Vibrational Raman Optical Activity of DNA and RNA

A. F. Bell, L. Hecht, and L. D. Barron*

Chemistry Department, University of Glasgow
Glasgow G12 8QQ, U.K.

Received March 16, 1998

The study of solution structure and dynamics of biopolymers remains at the forefront of biomolecular science. A promising technique for such studies is Raman optical activity (ROA), which measures vibrational optical activity by means of a small difference in the intensity of Raman-scattered light from chiral molecules in right- and left-circularly-polarized incident light.[1,2] Thanks to new instrumentation developed over the past few years, ROA can now be measured routinely on a wide range of biological molecules in aqueous solution.[3] Following ROA studies of pyrimidine nucleosides[4] and synthetic polyribonucleotides,[5] we report here the first observations on naturally occurring DNA and RNA samples which demonstrate that, on account of its ability to probe directly the central chiral elements of biomolecular structure, ROA may enhance the already considerable value of conventional Raman spectroscopy[6] in the study of nucleic acids.

Figure 1 shows the backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of calf thymus DNA (top pair), phenylalanine specific transfer RNA (tRNA^{Phe}) from brewers yeast (middle pair), and the same tRNA^{Phe} but with the Mg^{2+} ions removed (bottom pair). The instrument used was that described earlier.[5] These spectra are generally more complex than the synthetic polyribonucleotide ROA spectra published previously:[5] one reason is that there are now four different bases present compared with one in the single- and two in the double-stranded synthetic polyribonucleotides; another is the possible presence of more conformational substates. We shall confine the discussion to a few general remarks since to date we have only obtained definitive ROA spectra of model polynucleotides in A-type conformations. A more detailed analysis of the data will follow from further studies on oligo- and polynucleotides in canonical B- and Z-conformations as well as less regular structures.

Although at first glance the ROA spectra of the DNA and the two RNAs look rather similar, there are many differences of detail. The largest differences originate in the DNA taking up a B-type double helix in which the sugar pucker is predominantly C2′-endo, and the RNAs taking up A-type double helical segments in which the sugar pucker is predominantly C3′-endo, together with less regular structures,[7] with smaller differences originating in the replacement of thymine in DNA by uracil in RNA and by the presence of the O2′-H hydroxyl group on the RNA sugar rings. The smaller differences between the two RNAs are of special interest because it is well-known that Mg^{2+} ions are necessary to hold active RNAs in their specific tertiary folds:[8,9] for tRNA^{Phe},

