Myosin subfragment-1: structure and function of a molecular motor

Ivan Rayment and Hazel M Holden

University of Wisconsin, Madison, USA

The ability for directed movement is a fundamental process of all living systems. Molecules designed for such purposes are ubiquitous in eukaryotic cells and have been the focus of intense investigations for many years. Highlighted in this report is the three-dimensional structure of the myosin motor domain—the first such motor protein to be examined by single-crystal X-ray diffraction analysis.

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Introduction

Directed movement is a hallmark of living organisms and involves the transformation of chemical energy into mechanical energy. At the molecular level, it appears that a very limited number of strategies have evolved to accomplish this task. Presently, three major classes of molecular motors have been identified: myosin, dynein, and kinesin [1]. The most abundant of these three proteins is myosin, which plays both structural and enzymatic roles in muscle contraction and intracellular motility.

Myosin has been found in most eukaryotic cells and is far more abundant than was once believed. It is now thought to be involved in cellular locomotion, phagocytosis, and vesicle transport [2-4]. The role of myosin in movement has however been most clearly defined from the study of cross-striated skeletal muscle because this system shows a high degree of structural organization and is well suited to biochemical, biophysical and physiological measurements. Pioneering studies demonstrated that during muscle contraction, overlapping arrays of thick and thin filaments, which are composed primarily of myosin and actin, respectively, slide past one another [3,4]. Numerous studies have demonstrated that the component responsible for the generation of force is a globular portion of the myosin molecule [5].

Skeletal muscle myosin consists of two heavy chains of molecular weight 220kDa each and two pairs of light chains that have molecular weights in the range 15–22 kDa [6,7]. The molecule is highly asymmetric, consisting of two globular heads attached to a long tail. Each head contains an ATP- and an actin-binding site. The heads can be cleaved from the rest of the molecule by gentle proteolytic digestion to yield soluble fragments that consist of a heavy-chain segment of molecular weight 95 kDa and two light chains with a combined molecular weight of 130 kDa. The two light chains differ in their structures and properties and will be referred to as the ‘regulatory’ and ‘essential’ light chains. Although an enormous wealth of biochemical and physical information has accumulated for myosin during the past 40 years, until recently there has been a lack of detailed structural knowledge of this protein or any other molecular motor. This situation has changed with the recent report of the X-ray structure of chicken skeletal myosin subfragment-1 determined to 2.8 Å resolution [8-9]. As summarized below, this molecular model provides enormous insight into the molecular basis of motility.

X-ray structural determination of myosin subfragment-1

Myosin is an abundant protein in muscle and can be easily isolated in gram quantities. Likewise, the myosin head or subfragment-1 can be prepared in very large quantities. This soluble fragment has been known for more than 30 years and has been the subject of numerous crystallization attempts. Thus, the fundamental problem in determining the structure of the myosin head was obtaining crystals suitable for an X-ray diffraction analysis. This problem was overcome through a novel approach in which most of the lysine residues in the molecule were chemically modified by reductive methylation [9-10]. By this gentle procedure, the subfragment-1 was readily crystallized. Although

Abbreviation
MLCK—myosin light-chain kinase.

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this might appear to be a radical approach, reductive alkylation has long been used as a mild procedure for introducing radioactive labels into proteins [10].

On account of the unusual approach for obtaining the myosin subfragment-1 crystals, considerable effort was expended toward determining the optimal procedure for modifying a protein by reductive methylation and characterizing its chemical and physical properties. Accordingly, a control study was conducted in which the structure of hen egg white lysozyme was reetermined to 1.8 Å resolution after methylation [9**]. That investigation demonstrated that methylation caused essentially no changes in the three-dimensional structure of lysozyme except for the modified lysine residues. In contrast, alkylation of the lysine residues dramatically changed the crystallization properties of the protein. Likewise, a kinetic study of the modified myosin subfragment-1 revealed that the enzyme was still active as an actin-activated ATPase although with altered rate constants [11*]. Taken together, these two investigations suggest that no major changes in the overall conformation of the myosin subfragment-1 occur as a result of reductive methylation. Furthermore, myosin from most sources already contains several posttranslationally modified amino acid residues. For example, in chicken skeletal myosin subfragment-1, Lys35 is monomethylated, Lys130 and -551 are trimethylated, and His757 contains a 3-N-methylated side chain [12]. It has been suggested that methylation of Lys130, which lies close to the ATP-binding site, provides a permanent positive charge that may become buried when nucleotide is bound [13].

