Evidence for Interactions among Soybean Protein, Actin and α -Actinin

Michio MUGURUMA*, Liang-Chuan LIN** and Tatsumi ITO*

* *Department of Animal Science, Faculty of Agriculture, Kyushu University,
6-10-1, Hakozaki, Higashi-ku, Fukuoka 812*

** *National Chung Hsing University, 250, Kuokuang Road Taichung,
Taiwan, Republic of China*

To improve the utilization of soybean proteins in meat products, the interaction of α -actinin and soybean protein with actin was investigated by cosedimentation and viscosity experiments. Analyses of the sedimented proteins clearly demonstrated that soybean proteins can bind to F-actin filaments to some extent under neutral (7.5) or slightly acidic (5.5) pH conditions at 25°C. However, no interaction between α -actinin and soybean proteins was observed. In the presence or absence of soybean protein, no appreciable differences were observed in the amount of α -actinin that bound to F-actin under neutral pH condition. However, the amount of α -actinin that bound to F-actin in the absence of soybean proteins was greater than that of α -actinin that bound to F-actin in the presence of soybean proteins under acidic pH condition. These results indicated that α -actinin and soybean protein can bind to actin filaments simultaneously. By viscometry it was confirmed that α -actinin and soybean proteins can bind to F-actin.

Soybeans are recognized as a high-protein human food source. Recently, soybean protein has been used in fabricated foods such as meat patties and emulsion type sausage in order to modify the functional properties and nutritional value¹⁾. To develop technology for effective utilization of soybean protein in meat industry, it is very important to understand the mechanism of interaction between soybean proteins and muscle proteins. Although the binding of soybean protein to myosin B or myosin has been studied extensively^{2)~6)}, very little is known about the binding of soybean protein to purified myofibrillar proteins, such as actin, which constitutes about 22% of the myofibrillar protein mass⁷⁾, and α -actinin, that cross-links to actin filaments⁸⁾ and accelerates the polymerization of actin from the globular to the fibrous form⁹⁾.

We reported previously¹⁰⁾ that α -actinin was released from myosin B complex when myosin B was mixed with soybean protein, therefore, we suggested that soybean protein had greater

affinity for actin than for α -actinin. In the present study purified actin and α -actinin were used to further examine the interaction of soybean protein with actin and the effect of α -actinin on the interaction between them.

Materials and Methods

Preparation of proteins

G-actin was prepared from acetone powder of rabbit skeletal muscle by the method of SPUDICH and WATT¹¹⁾ and was purified further on a Sephacryl S-200 column. α -Actinin was purified from rabbit skeletal muscle according to the method of SUZUKI *et al.*¹²⁾. Soybean 7S and 11S components were prepared by the method reported previously¹⁰⁾. F-actin was prepared from G-actin by polymerization at 25°C for 30 min in a solution containing 10 mM Tris-acetate (pH 7.5), 100 mM KCl, 1 mM ATP, 0.2 mM CaCl₂ and 5 mM β -mercaptoethanol. Soybean protein was prepared by mixing the 7S and 11S components at 1 : 1 weight ratio.

Binding assay of α -actinin and soybean protein with actin

All protein solutions were centrifuged before use at $80\,000 \times g$ for 30 min at 2°C in order to remove aggregates. Protein solutions ($600\ \mu\text{l}$ total volume) containing constant amounts of α -actinin (0.6 mg), soybean protein (3 mg) and F-actin (0.6 mg) were incubated in a solution containing 400 mM KCl, 1 mM NaN_3 , 10 mM β -mercaptoethanol and 10 mM Tris-acetate (pH 7.5) or 10 mM acetate buffer (pH 5.5) at 25°C for 30 min, then centrifuged with a Hitachi 65 P-7 ultracentrifuge at $80\,000 \times g$ for 30 min or $10\,000 \times g$ for 20 min at pH 7.5

or pH 5.5, respectively. Then $30\ \mu\text{l}$ of supernatant was added to $100\ \mu\text{l}$ of a tracking dye, which contained 8% SDS, 60% glycerol, 0.4% bromophenol blue, 200 mM Tris-HCl, pH 6.8. Subsequently, $25\ \mu\text{l}$ of β -mercaptoethanol was added, and the solution was immediately boiled for 3 min. The precipitates were homogenized in $600\ \mu\text{l}$ of 400 mM KCl, 1 mM NaN_3 , 10 mM β -mercaptoethanol, 10 mM Tris-acetate (pH 7.5) or 10 mM acetate buffer (pH 5.5), plus $200\ \mu\text{l}$ of the tracking dye and $50\ \mu\text{l}$ of β -mercaptoethanol, and the solution was boiled for 3 min. Volumes were kept constant so that precipitate to precipitate, supernatant to

