Potentiation of actomyosin ATPase activity by filamin

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Received 4 September 1984; revised version received 16 October 1984

It was found that thin filaments from chicken gizzard muscle activate skeletal muscle myosin Mg²⁺-ATPase to a greater extent than does the complex of chicken gizzard actin and tropomyosin. The protein factor responsible for this additional activation has been now identified as the high $M_r$ actin binding protein, filamin.

Actomyosin ATPase activator  Filamin  (Chicken gizzard muscle)

1. INTRODUCTION

The protein composition of thin filaments from chicken gizzard smooth muscle, as revealed by SDS-polyacrylamide gel electrophoresis, is relatively simple [1,2]. The major components are actin and tropomyosin, the minor ones are usually $\alpha$-actinin, myosin light chain kinase, caldesmon and a high-$M_r$ protein (possibly filamin or fodrin). Although all these proteins have been found to interact with F-actin [3–7] only some of them seem to be integral constituents of thin filaments. Others are contaminants and their appearance in thin filaments depends on the method of thin filament preparation.

The minor proteins, independent of their origin, could influence the interaction of thin filaments with myosin. Here we show that a high-$M_r$ protein present in thin filament preparations from chicken gizzard muscle significantly increases the ATPase activity of the complex of thin filaments with myosin. Evidence is presented for the identity of this protein with filamin.

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Abbreviations: DTT, dithiothreitol; SBTI, soybean trypsin inhibitor

2. MATERIALS AND METHODS

Thin filaments were obtained from the myofibril-like fraction [8] of fresh chicken gizzard muscle as in [1]. Chicken gizzard actin was obtained according to [9], and chicken gizzard tropomyosin and rabbit skeletal muscle actin as in [10]. Chicken gizzard filamin was obtained as in [11]. Rabbit skeletal muscle myosin was prepared according to [12].

Mg²⁺-ATPase activity of chicken gizzard thin filament–rabbit skeletal muscle myosin complex and that of rabbit skeletal muscle actomyosin was assayed in a medium containing 50 mM KCl, 5 mM MgCl₂, 2 mM ATP, 2 mM EGTA, and 10 mM Tris-acetate, pH 7.0. The concentrations of proteins are given in the figure legends. The ATPase reaction was started by addition of ATP and stopped after 5 min incubation at 30°C by addition of SDS (final concentration 1%). The amount of inorganic phosphate liberated was measured as in [13].

Tryptic digestion of the activator protein was carried out in a solution containing 60 mM NaCl, 0.1 mM DTT and 20 mM Tris-HCl, pH 7.5, at 20°C. The enzyme to substrate ratio was 1:1000 (w/w). The digestion was stopped by addition of SBTI (twice the weight of trypsin).

SDS-gel electrophoresis was performed on 8%
acrylamide, according to [14]. Protein concentration was determined as in [15].

3. RESULTS AND DISCUSSION

As shown in fig.1, the ATPase activity of the complexes of chicken gizzard thin filaments and rabbit skeletal muscle myosin significantly exceeds that of the complexes of gizzard actin-skeletal muscle myosin or gizzard actin-gizzard tropomyosin-skeletal muscle myosin at weight ratios of actin to myosin greater than 1:1. The protein factor responsible for this additional activation is quantitatively released from the thin filaments at high ionic strength, i.e., 0.5 M KCl (in the original procedure of thin filament preparation [1] the KCl concentration was 0.125 M). To identify this factor, we have purified it from the supernatant, obtained by ultracentrifugation of the thin filaments in a buffer solution containing 0.5 M KCl, 2 mM EDTA, 0.1 mM DTT and 20 mM imidazole-Tris, pH 7.0, sequentially by fractionation with ammonium sulfate, chromatography on anionic exchanger, and gel filtration.

During ammonium sulfate fractionation of the supernatant the active fraction precipitated at 0–40% ammonium sulfate saturation. Potentiation of the Mg²⁺-ATPase of skeletal muscle actomyosin by this fraction was Ca²⁺-independent. The fraction salted out at 0–40% ammonium sulfate saturation did not affect the K⁺-EDTA and Ca²⁺-stimulated ATPase activities of myosin. This active fraction which contained denatured actin and a few proteins with Mᵣ values higher than that of actin (see inset to fig.1) was applied on a DEAE-cellulose column and eluted with a linear 0.06 0.3 M NaCl gradient. The activator of the actomyosin ATPase was present in the fraction eluted at about 0.1 M NaCl (fig.2A). This fraction contained mainly the protein of Mᵣ 250000 and a few proteins with lower Mᵣ values. The latter proteins were separated from the 250-kDa protein on a Sepharose 4B column equilibrated and eluted with a buffer containing 0.1 M NaCl, 1 mM EDTA, 0.1 mM DTT and 20 mM Tris-HCl, pH 7.5. Fractions which activated the actomyosin ATPase corresponded to the peak containing electrophoretically pure 250-kDa protein (fig.2B).

