ULTRAVIOLET ROTATORY DISPERSION OF ADENOSINE, INOSINE AND THEIR MONOPHOSPHATES

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The rotatory dispersion of adenosine and its phosphate derivatives has been determined to 320 mμ by Levedahl and James (1956). It was found that the observed plain curves could be described by a single term Drude equation. The effects of calcium, zinc and magnesium ions on the rotatory dispersion of both adenosine triphosphate (ATP) and inosine triphosphate (ITP) were investigated by McCormick and Levedahl (1959). The data were interpreted to be in agreement with a folded structure of ATP. The present communication reports the rotatory dispersion of adenosine and AMP in the ultraviolet region from 200 mμ to 320 mμ and of inosine and IMP in the range 210 mμ to 280 mμ. This wavelength range is particularly illuminating for these nucleosides and nucleotides. No anomolous dispersion is found at wavelengths greater than 320 mμ, whereas three Cotton effects may be noted at shorter wavelengths, centering approximately at 260 mμ, 210 mμ and 190 mμ. The effect of pH, temperature and solvent on these three Cotton effects is treated here. Preliminary results concerning metal ion effects on the rotatory dispersion curves of ATP will be noted.

The careful characterization of AMP is prerequisite to the determination of the conformation of ATP which plays such an important role in energy coupling in biological systems. Such a study is further warranted due to the recurrence of AMP as a unit in many nucleotides and coenzymes, e.g. flavine adenine dinucleotide, nicotinamide adenine dinucleotide and coenzyme A.

EXPERIMENTAL: The spectropolarimeter used was the Cary model 60. The instrument was operated with a response time of 30 sec. and scan speeds of 1 to 5 mμ/min. To reduce absorbance, solutions were not buffered. Three concentrations were employed depending on the spectral range: 2 μmole/ml between 320 and 280 mμ, 0.2 μmole/ml between 280 and 220 mμ and 0.06 μmole/ml below 220 mμ. Data obtained below 220 mμ were less precise due to low concentrations, high absorbance, and the limits of resolution of the instrument. The cell compartment of the spectropolarimeter was thermostated at approximately 27°C. The pH was determined with the use of a Beckman zeromatic pH meter, the samples being adjusted to extremes of pH immediately before running. The concentrations of HCl, $HClO_4$ or NaOH were such that no significant dilution occurred during adjustment of pH.

The biochemicals were purchased from Sigma Chemical Company and in each case were the highest purity there available.

RESULTS: The rotatory dispersion curves of AMP were determined at closely spaced pH intervals. A few representative curves are shown in Figure 1. It is seen that there are marked changes in the magnitudes of rotation of the 220 mμ and the 200 mμ extrema, whereas the 220 mμ extremum of AMP continues to decrease in magnitude as the pH is increased from 4.7. Figure 2 shows that adenosine does not exhibit this further change. Thus, in adenosine a decrease in the 220 mμ extremum is found in going from a pH of 1.3 to 4.3 and no significant change is found on increasing the pH to 7.2. However, in AMP there is a continued decrease in rotational strength at 220 mμ in going from pH 4.9 to 8.9. Ribose-5-phosphate does not show a significant pH effect in its rotatory dispersion even at a 2 μmole/ml concentration. Nor do IMP and inosine show such variation with pH (Figures 3 and 4).

The variation with temperature is not large for AMP. At a pH of 3.3, a seventy degree increase in temperature causes a 15 to 20% decrease in amplitude of the Cotton effects. Phosphate buffer does not

