5. Isolation of Native Gizzard Gamma-Actin

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Actin, a major contractile protein of most eukaryotic cells, has several isoforms. According to the sequence analysis done by Vandekerckhove and Weber, at least six isoactins are expressed in mammalian cells. When analyzed by electrofocusing, these isoactins are grouped up into three classes; alpha-, beta- and gamma-actins in descending order of acidity. Alpha-actin is sarcomeric isoactin which consists of I-filament in striated muscles. On the other hand, two other isoactins, beta- and gamma-species are detected from non-muscle cells. In non-muscle type of cells, actin filaments (microfilaments) display a variety of polymorphic figures as stress fibers, three-dimensional networks, and gel-like structure underneath plasma membrane. In developing muscle cells, the isoform-pattern of actin is more complex but interesting. Immature muscle cells contains non-muscle actin isoforms besides muscle-specific isoactins. The presence of different kinds of isoactins in a single cell tempts us to assume different function and spatial sorting between actin isomers. It is shown that non-muscle actin can be prepared by a similar method for muscle actin; extraction of acetone-dried tissue with low ionic strength buffer in the presence of ATP. Unfortunately, however, thus obtained actin preparations were always a mixture of beta- and gamma-actins. The situation is similar when we purify actin from smooth muscles. Gizzard gamma-actin is always contaminated with a small amount of beta-actin. Therefore, it is quite important to establish the method by which we can prepare single isoactin in a native form.

As a first attempt to separate single actin isomer from a mixture of different isoactins in native form, we here describe the purification procedure for native gizzard gamma-actin.

Materials and methods. Actin from chicken gizzards was extracted from the acetone-dried powder for 60 min on ice with 30 volumes of G-Buffer containing 0.2 mM CaCl₂, 0.2 mM ATP and 3 mM Tris-HCl, pH 8.0. After polymerization with 0.1 M KCl, the actin solution was treated with 0.6 M KCl to remove tropomyosin. F-actin was pelleted by centrifugation for 3 h at 100,000 ×g and then depolymerized by homogenization followed by dialysis for 48 h against G-Buffer. After brief sonication to facilitate depolymerization, the dialysate was clarified for 3 h at 100,000 ×g and then used as gizzard G-actin. G-ADP actin was prepared as described before. DEAE-cellulose (Whatman DE-52) column equilibrated with ADP was made as follows: To 50 ml of DEAE-cellulose, 0.1 g of ADP was added. After adjusting the pH at 8.0 with 0.1 M NaOH, the resin was packed into a column (2.6 cm×20 cm) and then equilibrated with 300 ml of ADP-Buffer containing 0.5 mM ADP, 0.2 mM CaCl₂ and 3 mM Tris-HCl, pH 8.0. DNase I-assay of actin was carried out according to Blikstad et al. 0.75 M guanidine-HCl was used for the depolymerization of F-actin. Flow birefringence measurements were made using an Edsall type apparatus.
SDS-polyacrylamide gel electrophoresis in the presence of 6 M urea (called as SDS/urea-gel) and high resolution two-dimensional gel electrophoresis were carried out as described before.\textsuperscript{8)} Protein concentrations were determined by biuret reaction standardized by bovine serum albumin. All the preparations were carried out at 4°C unless otherwise noted.

Hereafter “gizzard actin” refers to conventionally prepared actin which is a mixture of gamma- and beta-actin.

**Results and discussion.** As the classification of alpha-, beta- and gamma-actins is based on the difference in their isoelectric points, there is a possibility that each class of isoactin can be separated on an ion-exchange column chromatography. Using gizzard actin as a starting material, we tried various combination of ion-exchangers and solvent conditions and found that gizzard-gamma-actin could be successfully isolated in native form on the ADP-equilibrated DEAE-cellulose column. Gizzard G-actin (5 mg/ml X 10 ml) was firstly treated with Dowex-1 to remove free ATP and then polymerized with 0.1 M KCl. After complete polymerization, F-actin was pelleted by centrifugation for 3 hr at 100,000 \( \times \) g. G-(ADP)-actin was obtained by depolymerizing the pellet in ADP-Buffer with a Teflon-pestled homogenizer. The G-(ADP)-actin was immediately applied on an ADP-DEAE cellulose column. The column was washed with 100 ml of ADP-Buffer and then eluted by a linear gradient of KCl (Fig. 1). Two peaks were detected on the chromatographic pattern. The first peak did not contain protein and showed the absorption characteristic of nucleotide.

![Fig. 1. DEAE-cellulose column chromatography of G-(ADP)-actin.](image-url)
Actin isomers appeared in the second peak which was eluted between 20-25 mM KC1. The isomer composition of each fraction was examined by SDS/urea-gel electrophoresis (Fig. 2, a-b). As shown previously, non-muscle isoactins migrates faster than gizzard gamma-actin on the SDS/urea-gels. The peak portion of the peak contained purified gizzard gamma-actin, while the fractions eluted in latter half contained both beta- and gamma-species. Although the relative amount of beta-actin increased with the KC1 gradient, we did not yet succeed in the complete purification of beta-actin. It was found that actin partially polymerized during the elution with KC1. DNase I-assay on each fraction showed that 70-80% of purified gamma-actin was obtained in native form (refer to Fig. 1). Fractions containing purified gamma-actin were collected and polymerized with 0.1 M KC1. After pelleting by the ultracentrifugation, pelleted F-actin was depolymerized by homogenization followed by dialysis against G-Buffer. Denatured actin was removed by Sephadex G-200 column chromatography. The column was equilibrated and eluted with G-Buffer. Thus prepared gizzard gamma-actin showed a single spot on a two-dimensional gel with SDS/urea-polyacrylamide gel in the second dimension (Fig. 2c). When started from 50 mg of gizzard G-actin, 20-25 mg of purified gamma-actin was obtained.

As long as we have examined so far, we could not detect substantial difference in the polymerization characteristics between the purified gizzard gamma-actin and conventional gizzard actin. Critical concentrations were quite similar; those of gizzard actin and purified gamma-actin polymerized with 0.1 M KC1 in G-Buffer were 0.050 mg/ml and 0.045 mg/ml, respectively (Fig. 3). Flow birefringence properties were also similar, indicating the presence of long filaments.
of various lengths (data not shown). Strzelecka-Golasewska et al.\textsuperscript{12} showed that presence of Ca\textsuperscript{2+} was necessary for maintaining the polymerizability of gizzard G-actin. Even in the case of gizzard gamma-actin, addition to 0.2 mM CaCl\textsubscript{2} to Buffer was necessary to get native isomer. Removal of Ca from purified gamma-G-actin resulted in gradual denaturation of actin.

Isolation of gamma-isoactin was successful only when ADP-actin was separated on ADP-equilibrated DEAE-cellulose with ADP-containing elution buffer. Substitution of ATP for ADP at any step of purification resulted in the contamination of beta-actin in gamma-actin fractions. We speculate that surface charge of ATP-actin would be different from that of ADP-actin. In addition, as pointed out previously, actin was eluted in partially polymerized form from the DEAE-column. Therefore, it is quite probable that separated isoactins copolymerize in the column when ATP was used.

As gizzard actin contained gamma and beta-actin at ratios of around 9:1, it would be easier to purify single isoactin than from non-muscle sources. Preliminary experiments have shown that a similar preparation procedure can be applicable to the isolation of non-muscle type beta- and gamma-actin from brain. The report will soon be shown elsewhere.

References