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RAPID PURIFICATION OF CHICKEN GIZZARD α-ACTININ

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Summary: A method of purification has been developed which yields highly purified α-actinin and requires less than one day to complete. The α-actinin is extracted from washed chicken gizzard muscle with water at 37°. Actin and a 55,000 dalton protein are quantitatively precipitated from the extract with 20 mM $MgCl_2$. The α-actinin is subsequently precipitated from the extract by 30% $(NH_4)_2SO_4$ and fractionated on DEAE cellulose. Spontaneous protein aggregation is prevented by adding 10% glycerol.

α-Actinin is a component of the Z band of skeletal muscle (1) and has been shown to increase Mg^{2+}-dependent actomyosin ATPase, alter actomyosin superprecipitation and accelerate the conversion of G to F actin (2,3). Additionally, its presence in cardiac and smooth muscle (4), as well as in non-muscle cells, has been established (5). Investigation of α-actinin has been hindered by the rather time-consuming purification methods presently available. The method of Suzuki et. al. (6) yields highly purified α-actinin but requires 3 column chromatographic fractionation steps and at least 7 days to complete. Here we report a procedure which reduces the extraction time from 3 days to 30 minutes and, by a subsequent $MgCl_2$ precipitation step, eliminates 2 of the 3 column chromatographic procedures, thereby decreasing the time required for purification to one day.

Methods: Fresh chicken gizzards were obtained from a local poultry farm. Gizzard muscle, dissected free of fat and fascia, was ground twice through a hand meat grinder (Figure 2a). 75 g. of ground gizzard were suspended in 10 volumes of buffer (0.6 M KCl, 40 mM imidazole, 1 mM β-mercaptoethanol, 1 mM EGTA, 1 mM $MgCl_2$, 0.5% Triton X-100, pH 6 9) and homogenized for three, 30-second bursts in a Waring blender.

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The pellet collected by centrifugation (12,000 x g, 15 min.) was again homogenized in 10 volumes of the same buffer without Triton X-100. This procedure was repeated once. The pellet was then washed three times by homogenization with 20 volumes of water containing 1 mM $MgCl_2$, 1 mM EGTA and 1 mM β-mercaptoethanol, and the pellet was collected by centrifugation (12,000 x g, 15 min.) to yield 55 g. of white residue (Figure 2b).

The residue was then extracted with 3 volumes of water containing 1 mM β-mercaptoethanol and 1 mM $MgCl_2$ at 37° for 30 minutes with gentle agitation. The supernatant, collected by centrifugation (20,000 x g, 20 min.), contained 1.0 g of protein in 165 ml (Figure 2c). 1.0 M $MgCl_2$ was added dropwise to this extract at 37°, with stirring over 15 minutes, to a final concentration of 20 mM. A white, flocculent precipitate was removed by centrifugation (20,000 x g, 20 min.) (Figure 2f). The supernatant containing 66 mg of protein (Figure 2e) was cooled to 4° and solid $(NH_4)_2SO_4$ was added slowly to achieve a final concentration of 17.6 g/100 ml supernatant (30% saturation). After stirring for one hour, the pellet containing 52 mg of protein (Figure 2g) was collected by centrifugation (20,000 x g, 20 min.) and dissolved in 20 volumes of cold 20 mM Tris-Cl, 0.5 mM β-mercaptoethanol, 10% glycerol, pH 8.0. This protein solution can be dialyzed overnight against the same buffer, or it can be applied directly to a 30 ml DE 52 column equilibrated with 20 mM Tris-Cl, 0.5 mM β-mercaptoethanol, 10% glycerol, pH 8.0. α-Actinin elutes in the middle of a linear, 200 ml, 0 to 0.6 M KCl gradient (Figure 1). The peak fractions were pooled to yield 23.4 mg of highly purified α-actinin (Figure 2h).

SDS-polyacrylamide gel electrophoresis was done according to Fairbanks (7), modified to use 4% gels and 0.2% SDS. Protein determinations were by the method of Lowry et. al. (8).

Results and Discussion: The 30 minute, 37° extraction solubilizes most of the α-actinin present in the washed muscle. The density of the staining of the myosin heavy chain, which is not extracted under these conditions, can be used as an approximate standard between an SDS-gel of the washed muscle (Figure 2b) and an SDS-gel of the residue left after extraction (Figure 2d). Although the intensity of the myosin band is increased in the gel of the residue, α-actinin is virtually absent. This compares favorably with the degree of extraction obtained with the customary 3-day, 2° extraction method (6). Incubation for periods longer than 30 minutes leads to increased extraction of actin, without a further increase in α-actinin. Even after a 2 hour extraction there appeared to be no proteolysis as evidenced by the lack of production of new lower molecular weight protein bands on the SDS-gel (data not shown).

