Reversible Polymerization and Ultracentrifugal Purification of Actin*

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Actin is the second major structure protein of muscle. It is characterized by its ability to combine with myosin and to occur in two forms, globular and fibrous actin (9, 10, 3), which are indicated as G- and F-actin. The transition of G- into F-actin is brought about by addition of salt at neutral or weakly alkaline reaction (9, 10, 4).

The present paper describes a method of purifying actin by preparative ultracentrifugation of the fibrous protein, depolymerization of the sedimented and redissolved product, and repetition of this procedure. In the development of this method, it was found that reversible depolymerization is possible only in the presence of adenosinetriphosphate.

Experimental

Methods

The major method employed in this work was preparative ultracentrifugation. This was carried out in a rotor of the angle type, with twelve individual tubes having a total capacity of 150 ml., at 38,000 to 40,000 r.p.m. The tubes were placed at an angle of 64° with respect to the horizontal plane. The average radius of rotation was 5.5 cm.; the path of sedimentation from wall to wall along the diameter was 1.7 cm.; the acceleration in the middle of each tube amounted to about 140,000 × g in the indicated range of speeds. Acceleration required 4 minutes. This equipment (model L) was built by the Specialized Instruments Corporation, Belmont, California.

Protein nitrogen was determined by Kjeldahl analysis, with K₂SO₄ and a CuSO₄-selenium catalyst, and hydrogen peroxide towards the end of the digestion. A nitrogen content of 16 per cent was assumed in the case of unidentified proteins. Perchloric acid (5) was used as a protein precipitant to separate proteins from nucleotides in order to permit ultraviolet spectrophotometry of the supernatant solutions.

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Ultraviolet absorption spectra were determined in the Beckman quartz spectrophotometer, with cells of 10 mm. in optical depth.

The ATP used was a commercial preparation of the barium salt (supplied by the Sigma Chemical Company, St. Louis, Missouri). It was dissolved in the calculated amount of HCl, converted into the sodium salt, and freed of heavy metal impurities, by means of the cation exchange resin Amberlite IR-100 (6).

Crude actin solutions were prepared by extracting, with a 20-fold amount of water, the acetone powder of muscle prepared according to Straub (10) and Szent-Györgyi (12).

The transition of G- to F-actin was effected at an initial pH$^1$ of 7.5 to 8.5 by addition of KCl to a concentration of 0.1 M. The polymerization was judged qualitatively by observation of the birefringence appearing upon gently swirling a 1 cm. layer of solution in a 30 ml. beaker. Birefringence is very strong, and, in thicker layers and more concentrated solutions than those usually employed, extinction colors can be seen.

**Basic Observations**

Since, as will be shown in a forthcoming ultracentrifugal study,$^2$ polymerized actin has a sedimentation constant above $50 \times 10^{-13}$, as compared to about $4 \times 10^{-13}$ for the accompanying impurities, centrifugal separation may be expected to be successful.

When a solution of crude actin, after polymerization, is spun for 1 hour by the technique described, the supernatant solution is devoid of any birefringence. A small translucent pellet at the bottom of the tube contains the polymerized actin in a concentration of 5 to 10 per cent. If the pellet is disintegrated in 0.1 M KCl, its dissolution is extremely slow. After about 1 month in the cold, a major part of the gel has dissolved. The solution remains birefringent throughout this period.

Upon suspension of the pellet in water, dissolution proceeds more rapidly, and, after standing, the solution is no longer birefringent. The time required for this transition, depending on the pH and other factors, amounts to several hours. Renewed addition of salt to the solution does not cause repolymerization, even if magnesium, calcium, or ATP, singly or in combination, is added.$^3$ Hence, the depolymerization is irreversible.

If the pellet is dissolved in water containing ATP (100 mg. of ATP to 400 mg. of protein is a safe excess), dissolution and depolymerization are slower and require 1 to 2 days at pH 9. After the protein has returned to the globular state, addition of salt causes polymerization. Hence, ATP is required for reversible depolymerization.


Purification of Actin

Identity of Actin—Actin is the protein which, under influence of salt at neutral or weakly alkaline reaction, polymerizes from a globular into a fibrous form, with a concomitant increase in ultracentrifugal sedimentation velocity. Therefore, actin will be defined as the protein which, in a suitable electrolyte medium, becomes sedimentable in the centrifugal field employed. The protein thus identified has been regarded as pure actin in this work. It remains to be determined whether it represents a pure protein in the usual sense, or whether F-actin is a copolymer of several protein components.

Purity of Crude Preparations—Applying the above criterion, we tested the purity of actin in extracts prepared according to Straub (10). After inappropriate storage of the acetone powder, the polymerizing tendency of the resulting actin solution is decreased. It can then frequently be restored by dissolving ATP in the water used for extraction. Fresh preparations, or powders stored in perfectly dry condition, do not require addition of ATP.

The pH of the crude extract is adjusted to 8.0 ± 0.5, and KCl is added to a concentration of 0.1 M. After standing for several hours, the solution is ultracentrifuged. The protein contents of the pellet and of the supernatant solution are then determined; e.g., 48 ml. of extract centrifuged at 40,000 r.p.m. for 1 hour, 47 ml. of supernatant solution, 1.20 mg. of protein per ml., total 56 mg. of protein; sediment dissolved to 15 ml., 1.60 mg. of protein per ml., total 24.0 mg. of protein. Hence, 30 per cent of the protein became sedimentable under the influence of the added salt.

Experiments of this nature have shown, assuming that the sedimentation under the given conditions is not quite complete, that the purity of good preparations is of the order of 40 per cent.

Purification of Actin—The given example is illustrative for the method and its results. Some denaturation frequently occurs during the dissolution of the pellet, with the result that a fraction of the protein remains insoluble. The reason for this has not yet been discovered.

