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Minireview

The significance of myosin light chains in mechanochemical coupling in skeletal muscle*

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The molecular mechanism of mechanochemical coupling in muscle and other motile systems remains despite extensive investigations an open question. The proposed hypotheses are controversial and can neither explain all findings nor answer the questions raised [1 - 7]. The fundamental basis for elucidation of molecular mechanism of muscle contraction, the mechanism of ATP hydrolysis by myosin heads complexed with actin, has been well characterized [8 - 13]. It is generally accepted that force production is associated with phosphate release following ATP hydrolysis. However, molecular events related to force generation still remain insufficiently clear. Elucidation of the role of myosin heads, and their light chains, interacting with actin and ATP in the process of utilization of chemical energy for force production is required. The studies on the structure and function of myosin enabled characterization of actin and ATP binding sites on myosin heads. Two conformational states of myosin heads differing in structure and affinity towards actin have been described as intermediates of the ATP hydrolysis steps [10, 14]. The transition between these states may be associated with the force generation [14].

Myosin light chains localization and significance

Myosin heads formed by N-terminal parts of heavy chains contain two types of light chains. The primary structures of light chains [15, 16] and shape of the molecules have been described [17]. One of the two chemically homologous light chains of 20.7 kDa (A1) and 16.5 kDa (A2), called "essential" or "alkali" light chains and one with molecular mass 19.0 kDa, called "regulatory" light chain or (RLC)[1] are present in each head of the fast skeletal muscle myosin molecule [18].

The location of these chains within myosin heads was deduced from mapping experiments using antibodies specific to light chains or their fragments and electron microscopy [19 - 21], crosslinking studies [22], overlaying experiments on polyacrylamide gel [23, 24] and, finally, from measurements of energy transfer [25, 26]. The N-terminal parts of A1 and RLC are positioned in the "narrow neck" region of myosin molecule. It is also known that the N-terminus of RLC is located in the close vicinity of the head/rod junction, and that of A1 50 Å away of it. The C-terminal parts of A1 and RLC are located around the cysteine 707, in a more distal region of myosin heads. The C-terminal of A1 is located 57 Å from a nucleotide binding site and 50 Å from cysteine 707 [25, 26]. The N-terminal part of A1 displays a high degree of segmental mobility similarly as the N-terminus of RLC. This mobility of A1 N-terminal part is strongly reduced in the presence of actin. This points to the possibility of direct binding of A1

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[1]Abbreviations: DTNB, 5,5'-dithio-bis(2-nitrobenzoic)acid; EDTA, ethylenediaminetetraacetic acid; pPDM, *N,N'-p*-phenylenedimaleimide; RLC, "regulatory" light chain of myosin

with actin. In fact, the binding of N-terminal part of A1 with actin under rigor conditions was demonstrated by crosslinking techniques [22], and was characterized by Henry *et al.* [27]. A2, the shorter isoform of alkali light chains lacking 41 amino-acid residues of N-terminus, does not bind to actin [22]. Schematic illustration of myosin head complexed with actin is shown in Figure 1.

