POLYPEPTIDE CHAINS OF INTERMEDIATE MOLECULAR WEIGHT IN MYOSIN PREPARATIONS

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1. Introduction

The myosin molecule contains two heavy polypeptide chains (molecular weight 200,000), and also light chains (molecular weight in the range 14,000 to 32,000) whose number and function are still controversial [1–7]. For the preparation of these subunits and the study of their stoichiometry and properties, the myosin used should be rigorously pure. Assessment of purity is not easy for a fibrous protein like myosin, but the new technique of electrophoresis on polyacrylamide gels containing sodium dodecyl sulphate (SDS) [8, 9] allows the separation of all the polypeptide chains in a mixture of proteins according to their molecular weights. We have used this technique to examine the purity of myosin prepared in the usual manner and to test the usefulness of two purification procedures [10–13], which unfortunately are not widely used.

2. Methods

Rabbit skeletal myosin was prepared by the method of Perry [14], with an additional batch treatment with DEAE cellulose between the second and third precipitations to remove ribonucleoprotein impurities [12, 15]. The myosin was usually dissociated in 1% SDS–1% mercaptoethanol at 45° for 1 hr and electrophoresis performed according to the conditions of Weber and Osborn [9] on 5 mm diameter 6% polyacrylamide gels. The gels were fixed in 20% sulphosalicylic acid [8] and stained in Coomassie brilliant

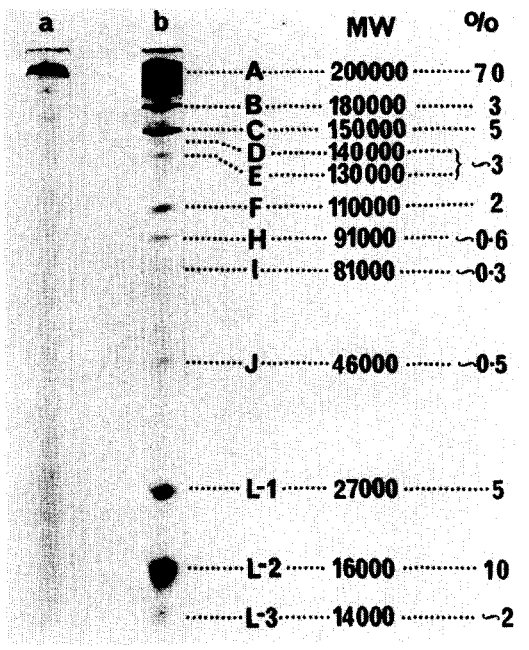

Fig. 1. Electrophoresis of myosin on SDS-polyacrylamide gels. (a) Loading of 1 μg myosin (b) loading of 10 μg myosin. At higher loadings band B is not clearly resolved from band A. The fastest light chain (L-3) is not always seen because it is often imcompletely resolved from L-2 and is easily lost during destaining. Denaturation of myosin at 100° rather than 45° does not change the pattern except that a further trace band (G) is seen between bands F and H. Chain weights shown were obtained by running markers of known molecular weight and plotting $\log_{10}$ mw versus mobility [9]. The approximate proportion of each component was the mean value obtained from densitometry of gels from two batches of myosin (a typical scan is shown in fig. 4b). It was assumed that the area under each peak was proportional to its protein content and that each component bound dye to the same extent.