Once satisfactory crystals had been obtained, the X-ray structure was determined by conventional multiple isomorphous replacement together with solvent flattening and phase combination. The present model contains 1072 amino acid residues out of a total of 1157, and has been refined to an R-factor of 22.3% for all measured X-ray data between 30 and 2.8 Å resolution [8**].

**Quaternary structure of the myosin head**

The myosin head is highly asymmetric, with a length of 165 Å, a width of 65 Å, and a thickness of ~40 Å. In Fig. 1, the heavy chain is depicted as three fragments that have been defined by proteolytic digestion of the myosin head at flexible loops [14]: an amino-terminal nucleotide-binding region of molecular weight 25 kDa
[15], a central segment of molecular weight 50 kDa, and a carboxy-terminal portion of molecular weight 20 kDa (see Fig. 1) [16,17]. The essential and regulatory light chains wrap around a segment of the heavy chain, but do not overlap each other to any significant extent. It can be seen that a key structural feature of the myosin head is a long α-helix (85 Å) that extends from the thick part of the molecule down to the carboxyl terminus of the heavy chain. This α-helix constitutes the light-chain-binding region of the heavy chain. Brief descriptions of the three polypeptide chains that constitute the myosin head are given below.

The light chains

Both the essential and regulatory light chains share considerable amino acid sequence similarity with troponin-C and calmodulin; however, of the eight possible divalent-cation-binding motifs, only one has been retained and resides in the regulatory light chain within the first helix-loop-helix motif [18]. As shown in Fig. 2, the regulatory light chain is located at the end of the subfragment-1 head and distal from the nucleotide-binding site. As expected, it consists of two domains that each share considerable structural homology with calmodulin and troponin-C. The regulatory light chain is arranged such that its amino-terminal domain wraps around the carboxy-terminal segment of the myosin heavy chain between amino acid residues Asn825–Leu842, whereas its carboxy-terminal domain interacts with the heavy chain in the region defined by amino acid residues Glu808–Val826. Note, however, that the long connecting α-helix observed in both troponin-C and calmodulin is distorted in the regulatory light chain. The Cα positions of the amino-terminal domains of the regulatory light chain and calmodulin superimpose with a root-mean-square deviation of 1.3 Å for 59 equivalent residues. By contrast, the carboxy-terminal domains of these two proteins show considerably larger deviations because of the movement of the F and G helices relative to E and H in the regulatory light chain to accommodate the myosin heavy chain.

The essential light chain wraps around an approximately linear section of the long α-helix of the myosin heavy chain in an arrangement similar to that recently observed for the interaction of calmodulin with a target peptide from myosin light-chain kinase (MLCK) [19,20]. The arrangement of the regulatory and essential light chains, relative to the nucleotide-binding pocket and actin-binding site of the subfragment-1 head, suggests that one of their functions may be to create a longer molecule, thereby amplifying the power stroke.

The heavy chain

The heavy chain constitutes the entire thick portion of the myosin head and contains both the nucleotide-binding site and actin-binding region, which are located on opposite sides of the protein. This part of the molecule consists of a complex arrangement of secondary structural elements centered mainly around a large, mostly parallel, seven-stranded β-sheet. The topology of this β-sheet is such that strands 1 and 6 run in the opposite direction to the other five strands. The organization of the heavy chain is described below relative to the three major tryptic fragments.

The 25 kDa segment

The 25 kDa segment of the heavy chain is located adjacent to the essential light chain in the center of the molecule and forms one side of the nucleotide-binding pocket. The first observed residue in the electron density map at the amino-terminus of the heavy chain is Asp4 and is located close to the essential light chain at the...
the approximate center of the entire myosin molecule (see Fig. 1). From here, the heavy chain crosses the width of the molecule and forms a small six-stranded antiparallel β-sheet motif bounded by residues Lys35 and Met80. This domain is fairly independent from the rest of the catalytic portion of the head and protrudes from the molecule as a whole. The function of this small β-sheet region is unknown. Following this motif, the heavy chain forms three strands of the large β-sheet motif, with these strands being connected by a series of α-helices. The fourth strand precedes the so-called ‘phosphate-binding loop’ or ‘P-loop’, as observed in adenylate kinase and the Ras protein [21*,22]. Following the P-loop is an α-helix, delineated by Lys185–Ile199, which forms the base of the nucleotide-binding pocket. A sulfate ion, as observed in the crystal structure, is shown as a space-filling model in Figs 1 and 3, and is embedded in the phosphate-binding loop. This sulfate is located close to the position of the β-phosphate, as observed in the complex between Ap5A (P1,P3,bis(adenosine-5’)-pentaphosphate) and adenylate kinase [21*]. A break in the electron density occurs between Glu204 and Gly216 at the far end of the active-site pocket. The missing residues are at the junction between the 25 and 50 kDa segments of the heavy chain and most likely form a constitutively flexible loop.

