THE PREPARATION OF MYOSIN ADENOSINETRIPHOSPHATASE FROM RAT MUSCLE

by

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INTRODUCTION

In the light of recent evidence it has become clear that the most reliable method for the assay of adenosinetriphosphate (ATP) is that of enzymic degradation by myosin adenosinetriphosphatase, sometimes combined with myokinase (BAILEY[1,2], ROWLES AND STOCKEN[3]). This method is to be preferred to the chemical method in which labile phosphorus is determined by hydrolyzing in *N* HCl for 10 minutes at 100° C.

This does not only apply to the assay of solutions of commercial or laboratory preparations of ATP, but is of even greater importance when ATP is to be determined in biological media. In the former case application of the 10 min. labile P test may give erroneous results due to the presence in the preparation of inorganic pyrophosphate and/or adenosinediphosphate (ADP), but in the latter case the analysis becomes problematic because acid hydrolysis can also liberate considerable amounts of phosphate from other phosphate esters present in the medium. The highly specific adenosinetriphosphatase test, although yielding results which are perhaps a trifle too low, is reliable in all cases.

BAILEY has preferably used rabbit muscle as the source of myosin adenosinetriphosphatase. Considering the limited keeping qualities of the preparation (3–4 weeks), rabbit becomes somewhat expensive when small amounts of muscle are regularly required.

We have experienced difficulties in preparing myosin from rabbit muscle according to BAILEY's prescription[1]; it often failed to precipitate properly. However, by lowering the p_H of the precipitation medium from 6.8–7.0 to about 6.2 we have been able to obtain active preparations, not only from rabbit muscle, but also from rat muscle. As our present method of preparation deviates a little from BAILEY's in some respects, we feel it may be of use to others to give a full description here.

PREPARATION OF MYOSIN ADENOSINETRIPHOSPHATASE

A fair-sized albino rat is killed by decapitation and the muscles of the hind-legs are rapidly removed and cooled in ice. All following operations are carried out in the cold room. After trimming, an amount of 25–40 g of tissue is minced coarsely with

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scissors and extracted with 8 volumes of ice-cold salt solution containing KCl (0.5 *M*) and $NaHCO_3$ (0.03 *M*), after homogenization in the chilled Waring Blendor during one minute with a little of the extractant. The extraction is carried out by stirring the suspension slowly during 20 minutes, adding solid $NaHCO_3$ if necessary, to maintain the p_H at 7.0–7.5. The suspension is then centrifuged and the supernatant fluid filtered through a layer of paper pulp, saturated with the extractant, under slightly diminished pressure.

After measuring its volume, the opalescent liquid is poured into 20 volumes of ice-cold glass-distilled water. The p_H will then be between 6.0 and 6.5, and the protein settles down fairly rapidly. The supernatant liquor is siphoned off and the myosin centrifuged down. The gel obtained is dissolved by adding an equal volume of *M* KCl and the p_H is adjusted to 7.0 by adding a little solid $NaHCO_3$ or dilute NaOH. The solution is again poured into 20 volumes of water. The p_H should then be about 6.2. After settling, the supernatant fluid is again siphoned off and the myosin centrifuged down. This time the protein gel is redissolved by adding solid KCl to a molarity of 0.5 and the p_H is again adjusted to 7.0. The myosin is reprecipitated by pouring the solution into 20 volumes of water as before, taking care that the p_H is again about 6.2. In all, the protein is precipitated 4 times and is then sufficiently pure. After the second and following precipitations, solution of the gelatinous precipitate is always effected by adding solid KCl to a molarity of 0.5.

The final solution is filtered after adjusting the p_H to 7.0 and stored at 0° C in the presence of a drop of toluene. The enzymic activity is preserved for several weeks.

ASSAY OF ADENOSINETRIPHOSPHATE WITH THE ENZYME PREPARATION

In Table I are given the chemical and enzymic analyses of some laboratory and commercial preparations of ATP.

TABLE I

CHEMICAL AND ENZYMIC ANALYSIS OF LABORATORY AND COMMERCIAL PREPARATIONS OF ADENOSINETRIPHOSPHATE

ATP prepared in the laboratory as monobarium salt; converted into the potassium salt with K_2SO_4 for analysis. For method of enzymic analysis see text.

