THE SEPARATION OF HEAVY MEROMYOSIN ISOENZYMES BY DIFFERENTIAL ACTIN BINDING

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1. Introduction

Myosin is a rod shaped molecule divided at one end into two globular head regions. Rabbit skeletal muscle myosin is hexameric [1] and comprises two heavy chains, which run throughout its length, two identical phosphorylatable light chains (P-LC) possibly located near the junction between the rod and head regions [2,3], and two different 'alkali' light chains associated with the heads [1]. Each head, or subfragment 1 (S-1) region, contains a single alkali light chain [4,5]. Densitometric and radiochemical studies have shown that the two phenotypically distinct types of alkali light chain, (the so-called A1 and A2 species [6]) exist in rabbit skeletal muscle myosin in a molar ratio A1/A2 of 1.35:0.65 [7,8]. Based upon this unequal alkali light chain distribution three myosin isoenzymes are predicted: the symmetrical homodimers, A1/A1 and A2/A2, and the heterodimer A1/A2.

Chymotryptic digestion of myosin can result in the production of heavymeromyosin (HMM) which contains both heads and half the rod portion of the myosin molecule; and which appears to retain a full complement of light chains [5]. Myosin isoenzymes with respect to the alkali light chains should appear therefore as isoenzymes of HMM. Immunological and other studies suggest the presence of both myosin homodimers, but their biological role remains undetermined [9,10]. In this report we present further evidence of myosin isoenzymes from two species of skeletal muscle; and we show how affinity chromatography techniques have been applied to study their actin and nucleotide binding properties. The A1/A1 homodimers exhibit a stronger affinity than the A2/A2 species for both the monomeric (G) and polymeric (F) forms of actin. This finding is consistent with data on the binding of the S1 species [11], and might be predicted from kinetic parameters obtained for the single head, S-1 fragments [5].

Abbreviations: S-1, subfragment 1; HMM, heavy meromyosin; A1, P-LC, A2 refer to the light chain components from rabbit and chicken skeletal muscle myosins of mol. wts 22 000, 18 000 and 16 000, respectively; SDS, sodium dodecyl sulphate; ATPase, adenosine 5′-triphosphatase

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2. Experimental

2.1. *Protein preparations*

Myosin was prepared from the longissimus dorsii muscles of New Zealand White rabbits and from the breast muscles of chicken [12]. HMM was obtained by digestion of the myosin with chymotrypsin in the presence of 2 mM $MgCl_2$ as described by Weeds and Taylor [5]. Actin from rabbit fast-twitch and bovine cardiac muscles was prepared from acetone-dried muscle powder by the method of Spudich and Watt [13].

2.2. *Preparation and operation of the affinity columns*

G-Actin was coupled to the CNBr-activated Sepharose-4B as described [14]. The glutaraldehyde cross-linked F-actin–tropomyosin complex was prepared and linked to CNBr-activated Sepharose-4B

as reported earlier [11]. The N^6-(6-aminohexyl)-ADP (N^6-ADP) ligand, and the pyrophosphate derivative (6-aminohexan-1-ol pyrophosphate: HAPP) were prepared and attached to Sepharose-4B by the method of Trayer et al. [15]. All 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*

Protein concentration was determined by measurement of absorbance at 280 nm or by the turbidometric microtannin method [11] when nucleotides were present. Polyacrylamide gel electrophoresis in urea, at pH 8.6, was performed according to the method of Perrie and Perry [16]. Polyacrylamide gel electrophoresis in 10% gels in the presence of 0.1% SDS and 0.1 M Tris–bicine, at pH 8.3, was carried out by the method of Weeds et al. [17]. Densitometric scans of the gels were obtained using a Gilford 2000 recording spectrophotometer fitted with a model 2410 gel scanner.

3. Results

Preliminary experiments with HMM from rabbit fast-twitch muscle myosin showed that it bound more tightly to the Sepharose–G-actin than did corresponding S-1 preparations from the same myosin; and the addition of 2 mM $MgCl_2$ to the buffer, which reduces S-1 binding [11], did not affect the HMM–immobilized actin interaction.