Fig. 1 Electrophoretic analysis of binding of α -actinin and soybean protein to actin filaments at pH 7.5

Proteins were added to incubation tubes in the order listed below and centrifuged at $80\,000 \times g$ for 30 min at 25°C . Odd numbers and even numbers represent precipitates and supernatant fractions respectively. Tracks 1 and 2 contained F-actin. Tracks 3 and 4 contained α -actinin. Tracks 5 and 6 contained soybean protein. Tracks 7 and 8 contained mixture of α -actinin and F-actin. Tracks 9 and 10 contained the mixture of soybean protein and F-actin. Tracks 11 and 12 contained the mixture of α -actinin and soybean protein. Tracks 13 and 14 contained the mixture of α -actinin, soybean protein and F-actin. Track M contained myofibrils as a standard.

MHC; myosin heavy chain, 7S; soybean protein 7S component, 11S; soybean protein 11S component

supernatant and precipitate to supernatant comparisons was able to be made.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 7.5~17.5% acrylamide gradient slab gels according to the method of LAEMMLI¹³⁾. Samples were applied to gels in 10 μ l aliquots. Densitometric scan of the gels was performed with a Hitachi 557 Double Wavelength-Double Beam Spectrophotometer.

Viscometry

The viscosities of protein solution were determined with an Ostwald viscometer, with a flow time of 30 sec for buffer. The temperature was maintained at 25°C with a water bath. The specific viscosity was calculated as follows: (sample flow time/buffer flow time) - 1.

Protein concentration

protein concentrations were determined by the biuret method¹⁴⁾. Bovine serum albumin

was used as a standard.

Results

Interaction of α -actinin and/or soybean protein with F-actin filaments in sedimentation assays as assessed by SDS-PAGE

Fig. 1 shows the interaction of α -actinin and soybean protein with F-actin filaments at pH 7.5. Approximately 90% of the F-actin sedimented (compare tracks 1 and 2), while α -actinin and soybean proteins remained in the supernatants (tracks 4 and 6). Under the conditions employed, a significant portion of 11 S components of the soybean proteins was bound by F-actin and precipitated (track 9). When α -actinin was present, the amount of soybean proteins that cosedimented with F-actin increased (compare tracks 9 and 13), especially the amount of 7 S component and 11 S component that coprecipitated increased and almost all of the F-actin sedimented (compare tracks 10 and 14). Mixing of α -

Fig. 2 Electrophoretic analysis of binding of α -actinin and soybean proteins to actin filaments at pH 5.5

Proteins were added to incubation tubes as described in the legend to Fig. 1 and centrifuged at $10\,000\times g$ for 20 min at 25°C. All indications and abbreviations are the same as in Fig. 1.

actinin and soybean protein in the absence of F-actin did not produce a sedimentary complex (track 11). At pH 5.5 (Fig. 2), most of the F-actin sedimented (track 1), while almost all of α -actinin and soybean proteins remained in the supernatants (tracks 4 and 6). Mixing of α -actinin and soybean proteins at pH 5.5, as in the case of pH 7.5, did not produce a sedimentary complex. The amount of α -actinin that cosedimented with F-actin markedly increased with the decrease of pH value (compare Fig. 1-7 and Fig. 2-7). However, the amount of α -actinin that cosedimented with F-actin decreased when soybean protein was added to the mixture of α -actinin and F-actin (compare tracks 7 and 13). Changing the order of the

addition of α -actinin, soybean protein and F-actin had no effect on the results obtained throughout the experiments.

The amount of α -actinin that cosedimented with F-actin in the presence or absence of the soybean proteins was determined by densitometry. The results obtained at pH 7.5 are shown in Fig. 3. The molar ratio of α -actinin (native molecular weight of 200 000) to G-actin (molecular weight of 42 000) in each incubation tube before centrifugation was 1 : 4.7. The molar ratio of sedimented α -actinin to G-actin was 1 : 40 in the absence of soybean protein (Fig. 3-a). When soybean protein was present (Fig. 3-b), however, the ratio of α -actinin cosedimented with the F-actin precipitate slightly increased (the molar ratio of

Fig. 3 Densitometric scans of SDS-PAGE slab gel of precipitated proteins after the centrifugation of the mixture of actin and α -actinin with and without addition of soybean proteins at pH 7.5

(a), densitometric scan of gel 7 in Fig. 1.
(b), densitometric scan of gel 13 in Fig. 1.
7S; soybean protein 7S component, 11S; soybean protein 11S component

Fig. 4 Densitometric scans of SDS-PAGE slab gels of precipitated protein after the centrifugation of the mixture of actin and α -actinin with and without addition of soybean proteins at pH 5.5

(a), densitometric scan of gel 7 in Fig. 2.
(b), densitometric scan of gel 13 in Fig. 2.
All abbreviations are the same as in Fig. 3.