The preparation of myosin ATPase used for analyses 2–5 is different from that used in analysis 1.

Results expressed in γ P per ml of solution, molarity approximately 0.002–0.0025. Labile P is corrected for inorganic phosphate.

No.	Source	Chemical Analysis			Enzymic Analysis			
		Total P γ	Free P γ	Labile P (10 min in *N* HCl at 100°) γ	Rat ATPase γ P	% of labile P	Rabbit ATPase γ P	% of labile P
1	Authors (fresh sample)	190	5	116	56	48	54	46.5
2	As above	—	5	172	78	45.4	—	—
3	As above	—	4	149	66	44.5	—	—
4	Commercial	—	20	121	35	29	—	—
5	As above, purified*	—	4	129	44	34	—	—

* Purified by precipitation as dibarium salt and reprecipitation as monobarium salt from 0.1 *M* HCl with aethanol; it still contained labile P other than ATP, which later proved to be fructose-diphosphate.

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The ATP was tested in solution as the potassium salt. The enzymic degradation took place at 28° C during 2 hours in the following reaction mixture: 0.1 *M* glycine buffer, p_H 9.1, 1 ml; 0.1 *M* $CaCl_2$, 0.1 ml; myosin, 0.1 or 0.2 ml (0.2 ml if the preparation was a few weeks old); 0.005 *M* K-ATP, 0.5 ml. The reaction was stopped by adding 0.5 ml 10% trichloroacetic acid, the mixture was placed in ice-water for 30 minutes, and after centrifugation an aliquot of the supernatant liquid was taken for the determination of inorganic phosphate according to SUMNER[4].

The results obtained with our preparations of rat adenosinetriphosphatase compare favourably with those published by BAILEY[1,2] and ROWLES AND STOCKEN[3] for rabbit adenosinetriphosphatase.

DISCUSSION

The reader cognizant with BAILEY's method of preparation of myosin adenosinetriphosphatase will have noticed that the most important modifications have been introduced by us at the following points:

1. The time of extraction and the volume of the extractant relative to the amount of muscle.

The time ot extraction of the muscle pulp should be as short as possible to avoid the solution of undesirable amounts of actin. In order to still extract a reasonable amount of myosin the volume of the extractant must then be augmented. We have obtained the most satisfactory results by extracting for 20 minutes with 8 volumes of salt solution.

2. The p_H of the medium in which the myosin is precipitated.

The crucial stage in the preparation of myosin is its precipitation by dilution with 20 volumes of water. The time required for the precipitate to settle is very sensitive to the p_H. In this respect rat myosin is even more exacting than rabbit myosin. Except for the first precipitation from the crude extract, the protein fails to separate properly when the p_H of the medium is kept at 6.8–7.0, as recommended by BAILEY. At a p_H of approximately 6.2, however, we found that the myosin always precipitates within a reasonable time. This agrees with the observations of GREENSTEIN AND EDSALL[5], who remarked that upon dilution with water the precipitate will not settle unless the p_H is under 6.5. According to these authors care must be taken not to descend below 6.0, as the myosin may then be denatured. So we always keep the p_H between 6.1 and 6.3. This p_H is usually attained when the myosin solution, adjusted to p_H 7.0, is poured into the always slightly acid distilled water, but if necessary the p_H can be corrected with a little dilute NaOH or HCl.

Fresh glass-distilled water must be used throughout the preparation to avoid inactivation of the adenosinetriphosphatase by heavy metal ions (BINKLEY *et al.*[6]).

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SUMMARY

A method is described for the preparation of myosin adenosinetriphosphatase from rat muscle. The most important conditions to be observed for a successful preparation are discussed.

RÉSUMÉ

Les auteurs décrivent une methode de préparation de myosine-adénosine-triphosphatase à partir de muscle de Rat. Ils discutent les conditions les plus importantes à observer pour la réussite de la préparation.

ZUSAMMENFASSUNG

Eine Methode zur Darstellung von Myosin-Adenosintriphosphatase aus Rattenmuskel wird beschrieben. Die wichtigsten Bedingungen für eine erfolgreiche Darstellung werden erörtert.

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