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Role of the essential light chain in the activation of smooth muscle myosin by regulatory light chain phosphorylation

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ABSTRACT

The activity of smooth and non-muscle myosin II is regulated by phosphorylation of the regulatory light chain (RLC) at serine 19. The dephosphorylated state of full-length monomeric myosin is characterized by an asymmetric intramolecular head–head interaction that completely inhibits the ATPase activity, accompanied by a hairpin fold of the tail, which prevents filament assembly. Phosphorylation of serine 19 disrupts these head–head interactions by an unknown mechanism. Computational modeling (Tama et al., 2005. J. Mol. Biol. 345, 837–854) suggested that formation of the inhibited state is characterized by both torsional and bending motions about the myosin heavy chain (HC) at a location between the RLC and the essential light chain (ELC). Therefore, altering relative motions between the ELC and the RLC at this locus might disrupt the inhibited state. Based on this hypothesis we have derived an atomic model for the phosphorylated state of the smooth muscle myosin light chain domain (LCD). This model predicts a set of specific interactions between the N-terminal residues of the RLC with both the myosin HC and the ELC. Site directed mutagenesis was used to show that interactions between the phosphorylated N-terminus of the RLC and helix-A of the ELC are required for phosphorylation to activate smooth muscle myosin.

1. Introduction

Phosphorylation of the regulatory light chain (RLC) of smooth muscle and non-muscle myosin affects two major properties of these class II myosins: it enhances the actin-activated ATPase activity (Sellers, 1985), and it affects the solubility of myosin by favoring filament formation (Trybus, 1991). Biochemical studies have shown that the inhibited state requires two myosin heads, because single-headed species are constitutively active (Cremo et al., 1995; Sweeney et al., 2000). Dephosphorylation reduces the actin-activated ATPase activity of smooth muscle heavy meromyosin (smHMM) by more than 25-fold, while only slightly reducing actin binding (Sellers et al., 1982), suggesting that ATPase inhibition primarily affects product release.

Much of the biochemistry can be explained by an asymmetric, intramolecular interaction between the two-myosin heads, first visualized by cryoelectron microscopy (cryoEM) of 2-D arrays of dephosphorylated smHMM (Wendt et al., 2001). Subsequently, this motif was also identified in thick filaments from three striated muscles (Woodhead et al., 2005; Zhao et al., 2009; Zoghbi et al., 2008), as well as in electron micrographs of negatively stained single molecules of myosin II isoforms from several species (Burgess et al., 2007; Jung et al., 2008a). The near ubiquitous presence of this intramolecular myosin head–head interaction has led to the suggestion that it is both an ancient and general mechanism for myosin II inhibition (Jung et al., 2008a,b).

In the dephosphorylated state (Fig. 1A), the upper 50 kDa domain of one myosin head ("blocked" head) is juxtaposed with the upper 50 kDa domain, converter domain and essential light chain (ELC) of the other head ("free" head). In this conformation, the "blocked" head cannot be docked onto actin without a steric clash with the "free" head. Conversely, the "free" head can be docked onto actin without steric interference from the "blocked" head, thereby explaining the retention of actin binding in the inhibited state (Sellers et al., 1982). This head–head interaction provided a convincing mechanism for stabilizing motions within the myosin heads that are required for phosphate release.

The normally extended α-helical coiled-coil rod in dephosphorylated smooth and non-muscle myosin II isoforms bends in the

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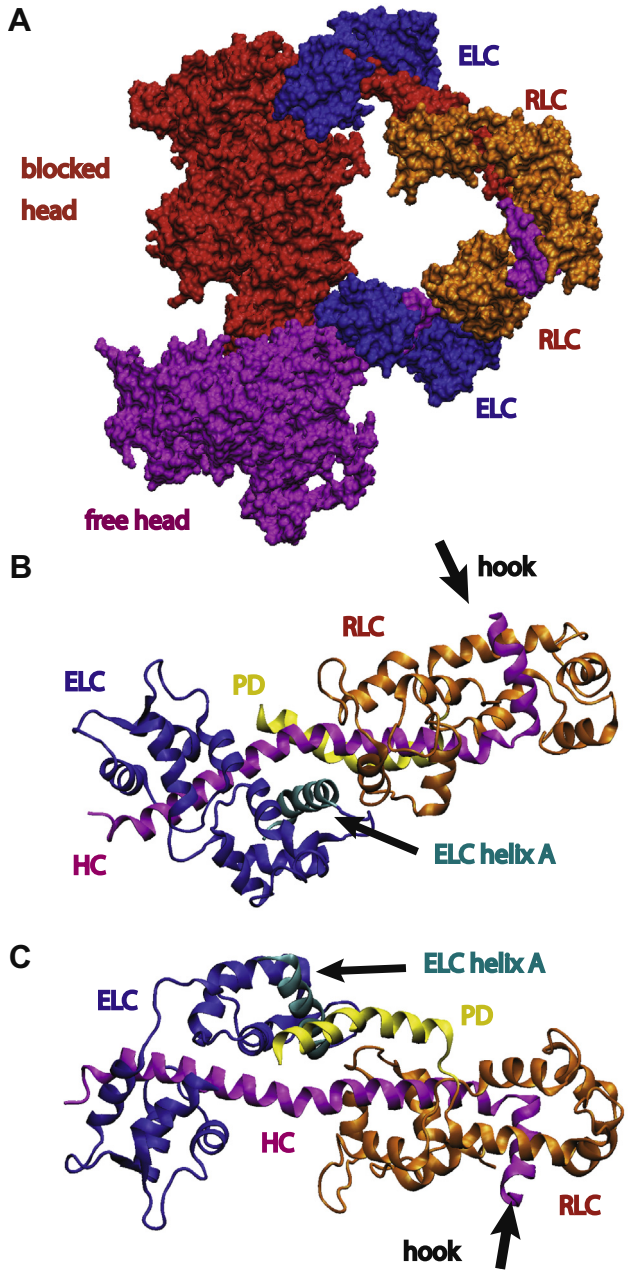