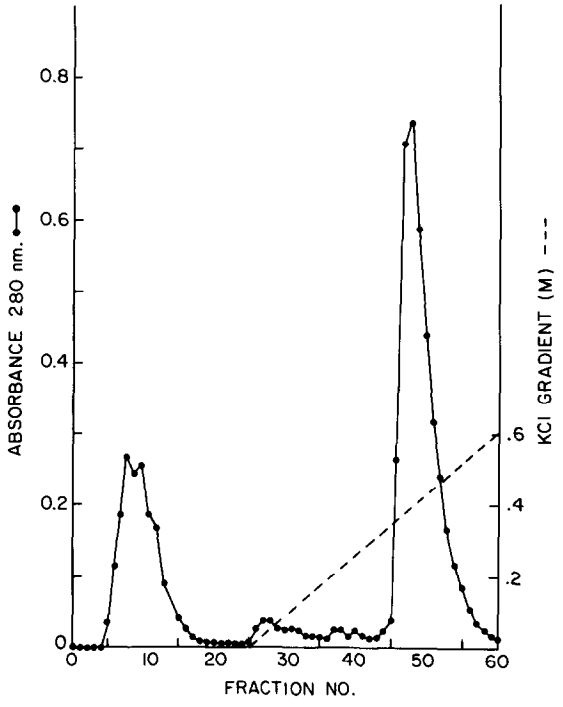

Figure 1. Elution profile of the 30% $(NH_4)_2SO_4$ pellet on a 30 ml DE 52 column. The protein solution and DE 52 were equilibrated with 20 mM Tris-Cl, 1 mM β-mercaptoethanol, and 10% glycerol. 45 mg. of protein were applied in 4.1 ml, and 5.5 ml fractions were collected. α-Actinin was eluted with a 200 ml, linear 0 to 0.6 M KCl gradient (----). Protein was estimated by absorbance at 280 nM (●—●). Fractions 47 to 51 were pooled.

20 mM $MgCl_2$ quantitatively and specifically precipitates actin and a 55,000 dalton protein (Figure 2f). This leaves α-actinin and tropomyosin as the predominant proteins remaining in solution (Figure 2e). Quantitative removal of actin at this step eliminates the need for a later hydroxyapatite column chromatographic separation (6). The $MgCl_2$ precipitate can be partially solubilized by dialysis against 1 mM Tris-Cl, 0.5 mM β-mercaptoethanol, pH 7.0, to yield a highly purified actin preparation free of tropomyosin and myosin contamination (Figure 2j). When $(NH_4)_2SO_4$ fractionation was done prior to $MgCl_2$ precipitation, the resultant pellet was "rubbery" and was difficult, occasionally impossible, to dissolve in non-denaturing buffers.

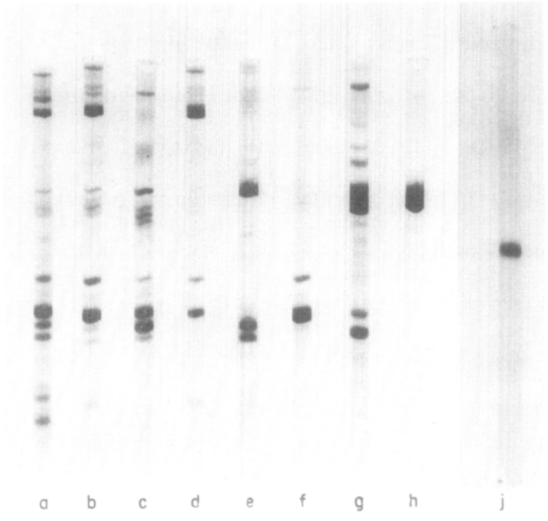

Figure 2. SDS-polyacrylamide gel electrophoresis of chicken gizzard α-actinin preparation. The method of Fairbanks (7) with 4% gels and 0.2% SDS was employed. Gel a, ground chicken gizzard (38 μg). Gel b, washed gizzard muscle (28 μg). Gel c, soluble 37° extract (40 μg). Gel d, insoluble 37° residue (22 μg). Gel e, 20 mM $MgCl_2$ supernatant (25 μg). Gel f, 20 mM $MgCl_2$ pellet (28 μg). Gel g, 30% $(NH_4)_2SO_4$ pellet (66 μg). Gel h, pooled DE 52 fraction (56 μg). Gel j, partially purified gizzard actin (8 μg). Gels a-h were run simultaneously. Electrophoresis was stopped when the tracking dye reached the end of the gel. Electrophoresis of gel j was stopped when the tracking dye was 2 cm from the end.

$(NH_4)_2SO_4$ fractionation effects little purification, but serves to concentrate the protein solution prior to DEAE chromatography (Figure 2g). The concentrated protein solution obtained at this point aggregates and readily precipitates irreversibly. 10% glycerol prevents this aggregation. Possibly, the $MgCl_2$ supernatant could be applied directly to an appropriately equilibrated DEAE column without a decrease in the purity of the final product.

When sufficiently diluted with the equilibration buffer, the $(NH_4)_2SO_4$ pellet could be applied directly to the DEAE column without prior dialysis. Tropomyosin elutes in the breakthrough peak whereas α-actinin is the major component of the large protein peak which elutes at approximately 0.25 M KCl (Figure 1). When 28 μg of α-actinin pooled from this DEAE column are analyzed by SDS-polyacrylamide gel electro-

phoresis, no contaminating proteins are evident. However, when 56 μg of protein are analyzed (Figure 2h), a faint double band at ca. 220,000 daltons, and single bands at ca. 85,000 daltons and 55,000 daltons are just barely discernible. This degree of purity of gizzard α-actinin exceeds (6) or equals (3) the purity of the best reported skeletal muscle preparations.

Although we have not as yet applied this method to the purification of α-actinin from non-muscle sources, the high yield at each step and the small number of steps offers hope for success.

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