Mechanism of Formation of Actomyosin Interface

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Force generation in muscle results from binding of myosin to F-actin. ATP binding to myosin provides energy to dissociate actomyosin complex while the hydrolysis of ATP is needed for re-binding of myosin to F-actin. At the end of each cycle myosin and actin form a tight complex with a substantial interface area. We investigated the dynamics of formation of actomyosin interface in presence and absence of nucleotides by quenched flow cross-linking technique. We showed previously that myosin head (subfragment 1, S1) directly interacts with at least two monomers in the actin filament. The quenched flow cross-linking experiments revealed that the initial contact (in presence or absence of nucleotides) occurs between loop 635–647 of S1 and 1–12 N-terminal residues of one actin and, then, the second contact forms between loop 567–574 of S1 and the N terminus of the second actin. The distance between these two loops in S1 corresponds to the distance between N termini of two actins in the same strand (53 Å) but is smaller than that between two actins from the different strands (102 Å). The formation of the actomyosin complex proceeds in ordered sequence: S1 initially binds to one actin then binds with the second actin located in the same strand but probably closer to the barbed end of F-actin. The presence of nucleotides slows down the interaction of S1 with the second actin, which correlates with recently proposed cleft movement in a 50 kDa domain of S1. The sequential mechanism of formation of actomyosin interface starting from one end and developing towards the barbed end might be involved in force generation and directional movement in actin–myosin system.

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Abbreviations used: S1, myosin subfragment 1; A1 and A2 myosin, alkali light chain 1 and 2, respectively; EDC, N-(3-dimethylaminopropyl)-N-ethylcarbodiimide.

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Quenched flow cross-linking studies

The steady-state cross-linking of myosin subfragment 1 (S1), containing only alkali light chain 2 (A2) to F-actin with zero-length cross-linker (EDC), produced three major complexes with apparent molecular masses of 150, 160 and 210 kDa (Figure 1). The 150 kDa and 160 kDa complexes consist of one S1 cross-linked to the N terminus of one actin via either loop 635–647 or loop 567–574, respectively. Cross-linking of one S1 with two actins via both loops forms a 210 kDa complex. S1 with alkali light chain 1 (A1) was not used in the experiments to avoid complications associated with cross-linking of A1 to actin. The cross-linking under steady-state conditions, when binding of S1 and F-actin reached equilibrium, did not reveal any predominance in formation of 150 kDa or 160 kDa complexes at physiological molar ratios of S1 to actin (<0.5) in presence or absence of nucleotides. The quenched-flow experiments, which were performed at room temperature with high protein concentrations and cross-linking times of more than 300 ms, showed the same results as steady-state cross-linking, which is not surprising since under these conditions equilibrium binding is usually reached within 40 ms. When the cross-linking reaction proceeded longer than the time needed to reach equilibrium binding, the transient acto-S1 complexes could not be detected. To resolve the intermediate states we performed the cross-linking reaction at 10 °C in 100 mM KCl (pH 7.5) and final concentrations of 2 μM and 6 μM of S1 and actin, respectively. The equilibrium of binding of S1 and actin under these conditions is reached within 120–150 ms. The reaction was initiated by rapid mixing of S1 and F-actin (pre-activated with cross-linker) in a stopped-flow apparatus and terminated after 50 ms by a three times excess volume of boiling solution of 10% (w/v) SDS, 100 mM β-mercaptoethanol (BME), 50 mM Tris–HCl (pH 6.8), 20% (v/v) glycerol and 10 mM hydroxylamine. At random or simultaneous binding of S1 via both sites the probabilities of their cross-linking would be equal and, therefore, the equal amounts of 150 kDa and 160 kDa complexes would be formed. The difference in the production of these two complexes would reveal which myosin site binds first. The cross-linked complexes were separated by gel electrophoresis and analyzed by Western blot using antibodies against S1. Coomassie blue staining and Western blot methods showed similar results when tested in steady-state cross-linking experiments (Figure 1). In contrast to the Coomassie blue staining method used in previous quenched-flow studies the Western blot method we used had thousands times higher sensitivity and allowed detection of a very small amount of protein complexes. The results indicated that 150 kDa was the predominant complex (Figure 2), which means that S1 initially binds via loop 635–647 to actin and later via loop 567–574 to the second actin. The relative difference in the amounts of 150 kDa and 160 kDa complex was highest in the
presence of ATP, intermediate in the presence of ADP and lowest in the absence of nucleotides. Recently it has been proposed that binding of S1 with F-actin induces movement of the cleft in the central domain and release of nucleotide from active center.\textsuperscript{18,19} It seems that in the absence of nucleotide the transition from weak to strong bound states is much faster than in the presence of ATP hydrolysis products in the active center of S1, which correlates with kinetic studies of cleft movement in the 50 kDa domain in the presence and absence of nucleotides.\textsuperscript{19}

**Structural analysis**

We analyzed the atomic structures of chicken skeletal muscle S1\textsuperscript{20} and reconstructed F-actin.\textsuperscript{21} In the S1 structure the methylated lysine residues were replaced by lysine and the missing loops 627–646 and 572–574 were constructed using the Swiss-PdbViewer program.\textsuperscript{22} The GROMOS 96 force field was used to evaluate the energy of repaired geometries. EDC is a “zero length” cross-linker and mediates cross-linking between lysine residues of S1 and aspartic or glutamate residues of actin. The distance between two cross-linking sites on S1 is about 53 Å (Lys642<>Lys574), which is comparable with the distance between N termini of two actins (53 Å from Asp1 of actin 1 to Glu4 of actin 2) in the same strand of F-actin. The shortest distance between N termini of two actins located on different strands of an actin filament is 102 Å, and it rules out the possibility of cross-linking of S1 with two actins from different strands.

**Conclusions**

The results indicate that initially S1 binds via loop 635–647 to the N terminus of one actin and then via loop 567–574 to the N terminus of the second actin located in the same strand as the first actin but probably closer to the barbed end of F-actin. The nucleotides slow down the binding of S1 with the second actin, which might be related with the conformational changes in the cleft in the 50 kDa domain. The model by Simmons and Huxley assumes that myosin rolls in the direction of stronger contacts and formation of new a contact is accompanied with breaking of a previous one.\textsuperscript{4} In contrast to that our model assumes that the formation of new contacts occurs without breaking previous contacts so the total number of contacts between myosin and actin increases in one direction. The directional development of interface by a zipper-like mechanism pulls myosin closer to the F-actin and locks it in a tight complex called rigor complex, which is formed at the end of the power stroke. Recent studies of contracting muscle fibers by low-angle X-ray diffraction led to the “roll and lock” model of interaction of the myosin head with F-actin.\textsuperscript{23}
work we provide a possible molecular mechanism of such a movement of myosin head on the actin filament. The formation of the actomyosin interface is accompanied with a release of free energy, and it seems unlikely that all this energy would be wasted during muscle contraction. Indeed, it was demonstrated recently that the force exerted by the muscle cross-bridge depends directly on the strength of the actomyosin bond.24

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References


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