Effect of Polydispersity—It will be observed that after purification a small fraction of the protein does not sediment under the standard conditions. This is not due to remaining impurities, and only partly, if at all, to partial inactivation of the protein. Further ultracentrifugation decreases the quantity of protein remaining in the supernatant solution. The explanation is that the polymerized protein is strongly heterodisperse. For the purpose of purification, the slower sedimenting part is sacrificed to obtain sharper separation from the slowly sedimenting impurities.

Purification of Actin

Crude solution 450 ml., 1300 mg. protein; polymerized at pH 8 with 0.1 m KCl; ultracentrifuged for 1 hr.

Supernatant fluid with 930 mg. protein

Sediment dissolved in 100 ml. water with 100 mg. ATP; dialyzed 40 hrs. vs. 400 ml. water with 15 mg. ATP; 11.9 mg. insoluble protein; 360 mg. dissolved protein; polymerized with KCl

270 mg. protein in 120 ml.; ultracentrifuged 1 hr.

Supernatant fluid with 45.6 mg. protein

Ultracentrifuged 2 hrs.; supernatant with 15.4 mg. protein

Sediment dissolved in 100 ml. water with 60 mg. ATP and dialyzed as before; 17.0 mg. insoluble protein; 200 mg. dissolved protein; polymerized with KCl

150 mg. protein in 96 ml.; ultracentrifuged 1 hr.

Supernatant fluid with 26.3 mg. protein

Ultracentrifuged 2 hrs.; supernatant fluid with 18.7 mg. protein

Sediment dissolved in water; 22 mg. insoluble protein; 99.8 mg. dissolved protein

Role of Nucleotides

Occurrence of Nucleotide in Original Extract—The previous experiments show the necessity of ATP for the transition of G- to F-actin. Yet, it is not necessary to add ATP to the extract of the acetone powder. The assumption is obvious that ATP, or another nucleotide, is present in the crude extract. In view of the large number of washings to which the minced muscle is subjected in the preparation of the acetone powder, this assumption needs corroboration. To this end, ultraviolet absorption spectra were measured.

In Fig. 1 absorption spectra of the extract are given, corrected for the dilutions to which the samples were subjected. The spectrum of the whole extract allows no conclusions, but, after deproteinization with perchloric
Fig. 1. Ultraviolet absorption spectra of an initial extract after ultracentrifugal removal of the actin. Curve I, spectrum of the whole extract; Curve II, spectrum after removal of the protein; Curve III, spectrum of the protein (by difference).

Fig. 2. Ultraviolet absorption spectra of purified actin with excess ATP after a centrifugal sedimentation of the actin. Curve I, spectrum of the supernatant solution; Curves II, III, and IV, spectra of the solutions of the sediment, Curve II as such, Curve III after deproteinization, Curve IV spectrum of the protein (by difference).
acid, a spectrum typical of an adenine compound appears. The protein spectrum, resulting from graphical subtraction, is typical for average proteins (maximum at 2800 Å; optical density 5.6 at the wave-length of maximum absorption for 1 per cent protein concentration).

The value of the extinction at the maximum of the adenine spectrum, assuming (1) a value of $1.50 \times 10^4$ for the molar extinction coefficient, suggests a concentration of about 75 mg. of ATP per liter of extract. However, the substance has not yet been definitely identified as ATP.

**Distribution of Nucleotides in Centrifugal Separation**—Fig. 2 gives the spectra of the deproteinized supernatant solution of an ultracentrifugal separation experiment with a pure preparation and of the sediment after dissolution into the original volume. Of the large excess of ATP added, the major part remains in the supernatant solution. The amount contained in the sediment, however, is more than can be accounted for as due to inclusion of fluid in the pellet. Therefore, a binding of ATP by F-actin occurs, which is being investigated at present.

**Preliminary Analysis of Actin**

Actin was purified by 3-fold repetition of the sedimentation and depolymerization procedure. After the last separation, the protein was dissolved in water and dialyzed. The pH of the solution was then adjusted to 4.5 to precipitate the protein. The precipitate was washed with water and dried with alcohol and ether. It contained 14.6 per cent nitrogen and 1.37 per cent sulfur (corrected for ash).

**DISCUSSION**

The two main results of the present work are the purification of actin and the demonstration that ATP, or an adenosine nucleotide which can be replaced by ATP, is essential for its polymerization.

Purification of actin had not been achieved previously. The results of this investigation show that preparations made by Straub's method may be up to 40 per cent pure. On the basis of indirect evidence, a higher degree of purity has been estimated by Spicer and Gergely (8). A claim for purification has been made by Price (7), but his descriptions are at variance with known properties of this protein. At present, the purity of actin is judged from its ultracentrifugal sedimentation after polymerization, by which complete purity is suggested. Other criteria will be applied in future work.

The present study has yielded an independent confirmation of the necessity of ATP for the polymerization of actin. Indications for the participation of ATP in the molecular transformations have been found before. However, the capacity to polymerize could not be restored after removal.
of ATP. It appears now that ATP must be present in the actin solution at all times; otherwise the protein loses its specific properties irreversibly. This was first found in the recent work of Straub and Feuer (11) and of Laki et al. (2), and it was only after their significant discovery that the present investigation became successful.

The relation between the rôle of ATP in the polymerization of actin and the effect of ATP on actomyosin (4) remains to be investigated.

SUMMARY

Polymerized actin can be separated from accompanying proteins by ultracentrifugation. The separated actin, after dissolution and depolymerization in water, does not polymerize again upon addition of salt, unless ATP is present during the entire depolymerization process.

The properties have been used to achieve apparently complete purification of actin by repeated polymerization, ultracentrifugation, and depolymerization.

BIBLIOGRAPHY

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