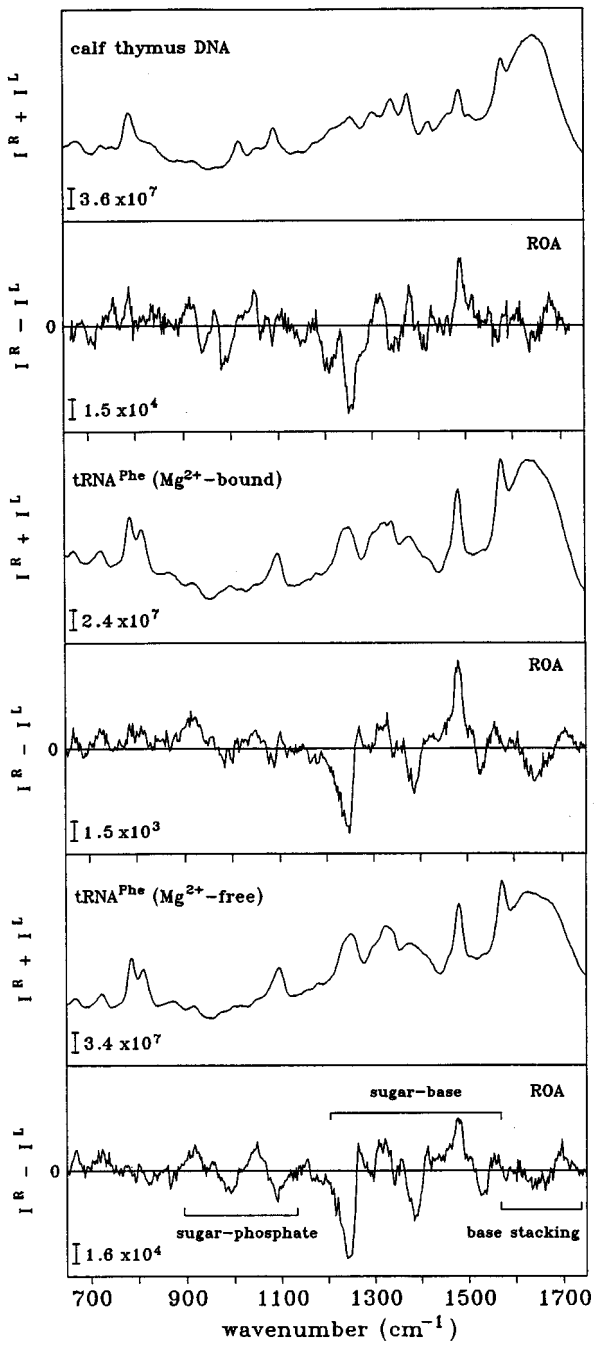

Figure 1. Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of DNA (top pair), Mg^{2+}-bound tRNA^{Phe} (middle pair), and Mg^{2+}-free tRNA^{Phe} (bottom pair). The DNA sample (from calf thymus) was supplied by Fluka Chemie AG and the tRNA^{Phe} (from brewers yeast) by Sigma Chemical Corp. The DNA sample was subjected to ultrasonication followed by the use of a Centricon filter to reduce the average molecular weight to the range ca. 10000−30000 in order to increase the solubility. The Mg^{2+}-free tRNA^{Phe} sample was prepared by dialysis against buffered EDTA. All were dissolved in sodium phosphate buffer (pH 6.8, 20 mM), the DNA at ∼30 mg/mL and the RNAs at ∼50 mg/mL with the Mg^{2+}-free sample containing additional 100 mM NaCl. Experimental conditions: laser wavelength 514.5 nm; laser power, 600 mW; spectral resolution, ∼10 cm^{-1}; recording time ∼16−20 h. The ROA measurements were not affected by the strong conventional optical activity of the rather concentrated nucleic acid solutions since the use of circularly polarized incident light at a transparent wavelength obviates any potential problems from optical rotation and circular dichroism.

(1) Hecht, L.; Barron, L. D. In *Modern Techniques in Raman Spectroscopy*; Laserna, J. J., Ed.; Wiley: Chichester, U.K., 1996; pp 265−304.
(2) Nafie, L. A. *Appl. Spectrosc.* **1996**, *50*, 14A-26A.
(3) Barron, L. D.; Hecht, L.; Bell, A. F. *Appl. Spectrosc.* **1996**, *50*, 619−29.
(4) Bell, A. F.; Hecht, L.; Barron, L. D. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 553−62.
(5) Bell, A. F.; Hecht, L.; Barron, L. D. *J. Am. Chem. Soc.* **1997**, *119*, 6006−13.
(6) Peticolas, W. L. In *Methods in Enzymology*; Lilley, D. M. J., Dahlberg, J. E., Eds.; Academic Press: London, 1995; Vol. 246, pp 389−416.
(7) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(8) Kazakov, S. A. In *Bioorganic Chemistry: Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: New York; 1996; pp 244−87.
(9) Tinoco, I., Jr.; Kieft, J. S. *Nature Struct. Biol.* **1997**, *4*, 509−12.

S0002-7863(98)00851-8 CCC: $15.00
Published on Web 05/28/1998

Mg^{2+} ions induce a change in structure from an open cloverleaf form (secondary structure) with an apparent angle between the anticodon and acceptor stems of ∼150° to a compact L-shaped form (tertiary structure) with an interstem angle of ∼80−90°.[10] Noncanonical local conformations, where changes in sugar puckers and backbone torsion angles facilitate turns and kinks in the RNA backbone allowing nonstandard base pairing or base intercalation, are the key to understanding higher-order RNA folding.[11] The L-shaped folded conformation of $tRNA^{Phe}$ provides well-characterized examples of these crucial conformation changes relative to the cloverleaf secondary structure and how they are stabilized by the Mg^{2+} ions.[7,8]

The earlier study of polyribonucleotides provided detailed band assignments which revealed that the ROA spectra can be divided approximately into three spectral regions containing information about distinct structural elements.[5] The base stacking region (∼1550−1750 cm^{-1}) contains bands characteristic for each base which are sensitive to the base stacking arrangement; the sugar-base region (∼1200−1550 cm^{-1}) contains bands associated with normal modes involving mixing of vibrational coordinates from both the base and sugar rings which reflect the mutual orientation of the sugar and base rings and perhaps the sugar ring conformation; and the sugar−phosphate region (∼900−1150 cm^{-1}) contains bands from vibrations localized mainly in the sugar rings which reflect the sugar ring and phosphate backbone conformations (the results of the present study have led us to extend the lower wavenumber limit of this region from ∼950 to 900 cm^{-1}).

