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Apparent differences in enzymic rates of hydrolysis of commercial adenosinetriphosphate preparations

We wish to draw attention to the finding that commercial crystalline disodium ATP preparations differ greatly in their susceptibilities to hydrolysis by myofibrils or actomyosin and that the differences are likely to be attributable to slight contamination of ATP with Ca^{2+}.

Table I shows the differences in the rates of P_i production between two lots of ATP's purchased from Sigma Chemical Co., St. Louis, using myofibrillar ATPase

TABLE I

VARIATIONS IN MYOFIBRILLAR ATPASE ACTIVITY USING DIFFERENT LOTS OF ATP

The 2.5-ml reaction mixtures at 25° contained 40 mM Tris–acetate buffer (pH 7), 4 mM magnesium acetate, 4 mM ATP (as shown) and 1 mg of myofibrillar protein[1,2]. The ionic strength was adjusted to 0.15 by a mixture of 0.13 M KCl and 0.02 M potassium acetate solution. After 5-min reactions, 2.5 ml of 8% trichloroacetic acid was added, and P_i was measured by the method of FISKE AMD SUBBAROW[10] after removal of precipitate by centrifugation.

Myofibrillar preparation	*ATP*	*ΔP_i (μmole/min per mg protein)*
A	Sigma 52B728	0.381
A	Sigma 52B728	0.381
A	Sigma 22B721	0.126
A	Sigma 22B721	0.139
B	Sigma 52B728	0.196
B	Sigma 22B721	0.070

(EC 3.6.1.3). Even though the specific activities varied from one myofibrillar preparation to another, the percentage differences between the two ATP's remained the same. Further, as seen in Table II, the differences among various ATP's persisted even when the myofibrillar ATPase was coupled to the creatine kinase (EC 2.7.3.2) system.

TABLE II

VARIATIONS IN MYOFIBRILLAR ATPASE ACTIVITY COUPLED TO PHOSPHOCREATINE AND CREATINE KINASE, USING DIFFERENT LOTS OF ATP

The 2.5-ml reaction mixtures at 25° contained 40 mM Tris–acetate buffer (pH 7), 4 mM magnesium acetate, 4 mM ATP (as shown), 3 mM sodium phosphocreatinate[11], 20 units of creatine kinase[3] and myofibrils as shown. The ionic strength was adjusted to 0.15 by a mixture of 0.13 M KCl and 0.02 M potassium acetate solution. After 5-min reactions, 0.2-ml aliquots were mixed with 1 ml of 2 N NaOH for creatine measurement[4].

Myofibrils (mg protein)	*ATP*	*ΔCreatine (μmole/min per mg protein)*
1.0	Sigma 52B728	0.22
1.0	Sigma 22B721	0.14
3.0	Pabst 133A	0.200
3.0	Sigma 22B721	0.073

With the slowly hydrolyzing ATP's, $CaCl_2$ added at a concentration of about 0.02 mM increased the rate of ATP utilization to a level which was observed with some other ATP's without any added Ca^{2+} (Fig. 1).

The myofibril-catalyzed ATP hydrolysis was also studied in the pH'stat at pH 7 at varying substrate concentrations (always with equimolar Mg^{2+} and ATP concentrations). As seen in Fig. 2, with Pabst lot 133-A, a saturation type of curve is obtained, whereas with Sigma 22B721 an apparent inhibition sets in above 0.5 mM ATP. Addition of 0.03 mM $CaCl_2$ to the experiment using 4 mM of the slowly hydrolyzing Sigma ATP raised the rate of hydrolysis exactly to that obtained with the rapidly hydrolyzing Pabst lot at identical substrate concentration but without added Ca^{2+}. On the other hand, addition of 0.03 mM $CaCl_2$ to the experiment, using 4 mM of the rapidly hydrolyzing Pabst ATP, failed to further augment the rate of hydrolysis.

The differences in hydrolytic rates of various ATP preparations could also be observed with actomyosin as a catalyst at low ionic strength. Sigma lot 52B728

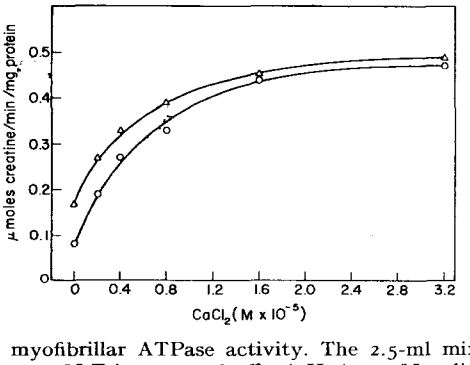

Fig. 1. Effect of Ca^{2+} on myofibrillar ATPase activity. The 2.5-ml mixtures at 27° contained 4 mM magnesium acetate, 40 mM Tris–acetate buffer (pH 7), 3 mM sodium phosphocreatinate[11], 10 units of creatine kinase[3], 4 mM ATP, 2.15 mg of myofibrillar protein and $CaCl_2$ as shown on the abscissa. The ionic strength was adjusted to 0.15 by a mixture of 0.13 M KCl and 0.02 M potassium acetate solution. After 5-min reactions, 0.2-ml aliquots were mixed with 1 ml of 2 N NaOH for creatine measurement[4]. ○—○, Mann G1907 ATP; △—△, Pabst 133-A ATP.