Digestion of the purified activator protein by trypsin at an enzyme to substrate weight ratio of 1:1000, in parallel with the degradation of the protein, decreased its ability to activate the actomyosin ATPase (fig.3).
Fig. 2. Purification of the activator of actomyosin ATPase. (A) DEAE-cellulose chromatography of the fraction of supernatant (see text) salted out at 0-40% ammonium sulfate saturation. Approx. 30 mg protein was applied to a 1.5 x 20 cm column of DEAE-cellulose DE-52 equilibrated with 60 mM NaCl, 0.1 mM DTT, and 20 mM Tris-HCl, pH 7.5. A linear gradient of 0.06-0.3 M NaCl was applied as indicated. Flow rate 20 ml/h. Inset shows SDS-gel electrophoretic pattern of the active fraction.

(B) Sepharose 4B chromatography of the fraction of the activator eluted from DEAE-cellulose column. Approx. 15 mg protein was applied to a 0.7 x 70 cm column of Sepharose 4B equilibrated and eluted with 100 mM NaCl, 0.1 mM DTT, 1 mM EDTA, and 20 mM Tris-HCl, pH 7.5. Flow rate 15 ml/h. Inset shows SDS-gel electrophoretic pattern of (a) active fraction, (b) filamin, (c) active fraction plus filamin. The effect of each fraction on actomyosin ATPase was measured as described in section 2 at 60 μg/ml rabbit skeletal muscle myosin, 40 μg/ml rabbit skeletal muscle F-actin and 50 μl sample of each fraction.

The Mr value of the activator suggested that it might be either filamin or a spectrin-like protein, fodrin. It has found out that both these actin-binding proteins are present in smooth muscle [16–18]. Fodrin seemed to be a more likely candidate, since it has been reported that partially purified fodrin obtained from porcine brain increases several times the ATPase activity of the skeletal muscle actomyosin [19,20].

Filamin and fodrin have very similar hydrodynamic properties (size and shape) and both are oligomers composed of two subunits [6]. However, whereas filamin is a homodimer (composed of subunits of Mr, 250000) fodrin is a heterotetramer (with subunits of Mr, 240000 and 235000) [6,21]. Moreover, one of the fodrin subunits, but not that of filamin, binds to calmodulin in a Ca2+-dependent way [22]. The lack of binding of the activator in the presence of Ca2+ to calmodulin coupled to Sepharose 4B as well as the failure to resolve it into two different subunits during prolonged SDS-gel electrophoresis on 5% acrylamide in the absence and presence of urca suggested that the activator is distinct from fodrin.

On the other hand, co-electrophoresis of the activator with filamin prepared from chicken gizzard muscle with a routine procedure [11] suggested the identity of these two proteins (fig.2B). This was confirmed by showing that the latter protein can
potentiate ATPase activity of skeletal muscle actomyosin to exactly the same extent as does the activator (fig.4).

It was previously shown that filamin inhibits ATPase activity of acto-heavy meromyosin [23]. This effect was observed at a molar ratio of filamin to actin of 1:25 and above (maximum inhibition occurred at a molar ratio of 1:3) and was probably due to blocking by filamin the sites on F-actin which interact with myosin. The activation by filamin of the ATPase of actomyosin observed here takes place only at very low molar ratios of this protein to F-actin; the maximum activation of the ATPase occurs at a ratio of about 1:100 (fig.4).

Activation of actomyosin ATPase by filamin seems to be attributed to the cross-linking of F-actin filaments and gelation of F-actin solutions by this protein [16,17]. This idea is supported by the observation that other proteins which cause gelation of F-actin solution, like α-actinin or fodrin, also activate the actomyosin ATPase [3,19].

![Graph](image-url)

**Fig.4.** The effect of the activator and filamin on actomyosin ATPase activity. ATPase activity was assayed as described in section 2 at 60 μg/ml rabbit skeletal muscle myosin, 40 μg/ml rabbit skeletal muscle F-actin and chicken gizzard activator of filamin in amounts indicated on the abscissa.

ACKNOWLEDGEMENT

This study was supported by the Polish Academy of Sciences within project MR II.1.2.4.

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