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Myosin light chain functions

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Summary

The two regulatory light chains (RLC) of fast-twitch skeletal muscle myosin from rabbit are digested proteolytically at different rates. In purified actomyosin where the heads bind to actin in rigor, both RLC are digested at the same rate. Removal of both RLC does not affect the ATPase activities of myosin. Morphological studies by the electron microscope on spread and rotary shadowed myosin preparations as well as hydrodynamic studies by gel filtration technique revealed that upon removal of both RLC the shape of the head portions changes, the heads of one molecule tend to form intramolecular aggregation and, in addition, intermolecular aggregates, mostly dimers, are formed. These interactions are hydrophobic in nature and cannot readily be dissociated. These results could imply that one of the functions of the RLC is to keep the two heads of an individual myosin molecule apart from one another in muscle.

Introduction

Myosin is a hexamer consisting of two heavy chains (HC) of around 200 kD and 4 light chains (LC) in the range of 16.5-22.0 kD. The alpha-helical HC are arranged in a coiled-coil rod of 140 nm length, and at its end uncoil to form the two globular head portions bearing the ATPase sites. The HC as well as the LC exist in a variety of isoforms according to the muscle type and the animal species. The ATPase activity of a particular myosin is determined by the HC isoform exclusively (1,2). The LC exist in two forms, the alkali or essential type (ALC) and the regulatory or DTNB type (RLC). Each myosin head bears one ALC as well as one RLC, thus two ALC and two RLC per myosin molecule. While some involvement in regulation of contraction has been established for the RLC, the function of the ALC remains elusive at present. The ALC have been implicated in skeletal muscle to modulate the binding of the myosin crossbridges to actin (3,4). In all types of muscles the RLC are participating in binding of either a Ca or Mg ion per myosin head. In molluscan striated muscles binding of Ca to the

RLC provides the trigger for contraction (5). In vertebrate smooth and non-muscle cells contraction is triggered by Ca-calmodulin-dependent phosphorylation at serine-19 of the RLC (6). In sarcomeric muscles (skeletal and cardiac) the RLC can also be phosphorylated reversibly at serine-15 in dependence on Ca-calmodulin and can also bind Ca or Mg ions with high affinity, its function, however, is less clear (7,8). Both phosphorylation and binding of divalent metal ions of RLC do not represent the dominant regulation of contraction, but may rather have modulatory functions. In general, phosphorylation of RLC leads in sarcomeric muscles to a decreased rate of ATP hydrolysis during isometric tension development, presumably, resulting in a slower cycling of the crossbridges (9). Most recently it has been suggested that the neck region of the double-headed myosin molecule including the RLC is required for regulation of contraction by Ca ions via the troponin-tropomyosin system on the actin filament (10). We report here on the involvement of the RLC in the disposition of the two heads in isolated myosin molecules.

Materials and methods

Myosin (11) and purified actomyosin devoid of troponin and tropomyosin (12) were prepared from rabbit fast-twitch skeletal muscles. Both RLC could be removed from myosin by partial digestion of the actomyosin system with alpha-chymotrypsin (ratio of proteinase to RLC = 1:1'000) in the presence of 0.5-1.0 mM dithiobis-nitrobenzoic acid (DTNB), 5 mM EDTA at low salt concentration and pH 7.5, followed by several washings, reduction with dithiothreitol, dissociation from actin by Mg-ATP at 0.7 M KCl and fractionation between 35 and 68 % ammonium sulfate saturation. The K-EDTA- and the Ca-ATPase activities were determined (11). Gel filtration was performed on Sepharose 4B columns (1.5 x 90 cm) in 0.7 M KCl and 2 mM EDTA. Electron microscope graphs of myosin spread on mica sheets were taken after air-drying and rotary shadowing with platinum and evaluated as described elsewhere (13). All preparations were subjected to electrophoresis in sodium dodecyl sulfate and analysed by quantitative densitometry (14).

Results and discussion

It was found difficult to remove or to recombine both RLC from isolated myosin. Analysis of the proteolytic kinetics revealed that in isolated myosin the RLC are digested at two different rates, both being 10-25

times faster than the rate for proteolytic attack of the HC (Table 1). Similar results were obtained during digestion at high salt concentration where myosin is dissolved. Quantitative evaluation indicated that always 50 % of the RLC were digested faster than the remaining. In actomyosin, however, a homogeneous digestion rate was observed indicating that both RLC are equally susceptible to the proteinase when the myosin heads are bound to actin in rigor. The presence of DTNB accelerates the digestion rate of both RLC without affecting the proteolytic susceptibility of the HC (Table 1). These results suggest that when the crossbridges interact with actin in rigor the two myosin heads are kept in a disposition where both RLC are equally exposed to the proteinase, while in isolated myosin this is not the case.

Table 1. Digestion rates of RLC and HC of isolated myosin and of purified actomyosin by alpha-chymotrypsin (rates are normalized per minute and per 100 µg alpha-chymotrypsin under conditions as indicated in materials and methods).