The polypeptide chain resumes at Gly216 as the start of an α-helix. This helix forms part of the nucleotide-binding pocket. Thereafter, the chain loops around close to the phosphate binding site and connects up to β-strands 6 and 7 of the large β-sheet motif. Strand 7 terminates in a domain composed of random coil and several short helices and comprising Glu271–Asp327. This region is located close to the nucleotide-binding site (Fig. 3) and contains Ser324, which had been previously identified by photolabeling studies to be an active-site residue [23]. An α-helix extending from Asp327 to Ile340 forms the top of the nucleotide-binding pocket. Following this domain, the polypeptide chain forms the end of the myosin head through a series of long α-helices and returns to form β-strand 5. This strand terminates in a random coil that drops from the upper domain into the lower domain of the 50 kDa segment. The midpoint between the upper and lower domains occurs in a region of the amino acid sequence (Tyr457–Gly516) that is highly conserved in all myosins [24]. Indeed, the cleft itself contains many individual highly conserved residues that extend into the space between the two domains.

The lower domain is built from several long α-helices, the last of which contains a hydrophobic bulge at Pro529. The segment between Pro529 and Lys553 is believed to be one component of the actin-binding surface [25**]. A single segment of random coil (Lys600–Leu603) passes from the lower domain and across the cleft to form a helix-loop-helix motif lying on the outer face of the upper domain of the 50 kDa segment and terminating at Tyr626. There is no electron density corresponding to amino acid residues Gly627–Phe646. This particular stretch contains the second major site of trypsin proteolysis, referred to as the 50/20 kDa junction. The primary sequence in this disordered region contains nine glycine and five lysine residues, suggesting that it may be a flexible region.

The 50 kDa segment

The 50 kDa segment has a complex topology that can be described as two major domains separated by a long narrow cleft. This cleft divides the distal one third of the myosin head into two regions, which will be referred to as the upper and lower domains of the 50 kDa segment according to their orientation in Fig. 3.

**Fig. 3.** Stereo view of the active site of myosin and its interaction with actin, as deduced from fitting the molecular model for actin [42] with the image reconstruction of the actomyosin complex [43]. In this view, the interaction between myosin and actin is seen approximately from the z-line towards the center of the sarcomere. This reveals the relationship between the nucleotide-binding pocket and the actin–myosin interface. In addition, it shows the close contact between the lower domain of the 50 kDa tryptic fragment and actin which could be relieved by closure of the prominent cleft that separates the upper and lower domains.
in the molecule. This segment has been implicated in actin binding from proteolytic protection, cross-linking, and kinetic studies of proteolytically cleaved protein [16,17,26].

The 20 kDa segment

The 20 kDa segment of the heavy chain is the most extended of the three proteolytic fragments. It starts as a long α-helix close to the thick end of the molecule and proceeds, via an excursion close to the active site pocket, through the light-chain-binding region to the carboxy-terminal end of the heavy chain of the myosin subfragment-1. The 20 kDa segment forms the third strand of the mixed β-sheet. Thus, the major tertiary motif of the head contains segments from all three of the tryptic fragments. After leaving the β-sheet, the polypeptide chain proceeds through a large surface loop that caps one end of the nucleotide-binding site pocket and then forms two α-helices lying under the nucleotide-binding site. This highly conserved segment in the amino acid sequence contains the two reactive sulphydryl groups Cys707 and Cys697, which are referred to as SH1 and SH2. These two thiols can be crosslinked, but only in the presence of nucleotide, by oxidation and by a wide variety of bifunctional chemical reagents differing in length from 14 to 3 Å [27–29]. Indeed, formation of a covalent link between these two groups serves to trap Mg2+-ATP in the active site. The observation that the α-carbons of Cys697 and Cys707 are ~18 Å apart in the absence of nucleotide suggests that a rearrangement or conformational change in this area must occur upon nucleotide binding. This point is further emphasized by the observation that SH1 and SH2 lie in small clefts that face out toward the solvent on opposite sides of the molecule. Following the reactive sulphydryl groups, the heavy chain forms a small three-stranded antiparallel β-sheet domain and then continues as an α-helix which is 85 Å in length and shows distinct curvature beginning at Leu771 and ending at Val826. The heavy chain terminates at Lys843 after a small α-helix that lies nearly at right angles to the preceding long helix.

