HOMO-DIMERS IN RABBIT SKELETAL MYOSIN

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1. Introduction

The myosin of rabbit skeletal muscle, which has been the material for the great majority of structural and kinetic studies, contains three polypeptide species of low molecular weight (light chains). Two of these are the so called alkali light chains, and cannot apparently be removed without loss of enzymic activity; the remaining species, the DTNB light chains, are not essential for ATPase. Each myosin head is supposed, on the basis of the light-chain stoichiometry, to contain one DTNB and one alkali light chain. The proportions of the two alkali light chains, as reported from different laboratories, vary appreciably, but it seems now to be generally agreed [1–3] that the one of higher molecular weight, A_1, is present in appreciable molar excess over the other, A_2, and indeed this relationship obtains in myosin isolated from single fibres of different types [2]. Only one alkali light chain is synthesised during embryogenesis [4], and the characteristic isozyme pattern develops subsequently. The A_1 and A_2 chains differ only by an additional segment of polypeptide chain present in A_1 [5]. It has been shown [6–8] that the A_1 and A_2 chains are present in different populations of heads (subfragment-1), and these have been separated. In functional terms, the A_1 and A_2-containing subfragments appear to differ only in respect of actin-stimulated ATPase activity [7]. On the basis of the apparently unequal molar concentrations of A_1 and A_2 in preparations of skeletal myosin, it has been inferred that at least some myosin molecules exist which are homo-dimeric in respect of A_1 light-chains.

We show here that myosins containing A_1 and A_2 chains differ in their affinity for actin, as judged by the relative extents to which they are dissociated from F-actin by pyrophosphate. On this basis fractions enriched in A_1 and A_2 can be obtained. This provides direct evidence for the existence of homo-dimers in respect at least of light chains in rabbit skeletal myosin.

2. Experimental

Myosin from rabbit leg and back muscle was prepared by the method of Perry [9]; preparations were purified by fractional precipitation with ammonium sulphate, and some by ion-exchange chromatography [10]. G-actin was prepared by the procedure of Spudich and Watt [11]. Protein preparations were screened for purity by gel electrophoresis in the presence of sodium dodecyl sulphate (SDS). After polymerisation of the actin, unbound ATP was removed by repeated suspension and centrifugation, until its concentration in the supernatant was less than 1 μM. When the myosin preparations were added to F-actin, spectrophotometric analysis of the supernatant after centrifugation showed that more than 95% of the protein was in the precipitate.

Mixtures of 2–3 mg myosin with a 10–15-fold molar excess of actin, suspended in 1.5 ml of 0.35 M sodium chloride, 5 mM phosphate, 10 mM imidazole, pH 7.5, were made up to a desired concentration of pyrophosphate within the range 5 μM–10 mM, and agitated at 4°C for some minutes to disperse the precipitate. Either EGTA (0.3 mM) or added calcium ions (0.1–0.15 mM) were present. The suspension was then centrifuged at 10^5 *g* for 90 min, and the supernatant removed. The concentration of myosin in the supernatant was determined spectrophotometrically. After exposure of the actomyosin to a given concentration of pyrophosphate, the precipitate was dispersed in 0.1 M pyrophosphate and the

residual myosin collected after centrifugation as before. It was then dialysed against the electrophoresis buffer. In some experiments the actomyosin precipitate was dissolved directly in buffer containing 1% SDS with heating. This proved a less satisfactory procedure because of the intense broad bands produced in the gels by the actin. The samples (100–200 μg) were equilibrated in 0.05 M phosphate, pH 7.0, containing 1% SDS and 1% β-mercaptoethanol in a boiling water bath for 5 min, and applied to cylindrical gels of 6 mm diameter (10% acrylamide, 0.27% methylenebisacrylamide). The electrophoresis, staining with Coomassie Brilliant Blue G250, and destaining followed the procedure of Weber and Osborn [12]. After complete destaining, the gels were scanned at 600 nm in a Unicam SP 1800 instrument, using a slit of 0.1 mm. Tests for adherence of the system to Beer's law were performed by loading varying quantities of myosin on identical gels, and loadings were throughout in the range within which Beer's law had been shown to hold. The zones associated with the three light chains were well separated, and their areas were determined by planimetry.

