29] However, another possibility is that vibrational coupling between the carbonyl groups in the guanine and cytosine bases is responsible for the normal mode. Such *inter*strand vibrational coupling has been demonstrated for vibrational bands above 1640 cm^{-1} from the IR spectra of isotopically labeled G·C base pairs.[30] Both factors may be affecting the ROA and to help clarify this situation the ROA spectrum of single-stranded poly(rG) or some equivalent such as poly(rI) is required.

Finally, both poly(rA)·poly(rU) and poly(rG)·poly(rC) exhibit a positive ROA signal at ∼1712 cm^{-1} that disappears upon deuteration and is not observed in any of the single-stranded polyribonucleotides. An IR marker band at ∼1710 cm^{-1} has been attributed to right-handed double-stranded structures (A- or B-type conformation) with either A·U or G·C base pairs,[31] and the ROA signal observed at this wavenumber may have a similar origin.

Sugar−Base Region (1200−1550 cm^{-1}). This region of the conventional Raman spectrum is dominated by the strong in-plane vibrations of the nucleic acid bases.[13−17] However, significant coupling between the vibrational coordinates in the base and sugar rings is predicted by normal coordinate analyses[32,33] and provides an explanation for the observed sensitivity of conventional Raman marker bands in this region to sugar ring and glycosidic link conformation.[13−17] This coupling has been demonstrated experimentally from resonance Raman spectra of purine nucleosides with the anomeric C(1′) carbon selectively deuterated.[34] Further support is available from our ROA study of pyrimidine nucleosides which revealed that, without this coupling, the achiral planar base rings would be unable to generate any ROA in this region.[10] In addition, this study also demonstrated that certain signals in this region undergo a sign inversion when the absolute configuration of the anomeric carbon is switched, which emphasizes the importance of the anomeric C(1′) chiral center and the glycosidic link for the generation of these ROA signals. For the polyribonucleotides, another possibility is that, as discussed in the previous section, ROA may be generated through base stacking interactions which provide another source of chirality in the polymers. However, the ROA sign patterns we have observed in this study have a high degree of similarity to those in the monomers[10] (although the Δ-values are much greater in the polymers and small wavenumber shifts are evident) which indicates that the same mechanism is responsible for their generation. These results imply that the ROA spectra in this region carry information on the mutual orientation around the glycosidic link of the sugar and base rings and/or the sugar ring conformation in both the monomers and the polymers.

The polyribonucleotide ROA spectra exhibit little change upon deuteration in this region. A number of ROA signals undergo small wavenumber shifts and/or small intensity changes, but the basic sign pattern is retained in each case. This means that deformations of the exchangeable groups, namely OH in the sugar rings and NH and NH_2 in the base rings, are not involved to any significant extent in the generation of ROA. In the following discussion, we shall therefore only consider the spectra recorded in H_2O.

The Δ-values for a number of the more prominent ROA signals in this region are listed in Table 2 for the purpose of

(28) Johnson, W. C., Jr. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum: New York, 1996; pp 433−68.

(29) Simard, G.; Savoie, R. *Biopolymers* **1994**, *34*, 91−100.

(30) Howard, F. B.; Frazier, J.; Miles, H. T. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *64*, 451−8.

(31) Liquier, J.; Akhebat, A.; Taillandier, E.; Ceolin, F.; Huynh Dinh, T.; Igolen, J. *Spectrochim. Acta* **1991**, *47A*, 177−86.

(32) Letellier, R.; Ghomi, M.; Taillandier, E. *J. Biomol. Struct. Dyn.* **1986**, *3*, 671−87.

(33) Letellier, R.; Ghomi, M.; Taillandier, E. *J. Biomol. Struct. Dyn.* **1987**, *4*, 663−83.

(34) Toyama, A.; Takino, Y.; Takeuchi, H.; Harada, I. *J. Am. Chem. Soc.* **1993**, *115*, 11092−8.