The active site

The catalytic site of the myosin head was identified by analogy to the phosphate-binding loop observed in both the Ras protein and adenylate kinase and by the position of the amino acid residues previously identified by chemical studies with ATP analogues [30*]. The nucleotide-binding pocket is in an open conformation, as can be seen in Figs 1 and 3. The view in Fig. 3 shows the position of the sulfate ion in the phosphate-binding loop and a few of the amino acid residues that have been chemically labeled, including Trp131, Ser181, Ser243, and Ser324 [13,23,30*]. The width of the nucleotide-binding pocket at its surface is ~15 Å, as measured between α-carbons. As the binding constant of myosin for Mg2+-ATP is ~3 × 1011 M−1 [31] and amino acid residues on both sides of the cleft have been photochemically labeled, it is likely that the pocket closes when nucleotides bind in the active site. Assuming that the binding face to actin remains essentially stationary, it is estimated that closure of the nucleotide-binding cleft could produce a movement at the carboxyl terminus of the myosin head of ~50 Å. How this rearrangement is actually accomplished cannot be easily predicted from the current X-ray model.

Actin-binding site: The structure of the actomyosin complex

The location of the actin-binding site on myosin was determined by combining the X-ray structure described above with a model for the actin filament and image reconstruction of myosin subfragment-1 bound to actin [25**]. The resulting model for the actomyosin complex (Figs 3 and 4) reveals that the protein–protein interface includes components from both the upper and lower domains of the 50 kDa myosin segment and involves the interaction of exposed hydrophobic amino acid residues on both actin and myosin. The model also places exposed complementary ionic and polar residues in close proximity. Examination of the model reveals that the nucleotide-binding pocket lies ~35 Å from the actomyosin interface (Fig. 3).

Fig. 4. The interaction of myosin with actin, shown in a space-filling representation. The tryptic fragments and light chains are color coded as in Fig. 1. This figure was prepared with the graphics program MIDAS [44].
One of the most important features of the model is a stereochromic collision between the lower domain of the 50 kDa tryptic fragment of myosin and the exposed hydrophobic residues at the carboxyl terminus of actin. A better fit to the image reconstructions of subfragment-1-decorated actin [25**] would be obtained if the long narrow cleft between the upper and lower domains of the 50 kDa segment were to close. This cleft extends from under the nucleotide-binding pocket to the actomyosin interface and suggests that conformational changes associated with it, as induced by nucleotide binding, may be the basis for modulating the binding affinity between myosin and actin.

**Model for muscle contraction**

Muscle contraction consists of the cyclic attachment and detachment of the myosin head to the actin filament with the concomitant hydrolysis of ATP. There have been extensive kinetic studies on the interaction of myosin with actin [32–36]. Transient kinetic measurements originally demonstrated that transduction of the chemical energy released by the hydrolysis of ATP into directed mechanical force occurred during product release rather than during the hydrolysis step itself [36]. From these investigations, the following series of events have been shown to occur during the contractile cycle: first, Mg²⁺-ATP rapidly releases myosin from actin by binding to the ATPase site of myosin; second, myosin hydrolyzes ATP to form a relatively stable myosin–products complex; and finally, actin recombines with this complex and dissociates the products, thereby forming the original actin–myosin complex. Force is generated during the release of the hydrolysis products [37].

At present, there is limited structural information concerning the conformational changes that occur when myosin interacts with actin. Nonetheless, significant movement within the myosin head must occur during the ATPase activity because of the large change in distance observed between the two reactive cysteinyl residues upon nucleotide binding [27, 28]. Recent low-angle X-ray scattering investigations also suggest large-scale movements during ATP hydrolysis [38].

All of the current kinetic models for the mechanism of muscle contraction require a change in the binding affinity of myosin for actin when ATP binds to the active site. The X-ray structure suggests that changes in the relationship between the upper and lower domains of the 50 kDa segment of the heavy chain may be prompted by binding of the γ-phosphate. Examination of Figs 3 and 5 reveals that the potential binding site for the γ-phosphate would be located close to the confluence of the upper and lower domains of the 50 kDa region. These observations, together with the extensive kinetic and chemical data available, form the basis of
the hypothesis proposed in Fig. 5 for the contractile cycle.

