A NEW METHOD FOR THE PREPARATION OF ACTIN*

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The preparation of actin, as described by Straub (1), involves treatment of the muscle with acetone, after which the actin becomes extractable with water. The action of acetone is not understood. Possibly acetone acts by altering certain relations between lipides and protein. For this reason it seemed desirable to work out a method for the preparation of actin which avoids the use of anhydrous solvents and consists of steps which are well understood. In this paper such a method is described, based on the reversible depolymerization of actin by potassium iodide (2). This method can also be used for the preparation of actin from actomyosin, which is not possible by Straub's process.

Before the method could be established, it was necessary to work out a method for the quantitative determination of actin in small quantities and the determination of the amount of impurities present in the different actin preparations. The first part of the paper deals with these methods.

For the sake of convenience, a new symbol is used, the myosin number (M number) of actin, which designates the quantity of myosin in gm. which combines with an actin preparation containing 1 gm. of protein dry weight. A low M number means impure, a high M number means relatively pure actin.

Methods and Materials

Myosin was prepared according to Szent-Györgyi (3), as modified by Portzehl, Schramm, and Weber (4), and stored in 50 per cent glycerol at −20° (5). During 5 months, no change was observed in the capacity of such myosin to form actomyosin. The same myosin preparation was used during the whole course of these experiments. Straub’s method of actin preparation was used, as modified by Guba and Szent-Györgyi (6).

Viscosity was measured at 20° in an Ostwald viscometer in 0.6 M KCl, containing 0.0067 M phosphate buffer, pH 7.0. Actin was always made to polymerize in 0.1 M KCl before adding to 13.3 mg. of myosin in 6 ml. of final volume. Actin was determined at two or more concentrations in each case; the error in the measurements corresponded to 0.02 mg. of actin.

Nitrogen was determined by the Kjeldahl method, a factor of 6.2 being

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used to calculate the protein present. Adenosinetriphosphate (ATP) was kindly supplied by Armour and Company. The distilled water used was redistilled in glassware.

**EXPERIMENTAL**

*Determination of Actin*

The two specific reactions of actin are its capacity to polymerize in the presence of small concentrations of neutral salts and its capacity to combine with myosin to form the highly viscous actomyosin. This combination in the presence of high salt concentrations is disrupted by small amounts of ATP whereby the viscosity falls to that of the components. Both of these reactions can be followed viscometrically. The second reaction, that is the measurement of the actomyosin formation, is more advantageous. It is more sensitive, the formation of actomyosin being proportional to the actin present, and furthermore involves the use of polymerized actin (F-actin) which is easier to handle than globular actin (G-actin), the latter being stable only in the absence of salts or in 0.6 M KI.

The logarithm of the relative viscosity of actomyosin is directly propor-
tional to its concentration, as shown by Portzehl, Schramm, and Weber 
(4). If different amounts of actin are added to a constant amount of 
myosin, the amount of actomyosin formed is dependent on the amount of 
actin added until the myosin becomes saturated. The difference in the 
logarithm of the relative viscosities of the actomyosin formed before and 
after the addition of ATP (Δ log η_{rel.}) is directly proportional to the actin 
concentration (Fig. 1). The Δ log η_{rel.} is independent of the concentra-
tion of myosin, as measured with different amounts of myosin ranging 
from 1.1 to 4.4 mg. of ml. of concentration. Thus from the Δ log η_{rel.} 
value of an actin preparation the amount of actin can easily be determined, 
and the actin content of different actin preparations can be compared. If 
the myosin preparation used originally contained some actin, the Δ log 
η_{rel.} value of this myosin should be subtracted from the Δ log η_{rel.} measured 
after actin has been added.

The Δ log η_{rel.} values were proportional to the amount of actin added up 
to the value 0.28 in these experiments, in which the total amount of myo-
sin introduced was 13.3 mg. At 0.28 there was a sharp break in the curve 
which leveled off, and the results began to show scattering as a result of 
strong thixotropy. This value was taken as the saturation point of myo-
sin with actin. If the Δ log η_{rel.} and the protein concentration of an actin 
preparation are known, the M number can be calculated at any value be-
low the saturation point of myosin, owing to the direct proportionality 
between the Δ log η_{rel.} and the actomyosin formed. If the M number of 
pure actin is known, the impurity present in an actin preparation can be 
estimated.

