Vol. 26, No. 6, 1967 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

ASSOCIATION OF ADENOSINE-5'-PHOSPHATE IN AQUEOUS SOLUTIONS*

G. P. Rossetti and K. E. Van Holde
Department of Chemistry and Chemical Engineering
University of Illinois, Urbana, Illinois

Received February 16, 1967

The work of T'so and collaborators (T'so, Melvin and Olson, 1963; T'so and Chan, 1964; Chan, Schweizer, T'so and Helmkamp, 1964) has clearly demonstrated that purine and a number of its derivatives tend to form "stacked" aggregates in concentrated aqueous solution. Similar behavior is exhibited in the formation of single strand structures by oligo-and polyriboadenylic acids (see, for example, Holcomb and Tinoco, 1965; Van Holde, Brahms and Michelson, 1965; Leng and Felsenfeld, 1966; Brahms, Michelson, and Van Holde, 1966; Poland, Vournakis, and Scheraga, 1966.)

A next question of considerable biological significance is whether or not adenosine-5'-phosphate (AMP) will undergo such spontaneous association. The fact that AMP is charged at all pH values above about 2.5 (Alberty, Smith and Bock, 1951) would suggest that its tendency to associate, if any, would be considerably less than that of purine. Nevertheless, in the course of investigations of the physical and optical properties of nucleotides, we have obtained direct evidence for the association of AMP in aqueous salt solutions.

---

*This research was supported by a Public Health Service Grant, GM-13373.

EXPERIMENTAL

The association was studied by sedimentation equilibrium in the Beckman-Spinco Model E analytical ultracentrifuge. The instrument was equipped with an RTIC unit. Because of the high concentrations, phase-plate schlieren optics were employed. The rotor speeds were so chosen as to yield an easily measurable gradient at each concentration. Solution columns about 6 mm in length were used.

Solutions for sedimentation equilibrium were prepared by dissolving the appropriate amount of AMP (Cal Biochem, A Grade) together with an equimolar amount of KOH and an equimolar amount of $KC\ell$ in the appropriate volume of distilled water. The pH values of these solutions ranged from 5.00 (0.6M) to 5.32 (0.05M). According to titration and electrophoresis data (Alberty, et. al., 1951; Bock and Alberty, 1951) the solute should be almost entirely in the AMP^- form under these conditions.

The partial specific volume of the potassium salt of AMP was determined pycnometrically with 0.02 M solutions; duplicate experiments gave values of 0.510 and 0.514.

RESULTS AND DISCUSSION

The apparent molecular weights obtained for electrolytes in the ultracentrifuge must be corrected for the effects of charge. In general, two effects are observed; a primary charge effect, which is proportional to the ratio of the concentration of the electrolyte in question to the concentration of low molecular weight supporting electrolyte, and a secondary charge effect, which persists in the limit of infinite dilution. It has been shown (Johnson, Kraus, and Scatchard, 1954; Johnson, Scatchard, and Kraus, 1959) that the theory is greatly simplified if one chooses to consider, instead of the 1:1 electrolyte AMP·K, a component $AMP{\cdot}K - \frac{1}{2} KC\ell$. We refer to this new component as component 2, and the appropriate amount of $KC\ell$ as component 3. By rearrangement of the equations of Johnson, et. al., one may obtain an expression for the apparent weight average molecular weight of the component AMP•K,

$$M^*_{wA} = \frac{RT}{\omega^2(1-\bar{v}_A\rho)\left\{1 - \frac{k_B M_B}{2k_A M_A}\right\}\left\{1 - \frac{M_B(1-\bar{v}_B\rho)}{2M_A(1-\bar{v}_A\rho)}\right\}} \frac{dC_2/dr}{rC_2}\left\{1 + \frac{C_2}{2C_3}\ \emptyset\right\}$$

where C_2 and C_3 are the molar concentrations of components 2 and 3, and subscripts A and B refer to the alternative components AMP•K and KCℓ. The quantities k_A and k_B are specific refractive index increments for AMP•K and KCℓ, respectively, and $\emptyset$ is a constant close to unity, given by

$$\emptyset = 1 - \frac{M_B(1-\bar{v}_B\rho)}{2(M_A(1-\bar{v}_A\rho)-M_B(1-\bar{v}_B\rho))}$$

The ratio $\frac{1}{C_2}dC_2/dr$ can be obtained at each point in the cell, from (corrected) wire image displacements and a (corrected) synthetic boundary experiment. The factor $\left\{1 + \frac{C_2}{2C_3}\ \emptyset\right\}$ can be approximated quite accurately by $\left\{1 + \frac{C_2}{2C_3^o}\ \emptyset\right\}$, where C_3^o is the initial concentration of 3. Details of the methods of calculation will be given in a later paper. The quantity M^*_{wA} obtained is the apparent weight average weight of the associating mixture, with AMP•K taken as monomer. It has been corrected for charge effects, but not for other sources of non-ideality.

