PREPARATION OF RABBIT MUSCLE MYOSIN OR ACTOMYOSIN FREE OF 5'-ADENYLIC ACID DEAMINASE

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The procedures in common use today for the preparation of myosin (myosin A) and actomyosin (myosin B) are essentially variation of that developed by Szent-Gyorgyi (1951). These preparations including subsequent variations (Portzehl, et al., 1950; Kielley and Bradley, 1956; Mommaerts, 1958; Tonomura, et al., 1961; Barany, et al., 1964) yield myosin or actomyosin which is contaminated with 5'-adenylic acid deaminase (AMP deaminase). While the percentage of the total protein that represents AMP deaminase is probably small, it is estimated that 95% or more of the muscle deaminase is associated with the myosin prepared in this manner.

Currie and Webster (1962) observed that the dialysis of rat muscle actomyosin against 2 changes of 10 volumes each of phosphate buffer (μ = 0.05, pH 7.2) resulted in the dissociation of AMP deaminase from the actomyosin. This procedure was not successful with rabbit muscle actomyosin. Ashley (1952) reported that rabbit skeletal myofibrils had no deaminase activity after repeated washing with phosphate-citrate buffer (pH 8, μ = 0.154). No other details have been reported.

This communication describes a simple procedure for the preparation of rabbit muscle actomyosin or myosin which is essentially free of AMP deaminase.

METHODS AND MATERIALS

Adenosine triphosphate and 5'-adenylic acid were purchased from Sigma Chemical Company or Calbiochem and were used without further purification. AMP deaminase was assayed by the method of Kalckar (1947) at 5×10^{-5} M AMP in 0.1 M potassium succinate (pH 6.5) at 30°C. The results are expressed as μmoles of AMP deaminated per min per mg of protein. This was calculated from the change in the molar absorbance at 265 mμ (8.86×10^{3} M^{-1} cm^{-1}).

ATPase activities were determined with a Radiometer pH stat at pH 7. The assay solutions contained either 10^{-3}M ATP, 10^{-3} M Ca^{2+}, and 0.1 M KCl or 10^{-3}M ATP, 10^{-3}M Mg^{2+}, and 0.1 M KCl adjusted to pH 7. The reaction was started by adding about 0.1 mg protein to 1 ml of the appropriate assay solution. The ratio of hydrogen ion uptake to inorganic phosphate released was found to be 0.5.

PROCEDURE

The procedure for the preparation of myosin or actomyosin free of AMP deaminase first involves the isolation of actomyosin as described by Szent-Gyorgyi (1951). The fresh muscle homogenate was filtered through cheesecloth to remove debris and allowed to stand at 2-4°C for at least 24 hrs. The actomyosin extract was diluted with an equal volume of water and centrifuged to precipitate the actomyosin. The precipitated actomyosin was suspended, preferably with a Potter-Elvehjem homogenizer in an excess of a solution containing 0.225 M KCl, 0.015 M $NaHCO_3$, and 0.00375 M Na_2CO_3. This solution was made by diluting Weber-Edsall solution (Szent-Gyorgyi, 1951) to $\mu = 0.25$. The suspension was again centrifuged to precipitate the actomyosin. This washing procedure was repeated twice. The washed actomyosin was subsequently dissolved in 0.6 M KCl and 0.01 M $MgCl_2$ to give a protein concentration of less than 0.5%. ATP (1 mg/ml) was added and the dissociated actomyosin was immediately centrifuged at 38,000 RPM for

two hours in a No. 40 rotor of a Spinco Ultracentrifuge to sediment the actin. The myosin, obtained as the supernatant from the centrifugation, was precipitated by dilution to 0.05 M KCl or by dialysis against ten volumes of distilled water to remove nucleotides. The precipitate was redissolved in 0.5 M KCl.

RESULTS AND DISCUSSION

The results of a typical preparation are noted in Table 1. The ATPase activities of the deaminase free myosin and actomyosin are in good agreement with values reported by Barany, et al. (1964). For the purpose of comparison, myosin was prepared as described by Szent-Gyorgyi (1951) taken through the low salt precipitation and redissolved in 0.5 M KCl. This preparation had a deaminase activity of 0.67 μmole AMP deaminated/min/mg protein and a Ca^{2+} activated ATPase activity of 0.20 μmole Pi released/min/mg protein.

TABLE 1

PREPARATION OF MYOSIN FREE OF AMP DEAMINASE

	ATPase (μmoles Pi/mg-min)		AMP Deaminase (μmole AMP/mg-min)
Preparation	Ca^{2+} activated	Mg^{2+} activated	
Crude Extract	*	*	0.30
Actomyosin (unwashed)	1.1	0.30	0.12
Actomyosin (3x washed)	0.35	0.20	0.001
Myosin	0.60	0.14	0.005

*ATP activities were not determined. Assay conditions are given in the text.

AMP deaminase appears to be strongly associated with myosin obtained from many muscles. This association is sufficiently firm in rabbit muscle myosin such that myosin preparations contain 95% or more of the total deaminase activity. The best methods reported to date for the separation of the two proteins have used differential heat coagulation (Engel-

hardt, et al., 1952; Locker, 1956, 1959; Lee, 1957). This procedure results in the complete denaturation of myosin. No preparation of rabbit muscle myosin has been reported that is free of 5'-adenylic acid deaminase. The procedure described herein gives an active preparation of myosin or actomyosin which is essentially free of the deaminase activity. Furthermore, the actomyosin or myosin from which AMP deaminase has been removed appears to retain its normal ATPase characteristics.

The AMP deaminase was removed from actomyosin by washing with a diluted Weber-Edsall solution (pH 9.3, μ = 0.25). The deaminase can not be removed by washing actomyosin with 0.25 M KCl or with Weber-Edsall solution diluted to μ = 0.05. Furthermore, the deaminase is not removed from myosin by washing with 0.05 M ammonium acetate, pH 8,

Table 2

RECOMBINATION OF MYOSIN AND AMP DEAMINASE

	AMP Deaminase μmol IMP/min/mg	Total Units μmol IMP/min
Myosin + AMP Deaminase[a]	0.83	0.21
Precipitate[b]	0.97	0.16
Supernatant[c]	3.65	0.03

[a] 0.22 mg Myosin + 0.03 mg AMP deaminase in 0.1 ml 0.5 M KCl.
[b] Precipitate from dilution of a to 0.5 M, redissolved in 0.1 ml 0.5 M KCl.
[c] Supernatant from dilution of a to 0.05 M: 1.0 ml.

or Weber-Edsall solution diluted to μ = 0.05. Thus the ionic strength and pH of the wash solution is critical.

An important aspect of this study is the observation noted in Table 2 in which it is shown that the deaminase-free myosine is capable of recombining with purified deaminase. Thus when AMP deaminase-free myosin and homogeneous AMP deaminase were mixed in 0.5 M KCl and precipitated by dilution to 0.05 M KCl, about 80% of the deaminase remained associated with the myosin. Homogeneous deaminase is soluble in 0.05

M KCl (Byrnes, Winely, and Suelter, 1965). It should be noted that the specific activity of the deaminase-myosin complex is larger than that obtained directly from muscle. Thus, it appears that there may not be a specific stoichiometry between the two proteins. The reversibility and properties of the deaminase-actomyosin or deaminase-myosin interaction is consistent with an electrostatic mechanism for the interaction. Further work is necessary to clarify this phenomenon.

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