THE OPTICAL ROTATORY DISPERSION OF ADENYLYL (3'-5') ADENOSINE AND ITS SIGNIFICANCE FOR POLYNUCLEOTIDE ROTATIONS

Myron M. Warshaw, C. Allen Bush and Ignacio Tinoco, Jr.

Chemistry Department
University of California,
Berkeley, California

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Recently, Lin, Urry and Eyring (1964) have suggested that the optical rotatory dispersion (ORD) of (5') adenylic acid is dependent upon the position of the phosphate group relative to the base. They point out that an octant rule analogous to that used for steroids (Djerassi, 1960) may be applicable to the nucleoside in explaining the effect of the position of the phosphate group. Thus, they explain the change in sign of the long wavelength rotation from negative for adenylic acid to positive for RNA by a change in position of the phosphate group. Although the orientation of the phosphate group may be important for the ORD of the mononucleotides, we have strong evidence that it is negligible for the polynucleotides.

Figure 1 shows the ORD per residue of adenylyl (3'-5') adenosine (ApA) and the average ORD of equal amounts of (5') adenylic acid and adenosine in .01M phosphate buffer, pH 7, and 0.08M $KClO_4$. The ORD for adenylic acid and adenosine are in good agreement with the published values (Lin, et al., 1964), and the rotation of ApA is positive at long wavelengths. However, the ORD of ApA exhibits two Cotton effects (one positive and one negative) near

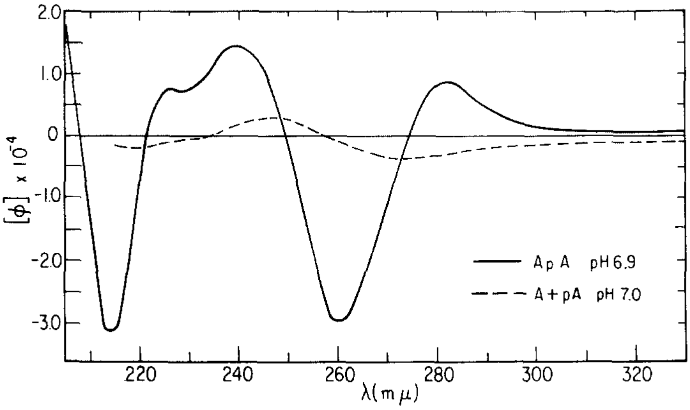

Figure 1. The rotation of adenylyl (3'-5') adenosine (ApA), 5' adenylic acid (pA) and adenosine (A) were measured on a Cary 60 spectropolarimeter and plotted as rotation per mole of residue. The buffer was 0.01M phosphate and 0.08M $KClO_4$ (neutral pH, 0.1 ionic strength); the temperature was 25°C. A 1 cm. cell was used with the absorbance at 260 mμ less than 1.

260 mμ, while adenylic acid and adenosine have only one. Furthermore, the rotation of ApA is an order of magnitude larger than its constituents. The change in conformation of the phosphate group would not be expected to introduce a new Cotton effect or to produce such a large change in the magnitude of the rotation.

The ORD of ApA can be qualitatively explained in terms of the interaction between the two adenines (Tinoco, et al., 1963). This interaction leads to a splitting of the 260 mμ absorption band of each adenine into two bands separated by a few millimicrons. The splitting is small enough so that two bands are not expected in the ApA absorption; in fact, ApA shows only a slight broadening of the 260 mμ band. However, these two bands must have Cotton effects which are opposite in sign and are of nearly equal magnitude. This leads to an ORD curve similar in shape to that of ApA in Figure 1.

A quantitative calculation of the contribution of the 260 mμ band of adenylic acid to the ORD of ApA can be made using exciton theory. The electric transition moment for this band has a magnitude of 4.1 Debye and its direction in 9-methyladenine is along the line joining C_4 and C_5 (Stewart and Jensen, 1964). To calculate the exciton splitting, we used point transition monopoles placed at the carbon and nitrogen nuclei in each adenine. The monopoles were calculated from LCAO-SCF-CI molecular orbitals which gave transition moments consistent in magnitude and direction with the experimental values for the 260 mμ transition. The geometry assumed for ApA is that for successive purines in the same strand of Watson-Crick DNA (Langridge, et al., 1960). This leads to a splitting of 2000 cm^{-1}, which is 14 mμ at 260 mμ. The calculated rotational strengths are $+193 \times 10^{-40}$ cgs for the 267 mμ band and -193×10^{-40} for the 253 mμ band. Therefore, the calculated signs of the Cotton effect are in agreement with experiment. To obtain the ORD curve, we assume that the circular dichroism bands have the same shape and position as the absorption bands (Moffitt and Moscowitz, 1959). A Kronig-Kramers transform on the bands leads to the ORD curve (Moscowitz, 1960). The calculated molar rotation per residue and wavelength at the trough of the ORD is -91,200 at 261 mμ as compared to -28,600 at 260 mμ for the experimental values. As the calculation is for a rigid molecule, while the experimental value is for a molecule which is not rigidly held in the DNA geometry, we would expect a larger magnitude for the calculation. Normalizing the magnitudes of the experimental and calculated ORD at their troughs gives the results shown in Figure 2; a normalized circular dichroism curve is also shown. For this comparison,