Preparation of Purified Actin

Actin prepared according to Straub contains considerable amounts of 
impurity, as was shown by Spicer and Gergely (10) and by Mommaerts 
(11). In order to obtain purified actin, advantage can be taken of the 
high sedimentation constant of F-actin and great centrifugal force can be 
used, as was used by Mommaerts (11). The $s_{20}$ of F-actin is about 55 to 
65 (4). If it is only the F-actin which sediments quickly, then the protein

1 Straub (1) was the first to describe a method for the quantitative determination 
of actin. He used the expression $(\eta_{rel.} - \eta_{rel. \text{ ATP}})/\eta_{rel. \text{ ATP}} \times 100$, in which $\eta_{rel.}$
is the viscosity before the addition of ATP, $\eta_{rel. \text{ ATP}}$ after the addition of ATP. 
This "activity" was compared with the "activity" of "myosin B" (7), which is an 
actomyosin as extracted from muscle by the Weber-Edsall (8, 9) solution. The 
activity of myosin B was arbitrarily taken as 100 per cent activity and thus allowed 
the calculation of the actin content of different preparations. However, the activity 
of myosin B, as was pointed out by Portzehl, Schramm, and Weber, is not a constant 
value and the expression used above depends on the myosin concentration. Be-
side, the measurements are limited to small concentrations of myosin (about 1 mg. 
per ml.) which reduces the sensitivity of the measurements.
left in solution consists of the impurities present. If the actomyosin formation of the sedimented and those of the untreated actin are compared, the same degree of actomyosin formation could be expected at a smaller protein concentration in the first case, provided no denaturation occurred. In the experiments described here, actin was made to sediment and the protein concentrations and $M$ numbers were determined before and after centrifugation in the supernatant fluid and in the sedimented protein, redissolved in 0.1 M KCl (Table I and Fig. 2).

In one series of experiments, actin (Preparations 1 and 2, Table I) was sedimented in the preparative ultracentrifuge at 140,000 $\times$ g in the presence of 0.1 M KCl, 0.0067 M phosphate buffer, pH 7.0, and $10^{-4}$ M ATP. In another series, actin (Preparations 3, 4, and 5, Table I) was sedimented in the high speed attachment of a refrigerated centrifuge at 25,000 $\times$ g in the presence of 0.1 M KCl and $10^{-4}$ M ATP by centrifuging for 90 to 120 minutes at 0°. The actin pellet was redissolved by storage overnight in 0.1 M KCl, containing 0.0067 M phosphate buffer, pH 7.0, and $10^{-4}$ M ATP. The final yield was 45 to 55 per cent of the original actin, the loss being due to the failure of the sedimented actin to dissolve completely.

The highest $M$ numbers were obtained with the redissolved actin separated in the ultracentrifuge (see the upper curve in Fig. 2). The value 4.3 closely agreed with the value obtained by Spicer and Gergely, whose cal-

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**Table I**

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>Actin untreated</th>
<th>Supernatant fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein</td>
<td>Actin viscometrically</td>
</tr>
<tr>
<td></td>
<td>mg. per ml.</td>
<td>mg. per ml.</td>
</tr>
<tr>
<td>1</td>
<td>2.30</td>
<td>1.265</td>
</tr>
<tr>
<td>2</td>
<td>2.30</td>
<td>1.403</td>
</tr>
<tr>
<td>3</td>
<td>3.211</td>
<td>1.94</td>
</tr>
<tr>
<td>4</td>
<td>6.152</td>
<td>3.814</td>
</tr>
<tr>
<td>5</td>
<td>3.221</td>
<td>2.01</td>
</tr>
</tbody>
</table>

Actin was determined viscometrically, assuming that the sedimented actin was pure. In the case of untreated actin, the amount of impurity was calculated from the actomyosin formation values of the original actin; in the case of the supernatant fluid, from the amount of protein left after centrifugation, after the actin present was subtracted.