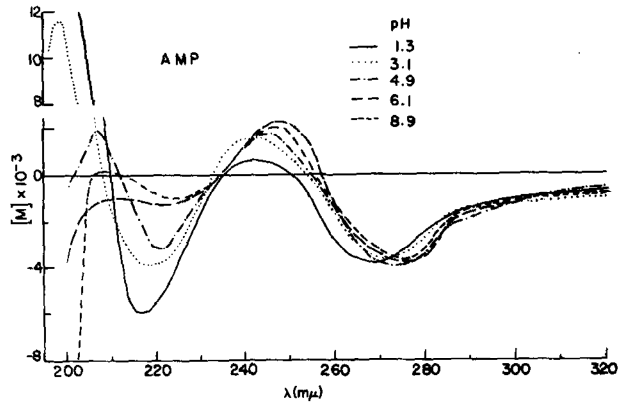


Fig. 1. Rotatory Dispersion Curves of AMP

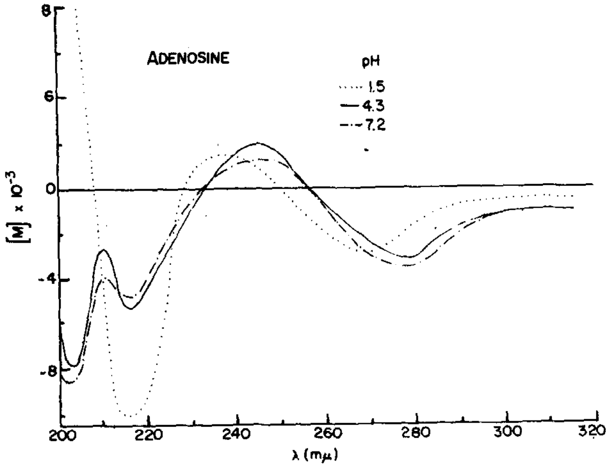


Fig. 2. Rotatory Dispersion Curves of Adenosine

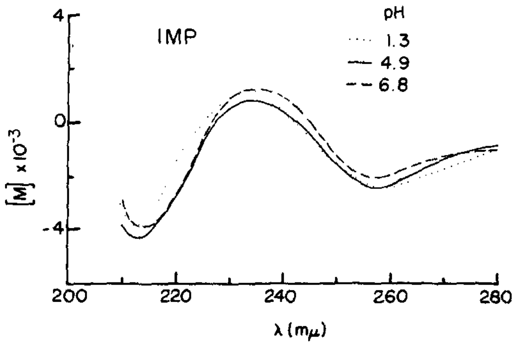


Fig. 3. Rotatory Dispersion Curves of IMP

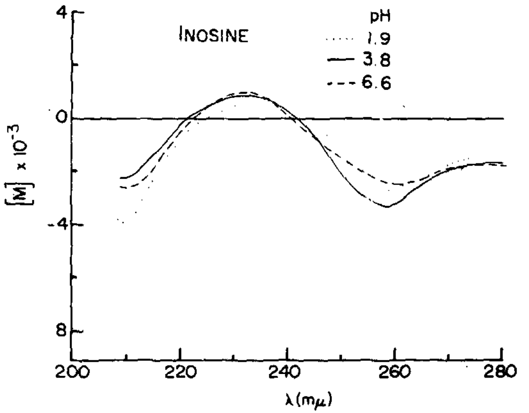
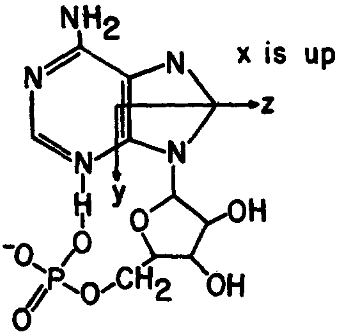


Fig. 4. Rotatory Dispersion Curves of Inosine

alter the anomolous dispersion of AMP nor do 10% and 50% ethanol solutions.

DISCUSSION: The integrated absorption coefficient is proportional to the square of the total electric dipole moment for a transition, whereas the rotational strength is the dot product of the electric dipole and magnetic dipole moments for that transition. Thus, a decrease in absorbance without a change in conformation would result in a decrease in the rotational strength. However, if the integrated absorption coefficient increases while the rotational strength decreases, one is led to consider conformational changes. As may be seen by referring to Figure 5, an increase in pH results in an increase in the absorption peak at about 210 mμ for AMP, whereas reference to Figures 1 and 2 indicates that the rotational strength decreases over this same interval. Adenosine and AMP have a proton function associated with the adenine moiety with a pk of 3.3. In addition, AMP has a second proton function in the phosphate group with a pk of 6 (Taylor, 1948). It is this proton function which is involved in the differing behavior of the rotational extrema at 220 mμ. Upon examination of molecular models of AMP, the foregoing considerations have suggested the possibility of the following hydrogen bonded structure for AMP at a pH of 5:



However, as there is only a small temperature effect, the conformation cannot be maintained solely by hydrogen bonding, i.e., other stabilizing effects are required.

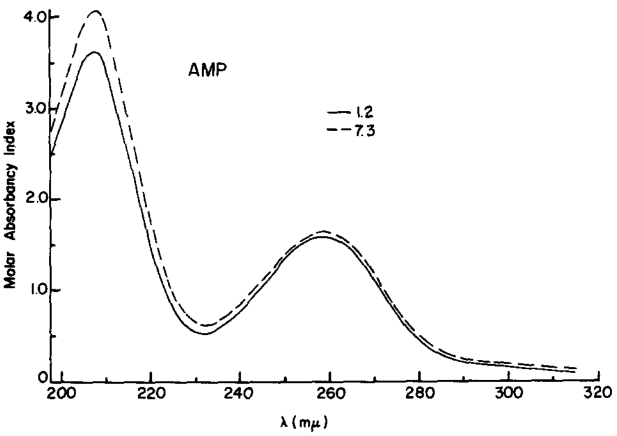


Fig. 5. Absorption Curves of AMP

It is of interest to note that the longest wavelength Cotton effect is reversed in sign in RNA (Samejima and Yang, 1964) as compared with the monomer nucleosides and nucleotides. While interactions with other identical units in the polymer greatly effect the rotation exhibited by the polymer, it may be pointed out that the phosphoribose moiety is extended and would lie in a different octant than the adenine ring system. If there is an octant rule operative, this would explain the reversal in sign of the Cotton effects. Thus, if one considers the adenine moiety as a gross chromophoric ellipsoid and sets up the coordinate system indicated in the figure drawn above, following the approach of Jones and Eyring (1961), a negative Cotton effect is predicted for the long wavelength absorption of AMP and a positive Cotton effect is predicted for the polymer conformation, that is, the sign of the Cotton effect has the same sign as -xyz and further x for the ribose moiety is positive for AMP and negative in the polymer.

Preliminary investigations of the effect of Zn(II) on ATP indicate a negative displacement of the anomolous dispersion curves at pH 7. Careful characterization of this effect in light of the AMP data could well support ideas of a folded conformation for ATP metal complexes (Szent Gyorgyi, 1936).

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