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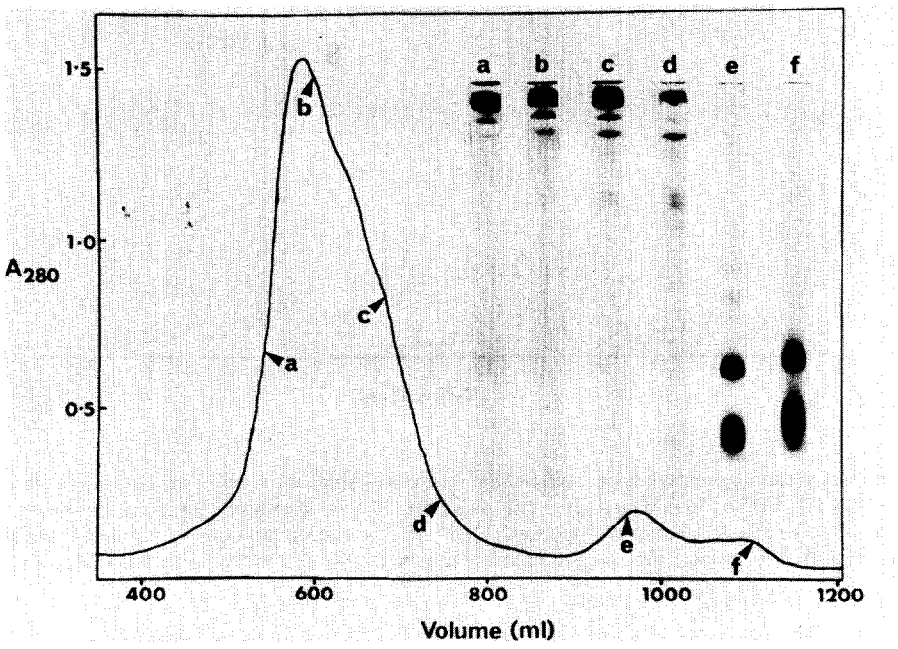

Fig. 2. Separation of the polypeptide components of myosin by gel filtration in SDS. 550 mg myosin denatured in 100 ml 1% SDS, 1% mercaptoethanol, 100 mM potassium phosphate pH 7.2 were applied to a 5 × 94 cm column of G-200 Sephadex equilibrated with 0.1% SDS, 100 mM potassium phosphate and eluted with this solvent at 25° with a flow rate of 24 ml/hr. The results of SDS electrophoresis performed on fractions eluted at a, b, c, d, e, f are shown.

blue in methanolacetic acid [9]. Estimates of the relative amounts of each component were made by densitometry of the gels at 600 nm with a Gilford scanning attachment.

3. Results

At low loadings (1 μg) myosin gives one sharp band (A) due to the heavy chains, which just enter the gel (fig. 1a). At higher loadings (5 to 30 μg) further bands are seen. The three fastest (L-1, L-2 and L-3 with molecular weights approximately 27,000, 16,000 and 14,000 respectively) correspond to light chains [4, 5, 9, 16, 17]. In addition we observe a number of bands in the intermediate weight range (40,000 to 180,000) which have not been discussed previously. Three major bands (B, C, F in fig. 1b) were present in all seven batches of myosin examined. Up to five trace bands (D, E, H, I, J in fig. 1b) were also seen but their appearance in different batches was more variable. The fastest of these trace bands (J) has the same mobility as actin, and indeed traces of F-actin were detectable in our preparations by electron microscopy. The other major myofibrillar proteins (tropomyosin and the several components of troponin) have higher mobilities than actin and cannot account for bands B-I. These bands could be due to other impurities; alternatively, they could be formed from heavy chains by proteolysis, or from light chains by aggregation. The following evidence shows that they are impurities.

We obtain the same band pattern from the first dilution precipitate run within three hours of the rabbit's death and from myosin stored in 0.5 M KCl at 4° for two weeks. It is therefore unlikely that the chains of intermediate molecular weight arise by proteolysis.

Gel filtration in the presence of 0.1% SDS (a modification of the method of [18]) gave a clear separation of the heavy and light chains (fig. 2). SDS electrophoresis of column fractions showed that the