α -actinin to actin was 1:32). Fig. 4 also shows densitometric scans of gels at pH 5.5. The molar ratio of α -actinin to actin was 1:6.75 in the absence of soybean protein but it was decreased to 1:32 in the presence of soybean protein. The amount of α -actinin that cosedimented with F-actin in the absence of soybean protein at pH 5.5 was about 6 fold greater than that at pH 7.5. However, the amount of sedimented α -actinin with F-actin in the presence of soybean protein at pH 7.5 was almost the same as at pH 5.5. Thus, soybean proteins had a stronger effect on the binding of α -actinin with F-actin at a slightly acidic pH value of 5.5.

Effect of α -actinin and soybean protein on the polymerization of actin

Although the optimum concentration of KCl for polymerization of skeletal muscle actin is around 0.1 M¹⁵⁾, soybean protein dissociates

into subunits under such low ionic strength¹⁶⁾¹⁷⁾. In the present study, therefore, the polymerization of actin was induced by the addition of KCl to a final concentration of 0.4 M. α -Actinin accelerates polymerization of G-actin⁹⁾. The effect of soybean protein on the polymerization of actin accelerated by α -actinin at pH 7.5 was examined with an Ostwald viscometer, and the result is shown in Fig. 5. α -Actinin caused a marked increase in the rate of actin polymerization in the presence of high concentration of KCl (filled triangles), whereas soybean protein caused little increase in polymerization rate (filled squares). In addition, soybean proteins did not interfere the effect of α -actinin in accelerating the polymerization of actin (filled circles).

At pH 5.5 (Fig. 6), addition of high concentration of KCl (0.4 M) to the actin solution resulted in a relatively small increase in

Fig. 5 Effect of soybean protein on the polymerization of G-actin accelerated by α -actinin at pH 7.5

All experiments were carried out in a solution of 0.5 mM ATP, 2 mM dithiothreitol, 1 mM NaN_3 , 0.5 mM CaCl_2 and 10 mM Tris-acetate (pH 7.5) at 25°C. Polymerization was induced by the addition of KCl to a final concentration of 0.4 M, and the viscosity was measured with an Ostwald type viscometer at 25°C.

○, G-actin (0.75 mg/ml); △, α -actinin (0.17 mg/ml); □, soybean protein (0.61 mg/ml); ▲, G-actin + α -actinin; ■, G-actin + soybean protein; ●, G-actin + α -actinin + soybean protein

Fig. 6 Effect of soybean protein on the polymerization of G-actin accelerated by α -actinin in pH 5.5

All experiments were carried out in a solution of 0.5 mM ATP, 2 mM dithiothreitol, 1 mM NaN_3 , 0.5 mM CaCl_2 and 10 mM acetate buffer (pH 5.5) at 25°C. Polymerization was induced by the addition of KCl to a final concentration of 0.4 M, and the viscosity was measured with an Ostwald type viscometer at 25°C.

○, G-actin (0.9 mg/ml); △, α -actinin (0.2 mg/ml); □, soybean protein (0.73 mg/ml); ▲, G-actin + α -actinin; ■, G-actin + soybean protein; ●, G-actin + α -actinin + soybean protein

viscosity when it was compared with pH 7.5 (open circles). Actin polymerization was accelerated by α -actinin (filled triangles). The effects of soybean proteins on the rate of actin polymerization in the presence of α -actinin were similar to those at pH 7.5 (filled circles). However, the final viscosity reached in the presence of soybean proteins was slightly higher than that in its absence (filled circles). α -Actinin alone had a detectable viscosity (open triangles).

Discussion

Although thermal treatment above 75~85°C is required for the interaction between soybean protein and myosin^{4,5)}, electrophoretic analyses of the sedimented proteins clearly demonstrates that soybean proteins can bind to F-actin filaments to some extent under neutral (7.5) or slightly acidic (5.5) pH conditions at 25°C. HAGA¹⁸⁾ also suggested that the interaction might occur between actin and soybean protein. However, no interaction between α -actinin and soybean proteins was observed under the present experimental conditions. GOLL *et al.*¹⁹⁾ showed that the amount of α -actinin that combined with F-actin depended on temperature. The amount of α -actinin that cosedimented with F-actin at 25°C and pH 7.5 was similar to their results, whereas the amount of α -actinin that cosedimented with F-actin at 25°C and pH 5.5 was about 6-fold of that at pH 7.5.