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culated $M$ number was close to 4.1. The fact that in all cases identical $M$ numbers were obtained, regardless of the magnitude of the centrifugal force employed, indicates that the actin thus obtained was pure and that its $M$ number corresponds to the stoichiometric relation of actin and myosin.

The $M$ number of pure actin being known, the amount of impurity in the original preparation could also be calculated from it. This quantity of impurity agreed with the quantity of protein found in the supernatant fluid after centrifugation and the deduction of the quantity of actin still present (Table I).

The lowest curve of Fig. 2 was obtained with actin prepared according to Straub. The viscosity measurements and the centrifugation both show it to contain about 40 per cent impurity. This value is the same as that of Spicer and Gergely and is lower than the 60 per cent impurity found by Mommaerts. Strub (1) found an $M$ number of 2.5, which was confirmed by Snellman and Erdős (12) who found it to be 2.5 to 3.0. The $M$ number of the actin preparations used in the experiments described here and prepared according to Straub was 2.65, showing that our preparations had the same purity as Straub’s. The electrophoretic measurements of Spicer and Gergely and the presence of myokinase (13), deamidase, and creatine-

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*Laki, K., and Clark, A. M., personal communication.*

**Fig. 2. Purity of actin preparations.** •, actin purified by centrifugation; five different preparations. Ó, actin prepared by KI; five different preparations, one not represented, was 55 per cent pure. △, actin prepared by Straub’s method; six preparations, one not represented, was 45 per cent pure. Abscissa, protein added to 13.3 mg. of myosin in 6 ml. final volume; ordinate at left-hand side, difference in the logarithm of relative viscosities before and after ATP; ordinate at right-hand side, amount of myosin saturated by the actin introduced in mg. The broken line shows the value at which all the myosin present is saturated. Correction was taken for the contamination of actin originally present in myosin.
phosphophorase (14) in the actin preparations indicate that the impurity is not denatured actin. The large amount of impurity is not unexpected, since the preparation of actin does not involve any specific method of purification.

**Preparation of Actin from Muscle with KI**

The electron microscope pictures of Rozsa, Szent-Györgyi, and Wyckoff (15) suggest that actin is responsible for the continuity of the structure of the cross-striated muscle in which it is present in the polymerized state. Once it is depolymerized, it should become extractable easily. As shown elsewhere, the depolymerization of actin by potassium iodide is reversible in the presence of a small amount of ATP (2). In the method to be described here, the actin was extracted with KI, the myosin extracted at the same time (see Dubuisson (16)) being separated from the actin by adding 20 per cent alcohol.

The procedure used was as follows.

The fresh, minced rabbit muscle was extracted with 3 volumes of the cold KCl-phosphate for 10 minutes in order to extract the myosin (3). The suspension was diluted with 4 volumes of ice-cold distilled water and pressed through a cloth.

The muscle residue was reextracted with 3 volumes of cold KCl-phosphate in the presence of 10⁻⁴ M ATP for 10 minutes, diluted with 4 volumes of cold water, and pressed through a cloth.

The residue was washed in 10 volumes of cold water and pressed through a cloth. The residue obtained could be stored in the deep freezer.

To every 33 gm. of the muscle residue, cold water was added to make up the volume to 100 ml., then 1 ml. of 2 × 10⁻² M ATP and 11 ml. of 6.0 M KI containing 0.06 M sodium thiosulfate. The suspension was kept in an ice water bath and stirred for 10 minutes; then 110 ml. of cold water were added and the insoluble part was quickly centrifuged in a refrigerated centrifuge.

The supernatant fluid was measured, kept below 5°, and 0.25 volume of 96 per cent alcohol at −25° was added. The precipitate was quickly centrifuged and the alcohol and KI were removed by dialysis against 0.0067 M phosphate buffer, pH 7.0, and 5 × 10⁻⁵ M ATP at 0°. The dialyzing tube was constantly shaken and the small volume of dialyzing liquid was changed every half-hour. After 4 hours dialysis the alcohol concentration in the dialysate was less than 1 per cent. The precipitate was eliminated by 5 minutes high speed centrifugation. The actin obtained was mostly in its F form. To attain complete polymerization, 2 ml. of saturated KCl were added to every 100 ml. of fluid and the solution was kept at room temperature for 30 to 60 minutes.
According to Balenovic and Straub (17), muscle contains 2.5 to 3.0 per cent actin calculated to the wet weight. Because their calculations were based on an actin of about 60 per cent purity, the muscle must have contained 1.5 to 1.8 per cent actin. The yield of the actin obtained with the method described here was somewhat over 50 per cent of this value. The amount of impurity present was usually constant, five preparations being 73 to 75 per cent pure and one 55 per cent pure (Fig. 2).