Starting at the rigor complex (myosin bound to actin the absence of nucleotide; Fig. 5a), it is assumed that the narrow cleft between the upper and lower domains of the 50 kDa segment of the myosin heavy chain is closed. Nucleotide binding is thought to occur via a two-step process. In the first stage, it is hypothesized that only the α-, β-, and γ-phosphates and perhaps part of the ribose moiety of the nucleotide bind to the protein in the P-loop at the base of the active site pocket. This might then cause the narrow cleft between the upper and lower domains of the 50 kDa segment to open, thereby disrupting the strong-binding interaction between myosin and actin (Fig. 5b). In the second stage of ATP binding, closure of the nucleotide-binding pocket might then cause the molecule to undergo a major conformational rearrangement, leading to a net change in the curvature of the molecule. At this stage, hydrolysis of the nucleotide would result in a metastable state with bound product and a conformationally bent molecule (Fig. 5c). Implicit in this hypothesis is the concept that the molecule must structurally rearrange in order to attain a tight complex with the nucleotide and to orient the amino acid residues in the active site such that hydrolysis of ATP can occur. The release of products from the enzyme would then be catalyzed by actin.

Rebinding of myosin to actin is believed to also consist of a multistep process (Fig. 5d and e). In the first stage, binding of the upper and lower domains to actin would result in release of the γ-phosphate by closure of the narrow cleft. This loss of the γ-phosphate would trigger the start of the power stroke and allow the myosin molecule to reverse the conformational changes induced by the binding of the adenine portion of the nucleotide (Fig. 5d). As a result, the active-site pocket would reopen and the myosin head would return to its rigor state (Fig. 5i). After this process, ADP would be released, whereupon ATP could rapidly rebind. In the muscle fiber, the myosin head is tethered to the thick filament through the subfragment-2 region of the molecule, such that the rate of this conformational change would be determined by the actomyosin lattice movements. One of the key implications of this hypothesis is that formation of the tight-binding conformation, which serves to initiate the power stroke, will only occur when the myosin head is in a strespecific orientation with respect to the actin filament.

This is probably necessary for the efficient transduction of force to thick and thin filament arrays.

Conclusions

The structure of myosin subfragment-1 described above represents a turning point in the study of the molecular basis of motility. It is now possible to propose a mechanism for the contractile cycle that is based on the tertiary structure of the proteins involved, whereas before it was only possible to suggest models based on the analogous properties of ratchets and springs. Although the earlier models were both insightful and capable of accounting for many of the mechanical and thermodynamic properties of muscle contraction [39], they were unable to provide a connection with the biochemical studies. The hypothesis presented here provides a framework based on the general three-dimensional properties of proteins and can account for the vast literature that describes the biochemical, physical, and physiological properties of muscle contraction. The structure of myosin subfragment-1 does not answer all of the questions. Rather, it restates the problem at a more fundamental biochemical level. Given this structural framework, it is now possible to examine the molecular basis of motility through a combination of molecular biology, in vitro motility assays, and chemical and kinetic studies.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


This paper relates the recent discovery and classification of an expanded family of myosin molecules.


This structure determination of chicken skeletal muscle myosin subfragment-1 provides enormous insight into the molecular basis for motility.

As described in this control study, reductive methylation of lysozyme does not alter the three-dimensional structure of the protein. This paper also contains the details of the protocol for chemical modification used to obtain crystals of myosin subfragment-1.


This paper demonstrates that reductive alkylation of myosin subfragment-1 changes its enzymatic properties but that the protein still retains many of the key features of the kinetic cycle.


This is the first NMR structure determination of calmodulin bound to its target peptide in MLCK.


A discussion of the refined X-ray structure of calmodulin bound to its target peptide in MLCK.


This structural study investigates the binding of ATP and AMP to adenylate kinase and defines how nucleotides bind to this particular class of enzymes.


This paper combines information from image analysis with the three-dimensional structure of the myosin head and the molecular model of the actin filament to suggest a molecular basis for muscle contraction.


This is a review of the chemical-mapping studies of the active site of myosin. These investigations were critical for establishing the location of the nucleotide-binding pocket and for indicating the necessity of a large conformational change in the myosin head during the contractile cycle.


This report describes the first definitive physical evidence for a conformational change in the myosin head when it binds nucleotide in solution.


I Rayment and HM Holden, Department of Biochemistry and Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705, USA.