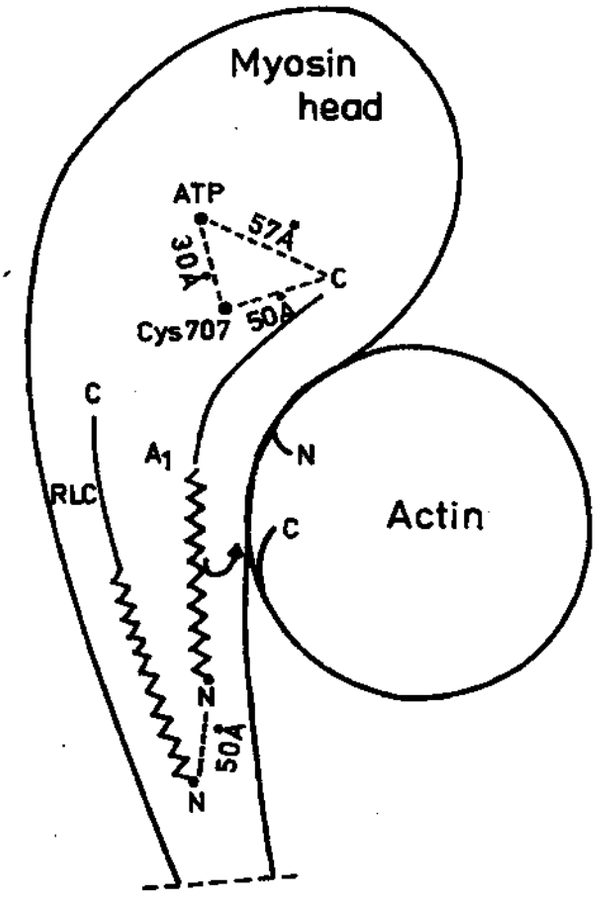

Fig. 1. Schematic organization of the myosin head-actin complex showing the vicinal localization of N-terminal parts of RLC and A1.
The highly mobile parts of light chains are shown as zig-zag lines. The distances between the C-terminus of A1 and highly reactive Cys 707 and the putative nucleotide (ATP) binding site are shown according to [25, 26].

The importance of alkali light chains of myosin for its catalytic properties remains still unclear, although their influence on actin-activated ATPase has been demonstrated [28]. It is very probable that isoforms of alkali light chains differently influence the myosin cross-bridges interaction with actin filaments during the contractile cycle, and affect the force production [27]. Recently, Lowey *et al.* [29] reported that both alkali and regulatory light chains affect the movement of actin filaments in a motility assay, and suggested that myosin light chains – heavy chains interactions are essential for conversion of chemical energy into movement. The high degree of segmental mobility of N-terminal parts of both A1 and RLC and their proximity to each other make possible the influence of RLC on the interaction between alkali light chain and heavy chain in myosin head [19]. Wagner & Stone have observed influence of RLCs on exchange of alkali light chains, and suggested that RLCs probably affect positioning of alkali light chains [30].

In the studies on isolated contractile proteins we have shown that lowering of the divalent cations concentration leads to the dissociation of RLCs from fast skeletal muscle myosin [32]. About 50% of these chains can be removed by treatment with ethylenediaminetetraacetic acid (EDTA). Treatment with 5,5'-dithio-bis(2-nitrobenzoic)acid (DTNB) and EDTA has been used for partial removal of RLCs [33] and in our hands total removal of RLC was possible when the DTNB and EDTA treated myosin was subsequently treated with EDTA [34, 35]. Myosin devoid of these chains differs in some of its properties from intact myosin ([34, 35], for review see [36]). The difference was observed both, in the interaction with actin [33 - 35] and in the ability to form synthetic filaments [34]. Changes in properties of muscle fibers and isolated contractile proteins after removal of RLCs were also observed. Metzger & Moss [31] have reported a reversible reduction of maximal velocity of shortening as a consequence of removal of RLC from skinned muscle fibers.

In muscle cells, increased concentration of free Ca^{2+} may lead to a change in conformation of regulatory light chains by exchange of Mg^{2+} bound to these chains [37] and/or phosphorylation of their Ser 15 due to activation of myosin light chain kinase [38]. There are some observations suggesting the influence of calcium binding to RLC on contractile properties of skeletal muscle and myosin actin interaction [39 - 42]. Although, as shown by Bagshaw & Reed [37] the slow dissociation of divalent cations from myosin RLCs indicates that the exchange of bound Mg^{2+} for Ca^{2+} can not be involved in the activation of contraction, the demonstrated conformational changes of RLCs due to Ca^{2+} binding [43] may influence the

conformation of myosin head and its interaction with actin. Although the significance of high affinity Ca^{2+} binding sites of vertebrate skeletal muscle RLCs for the Ca^{2+} sensitivity of contraction is still controversial [37], the new findings of Metzger & Moss point to the role of RLCs in Ca^{2+} sensitive crossbridges transitions [31]. The authors show that at low concentration of Ca^{2+} the rate of force development increases after partial extraction of RLC. Reconstitution with myosin RLC restores native Ca^{2+} sensitivity in skinned single fibres.