Compared to that observed in double-helical polyribonucleotides,[5] the ROA in the base stacking region of all three samples is very weak, presumably on account of cancellation of bands from the large number of distinct stacking interactions between the four base types. Nonetheless, all three samples do show a weak ROA couplet in this region, negative at low wavenumber and positive at high, centered at ∼1660 cm^{-1} in the DNA and ∼1680 cm^{-1} in the RNAs, which may have a similar origin to that seen in double-helical poly(rA)·poly(rU):[5] the shift to lower wavenumber in the DNA might be due to the replacement of uracil by thymine. Some of these base stacking ROA bands might be boosted by measuring the spectra in D_2O solution since, for reasons still not understood, we have observed very large increases in certain polyribonucleotide ROA bands in this region in D_2O.[5]

In the sugar-base region the ROA band patterns in the RNA samples, which contain A-type double helical segments, look rather like a superposition of the patterns seen in this region in the poly(rA)·poly(rU) and poly(rG)·poly(rC) duplexes[5] which adopt A-type conformations. However, the ROA band pattern in the DNA sample, which contains mainly B-type double helices, is somewhat different. These observations are consistent with ROA bands in the sugar-base region reflecting mainly the torsion angles in the C−N glycosidic links and perhaps the sugar ring conformations, which are different in A- and B-type double helices. One potentially valuable feature is the strong positive ROA band associated with strong parent Raman bands at ∼1486 cm^{-1} in the DNA and ∼1481 cm^{-1} in the RNAs and previously assigned to guanine and adenine.[12] Although no changes are perceptible in the intensities and peak positions of the parent Raman bands, this ROA band is significantly stronger and sharper in the Mg^{2+}-bound than in the Mg^{2+}-free RNA, which might be associated with the different sugar-base torsion angles, or with different external perturbations of guanine and adenine from the nonstandard horizontal and vertical base−base interactions, including base intercalation, which are present in the turns and kinks stabilized by the metal ions in the noncanonical regions of $tRNA^{Phe}$ in its folded conformation.[7]

The sugar−phosphate region provides another example of the incisiveness of ROA for, although only very small differences can be seen in the (very weak) parent Raman bands of the Mg^{2+}-bound and Mg^{2+}-free RNA in this region, large differences are apparent in the ROA spectra which reflect differences in sugar ring and phosphate backbone conformation. Specifically, the Mg^{2+}-free sample shows a clear strong negative−positive−negative triplet with peaks at ∼992, 1047, and 1089 cm^{-1}, respectively, which coincides with an ROA triplet observed in the A-type polyribonucleotides and assigned to the associated C3′-endo sugar ring conformation,[5] whereas the Mg^{2+}-bound sample shows a weaker, more complex, pattern. Both show a positive ROA band at ∼920 cm^{-1}, which might monitor sugar rings with the C2′-endo pucker since calf thymus DNA shows a similar ROA band at this wavenumber and a band at ∼923 cm^{-1} in the conventional Raman spectrum of calf thymus B-DNA in aqueous solution has been assigned to bond stretching vibrations of the deoxyribose ring[12] (also this band is not seen in the ROA spectra of exclusively A-type polynucleotides[5]). If so, this would mean that the Mg^{2+}-free RNA contains a significant amount of C2′-endo sugar puckers in addition to C3′-endo (taking the ROA band pattern in the DNA in this region to be characteristic of the C2′-endo sugar pucker, it would tend to reinforce rather than cancel the C3′-endo ROA triplet). A change from C3′-endo to C2′-endo sugar puckering stretches the ribose−phosphate backbone and is known to occur in some regions of the tertiary fold of $tRNA^{Phe}$ such as positions where chain foldings switch abruptly from helical to looped;[7] our observations therefore indicate that such conformations are already nascent in our Mg^{2+}-free RNA sample, as might be expected for some regions of the cloverleaf secondary structure. The disappearance of the ROA triplet in the Mg^{2+}-bound RNA might originate in variations of the standard sugar puckers in the π-turns present in the loops stabilized by the Mg^{2+} ions within the tertiary fold[7] (or perhaps from new phosphate perturbations of the sugar rings due to the new P−O torsion angles in the π-turns).

We have shown that ROA spectra may now be measured routinely on naturally occurring DNA and RNA samples in aqueous solution and that they contain new information central to studies of the structure, folding, and function of these biomolecules. It is particularly encouraging that ROA appears to be sensitive to the different sugar ring and phosphate backbone conformations since these key elements of nucleic acid structure are difficult to detect by using conventional optical spectroscopic techniques.

Acknowledgment. We thank the Biotechnology and Biological Sciences Research Council for a Research Grant and the Engineering and Physical Sciences Research Council for a Senior Fellowship for L.D.B.

JA980851V

(10) Friederich, M. W.; Hagerman, P. J. *Biochemistry* **1997**, *36*, 6090−6099.

(11) Doudna, J. A.; Doherty, E. A. *Fold. Des.* **1997**, *2*, P65-P70.

(12) Thomas, G. J., Jr.; Benevides, J. M.; Overman, S. A.; Ueda, T.; Ushizawa, K.; Saitoh, M.; Tsuboi, M. *Biophys. J.* **1995**, *68*, 1073−88.