Table 2. Δ-Values of Some of the Prominent ROA Signals in the Sugar−Base Region (1200−1550 cm^{-1}) for Both Monomers and Polymers

	$\Delta \times 10^5$ (wavenumbers in cm^{-1})[a]
cytidine	18 (1252)
poly(rC)	118 (1249)
poly(rG)·poly(rC)	288 (1247)
uridine	8 (1230)
poly(rU)	22 (1230)
poly(rA)·poly(rU)	103 (1235)
5′-AMP	113 (1358)[b]
poly(rA)	139 (1337)
poly(rA)·poly(rU)	101 (1336)

[a] All the quoted ROA signals have a negative sign. [b] Band shifted and may have different origin.

comparing the normalized intensities observed for the single- and double-stranded polyribonucleotides with those for their corresponding monomers. The single-stranded polyribonucleotides containing pyrimidine bases, poly(rU) and poly(rC) (Figures 3 and 4), have ROA sign patterns that are similar to their corresponding nucleoside monomers although with much greater Δ-values. Therefore, for these polymers, the dominant conformation of the individual nucleotide repeating units is similar to that of the corresponding monomers. In addition, it is clear that the phosphate groups, which link the individual sugar rings in the polymers, do not contribute significantly to the generation of ROA in this region. One contribution to the increase in Δ-values is from the hypochromicity known to be exhibited by certain conventional Raman bands in this region.[35,36] The resulting loss of Raman intensity in the polymers on taking up ordered structures, as compared with their respective monomers, leads to a concomitant increase in the observed Δ-values for the polymers. However, there is also an increased tendency toward a single conformational range for the glycosidic link, ribose ring, and the sugar−phosphate backbone torsion angles upon polymerization.[19,37] This should also contribute to the increase in ROA intensity as there will be a reduction in the degree of cancellation of ROA signals arising from the individual conformers. From the data presented here, it is difficult to separate these two distinct mechanisms although, in principle, it should be possible to separate hypochromic and conformational effects with accurate measurements on the temperature dependence of polyribonucleotide ROA signals and their associated conventional Raman bands.

Having discussed the general features of the ROA spectra in this region, we turn to more specific assignments of the individual spectra starting with the single-stranded polyribonucleotides. For poly(rC), the Δ-values of two negative ROA signals at ∼1242 and 1519 cm^{-1} increase by approximately 6-fold relative to those observed in the corresponding monomer, cytidine (Table 2). In contrast, for the other ROA signals in this region, it is difficult to measure accurate Δ-values due to the weak intensity of the corresponding conventional Raman bands. However, it is clear that their ROA intensities relative to the two strong negative ROA signals are diminished when compared with the spectrum of cytidine.[10] Similarly, for poly(rU), we observe a negative ROA signal at ∼1230 cm^{-1} that appears to be selectively enhanced over the other ROA signals in this region. We noted in the previous section that the ROA signal intensities in poly(rU) were between 3 and 4 times weaker than those typical for the other single-stranded polyribonucleotides. This may be attributed to the reduction in base stacking evident in poly(rU) with the concomitant loss of ordered helical structure.[19,20]

The situation for poly(rA) is more complex as some important differences are evident between the ROA spectra of the polymer and its nucleotide repeating unit, adenosine 5′-monophosphate (5′-AMP) (not shown here). In poly(rA), the *anti* conformation of the glycosidic link dominates,[38] whereas in 5′-AMP both *syn* and *anti* conformers coexist.[39] Thus, the contribution to the ROA from the *syn* conformer in the monomer may be responsible for the observed spectral differences.

