ROLE OF THE MYOSIN LIGHT CHAINS IN BINDING TO ACTIN

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1. Introduction

Myosin from the fast twitch muscles of rabbit is hexameric and comprises two polypeptide chains of approx. mol. wt 200 000 (heavy chains) and 4 mol (per mol myosin) of light chains: two identical phosphorylatable polypeptides of mol. wt 18 000 (the P-LC) and two polypeptides of molecular weight 22 000 and 16 000 (the so-called alkali light chains, A1 and A2 respectively (see review [1] and references therein). The four light chain components reside in the globular 'heads' of the myosin molecule, the subfragment 1 (S-1) regions, which possess both the actin binding and ATPase activities of the myosin [1]. Densitometric and radiochemical methods have shown that there is an unequal distribution of the two alkali light chains which supports the hypothesis that myosin isoenzymes exist [2,3]. Myosin from chicken breast-muscle also contains three species of light chain and it is likely that this myosin is similar to that of the rabbit in overall structure and design.

Chymotryptic digestion of insoluble myosin filaments from rabbit fast-twitch muscle produces S-1 species without the P-LC and such fragments have been separated into two species by ion-exchange chromatography, each of which contains a single type of alkali light chain [4].

The precise role of the myosin light chains is still uncertain. In this report we present evidence, from affinity chromatographic procedures, that the alkali light chains from rabbit fast-twitch myosin are involved in actin binding and that the corresponding, non-phosphorylatable light chains from other myosin species possess similar roles in their respective muscles.

Abbreviations: S-1, subfragment 1; A1, P-LC, A2 refer to the light chain components from rabbit and chicken skeletal-muscle myosins mol. wt 22 000, 18 000 and 16 000 respectively; ATPase, adenosine 5′-triphosphate

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2. Experimental procedure

2.1. *Protein preparations*

Myosin was prepared from the longissimus dorsii muscles of New Zealand White rabbits and the breast-muscles of chicken [5]. S-1 was obtained by chymotryptic digestion of these myosins essentially as described by Weeds and Taylor [4]. Crude S-1 refers to the material released into solution by the action of chymotrypsin on the myosin filaments. Occasionally the rabbit protein was further fractionated on DEAE-cellulose into S-1 containing either A1 light chain only (S-1 (A1)) or the A2 light chain only (S-1 (A2)) [4]. Actin from rabbit fast-twitch muscle was prepared from acetone-dried muscle powder by the method of Spudich and Watt [6].

2.2. *Preparation and operation of the actin affinity columns*

G-Actin was coupled to CNBr-activated Sepharose-4B exactly as described previously [7]. F-Actin and F-actin plus tropomyosin (4:1, by weight) were cross-linked by the addition of glutaraldehyde by a modification of the method of Lehrer [8]. These cross-linked F-actins were also attached to CNBr-activated Sepharose-4B [7] such that the final matrices contained 2–3 mg actin/g wet wt gel. All subsequent chromatographic procedures were carried out at 4°C in

5 mM triethanolamine–HCl, pH 7.5, containing 10 mM β-mercaptoethanol.

2.3. *Analytical procedures*

The specific ATPase activities of the S-1 preparations were routinely estimated by measuring the P_i-liberated [9] after incubation at 25°C and pH 7.5 in the presence of 2.5 mM $CaCl_2$, 2.5 mM ATP and 50 mM KCl. They were found to be 3–3.5 μmol P/min/mg. Determination of protein concentration was made by the turbidimetric microtannin method [10]. Polyacrylamide gel electrophoresis in urea, at pH 8.6, was carried out according to the method of Perrie and Perry [11].

3. Results

Earlier reports from this laboratory demonstrated the interaction between Sepharose–G-actin and S-1 produced by digestion of rabbit myosin with papain [7]. In these experiments, inclusion of Mg^{2+} in the column buffers was found to improve binding. It was a surprise, therefore, that in our initial experiments with chymotryptic S-1 from rabbit fast twitch muscle myosin the presence of 2 mM $MgCl_2$ in the operating buffer inhibited the binding of this S-1 species to the Sepharose–G-actin matrices. In the absence of Mg^{2+}, this S-1 was tightly bound to the Sepharose–G-actin and could be dissociated from it stepwise by inclusion of 0.25 M KCl, ATP or ADP (either as the free nucleotides or the Mg-salts) in the operating buffer. Elution of the S-1 from the G-actin columns by a gradient of increasing $Mg.ATP^{2-}$ concentration produced a profile as shown in fig.1. Examination of the eluted material by polyacrylamide gel electrophoresis revealed a marked variation in the ratio of the A1 : A2 light chains across the peak (fig.1). Similar resolution of the S-1(A1) and S-1(A2) species based upon their actin-binding properties could also be achieved when the columns were eluted with increasing concentrations of $MgADP^-$, ATP^{4-}, ADP^{3-} or even KCl. Better resolutions were obtained, however, when the nucleotides were used as the eluting species rather than salt.