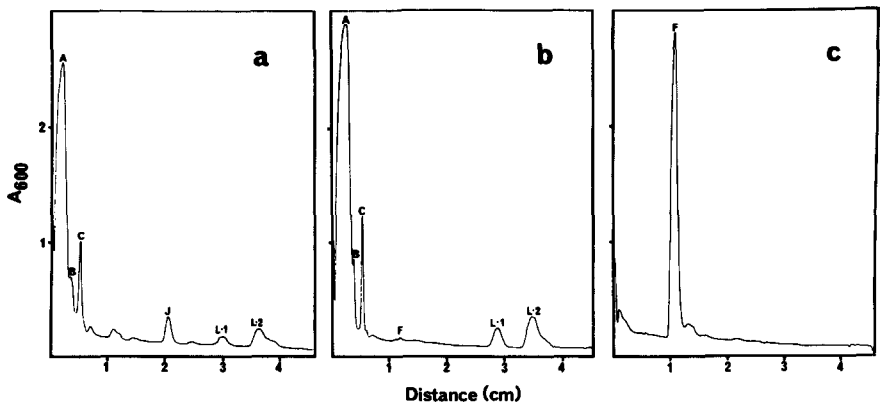

Fig. 3. The effectiveness of ammonium sulphate fractionation of myosin. Solid ammonium sulphate was added to 5 mg/ml fresh myosin in 0.5 M KCl, 1 mM EDTA, 50 mM potassium phosphate pH 7.0 at 4°. Densitometer traces are shown for SDS gels run of the following samples: (a) 30–35% saturation fraction; (b) 35–40% saturation fraction; (c) 45–55% saturation fraction. The peak in (c) was identified by running this fraction mixed with myosin. See fig. 4b for unfractionated myosin control.

polypeptide components were eluted in the order of their electrophoretic mobilities, and there was no evidence of any interconversion between them. In particular, fractions eluted on the trailing side of the main peak were rich in intermediate chain, B, C and F, while the light chain fractions did not give rise to these components. Therefore the intermediate chains are not formed by aggregation of light chains.

The best demonstration that the intermediate chains are impurities would be their removal by mild procedures. Ammonium sulphate fractionation has been proposed for the preparation of myosin [10, 11]. Under our conditions the small amount of protein (6% based on absorption at 280 nm) which precipitates at 35% saturation is enriched with actin (J of fig. 3a). Most of the protein (90%) precipitates in the 35–40% saturation range and is depleted of component F (fig. 3b). Nearly pure component F is obtained in the 45–55% fraction, which accounts for 3% of the total absorption at 280 nm (fig. 3c). Component F rapidly aggregates at room temperature.

Another method proposed for the preparation of myosin uses chromatography of DEAE-Sephadex with elution by phosphate or pyrophosphate buffers [13]. Although a substantial amount of protein is not bound to such columns, this unretarded material has been thought to be aggregated myosin [13]. However, electrophoretic examination shows that this unretarded protein is a mixture of components C and F, and contains no heavy or light chains (fig. 4c). Correspondingly the main peak contains nearly pure myosin, for only one component (B) is seen apart from the heavy and light chains (fig. 4d); (a similar pattern is seen in fig. 4e of [17]). In our experiments the unretarded protein reproducibly accounted for 14% of the total 280 nm absorbing material eluted. This corresponds to 8% by mass, in agreement with the proportions of C and F found from gel densitometry ($A^{1\%}_{280}$ was 11 based on a refractive index increment of 0.18). When myosin which had been purified by ammonium sulphate fractionation was chromatographed, the unretarded peak was reduced to 11% (based on absorption at 280 nm) and electrophoretic examination showed that this material was mainly component C (fig. 4e). In the analytical ultracentrifuge it gave one main peak with some trailing material. $S_{20,w}$ for the main peak was 4.3 S at A_{280} = 6.4 in 0.5 M NaCl, 1 mM EDTA, 10 mM KH_2PO_4–K_2HPO_4, pH 7.0. Component C, unlike myosin, remains in solution after dialysis against a low ionic strength medium (0.04 M NaCl, 1 mM EDTA, 10 mM potassium phosphate, pH 7.0).