Elution of HMM from the G-actin matrix could be achieved by increasing concentrations of ATP^{4-}, ADP^{3-}, PP_i^{4-} either free or as their Mg^{2+} salts, or even KCl; and in each case a partial fractionation was achieved. Analysis by urea gel electrophoresis confirmed that the fractionation coincided with variation in the alkali light chain content of the HMM. Improved fractionation was obtained using the Sepharose–F-actin tropomyosin matrix (fig.1). Fractions containing only the A1 and P-LC light chains, i.e. A1/A1 homodimers, bound actin more tightly than those which contained predominantly A2/A2 homodimers, and are therefore eluted from the matrix only at higher concentrations of, in this particular case, KCl. This is consistent with achieved separations of S-1 species [11]. The separation of homodimeric isoenzymes of HMM does not depend upon the actin species: quantitatively identical fractionations have been achieved using immobilized cardiac muscle actin. Quantitatively similar separations of HMM homodimers from chicken breast muscle have been obtained using the immobilized actin matrices, and again the homodimers with the larger alkali light chains (analogous to the A1/A1 of rabbit fast twitch muscle) showed a greater affinity for actin. The binding of HMM to the N^6-ADP and HAPP matrices again gave results which were consistent with the data on S-1 behaviour [18]. HMM eluted from the Sepharose–N^6-ADP column by an increasing concentration of ADP^{3-} was partially fractionated: the A2-rich species exhibited a greater affinity for the free nucleotide. A more complete fractionation was achieved using the Sepharose–HAPP matrix and elution by increasing concentration of sodium pyrophosphate (fig.2). Samples of eluted HMM were analysed by polyacrylamide electrophoresis. Densitometric scans of the

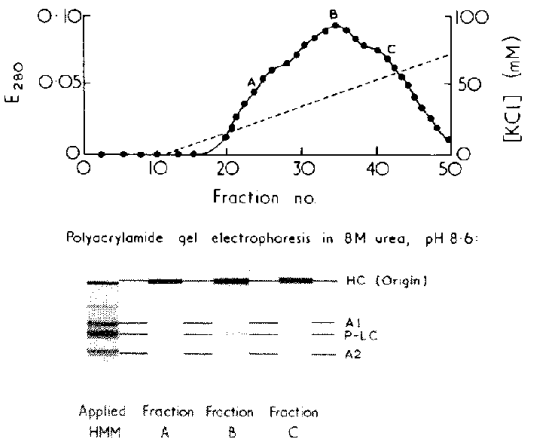

Fig.1. Chromatography of rabbit skeletal muscle HMM (6 mg) prepared by chymotryptic digestion on a Sepharose-bound F-actin–tropomyosin (cross-linked with glutaraldehyde) matrix. The column (20 × 0.8 cm, containing 2.2 mg cross-linked F-actin–tropomyosin complex/g wet wt gel) was operated in 5 mM triethanolamine–HCl buffer, pH 7.5, at 4°C. After application of the protein and subsequent washing with this buffer, the bound HMM was eluted with a linear gradient from 0–100 mM KCl (total vol. 80 ml) dissolved in the running buffer (- - -). Fractions, 1.4 ml, were collected and their absorbance at 280 nm measured (•). The applied HMM and fractions A,B and C were analysed by polyacrylamide gel electrophoresis in 8 M urea, pH 8.6.