It is known that paracrystals are formed by side-by-side association of F-actin particles in the presence of 0.4 M KCl at pH 5.0²⁰⁾. In this experiment, actin filaments were sedimented at pH 5.5 by low speed centrifugation (Fig. 2-1), probably due to the formation of F-actin paracrystals. KOBAYASHI and TASHIMA²¹⁾ have reported that the isoelectric point of skeletal muscle α -actinin is 5.9~6.1. Prolonged exposure to low pH causes side-to-side aggregation of α -actinin¹²⁾. The present results (Fig. 2-7 and 4-a) suggest that the binding of α -actinin to actin paracrystals and the conformational changes of α -actinin at low pH values may increase the cosedimentation of α -actinin with

F-actin. In the presence or absence of soybean protein at pH 7.5, no appreciable differences were observed in the amount of α -actinin that cosedimented with F-actin (Figs. 1-7 and 1-13). Evidently, soybean protein does not interfere the interaction of α -actinin and F-actin. In the presence of α -actinin at pH 7.5, the amount of soybean proteins that cosedimented with F-actin appreciably increased (Fig. 1-13). This may be due to the formation of F-actin bundles induced by α -actinin^{22,23)} following the binding of soybean proteins to F-actin. When soybean proteins were incubated with F-actin and α -actinin in the presence of 0.4 M KCl at pH 5.5, the amount of α -actinin that cosedimented with F-actin decreased to the level of pH 7.5. There were no differences in the amount of soybean proteins that cosedimented with F-actin (Fig. 2-13 and Fig. 4). These results suggest that the binding of soybean proteins to F-actin filaments inhibits the formation of actin paracrystals, so that the amount of α -actinin that cosedimented with F-actin may be decreased.

α -Actinin accelerates the rate of polymerization of G-actin⁹⁾. Because α -actinin cross-links F-actin filaments^{12,22)}, the addition of α -actinin to F-actin increases the viscosity of the F-actin solution¹⁸⁾. α -Actinin increased the viscosity of F-actin even under high ionic strength and low pH conditions (Fig. 5 and 6). Because soybean proteins did not have a significant effect on actin viscosity, soybean proteins evidently do not bind with F-actin by a cross-linking manner. By viscometry it was confirmed that soybean proteins did not interfere the interaction of α -actinin and F-actin under neutral condition. Thus, the binding of α -actinin to F-actin and of soybean protein to F-actin is not competitive in the presence of 0.4 M KCl. However, under slightly acidic pH condition, the amount of α -actinin that bound to F-actin in the absence of soybean protein was greater than that of α -actinin that bound to F-actin in the presence of soybean protein. Therefore, the present result supports the previous suggestion that the affinity of soybean protein for actin is

stronger than that of α -actinin under low ionic strength and low pH conditions. IIBUCHI and IMAHORI²⁴⁾ described that the 7S globulin was in a monomeric state at pH 3 or at an ionic strength higher than 0.8, while it was in a dimeric state at pH 6 and at an ionic strength below 0.2. Under the intermediate conditions it existed as a mixture of monomer and dimer. Thus, the effects of ionic strength and pH may not be small in the interaction between soybean proteins and α -actinin with F-actin. Furthermore, it is considered that the interaction between 7S component and 11S component may effect the interaction between soybean protein and muscle protein. Further investigations of the molecular mechanism for the interaction between soybean proteins and muscle proteins are required.

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大豆蛋白質とアクチンおよび α -アクチニン との相互作用

六車三治男*・林 亮全**・伊藤肇躬*

*九州大学農学部

(〒812 福岡市東区箱崎 6-10-1)

**国立中興大学

(中華民国台湾省台中市国光路 250)

大豆蛋白質の肉製品への有効利用に関する技術を改善するため、大豆蛋白質と筋肉蛋白質であるアクチンおよび α -アクチニンとの相互作用について遠心法および粘度測定法により検討した。その結果、大豆蛋白質は 25℃ 中性 (7.5) および弱酸性 (5.5) の pH 条件下で F-アクチンに結合することができた。しかし、中性および弱酸性の pH 条件下で、大豆蛋白質と α -アクチニンと

の相互作用は認められなかった。F-アクチンに α -アクチニンが結合することは良く知られているが、中性の pH 条件下では大豆蛋白質の存在に関わりなくそれらの結合量に変化は認められなかった。しかし、弱酸性の pH 条件下では大豆蛋白質が存在しない場合に F-アク

チンに結合する α -アクチニンの量は大豆蛋白質が存在する場合よりもかなり多かった。 α -アクチニンは F-アクチンを架橋結合することにより F-アクチン溶液の粘度を増加させるが、大豆蛋白質の添加は α -アクチニンの作用に影響を及ぼさなかった。