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Dissociation and association of AMP nucleosidase from *Azotobacter vinelandii*

AMP nucleosidase (AMP phosphoribohydrolase, EC 3.2.2.4) from *Azotobacter vinelandii*, strain O, catalyzes the hydrolysis of AMP to form adenine and ribose 5-phosphate. HURWITZ, HEPPEL AND HORECKER[1] demonstrated the absolute dependence on ATP for the catalytic hydrolysis of AMP, and that ATP was recovered unchanged from the reaction mixture.

In a previous report[2], evidence has been presented which characterized P_i as an allosteric inhibitor of AMP nucleosidase from *A. vinelandii*, and the physiological implications of the metabolic regulation of this enzyme have been discussed briefly.

The present paper reports experiments which suggest that AMP nucleosidase[2] dissociates into subunits in buffers with low sulfate concentrations and that the cofactor ATP or the substrate AMP, when present, participates in the association of the subunits. This is shown by the sucrose density gradient centrifugation.

The sedimentation coefficient of AMP nucleosidase depends strongly on sulfate concentrations of the buffer used for the measurements. Freshly prepared enzyme in 200 mM sulfate sediments in the sucrose gradients as a single component with an S value of approx. 10.6. However, after dialysis against the buffer with 50 mM sulfate, an *s* value of 6.3 was obtained. A graphic representation of the sedimentation pattern is shown in Fig. 1. The decrease in the sedimentation coefficient from 10.6 to 6.3 S

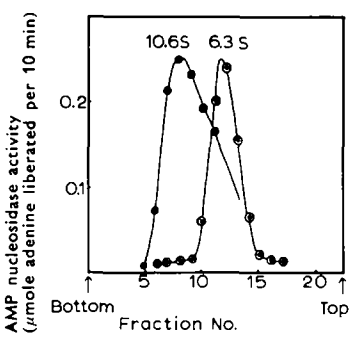

Fig. 1. Dissociation of AMP nucleosidase. The enzyme preparation in 200 mM or 50 mM K_2SO_4 was mixed with bovine serum albumin which served as a marker for the determination of the sedimentation coefficients[3]. The 0.2-ml samples were layered on to 4.2 ml of 5–25% sucrose gradients containing 200 mM (●– ●) or 50 mM K_2SO_4 (◉–◉), and 20 mM Tris HCl buffer (pH 7.8). After centrifugation for 16 h at 37 500 rev./min in a Hitachi RPS 40 rotor at 8°, fractions of approx. 0.2 ml were collected. The activities of AMP nucleosidase were measured as described previously[2].

suggests that AMP nucleosidase may exist as a small or a large form depending on the concentrations of sulfate present. The change from one to the other is reversible; the 10.6-S form can be converted to the 6.3-S form by dialysis into a buffer with sulfate concentrations of 50 mM and also the 6.3-S form can be converted to the 10.6-S form merely by raising the sulfate concentrations in the buffer to 200 mM. When the sulfate

concentrations are raised to 200 mM, however, sedimentation of the enzyme results in a major peak with 10.6 S and a minor peak or a shoulder with 8 S.

Moreover, when fresh, the enzyme sediments as a sharp peak with a sedimentation coefficient of 10.6 S, while after aging, the same preparation sediments as two peaks, one corresponding to the large form (10.6 S) and the other to the middle form (8.0–8.3 S).

ATP, a cofactor of this enzyme, re-associates the dissociated (6.3 S) AMP nucleosidase. Sedimentation of this enzyme in the presence of 2 mM ATP and 50 mM sulfate resulted in two peaks, one corresponding to the large form (10.6 S) and the other to "halves" (6.3 S). In the presence of 5 mM ATP and 50 mM sulfate only a single peak corresponding to the large form was observed (Fig. 2A). The presence of ATP has no effect on enzyme sedimentation at 200 mM sulfate.

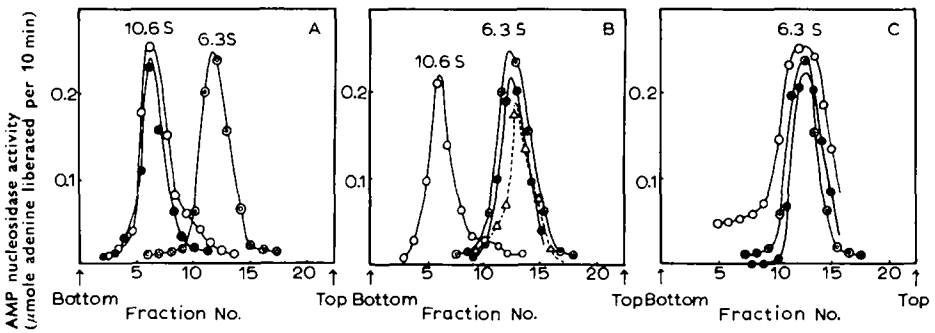

Fig. 2. Sedimentation patterns of AMP nucleosidase in sucrose gradients. AMP nucleosidase preparations in 50 mM K_2SO_4 were incubated for 1 h in additives (*e.g.* AMP, ATP, P_i, $MgCl_2$ *etc.*), and sedimented in 5–25% sucrose gradients containing 50 mM K_2SO_4 and additives at pH 7.8. The conditions of the centrifugation and enzyme assays were the same as those in Fig. 1. A. Sedimentation patterns obtained at 5 mM ATP (○- -○), 5 mM AMP (●—●) and no additives (◉--◉). B. Sedimentation patterns obtained at 5 mM IMP (○- -○), 5 mM UMP (●—●), 5 mM adenine (△- - -△), and no additives (◉—◉). C. Sedimentation patterns obtained at 5 mM $MgCl_2$ (○—○), 20 mM P_i (●—●) and no additives (◉—◉).

The substrate, AMP, re-associates the dissociated AMP nucleosidase. When 5 mM AMP is added to the gradients containing 50 mM sulfate, the enzyme sediments as a large form (10.6 S) (Fig. 2A). The addition of AMP (5 mM) to the gradients containing 200 mM sulfate has no effect on the sedimentation properties of this enzyme.

IMP, one of the allosteric inhibitors of this enzyme, also re-associates the dissociated enzyme. The addition of 5 mM IMP resulted in a single peak corresponding to the large form (Fig. 2B). However, the addition of 5 mM UMP showed no effect on the sedimentation patterns of this enzyme. The presence of 5 mM adenine, which is one of the reaction products, or P_i, an allosteric inhibitor, revealed no effect on enzyme sedimentation at 50 mM sulfate (Figs. 2B and 2C).

The re-association of the subunits was not dependent on the presence of Mg^{2+} in the sucrose gradients, although Mg^{2+} is absolutely required for the catalytic activity. As shown in Fig. 2C, the addition of 5 mM Mg^{2+} to the gradients containing 50 mM sulfate has no effect on the sedimentation patterns of the enzyme.

The above experiments are presented now because they do show quite clearly the evidence for the presence of two distinct forms of AMP nucleosidase. However,

these findings do not clarify the correlation between the subunit structure of this enzyme and its catalytic activity. The results presented here represent a start on a study of the regulatory mechanism of AMP nucleosidase. Work now is proceeding on the experiments to elucidate the relationship between two forms of AMP nucleosidase and its catalytic activities. Further work will especially be necessary to clarify the role of ATP.

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