Fig.1. Background on smooth muscle myosin regulation. Color scheme used throughout except where specifically noted has the free head heavy chain (HC) – magenta, the blocked head – red, ELC – blue, RLC – orange with the 24-residue phosphorylation domain (PD) colored yellow. Helix-A of the ELC is colored cyan. (A) In the inhibited conformation the blocked head motor domain (MD) binds the free head MD at a position consisting of parts of the converter domain, MD and ELC. In this position the free head sterically inhibits actin binding by the blocked head, but actin binding by the free head is not similarly affected. The myosin S2 domain would be positioned on the near side of the molecule and lies over the MD of the blocked head. (B) Atomic model of the LCD rendered in a ribbon diagram with the PD position as obtained in the present study. The LCD is oriented similar to that of the free head shown in (A). Within the LCD (on the right), the HC makes a nearly 90° bend to form the "hook" which connects the myosin head to the coiled-coil S2 domain. (C) View of the LCD after rotating about the horizontal axis by ~180°. All graphics produced using VMD (Humphrey et al., 1996). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presence of MgATP and low ionic strength to assume a folded hairpin monomeric conformation which sediments at 10S (Svedberg) (Craig et al., 1983; Trybus et al., 1982), and effectively inhibits product release (Cross et al., 1986). Addition of salt unfolds 10S myosin back to the typical 6S structure. In the 10S conformation, smooth muscle myosin heads are engaged in an intramolecular interaction virtually identical to that found in smHMM (Burgess et al., 2007; Liu et al., 2003). Salt-dependent conformational changes (from 9S to 7S) reflecting changes in head disposition are also observed with smHMM (Nag et al., 1987; Suzuki et al., 1985). Thus, ATPase inhibition and myosin solubility were shown to be dependent on the same head–head interaction, but how they are linked and the consequences of this on the physiology of smooth muscle are less clear.

The first 24 residues that comprise the N-terminus of the RLC have been dubbed the phosphorylation domain (PD), terminology that distinguishes these residues from the portion of the N-terminal domain that is visible in myosin crystal structures (Espinoza-Fonseca et al., 2008). Phosphorylation of the PD at S19 disrupts the head–head interaction of the inhibited state producing a conformation with the heads extended away from each other on opposite sides of the elongated coiled-coil rod (Craig et al., 1983; Trybus et al., 1982), a structure which is competent for filament assembly at physiological ionic strength. Recently, a structural analysis of phosphorylated smHMM by cryoEM showed an open conformation with heads disposed on opposite sides of the rod subfragment-2 (S2), but the interaction between heads from *different* molecules was similar to that observed in dephosphorylated smHMM (Baumann et al., 2012). This suggests that phosphorylation has a minimal effect on the motor domain (MD–MD) interfaces themselves, and mostly affects the ability to form a stable intramolecular interaction.

The PD is located distant from the site of the head–head interaction (Fig. 1B) and its structure and interacting partners in the phosphorylated state have not been determined. Despite a large number of studies probing the effect of phosphorylation on smooth muscle myosin regulation, no structural model has yet emerged that unifies the experimental observations. A recent modeling study that applied normal mode analysis to the conformational change from a putative "active" state to the folded inhibited state, found that head motions required to achieve the intramolecular head–head interaction can propagate distortions throughout the S2 and LMM regions (Tama et al., 2005). The coupled motion between the coiled-coil rod and myosin heads may explain some puzzling features of myosin and motor function, among them the effect of S2 length on regulation (Trybus et al., 1997). The modeling also indicated that a key stress point in the myosin HC occurs at a location between the ELC and the RLC, dubbed the "elbow" (Ni et al., 2012). This is one locus where the X-ray structure of the scallop myosin light chain binding domain (LCD) differed from the chicken skeletal myosin LCD (Houdusse and Cohen, 1996). A more recent comparison of cryoEM structures of both dephosphorylated and phosphorylated smHMM showed that the "blocked head" was more bent at this locus than the dephosphorylated "free head", the phosphorylated heads or even structures of isolated LCDs (Baumann et al., 2012).

These observations suggested that mechanical stress on the HC elbow resulting from the head–head interaction might be relieved by placement of the PD at this location. This report describes the resulting model (Fig. 1B, C) and its possible effects on the inhibited to active conformational change. The model makes specific predictions about interactions between amino acid residues that are needed to stabilize the PD when phosphorylated. The most significant of these interactions was tested by site directed mutagenesis. The results indicate that contrary to prior investigations, the ELC plays an important role in phosphorylation-based activation of smHMM through an interaction between the PD and helix-A of the ELC.