It is shown in fig.1 that a higher concentration of $Mg.ATP^{2-}$ is required to dissociate the S-1(A1) species from the immobilized G-actin than is needed to

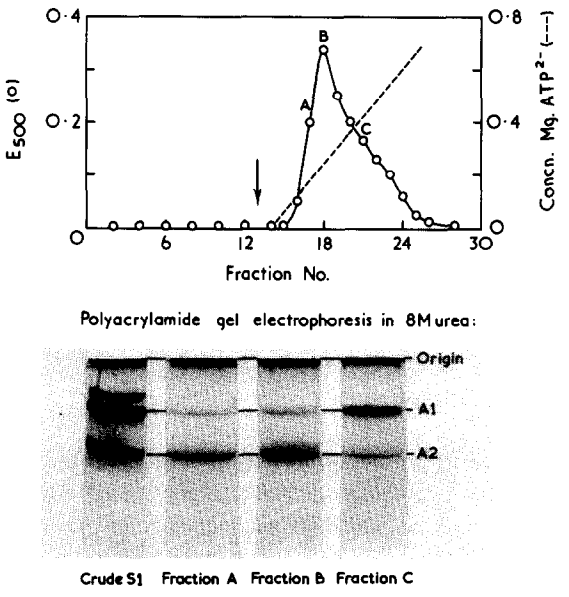

Fig.1. Chromatography of crude rabbit S-1 (1.5 mg) on Sepharose–G-actin. The column (6 × 0.8 cm, containing 2 mg G-actin coupled/g wet wt gel) was operated in 5 mM triethanolamine–HCl, pH 7.5, containing 10 mM β-mercaptoethanol. At the arrow, a linear-gradient formed with 20 ml of the above buffer and 20 ml of that buffer containing 2 mM $MgCl_2$, 1 mM ATP was applied and 1 ml fractions were collected. Aliquots (0.2 ml) were taken for turbidimetric analysis (○). Fractions A, B and C were analysed by polyacrylamide gel electrophoresis in 8 M urea.

dissociate the S-1(A2) species. The same effect was shown when the individual S-1 species, previously fractionated on DEAE-cellulose [4], were applied to the Sepharose–G-actin.

The coupling of F-actin to CNBr-activated Sepharose has been shown to produce an unstable matrix for affinity chromatography of the myosin fragments [7]. However, these matrices could be stabilized by glutaraldehyde crosslinking of the F-actin polymers (either in the absence or presence of tropomyosin; in the following experiments these different matrices were indistinguishable) prior to attachment to Sepharose. (The crosslinked F-actin readily stimulated the Mg.ATPase activity of myosin in free-solution.) The crude S-1 preparations could be fractionated on these F-actin matrices when operated exactly as above in the absence of Mg^{2+}. Both S-1 species bound more tightly, as judged by the con-

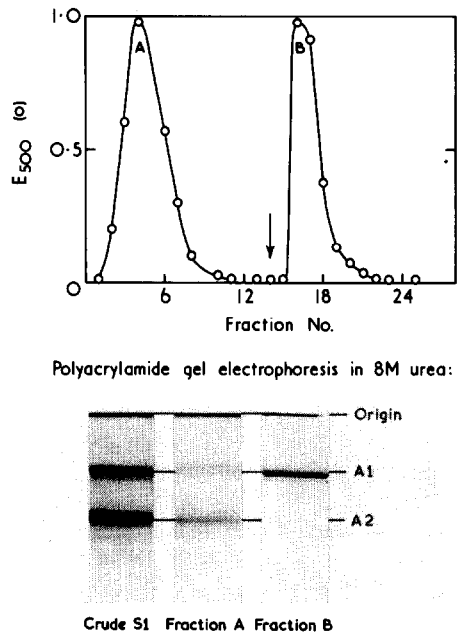

Fig.2. Chromatography of rabbit S-1 (5 mg) on Sepharose–F-actin–tropomyosin (crosslinked with glutaraldehyde). The column (6 × 0.8 cm, containing 2.5 mg of crosslinked F-actin coupled/g wet wt gel) was operated in 5 mM triethanolamine–HCl, pH 7.5, containing 10 mM β-mercaptoethanol and 2 mM $MgCl_2$. At the arrow, 0.25 M KCl was included in the buffer. Fractions (1.5 ml) were collected on 0.2 ml aliquots taken for turbidimetric analysis (○). Fractions A and B were analysed by polyacrylamide gel electrophoresis in 8 M urea.

