

Molecular Mechanism of Myosin Assembly

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Myosin of muscle (skeletal muscle, smooth muscle or cardiac muscle) has an intensive tendency to assemble with the axial staggers of 14.3 and 43 nm under the physiological conditions of ionic strength 0.05–0.20 M and pH 6–8. For the molecular mechanism of self-assembly by the myosin molecule, we first reported that the myosin molecule has a specific region which is responsible for the self-assembly. Later, Nyitray *et al.* showed that this region is located near the COOH terminus of the rod segment of the myosin molecule. The molecular mechanism of myosin assembly has been studied in more detail in order to elucidate how this region is related to axial stagger of 43 nm.

Key words ; Light meromyosin/ Axial stagger/ Solubility determining region

The myosin molecule of muscle cell is composed of two heavy chains and four light chains. Each heavy chain has an NH₂-terminal globular head with chain mass of ~95 kDa and a COOH-terminal long fibrous portion folded into an α -helix. The two α -helices wrap around each other to construct the α -helical coiled-coil known as the myosin tail or the myosin rod. It is well known that the myosin rod amino acid sequence is highly repetitive and has the characteristics of α -helical coiled-coil [1]. Myosin self-assembly results from interactions between the rods. Whether the myosin molecule prefers an assembled and polymerized form or a soluble form is strongly dependent on both ionic strength and pH [2]. The polymerized form of myosin *in vivo* is known as thick filament. The thick filament, together with the actin-containing thin filament, forms the smallest structural unit for tension generation in muscle [3]. In thick filament, myosin molecules assembled in a parallel arrangement are obliged to cause the axial staggers of

14.3 and 43 nm [4] at the rod segments of adjacent myosin molecules. Myosin heads projecting away from the thick filament surface can act as movable cross-bridges between the thick and thin filaments [5]. The axial spacings between heads on the filament surface are 14.3 and 43 nm, and these spacings result from the axial staggers between the rods.

McLachlan *et al.* reported excellent results on the molecular mechanism of myosin to explain how these axial staggers are caused between two parallel rods by an analysis of the amino acid sequence of the rod portion of a myosin heavy chain and by a computational analysis of electrostatic interactions between the two rods [1]. The important points of their conclusions are that the periodic charge distributions in the amino acid sequence of the rod are in good agreement with these axial staggers and that the electrostatic interactions between the rods play a crucial role in causing the axial staggers. However, what regions of the rod are crucial for the axial stagger of 43

MOLECULAR BIOLOGY AND INFORMATION – Biopolymer Structure –

Scope of research

Our research aims are to elucidate structure-function relationships of biological macromolecules, mainly proteins, by using physicochemical methods such as spectroscopic and X-ray diffraction methods. The following attempts have been mainly made in our laboratory for that purpose. (1) Peptide secondary or supersecondary structures in aqueous or hydrophobic environments are studied to get a principle of protein architecture, employing various spectroscopic methods. (2) X-ray diffraction studies on protein structures in crystal and in solution are carried out by crystallographic and/or small-angle X-ray scattering techniques to elucidate structure-function relationships of proteins. (3) Molecular mechanism for myosin assembly is studied by proteolytic method, electron microscopy, and computer analysis of the amino acid sequence.



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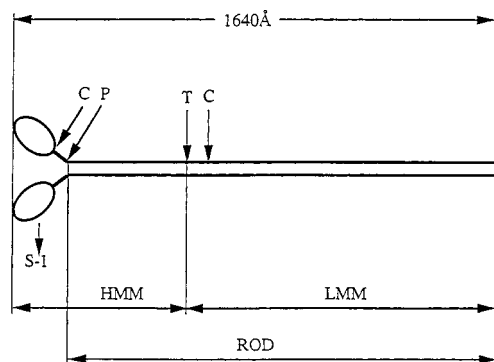


Figure 1. Model picture of myosin molecule. C→, T→, and P→ are chymotryptic, tryptic, and papainic cleavage sites, respectively. HMM is heavy meromyosin which is composed of the head and one-thirds of the rod in the myosin heavy chain, and LMM corresponds to the COOH-terminal two-thirds of the rod. The myosin head is called S-1.

nm has not been clarified yet.