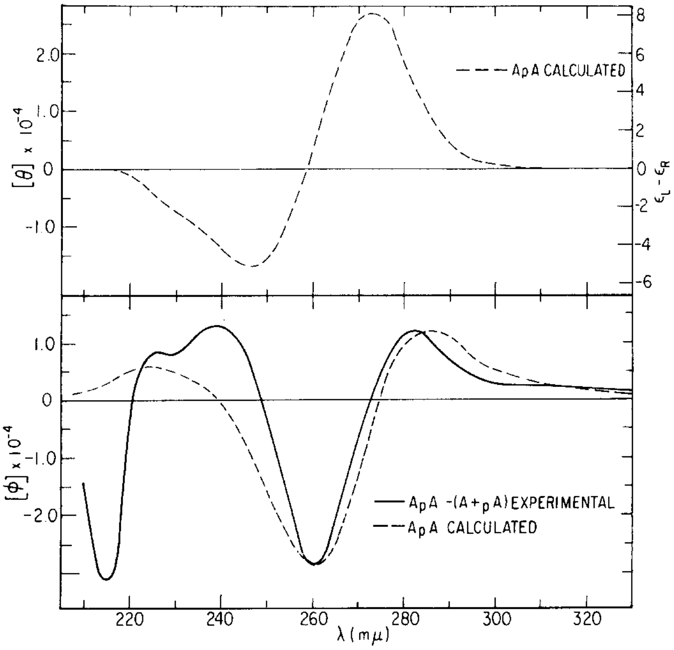

Figure 2. The upper dashed curve is the calculated circular dichroism of ApA plotted as the ellipticity per mole of residue (left ordinate) or as $\epsilon_L-\epsilon_R$ per mole of residue (right ordinate). The lower dashed curve is the calculated rotation per mole of residue of ApA. The solid curve is a plot of the difference between the two curves of Figure 1, $([\phi]_{ApA}-[\phi]_{A\ +\ pA})$. The calculated ORD is normalized to the magnitude of the experimental ORD at the first trough (approximately 260 mμ). The circular dichroism was normalized accordingly.

experiment is taken to be the difference between the measured ApA and the adenylic acid plus adenosine ORD curves of Figure 1. The wavelength positions of the calculated peaks, trough and crossovers are close to the experimental values, and the calculated ratio of the long wavelength peak to the trough agrees with experiment. Inclusion of the 206 mμ band of adenosine in the calculation would give a trough close to the experimental one seen at 216 mμ.

Calculation of the ORD with the base planes parallel and their vertical distance 3.4Å, but with the angle between the bases different from the Watson-Crick 36°, does not change the general shape of the ORD. The sign of the calculated rotation changes when this angle becomes greater than 90° or less than 0°. It seems clear that the agreement seen in Fig. 2 is not limited to the particular parameters chosen, and that ApA has a conformation similar to the bases in a single strand of DNA.

Polyadenylic acid at pH 7 in 0.15M KCl has an ORD curve nearly identical in shape to that of ApA, but increased in magnitude (Holcomb and Tinoco, 1965). Other dinucleoside phosphates and trinucleoside diphosphates of different compositions have ORD curves which are indicative of base-base interaction. Also the long wavelength Cotton effect for the 2' and 3' isomers of adenylic acid are identical to that of adenosine and 5' adenylic acid. Therefore, we are confident that the ORD of RNA can be interpreted as coming primarily from interaction among the four bases and that the phosphate orientation is not important.

Acknowledgments

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Erratum

Vol. 18, No. 1 (1965), in the communication "Peptide Maps of Antibodies against an Antigen Containing Two Different Determinant Groups", by Eugene F. Gold, Katherine L. Knight, and Felix Haurowitz, pp. 76-80:

Page 78, the following should be added to the legend for Figs. 1 and 2: "The peptide mixture was applied at the bottom of the vertical center band. The cathode in Fig. 1 is at the right, in Fig. 2 at the left side of the center band."