**Preparation of Actin from Actomyosin with KI**

The main difficulty with the purification of actin lies in the difficulty of precipitating it without denaturation. On the other hand, actomyosin can be washed and precipitated repeatedly without significant loss and without denaturation.

Actin was prepared from twice precipitated "myosin B" (7) and from actomyosin prepared with the use of KI. In the latter case, the actomyosin was extracted from rabbit muscle with 0.6 M KI, containing 0.6 M sodium-thiosulfate, and after the elimination of the muscle residue by centrifugation, it was precipitated at 0.04 M KI by the addition of water with constant stirring.

In order to increase the concentration of actin, 0.1 mg. of ATP per ml. was added to the actomyosin precipitate at room temperature to obtain the precipitate in a small volume. The centrifuged precipitate was cooled below 5° and dissolved in 2 \times 10^{-4} M ATP and 0.6 M KI by adding 6.0 M KI. After 10 minutes storage, the myosin was removed and the actin was prepared as described in the preceding section.

The yield of actin was over 50 per cent of the actin originally present in the actomyosin. The purity was mostly between 55 to 75 per cent starting with "myosin B," and 75 to 80 per cent starting with actomyosin obtained with KI. The final concentration of actin varied between 1.5 and 2.0 mg. per ml.

**Preparation of Actin-Free Myosin with KI**

Actin-free myosin was first prepared by Szent-Györgyi (3). His and the later methods (4, 10, 11) are all based on the different solubilities of myosin and actomyosin. Since the elimination of actin involves a great loss of myosin, these methods can be used only in cases in which the actin content is low. Dubuisson (16) found that, when KI was employed, the yield of the extracted muscle proteins was much larger than with KCl. Since the depolymerization of actin is irreversible in the absence of ATP, it seemed logical to try to use KI for the preparation of actin-free myosin. With the method described here, actin-free myosin can be prepared from washed muscle or from actomyosin.
The rabbit muscle was twice washed for 10 minutes in cold water, then comminuted in the Waring blender and washed twice in 0.05 M KCl in order to remove the ATP completely. The myosin was obtained by extraction for 10 minutes in cold 0.6 M KI, containing 0.006 M sodium thiosulfate. The muscle residue was eliminated by centrifugation and the myosin precipitated by adding cold water slowly with constant stirring to bring the KI concentration to 0.025 M. The water was neutralized with dilute NaHCO₃ or phosphate buffer, pH 7.0.

From 100 gm. of muscle, 3.5 to 4 gm. of myosin were obtained; thus the yield was three to four times higher than that obtained by conventional methods. The myosin was completely free of actin, as was ascertained from superprecipitation and viscosity measurements. It did not crystallize, but otherwise behaved in every respect in a similar manner to the myosin obtained by standard methods.

Myosin was also prepared from actomyosin. An actomyosin three times precipitated or washed was dissolved in 0.6 M KI and, after 10 minutes extraction, the myosin was precipitated by diluting the KI to 0.025 M. The yield of the actin-free myosin was 50 per cent or more of the myosin originally present in the actomyosin preparation.

SUMMARY

1. A method for the quantitative determination of actin and for the determination of its purity is presented.
2. The actin obtained by Straub's method was found to be about 40 per cent impure. 1 gm. of actin is capable of combining with about 4.3 gm. of myosin.
3. A new method for the preparation of actin from muscle and from actomyosin is described, based on the reversible depolymerization of actin by potassium iodide.
4. A method is described for the preparation of myosin with potassium iodide. The product is actin-free and its yield is three to four times higher than that obtained by earlier methods.

BIBLIOGRAPHY

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