# EXPERIENCE IN THE PREPARATION OF MYOSIN

by

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The SZENT-GYÖRGYI method of preparing myosin[1] has been modified so as to obtain with comparative ease preparations which are actin- and lipid-free and give the super-precipitation reaction which we have found to be characteristic of a fresh myosin. The procedure involves specific precipitation of the contaminating actomyosin by addition of adenosine-triphosphate (ATP) at 0.12 *M* KCl and crystallization of the myosin on dilution to 0.04 *M* KCl. This method has been described briefly[2], but elaboration seems desirable in view of the difficulty in obtaining a satisfactory product.

## METHOD

It is important to use very young rabbits as a source of muscle in order to minimize both the loss of myosin in combination with actin at the actomyosin precipitation step and the interfering turbidity due to lipids in the final product. Fasting the animals for 24 hours before using may also reduce the lipid content. The practice in this Laboratory is to utilize the back and hind leg muscle of two New Zealand, albino rabbits, weighing 1500 to 2000 g. All of the operations must be carried out as near 0° C as possible except the precipitation of actomyosin by ATP. Glass distilled water is used exclusively.

The muscle, removed as quickly as possible from the animal killed by severance of the cervical vessels, is ground immediately through a cold meat grinder into a weighed −2° pH 6.5 buffer solution. The buffer contains 227 ml of 0.6 *M* $KH_2PO_4$, 148 ml of 0.6 *M* $K_2HPO_4$, 375 ml of 1.2 *M* KCl and 750 ml of water. When 500 g of muscle have been added to the buffer, the mixture is stirred vigorously for 10 minutes, then diluted with 3 volumes (6 liters) of cold water with stirring. The suspension is strained through a cheese cloth, and the residue is discarded or used for the preparation of actin. The filtrate is diluted by addition of $1^1/_3$ volumes of water (9.6 liters) with vigorous stirring. The flocculate of myosin and actomyosin crystals, allowed to settle for ½ hour, is aspirated free of supernatant before centrifugation. The precipitate is dissolved in an equal volume of 1.2 *M* KCl. This solution is diluted to 0.12 *M* KCl by slow addition of 4 volumes of 25° water with stirring. Approximately 100 mg of ATP is added, and the actomyosin precipitate forming while the mixture stands 5–10 minutes at room temperature is centrifuged down in the cold. The precipitate may sediment poorly. Of the three layers that often form, the uppermost containing the least actin is separated by aspiration, the bottom fractions being retained for subsequent extraction of myosin as described below. Myosin precipitates from the aspirated supernatant upon dilution to 0.03 *M* KCl with

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3 volumes of water. The myosin precipitate, removed by centrifugation and dissolved in an equal volume of 1.2 *M* KCl, usually gives a cloudy solution. The contaminating actomyosin and lipids causing this turbidity can be largely removed by reprecipitation of actomyosin at 0.12 *M* KCl (without addition of ATP this time) and recrystallization of the myosin from the supernatant solution at 0.03 *M* KCl. Any remaining turbidity can be removed either by a third such treatment or preferably by centrifugation in the preparatory rotor of the ultracentrifuge or the high speed head of a refrigerated centrifuge.

Extraction of the actomyosin precipitate with a liter of 0.12 *M* KCl provides an increased yield of myosin. Myosin precipitates from this extract, after removal of the actomyosin in the centrifuge, on dilution with 3 volumes of water.

To prolong its usefulness *in vitro* the myosin solution is made 0.006 *M* with histidine buffer of pH 7.0, and is then stored at 0° C under a thin film of xylol.

## RESULTS

The procedure outlined yields approximately 200 ml of clear, 0.6 *M* KCl myosin solution containing 1–2% of protein. Appearance of a fine white turbidity upon addition of ATP to myosin diluted ten-fold at 0.10 to 0.18 *M* KCl—a sensitive test for actin contamination—has not been observed with these myosin preparations. As shown in Fig. 1, electrophoretic analysis reveals a monodisperse system. The ultracentrifuge pattern (Fig. 2) also indicates homogeneity. Myosin that has been exposed to temperatures greater than 0° C for a short time gives a superprecipitation reaction characteristic of aged myosin. Thus an aged myosin solution containing ATP at 0.13 *M* KCl forms on addition of unpolymerized actin (G-actin)[3] a reticular precipitate which contracts slowly to a plug. Fresh myosin on the other hand gives a finely granular, nonsettling precipitate under these circumstances[4]. Myosin prepared according to the method described reacts like a fresh preparation even after 2 or 3 weeks storage at 0° C. Since exposure to temperatures above 0° markedly reduces the longevity of myosin preparations, the method has the advantage of largely avoiding such exposure.

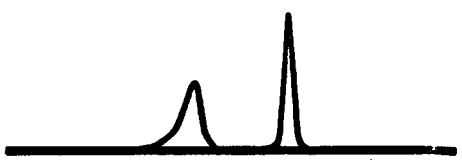

Fig. 1. Schlieren diagrams of descending (left) and ascending (right) boundaries of myosin in electrophoretic analysis. 1.2% myosin in 0.3 *M* KCl with 0.03 *M* pH 7.0 histidine. 19.5 hours at 0.80 volts/cm.

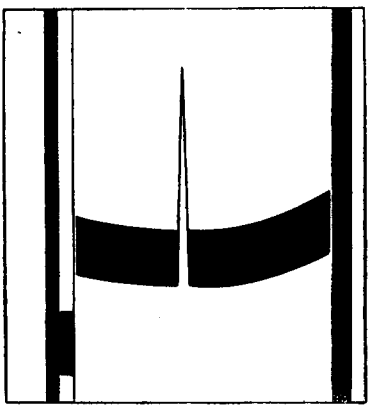

Fig. 2. Sedimentation diagram of myosin centrifuged at 260,000 *g* for 97 minutes. 0.2% myosin in 0.6 *M* KCl at 10.7° C. $S_{20} = 5.15$.

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SUMMARY

A detailed procedure for the preparation of myosin in a purified state has been described.

RÉSUMÉ

Les auteurs décrivent un procédé détaillé pour la préparation de myosine à l'état purifié.

ZUSAMMENFASSUNG

Ein ausführliches Verfahren zur Herstellung von Myosin im gereinigten Zustand wird beschrieben.

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