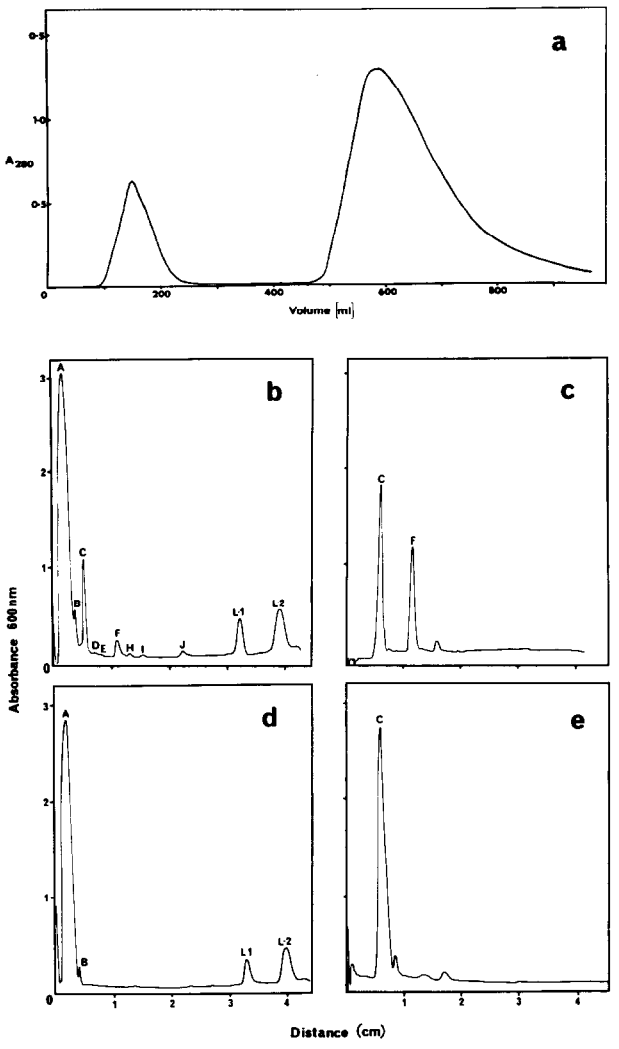

Fig. 4. The effectiveness of DEAE-Sephadex chromatography of myosin. (a) Elution profile of myosin chromatographed on DEAE-Sephadex according to the conditions of [19]. 120 ml 10 mg/ml myosin, dialysed against 0.15 M potassium phosphate, 10 mM EDTA, pH 7.5, was applied to a column 5 × 35 cm of DEAE-Sephadex equilibrated with this solvent. A linear gradient to 0.5 M KCl, 0.15 M potassium phosphate, 10 mM EDTA was started immediately after the sample was applied. (b–e) Densitometer traces of SDS gels. (b) myosin control (c) and (d) unretarded and main peak respectively from myosin. (e) unretarded peak from ammonium sulphate purified myosin.

4. Discussion

We have shown that myosin prepared by the customary procedure contains polypeptide chains intermediate in size between the heavy and light chains. All these intermediate chains, except B, may be removed by chromatography on DEAE-Sephadex [13, 19] without removing any of the light chain components. This purification is obviously highly desirable for routine use, because the impurities may affect the ATPase activity of myosin, its antigenic properties and its assembly to filaments.

The presence of these impurities (totalling approximately 15% of the protein) may influence significantly the interpretation of sedimentation equilibrium studies of myosin in dissociating media [1, 2, 7], which has hitherto been made solely in terms of heavy and light chains. It might also explain the non-integral values reported for the number of active sites of the myosin molecule [20, 21].

The following evidence suggests that proteins B, C and F are located in the myofibril. Chain and Sender [22] have examined the electrophoretic band pattern of myofibrils and noted components with similar mobilities to our B, C and F. α-Actinin was clearly distinguishable from all three. We have confirmed their results by running myofibrils with added C and F. Thus B, C and F differ from all the well known proteins of the thin filament and Z-line. Their presence in myosin preparations suggests instead that they are new proteins of the thick filament assembly, functioning perhaps as proteins of the M-line [23] or as the hypothetical core protein or length determining factor of the thick filament [24]. A protein working in any of these ways should bind to myosin. This is true of the C protein, which is soluble at low ionic strength but is precipitated in the presence of myosin. This may be physiologically important, since electron microscope studies show that the C protein influences the growth of synthetic myosin filaments (Moos, unpublished). Further investigation of proteins C and F is in progress.

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