If the AMP^- ions undergo association, the apparent molecular weight of AMP•K will increase with increasing solute concentration. Furthermore, experiments performed at different concentrations should yield points lying on a single curve. This latter criterion is of considerable importance, for unless the charge corrections have been properly made, such superposition will not be observed. The results are shown in the accompanying Figure, where they are expressed as the ratio of apparent weight average molecular weight to the known monomer weight of AMP•K. The data extrapolate, as they should, to the monomer weight at zero concentration. It is clear that the results do indicate increasing association with increasing concentration. The

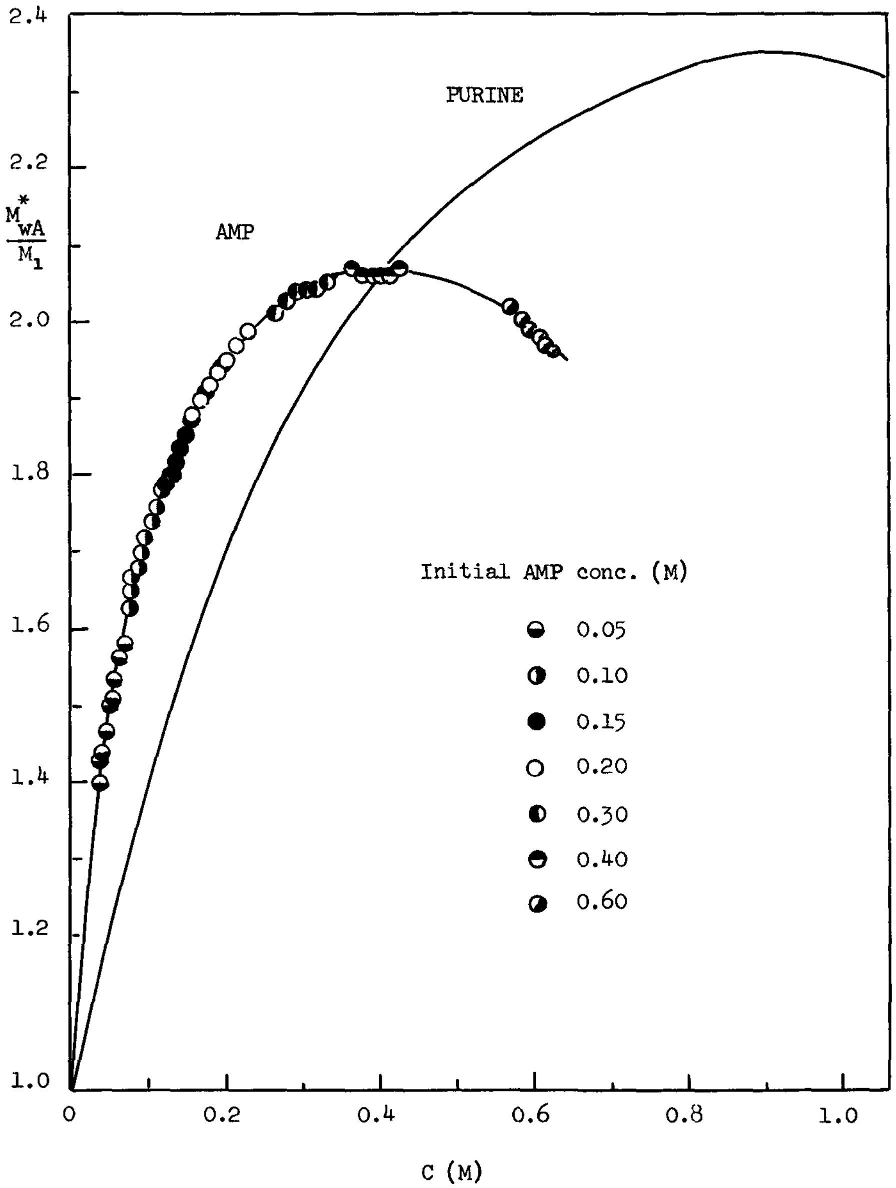

Figure 1. Weight average molecular weight over monomer weight for AMP and Purine. AMP data at 23.7°C, purine at 24.9°C.

decrease in M^*_{wA} at high concentrations apparently results from non-ideality (a positive second virial coefficient). We have observed a similar effect with very concentrated purine solutions, and have been able to separate the effects of non-ideality and association.

Even though the effect of non-ideality is to depress the apparent molecular weights at higher concentrations, the curve indicates apparent association of AMP past the dimer stage. In fact, the association is very similar to that of purine (see solid line) which we have also studied by sedimentation equilibrium. Our data on purine are consistent with those of T'so et. al., once corrections for non-ideality are made.

It should be emphasized that at this point we have no information on the mechanism of the AMP$^-$ association. In particular, we do not know to what extent "stacking" interactions and hydrogen-bonding may be involved. Experiments to elucidate this point are now in progress. More complete reports of both the AMP$^-$ and purine experiments are being prepared.

REFERENCES

Alberty, R. A., Smith, R. M., and Bock, R. M.; J. Biol. Chem. 193, 425 (1951).

Bock, R. M., and Alberty, R. A.; J. Biol. Chem. 193, 435 (1951).

Brahms, J., Michelson, A. M., and Van Holde, K.; J. Mol. Biol. 15, 467 (1966).

Chan, S. I., Schweizer, M. P., and T'so, P. O. P.; J. Am. Chem. Soc. 86, 4182 (1964).

Holcomb, D. N., and Tinoco, I., Jr.; Biopolymers 3, 121 (1965).

Johnson, J. S., Kraus, K. A., and Scatchard, G.; J. Phys. Chem. 58, 1034 (1954).

Johnson, J. S., Scatchard, G., and Kraus, K. A.; J. Phys. Chem. 63, 787 (1959).

Leng, M., and Felsenfeld, G.; J. Mol. Biol. 15, 455 (1966).

Poland, D., Vournakis, J. N., and Scheraga, H. A.; Biopolymers 4, 223 (1966).

T'so, P. O. P., and Chan, S. I.; J. Am. Chem. Soc. 86, 4176 (1964).

T'so, P. O. P., Melvin, I. S., and Olson, A. C.; J. Am. Chem. Soc. 85, 1289 (1963).

Van Holde, K. E., Brahms, J., and Michelson, A. M.; J. Mol. Biol. 12, 726 (1965).