The two double-stranded polyribonucleotides, poly(rA)·poly(rU) and poly(rG)·poly(rC), exhibit ROA spectra in this region that resemble a superposition of the spectra of the two individual single-stranded polymers. This indicates that there is little, if any, *inter*strand mixing of the normal modes in this region. In Figures 5 and 6, the conventional Raman and ROA signals are marked with the letters A, C, G, and U to indicate which strand of the polyribonucleotide they predominantly originate from. A dramatic increase in the Δ-value of the negative ROA signal at ∼1235 cm^{-1}, which is assigned to strands containing uracil bases, is observed in the spectra of poly(rA)·poly(rU) relative to single-stranded poly(rU). This can only partially be related to the hypochromicity of the conventional Raman band, as temperature-dependent Raman studies only indicate a 52% decrease in Raman intensity[35] which would result in an approximate doubling of the Δ-value, whereas in our spectra we observe a 5-fold increase. Thus, in this case, there is an important contribution to the intensity increase from the more ordered structure adopted by the poly(rU) strand in the double-stranded polymer. In contrast, the negative ROA signal at ∼1336 cm^{-1}, attributed to poly(rA) strands, has a similar value in both poly(rA) and poly(rA)·poly(rU). The corresponding conventional Raman band has an observed hypochromicity of only 10%,[35] and this may explain our observed values. No Raman hypochromicity data are available for poly(rG)·poly(rC) as this particular double helix does not melt until the temperature is raised above 100 °C.[36]

Sugar−Phosphate Backbone Region (950−1150 cm^{-1}). This region of the conventional vibrational spectrum of polynucleotides is dominated by normal modes localized in the sugar ring and the phosphate group.[13−17] This fact is highlighted by resonance Raman studies which reveal that few bands in this region involve contributions from the vibrational coordinates of the base.[40] One result of this vibrational localization is that the conventional Raman bands in this region are generally broad and of weak relative intensity. For this reason, they have not found as much use as bands in other spectral regions as conformational markers in polynucleotides,[13−17] despite the fact that many of these bands are expected to be sensitive to the sugar ring and sugar−phosphate backbone conformation.

In contrast, for the polynucleotides in this region, distinct base-independent ROA sign patterns that can be related to the particular sugar and sugar−phosphate backbone conformations are observed. For A-type polyribonucleotides, which adopt predominantly a C3′-endo sugar conformation,[19,20] the characteristic sign pattern is a positive, negative, positive triplet observed between ∼950 and 1150 cm^{-1}. This sign pattern is

(35) Lafleur, L.; Rice, J.; Thomas, G. J., Jr. *Biopolymers* **1972**, *11*, 2423−37.
(36) Small, E. W.; Peticolas, W. L. *Biopolymers* **1971**, *10*, 69−88.
(37) Danyluk, S. S. In *Nucleoside Analogues. Chemistry, Biology and Medical Applications*; Walker, R. T., DeClercq, E., Eckstein, F., Eds.; Plenum: New York, 1979; pp 15−34.
(38) Evans, F. E.; Sarma, R. H. *Nature* **1976**, *263*, 567−72.
(39) Davies, D. B. In *Progress in NMR Spectroscopy*; Emsley, J. W., Feeney, J., Sutcliffe, L. H., Eds.; Pergamon: Oxford, U.K., 1979; Vol. 12, pp 135−225.
(40) Tsuboi, M.; Nishimura, Y.; Hirakawa, A. Y.; Peticolas, W. In *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; Wiley: New York, 1987; Vol. 2, pp 81−134.

Table 3. Positions of the Three Components of the ROA Triplet Characteristic for the Sugar Ring Conformation between ~950 and 1150 cm^{-1} in H_2O of A-type Polyribonucleotides

polyribonucleotide	positive component (cm^{-1})	negative component (cm^{-1})	positive component (cm^{-1})
poly(rA)	991	1049	1107
poly (rC)	987	1044	1082
poly(rU)[a]	995	1055	1092
poly(rA)·poly(rU)	996	1050	1086
poly(rG)·poly(rC)	994	1050	1086

[a] Poly(rU) ROA spectrum was measured at ~2 °C.

evident in all the polyribonucleotides studied here, except for poly(rU), and the positions of these individual components of this overall ROA triplet are listed in Table 3. At 20 °C, poly (rU) adopts a predominantly random backbone structure, but when the solution is cooled to ~5 °C (in the presence of Mg^{2+} ions), an ordered backbone conformation is formed[19] and the ROA spectrum (not shown here) displays a triplet in this wavenumber range. The relative intensity of these ROA signals are somewhat weaker in the single-stranded structures than those in the double-stranded structures which presumably reflects the greater degree of order present in the double helices. There is no evidence for this triplet in any of the ROA spectra of the nucleosides or nucleotides that we have studied so far. Preliminary results on B- and Z-form DNA reveal different sign patterns in this region depending upon the sugar−phosphate backbone conformation.