The significance of RLC phosphorylation in skeletal muscle myosin was examined on intact muscle, on skinned fibers and on isolated contractile proteins. Experiments on skinned fibers and intact muscle showed a decrease of the crossbridge cycling rate [44] by phosphorylation of RLC. Manning & Stull [45], Klug *et al.* [46] and Moore & Stull [47] observed a correlation between the potentiation of isometric tension and myosin phosphorylation. There are conflicting reports concerning correlation between the shortening velocity of intact muscle and myosin phosphorylation reviewed by Butler *et al.* [48]. Thus the modulatory role of the myosin light chains kinase/phosphatase system in muscle contraction is still not sufficiently elucidated.

In our further studies on isolated proteins attempts were made to evaluate the significance of both phosphorylation and Mg^{2+} for Ca^{2+} exchange in myosin RLCs for the structure and function of fast skeletal muscle myosin. We have shown the difference in shape of arrowheads formed by decoration of actin filaments with either phosphorylated or dephosphorylated heavy meromyosin [49]. The early reports concerning the influence of RLC phosphorylation on the interaction of isolated contractile proteins in the presence of ATP were not fully convincing. Morgan *et al.* [50] and Perry [51] did not observe influence of RLC phosphorylation on the myosin or heavy meromyosin interaction with actin, whereas Pemrick [52] and our work [35, 53, 54] clearly showed the differences in the actin-stimulated Mg^{2+}-ATPase of myosin before and after phosphorylation of RLC. Pulliam *et al.* [39] have reported a correlation between calcium sensitivity of Mg^{2+}-ATPase activated by pure actin and phosphorylation of myosin RLC. Controversial results concerning the effect of RLC phosphorylation on actin activated Mg^{2+}-ATPase of myosin seem to be the consequence of differences in experimental conditions used in different laboratories. As shown by Stępkowski *et al.* [49] the magnitude of this effect depends mainly on ionic strength.

The increased Ca^{2+} sensitivity of skinned rabbit skeletal muscle fibers correlated with increased phosphorylation of myosin RLC was demonstrated by Persechini *et al.* [55] and that of glycerinated single fibers by Wrotek *et al.* [56]. The latter authors have introduced a method for preparation of glycerinated muscle fibers containing fully phosphorylated RLC [57]. They have shown that fibers containing myosin with phosphorylated RLCs developed tension at 0.6 μM Ca^{2+} concentration. Dephosphorylation of myosin light chains by specific phosphatase resulted in decrease of tension generated at 0.6 μM Ca^{2+} concentration. In parallel to the tension development measurements, they were able to demonstrate the structural changes of phalloidin-rhodamine labelled actin filament at contracted state of the fibers by measuring polarized fluorescence. They observed the difference in the angles between the actin filament long axis and absorption and emission dipoles of fluorophore in contracted as compared to relaxed fibers suggesting changes in actin filament conformations [56].

Exploiting ghost fibers (containing mainly native actin filaments) and the polarized microfluorometry method have been shown to be very convenient tools for studies on the significance of RLC phosphorylation and the exchange of Mg^{2+} for Ca^{2+}. The conformational changes in F-actin, myosin head and tropomyosin were reflected by the changes in polarized fluorescence parameters of intrinsic tryptophan of actin and extrinsic fluorophores attached to different regions of actin [58 - 61], native or modified myosin [62, 63], and tropomyosin [64, 65]. When ghost fibers were decorated with phosphorylated or dephosphorylated heavy meromyosin, different changes of the angles of emission and absorption dipoles of the fluorophore and the angle between the actin filament axis and the fibre axis were observed depending on whether Mg^{2+} or Ca^{2+} were bound with myosin, suggesting a different influence of these cations on the actin filament flexibility. Thus, myosin heads were able to induce various conformational changes in

actin filament dependent on the state of myosin heads ([61] and references therein). The changes were enhanced when ghost fibers were, prior to binding with myosin heads, reconstituted with tropomyosin complexed with troponin [59]. The above described conformational changes of actin filament can be induced only by myosin heads containing intact RLC. In the case of RLC lacking the 2 kDa N-terminal fragments the effect of heavy meromyosin binding on actin filament flexibility was independent of the kind of divalent cation bound to RLCs [61].