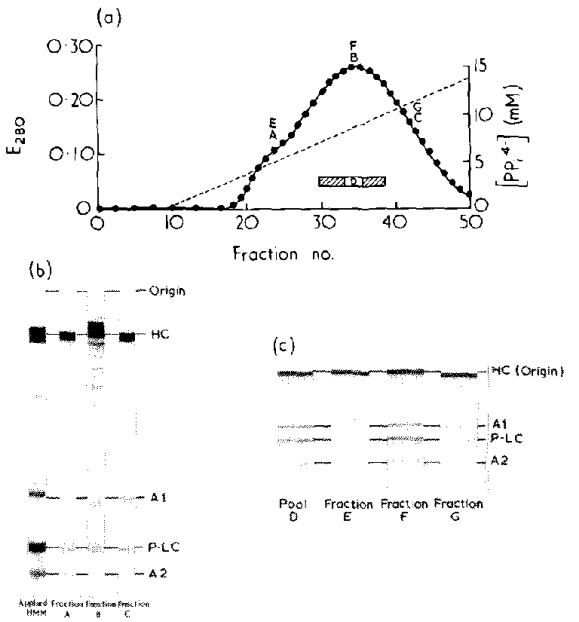

Fig.2. Chromatography of rabbit skeletal muscle HMM (12 mg) prepared by chymotryptic digestion on a Sepharose–6-aminohexan-1-ol pyrophosphate (HAPP). (a) The column (26 × 0.8 cm, containing 2.5 μmol HAPP/g wet wt gel) was operated in 5 mM triethanolamine-HCl, pH 7.5, at 4°C. After washing the column with this buffer, the bound HMM was eluted by a linear gradient from 0–15 mM sodium pyrophosphate (total vol. 80 ml) dissolved in the buffer (- - -). Fractions, 1.4 ml, were collected and their absorbance at 280 nm measured (•). (b) Fractions A, B and C were analysed by polyacrylamide gel electrophoresis in 10% gels run in the presence of 0.1% SDS and 0.1 M Tris–bicine, pH 8.3. The hatched area, D, was pooled, dialysed against 5 mM triethanolamine–HCl, pH 7.5, to remove any PP_i^{4-}, and re-applied to a smaller Sepharose–HAPP column (12 × 0.8 cm). The column was eluted as previously except that the total volume of the gradient was 60 ml. A column profile was obtained that was virtually identical to that shown above. (c) Fractions E, F and G from the chromatography of pool D on Sepharose–HAPP were taken at similar elution positions as fractions A, B and C, respectively and were analysed by polyacrylamide gel electrophoresis in 8 M urea, pH 8.6.

gels showed that although the ratio of A1 light chain to A2 light chain (A1:A2) increased across the peak of eluted material, at any point the sum total of A1 plus A2 material was approximately constant and equal to that of the P-LC. The unresolved material eluted from the column (fig.2a) was dialysed against running buffer and reapplied, whereupon a further fractionation into A1-rich and A2-rich species was observed (fig.2c). This strongly suggests that the unresolved HMM still contains homodimers and does not represent a sizeable population of A1/A2 heterodimers.

4. Discussion

Our results establish the existence of myosin homodimers, and the data from the reapplication experiments, while not eliminating the presence of heterodimers, suggest that they could be present only in small amounts. It has been demonstrated [19] that the heavy chains isolated from separate S-1 species containing either the A1 or the A2 light chain display the same chemical heterogeneity. Furthermore, the extensive sequence homology between the A1 or A2 light chains [6], and the ease with which the different S-1 species can be hybridized [19], suggest that there is little selectivity in the interaction between individual light chains and the different heavy chains. Therefore, a random subunit assembly of myosin in vivo might be expected to result in a high percentage of heterodimers. Since this does not appear to be so it is tempting to speculate that temporal or spatial control of gene expression is employed to ensure the production of homodimers.

We have separated HMM homodimers from two types of fast-twitch skeletal muscle, and have shown in both cases that the isoenzymes differ in their affinity for actin and the free nucleotide. In ascribing the relative affinities for actin of the two isoenzymic species our results are irreconcilable with those of Alexis and Gratzer [9] under whose experimental conditions (0.35 M NaCl) we can show no significant HMM–immobilized actin interaction.

From previous results with S-1 [18] we would predict that rabbit slow-twitch skeletal muscle myosin also contains isoenzymes which differ in their affinity for actin and possibly therefore in their potential to generate force. It appears that only vertebrate skeletal muscles possess two types of actin binding (alkali) light chains (in addition to the P-LC), thus the homodimeric isoenzymes discussed above may be characteristic of muscles in which both maintained muscle tone and variation in contractile speed are required. The exact location of the myosin iso-

enzymes is uncertain; there does not appear to be any difference in the ratio of the A1/A2 light chains within individual fibres from fast-twitch rabbit psoas muscle [17]. It is conceivable that they could occur within a single A filament, and so may be a vital factor in the mechanical functions of the sarcomere.

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