By combining the information given above, we can assign this ROA triplet to sugar ring vibrations in ordered sugar−phosphate backbones adopting a geometry associated with A-type right-handed helices. It is as yet not certain whether the phosphate groups which link the sugar rings in the polymers are involved in the generation of ROA.

All of the polyribonucleotide ROA spectra presented here actually extend down to ~650 cm^{-1}. However, below ~950 cm^{-1}, we observe only weak ROA signals that are difficult to assign. There are a number of important conventional Raman marker bands in this spectral region, for instance, a band at ~810 cm^{-1} that is assigned to a vibration of the furanose−phosphate ester linkage of A-type polyribonucleotides and the base-ring breathing modes between ~650 and 800 cm^{-1}.[13−17] But unfortunately, these particular bands are strongly polarized[41] and so are susceptible to artifacts in ROA measurements[42] (we observe little or no ROA intensity associated with these particular marker bands).

Conclusions

For polyribonucleotides, and nucleic acids in general, there are three central sources of chirality: the chiral arrangement of adjacent intrinsically achiral base rings, the mutual orientation of the base and sugar rings around the glycosidic link, and the inherent chirality associated with the asymmetric centers of the sugar rings. This first survey of polyribonucleotide ROA spectra has demonstrated that each of these sources dominates the generation of ROA signals in a separate well-defined spectral region.

The first region (~1550−1750 cm^{-1}), which we have named the base stacking region, contains sign patterns characteristic for each base that are highly sensitive to differences in base stacking arrangements. These sign patterns are particularly prominent in the deuterated forms of the polyribonucleotides with distinct ROA signals for each base (except for poly(rC)) undergoing an intensity increase relative to the undeuterated form. These enhanced signals appear at different characteristic wavenumbers for each base reflecting the different stretching vibrational coordinates that dominate the normal modes (C═O, C═N, C═C). However, each enhanced signal is negative at lower and positive at higher wavenumber. This conservation of sign may reflect a similar coupling in each and may be characteristic for right-handed polynucleotide structures. Our interpretation of the ROA data does not exclude the possibility that poly(rC) adopts a left-handed, non-base stacking helical structure as originally proposed by Broido and Kearns.[23] However, there are still a number of unexplained observations in this region which are the subject of ongoing ROA studies.

The second region (~1200−1550 cm^{-1}) we have named the base−sugar region as vibrational coordinates in both the base and sugar rings are mixing in the normal modes. The observed sign patterns reflect the mutual orientation of the sugar and base rings and/or the sugar ring conformation and are found to be similar to those observed for the corresponding monomers except for certain ROA signals that are selectively enhanced. Two contributions appear to be responsible for this enhancement: a hypochromic effect and a conformational effect. The hypochromic effect arises from the fact that there is a reduction in conventional Raman intensity when the polymers adopt base-stacked ordered structures with a concomitant increase in the observed Δ-value. The conformational effect reflects the decrease in ROA cancellation as the range of available torsion angles around the glycosidic link and the sugar ring is reduced upon polymerization.

Finally, the ROA in the third region (~950−1150 cm^{-1}), which we have named the sugar−phosphate backbone region, originates in normal modes that are localized in the sugar rings and as such reflect the conformation of this ring. It is not clear at present whether the phosphate backbone also contributes to the ROA in this region, but since the ROA is unlike that observed in the monomers, it may well be involved.

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(41) Ueda, T.; Ushizawa, K.; Tsuboi, M. *Biopolymers* **1993**, *33*, 1791−802.

(42) Hug, W. *Appl. Spectrosc.* **1981**, *35*, 115−24.