3. Results

The relative concentrations of the DTNB and alkali light chains in the fraction of myosin dissociated from actomyosin at each concentration of pyrophosphate, and of the fraction remaining bound to the actin, were determined from the areas under the peaks obtained by densitometry of the stained gels. The colour values of the alkali light chains are known [13] to be identical in the conditions of staining described above, and that for the DTNB chains at most only slightly different [3], and the ratios therefore reflect relative molar concentrations, when allowance is made for the differences in mol. wt. As between the most readily dissociated myosin fraction and the most strongly bound there is no clean fractionation in terms of the A_1 and A_2 light chains. However visually in the most favourable cases, and by densitometric evaluation, it is found that the supernatant is relatively enriched in A_1, particularly in experiments performed in the absence of free calcium ions. Under these conditions the mid-point of the dissociation profile is in the range 10^{-5}–10^{-4} M pyrophosphate. To take account of the appreciable variation found in the A_2:A_1 ratio between different myosin preparations, the concentrations in the fractions are expressed relative to those in the original myosin. The ratio of total alkali light chains (A_1+A_2) to DTNB light chains is always close to unity, and the concentration of A_1 and A_2 in the original myosin is conveniently referred to a value of total alkali light chains of 2.0. Typically for the supernatant and precipitate fractions in a single experiment at a pyrophosphate concentration of 10^{-4} M, the ratio for A_1 in the precipitate (normalised as described) was 1.42, whereas the same ratio for A_2 was 0.72. For a similar experiment at a pyrophosphate concentration of 5×10^{-5} M, the corresponding ratios were 1.24 and 0.77. In the presence of calcium ions this effect was diminished: at 10^{-4} M pyrophosphate, the ratios were respectively 1.10(A_1) and 0.88 (A_2), and at 5×10^{-5} M, 1.05(A_1) and 0.94 (A_2). Because the differences are not very large, and the precision of the experiments in their nature limited, the data were not adequate to demonstrate a clear trend in the change of

Table 1
Ratios of A_1 and A_2-light chain contents of myosin fractions obtained by partial dissociation of actomyosin with pyrophosphate

	Supernatant fractions					Precipitate fractions				
	$(A_1)/(A_{10})$[a]	σ_m[b]	$(A_2)/(A_{20})$	σ_m[b]	n[c]	$(A_1)/(A_{10})$[a]	σ_m[b]	$(A_2)/(A_{20})$[a]	σ_m[b]	n[c]
$-Ca^{2+}$	1.054	0.039	0.923	0.061	7	0.846	0.041	1.190	0.051	5
$+Ca^{2+}$	1.012	0.019	0.987	0.987	9	0.965	0.032	1.045	0.039	8

[a] (A_1) is concentration of A_1-light chain in the fraction, and (A_{10}) that in the corresponding unfractionated myosin preparation (both normalised to a total alkali light chain content of 2.0);
[b] Standard deviation of the mean value;
[c] Number of experiments.

ratios with pyrophosphate concentration. Instead the ratios obtained in experiments over the whole pyrophosphate concentration range explored were averaged and gave the results shown in table 1. The average differences were somewhat smaller than in the individual pairs of fractions, but the trend was maintained: the supernatant fraction was significantly enriched in the A_1 light chain, and the precipitate in the A_2. The difference appeared to be considerably diminished in the presence of calcium ions, which also had the effect of increasing the total dissociation somewhat at each pyrophosphate concentration. In some precipitate fractions the A_2:A_1 ratio was greater than unity.

4. Discussion

The results presented above strengthen the evidence for the existence of homo-dimers in skeletal muscle myosin. Because the separate A_1- and A_2-containing myosin heads are identical in their magnesium-activated ATPase activities, while differing in actin-activated ATPase [7], it is not surprising to find that partial fractionation of A_1- and A_2-containing species occurs under conditions that depend on the affinity for actin. The kinetic complexity of the actomyosin system [14] is too great to allow a prediction of the way in which a difference in actin affinity in the presence of pyrophosphate will affect the actomyosin ATPase.

Unfortunately, the degree of fractionation that we have been able to achieve is not sufficient to render analyses of the ATPase activities of the fractions meaningful. That the relative affinities of the two species should be affected by calcium ions is also not surprising in view of the known effect of calcium on the strength of the actin-myosin interaction, mediated by the DTNB-light chains [15].

Our results leave several relevant questions about myosin heterogeneity unanswered. It appears for example [16,17] that heterogeneity is also present in the heavy chains of rabbit skeletal myosin. As yet it is not known whether this is correlated with the light chain heterogeneity. Certainly, as between pure fast and slow-twitch muscles, differences in the light chains are accompanied by differences in the heavy chains [18,19], but in rabbit skeletal muscle, the A_1 and A_2 light chains are uniformly distributed between different fibre types [2]. In addition, the results do not answer the question whether rabbit skeletal myosin contains only homo-dimeric species, or whether all three possible isozymes, including the hetero-dimer, are present. Such a mixture would be consistent with the low degree of apparent fractionation that we have found it possible to achieve.

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