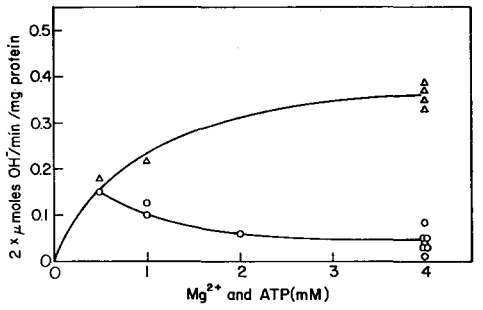

Fig. 2. pH-Static measurement of myofibrillar ATPase activity. The 9.8-ml mixtures at 25° and pH 7, contained 100 mM KCl, 1 mg of myofibrillar protein, equimolar Mg^{2+} and ATP concentrations as indicated on the abscissa. Titrant was 0.01 M NaOH with 0.04 M NaCl. Ordinate is presented as twice the amount of base used in titration in order to approximate the total amount of phosphate hydrolysed. ○—○, Sigma 22B721 ATP; △—△, Pabst 133-A ATP.

hydrolyzed 4-fold faster than Sigma lot 22B721. Further, it is of interest to note that the slowly hydrolyzing ATP failed to produce a visual superprecipitation of the actomyosin gel.

Since all ATP preparations gave identical ε_M values in the ultraviolet and chromatographed identically[5], and since the slow hydrolytic rates could be augmented by adding Ca^{2+}, it was suspected that a critical contamination of some of the ATP's with Ca^{2+} might be responsible for the observed differences. Direct Ca analyses (carried out by Chicago Spectro Service, Inc., Chicago, Ill.) did in fact reveal that the percentage Ca contents of crystalline disodium ATP preparations varied as follows: Mann G1907, 0.0009%; Sigma 22B721, 0.0010%; Pabst 133A, 0.0070–0.0100%; Sigma 52B728, 0.0090%; Sigma 102B646, 0.0070% and Pabst 140-A, 0.0200%. Furthermore, the slowly hydrolyzing ATP's seemed to contain about 0.001% Ca while the rapidly hydrolyzing lots ranged from 0.007% to 0.02%. It may be readily calculated that using 4 mM of the Ca-rich (0.02%) ATP, the extra Ca^{2+} carried into an experiment by ATP alone may amount to about 0.0125 mM. In view of what has been described earlier (*e.g.* Fig. 1), this Ca^{2+} concentration in itself may possibly be sufficient to account for the differences found. Actually, passage of Sigma ATP lot 52B728 through a cation-chelating column (Bio-Rad Chelex-potassium, 1 × 15 cm, flow rate of 0.2 ml/min at 5°) reduced the rate of ATP hydrolysis from 0.32 to 0.18 μmoles/min per mg of myofibrillar protein, when tested in a system similar to that described in the legend of Table I.

In general, our data (*e.g.* Fig. 1) support the conclusions of EBASHI[6] and WEBER *et al.*[7] that Ca^{2+} is essential for the characteristic hydrolysis of ATP by myofibrils. At times, the Ca^{2+} which contaminates some commercial disodium ATP preparations seems to be sufficient to provide the required concentration. A remark by WEBER AND HERZ[8] and a recent abstract by SEIDEL AND GERGELY[9] may be interpreted likewise.

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Effect of magnesium and calcium on the ATPase activity of actomyosin at low ionic strength

Since the pioneering work of BANGA AND SZENT-GYÖRGYI[1] and the subsequent detailed work of HASSELBACH[2], it has been well known that Mg^{2+} activates the ATPase activity of actomyosin (EC 3.6.1.3) at low ionic strength, where actomyosin is superprecipitated with ATP. However, exact data of the dependence of the ATPase activity upon the ionic strength have been lacking. In our previous report[3], it appeared that the Mg^{2+}-activated enzyme action is increased progressively as the ionic strength is decreased down to about 0.04. We have further attempted to investigate the ATPase activity at lower ionic strength with special reference to the activating effect of Mg^{2+} and Ca^{2+}. The experimental technique is referred to in the paper cited above[3].

As seen in Fig. 1, the ATPase activity of natural actomyosin (myosin B) is increased up to $I = 0.035$ and then at lower ionic strength decreased in presence of 1 mM $MgCl_2$ at pH 8.0 and 20°. Superprecipitation took place rather incompletely at very low ionic strength ($I < 0.03$). A notable decrease in the ATPase activity around $I = 0.08$ is due to the clearing response of actomyosin where actomyosin is dissociated[4]. It is of significance that the maximal ATPase activity in presence of 1 mM $MgCl_2$ ($I = 0.035$) is higher than that in presence of 1 mM $CaCl_2$ at its optimal ionic strength ($I \sim 0.15$), although the ATPase activity in presence of optimal concentration of Ca^{2+} ($\sim 10^{-2}$ M) at $I \sim 0.15$ is comparable with the maximal activity in presence of Mg^{2+}. At very low ionic strength ($I \sim 0.03$) Mg was the most