It is well known that myosin assembly occurs within the COOH-terminal two-thirds of the rod (light meromyosin or LMM). The LMM gene of rabbit leg muscle is cloned to give the LMM protein of the sequenced 676 amino acid residues [6]. However, LMMs obtained by proteolytic cleavage of myosin [7] have often been used for the study of LMM assembly, because the proteolytic method permits the isolation of different LMMs in length by selection of enzymes used [8]. LMMs produced by the proteolytic cleavage can also cause the axial staggers of 14.3 and 43 nm when they assemble by themselves, and produce LMM crystals [9]. It should be noted, however, that LMMs obtained by proteolytic cleavage are necessarily shorter than the recombinant LMM of 676 residues because LMM has some cleavage sites in the COOH-terminal portion. Akutagawa *et al.* first reported that the LMM molecule has a region which is responsible for the LMM assembly [8]. Later, Nyitray *et al.* showed that this region is located near the COOH terminus of the myosin rod [10], and called this region the solubility determining region (SDR). Their group and our group independently showed that the lack of this region induces the myosin molecule to cause neither the assembly nor the aggregation. SDR is probably related to molecular mechanism of the axial staggers of 14.3 and 43 nm because the myosin assembly accompanies these axial staggers.

Here we show that SDR is located near the COOH terminus of the LMM molecule by using LMM-T which was prepared by tryptic split of myosin. Fig. 1 shows a model picture of myosin molecule of skeletal muscle and a relationship between the segments in the myosin molecule. Fig. 2 shows the chain mass relationship among the fragments obtained from LMM-T by proteolytic cleavage using trypsin and chymotrypsin. Here LMM-CT is obtained by chymotryptic cleavage of LMM-T. SDR is obtained by enzymatic splits. As shown in Fig. 2, the chain mass of SDR-9k is determined to be 9 kDa corresponding to the difference of chain mass

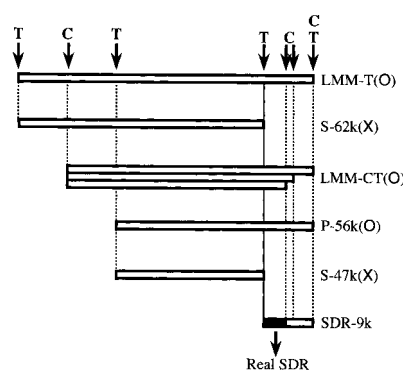


Figure 2. Positional relationships between proteolytic cleavage sites and fragments in LMM-T. The chain masses of fragments LMM-T S-62k, LMM-CT, P-56k and SDR-9k were determined to be 70, 62, 62, 56, 47 and 9 kDa, respectively. The black portion in the figure shows the position of SDR. The symbols ((O) and ((X) show whether fragments can assemble with the axial stagger of 43 nm or not, respectively. The former fragments have SDR, but the latter lack it. Therefore, SDR is located near the COOH terminus of the myosin rod, and the existence of SDR in fragments is crucial for myosin assembly.

between the precipitated fragment P-56k and the soluble fragment S-47k. The real chain mass of SDR shown as the black region in Fig. 2 is smaller than 9 kDa, because LMM-CT contains a small number of LMMs which are able to assemble in spite of difference in COOH terminus. Characterization of the real SDR is essential to know the molecular mechanism for the axial stagger of 43 nm, because the charge distributions in SDR possibly contributes to electrostatic interactions between parallel two rods. The axial stagger of 43 nm can not be caused by the single SDR per one rod when this stagger is caused between two parallel rods by using SDR. Plural SDRs for each rod are required to induce the stagger between the two parallel rods. In order to elucidate whether an axial stagger determining region exists in the rod, we have recently isolated LMM whose length is thought to be shortest to cause the axial stagger of 43 nm, and are characterizing the shortest LMM.

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