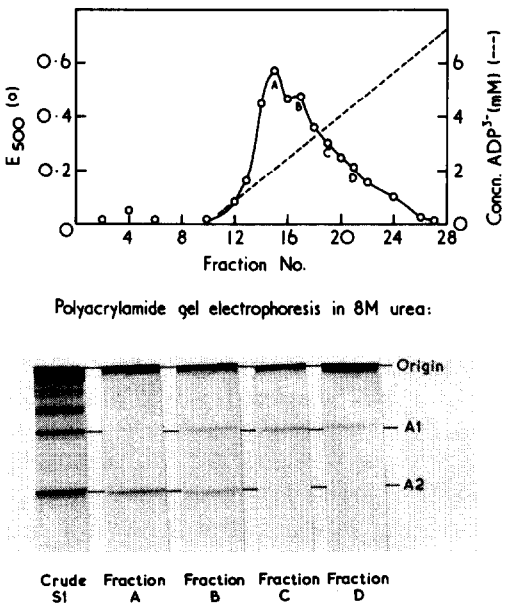

Fig.3. Chromatography of crude chicken S-1 (2 mg) on Sepharose–G-actin. The column was operated and labelled fractions analysed exactly as in the legend to fig.1 except that the limit gradient buffer contained 15 mM ADP.

centration of $Mg.ATP^{2-}$ required to achieve elution, but were resolved in the same order as above. A more complete fractionation of the two S1-types could be achieved when the columns were operated in the presence of 2 mM $MgCl_2$ (fig.2). Under these conditions the S-1 (A2) passed through the column unretarded whereas the S-1 (A1) bound to the column and could be eluted by steps of either 0.25 M KCl or 5 mM $Mg.ATP^{2-}$. The same effect was observed when the two S-1 species previously fractionated by DEAE-cellulose [4] were applied separately to the crosslinked actin matrix.

The results reported above are not unique to S-1 populations isolated from the myosin of fast-twitch rabbit muscle. Corresponding experiments have been carried out with S-1 populations isolated from chicken-breast myosin. Chymotrypsic digestion again resulted in loss of the P-LC (fig.3). For simplicity we have labelled the remaining light chains of chicken S-1 'A1' and 'A2' by analogy with the rabbit S-1. When the crude chicken S-1 preparation was applied to Sepharose–G-actin and the column developed by a gradient of increasing ADP^{3-} concentration, the S-1(A2) was again eluted before the S-1(A1) (fig.3). Similar experiments with the S-1 from mixed rabbit crureus and soleus muscle myosin also revealed two light chain species of S-1 with distinct actin binding properties (M. A. Winstanley and I. P. Trayer, unpublished observations).

4. Discussion

The results show different binding properties of the S-1s containing the two light chain types: in each case the S-1(A1) species exhibiting the greater affinity for both monomeric and polymeric actin forms. This is consistent with kinetic data which showed that the apparent K_m for actin for the S-1(A1) < that for S-1(A2) ([4] and unpublished observations). The experiments described here do not

distinguish between the relative roles played by the heavy and light chains in actin binding. However, a role for the alkali light chains in determining the strength of binding to actin is indicated when these results are taken in conjunction with the recent observations of Wagner and Weeds [12]. These authors showed that the differences observed in the actin-activated ATP activities of isolated native and hybrid rabbit S-1 populations appeared to be due to the particular alkali light chain present.

Amino acid sequence data show that the A1 and A2 light chains of rabbit myosin are almost identical over much of their sequence [13], but that the A1 possesses an additional 41 residues rich in proline and alanine at the N-terminal end. The amino acid composition of the chicken A1 light chain is also richer in proline and alanine than the A2 light chain [14]. It is tempting to speculate that this proline rich N-terminus region of the A1 light chain is involved in strengthening the interaction with actin, or in preserving within the myosin head the optimal configuration for interaction with actin.

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