Two conformational states of myosin heads can be obtained by modification of myosin with *N,N'-p*-phenylenedimaleimide (pPDM) in the presence of MgADP. Depending whether interthiol crosslinks were formed between the Cys 707 – Cys 697 or Cys 697 – Cys 540, modified myosin simulate conformation of myosin ATP or ADP·P_i, and myosin ADP state, respectively [65 - 69]. When ghost fibers labelled specifically with *N*-(iodoacetylaminoethyl)-5-naphthylamine-1-sulfonic acid, (1,5-IAEDANS) attached to Cys-374 of actin [70] or labelled with phalloidin-rhodamine, bound to the cleft between the two domains of actin monomers [71, 72], were decorated with pPDM modified myosin heads, information has been obtained about the conformational changes of actin filament induced by myosin heads simulating the conformational states of this head formed during ATP hydrolysis. It was observed that myosin heads resembling those complexed with ATP or ADP*P_i decreased the flexibility of actin filament and the heads resembling those after release of P_i (myosin*ADP) increased the actin filament flexibility showing some alterations of actin monomer organization and/or intermonomer structural changes [61]. The observed conformational changes of actin filament may accompany the interaction of myosin heads with actin filament during ATP hydrolysis.

As mentioned above, the increase or decrease of actin filament flexibility was induced by the binding of myosin heads (in rigor), depending on the phosphorylated or dephosphorylated form of RLC and on whether Mg^{2+} or Ca^{2+} were bound. In agreement with these findings the actin binding ability of heavy meromyosin in the absence of ATP depends both on phosphorylation of RLC and on the kind of divalent cations bound [73, 74]. Moreover, both the geometrical arrangement of tropomyosin and actin complexed with phosphorylated and dephosphorylated myosin heads, and the affinity of actin for tropomyosin depends on whether Ca^{2+} or Mg^{2+} were bound to RLCs [64].

The putative role of N-terminal parts of myosin light chains

Using the limited proteolysis method we were able to show the influence of RLC conformation on the alterations of myosin heads induced by actin. The proteolytic cleavage of myosin heavy chain [75] and the cleavage of N-terminal part of A1 (unpublished results) was dependent on Mg^{2+} for Ca^{2+} exchange and/or phosphorylation of RLC. Thus, it may be expected that the changes of Ca^{2+} concentration in excited muscle cell involve changes in structural organization of myosin heads as a consequence of conformational changes of myosin RLCs induced by exchange of bound Mg^{2+} for Ca^{2+} and phosphorylation of RLC by Ca^{2+} calmodulin dependent myosin light chains kinase [38]. As mentioned earlier, the N-terminal parts of both RLC and A1 are located close to each other are highly mobile, and the latter can bind to actin. Wagner & Stone [76] and Wagner [77] have observed that the Ca^{2+} sensitivity of binding of heavy meromyosin to regulated actin was lost when N-terminal part of RLC was cleaved. Borovikov & Kąkol [61] found that such cleavage caused desensitization of the heavy meromyosin-actin rigor complex conformation to the cation exchange in RLC and phosphorylation-dephosphorylation of these light chains. We have observed also, that the changes in RLCs conformation (due to phosphorylation of Ser 15 and Mg^{2+} for Ca^{2+} exchange) influence the internal organization of myosin [75] and positioning of N-terminus of A1 (unpublished results). It is very probable, that the A1 and RLC chains may communicate to each other through the N-terminal parts, the RLC would sense the calcium signal (through exchanging Mg^{2+} with Ca^{2+} and phosphorylation) and A1 conferr it to myosin head actin binding ability. Thus, the significance of the light chains in the conversion of chemical energy into movement postulated by Lowey *et al.* [29] seems to be not only strongly supported by our findings but our results additionally point to the significance of the interaction of the RLC

and A1 light chains N-terminal parts in the mechanochemical coupling in skeletal muscle.

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