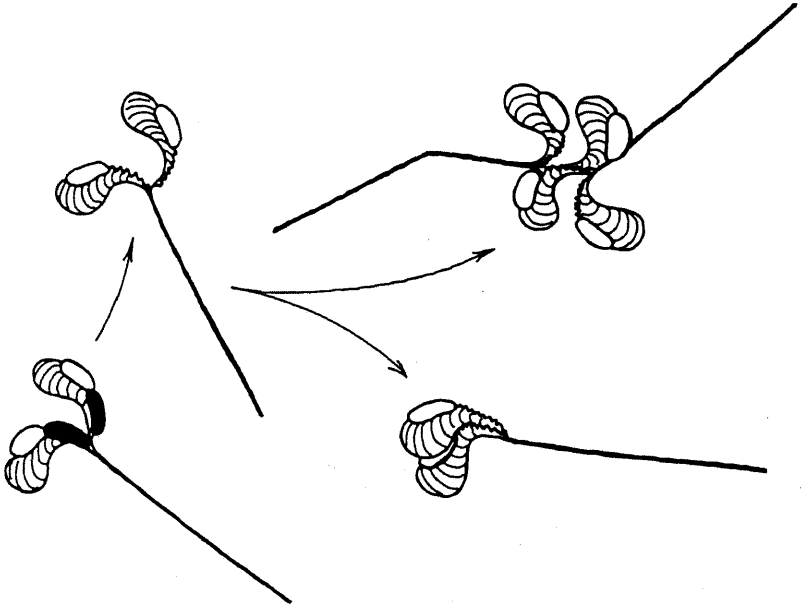
	Myosin	Actomyosin	Actomyosin plus 1 mM DTNB
First RLC	10.5	2.5	18.5
Second RLC	4.2	2.5	18.5
HC	0.4	0.4	0.3

The electron microscopic studies revealed that the removal of both RLC leads to a loss of material between the two heads in the neck region of isolated myosin. The individual heads appear slightly shorter, more circular, often closer together and pear-shapes are no longer observed. Measurements indicated that the width of the heads was not affected by the removal of both RLC but that the length was significantly reduced by 18 % ($p < 0.001$ with $n1 = 124$ and $n2 = 92$). Myosin devoid of RLC was indistinguishable in its dimensions from the controls after stoichiometric recombination with RLC. The localization of RLC in the neck region of mammalian skeletal muscle myosin corresponds to that observed in molluscan striated muscle myosin (15). Furthermore, the maximal distance perpendicular to the rod axis covered by the two projecting heads in each molecule is also significantly reduced by 15 % ($p < 0.001$ with $n1 = 65$ and $n2 = 32$) in myosin devoid of RLC.

Myosin devoid of both RLC has a molecular mass 8 % lower than intact myosin, yet, in gel filtration at high salt concentrations, it elutes slightly, but significantly, earlier like a somewhat larger molecule ($p < 0.001$ with $n1 = n2 = 12$). This elution behaviour was not affected by the presence of either ATP or reducing agents. Thus its hydrodynamic behaviour differs from that of intact myosin. The Stokes' radius was determined for both types of myosin by calibration with standard proteins. From this and the molecular mass the Perrin shape factor can be deduced which allows to estimate the maximal axial ratio (16). For intact myosin this ratio amounts to 53 and for myosin devoid of RLC to 66. The higher the axial ratio the higher is the molecular asymmetry. Since the dimensions of the rod moiety are identical for both types of myosin, the higher asymmetry in the case of the myosin devoid of RLC means that its mass distribution of both heads must be closer together, i.e. the total mass of both heads is more concentrated near the rod axis. Electrophoretic analysis of such myosin indicated that the HC were not covalently bonded together. Both the electron micrographs and gel filtration behaviour of myosin without its RLC suggest that intramolecular hydrophobic interaction takes place between the two head portions. It has been speculated that removal of RLC leads to an exposure of a sticky patch in molluscan striated muscle myosin (17).

In addition, the electron micrographs and gel filtration chromatography indicated that myosin devoid of RLC also tends to form intermolecular aggregates. These aggregates consist primarily of dimers and also result from association in the head region. Rod-rod interactions were never observed. These interactions are also non-covalent and hydrophobic in nature, and cannot readily be dissociated. The ATPase activities of monomeric intact myosin and myosin devoid of RLC were identical. In polymeric aggregates of myosin without RLC the ATPase activities were reduced by 20-30 %. In conclusion, our results could imply that one function of the RLC is to prevent hydrophobic interactions between myosin heads within individual molecules as well as between adjacent molecules in the filament. It could be further speculated that the RLC contribute in fact to repel the two heads from one another for the following reason. The head HC bears almost no net charge as judged from electrofocusing. On the other hand, the isoelectric point of the RLC lies around pH 5 or below. Since the RLC are

predominantly bound by hydrophobic interactions they are expected to introduce a number of net negative charges on both heads in the neck region. Such a repulsion could give rise to negative cooperativity between the two heads. Heads pointing away from one another have been observed in frog and tarantula myosin filaments under resting conditions (18,19). Functional cooperativity between the two heads of isolated myosin induced by binding of nucleotides to the active sites has also been observed (20,21). The precise role the RLC might play in cooperative functions of the myosin crossbridges remains to be elucidated. The putative repelling function of the RLC and the intramolecular and intermolecular aggregations occurring upon their removal are depicted in the scheme below.



Acknowledgements

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