Article

Cryo-EM structure of the inhibited (10S) form of myosin II

https://doi.org/10.1038/s41586-020-3007-0

Received: 2 July 2020

Accepted: 1 October 2020

Published online: 2 December 2020

Check for updates

Shixin Yang[1,3,5], Prince Tiwari[1,5], Kyoung Hwan Lee[1,4], Osamu Sato[2], Mitsuo Ikebe[2], Raúl Padrón[1] & Roger Craig[1]✉

Myosin II is the motor protein that enables muscle cells to contract and nonmuscle cells to move and change shape[1]. The molecule has two identical heads attached to an elongated tail, and can exist in two conformations: 10S and 6S, named for their sedimentation coefficients[2,3]. The 6S conformation has an extended tail and assembles into polymeric filaments, which pull on actin filaments to generate force and motion. In 10S myosin, the tail is folded into three segments and the heads bend back and interact with each other and the tail[3–7], creating a compact conformation in which ATPase activity, actin activation and filament assembly are all highly inhibited[7,8]. This switched-off structure appears to function as a key energy-conserving storage molecule in muscle and nonmuscle cells[9–12], which can be activated to form functional filaments as needed[13]—but the mechanism of its inhibition is not understood. Here we have solved the structure of smooth muscle 10S myosin by cryo-electron microscopy with sufficient resolution to enable improved understanding of the function of the head and tail regions of the molecule and of the key intramolecular contacts that cause inhibition. Our results suggest an atomic model for the off state of myosin II, for its activation and unfolding by phosphorylation, and for understanding the clustering of disease-causing mutations near sites of intramolecular interaction.

Myosin II consists of pairs of heavy chains, essential light chains (ELCs) and regulatory light chains (RLCs), which combine to form the two-headed molecule and α-helical, coiled-coil tail (Fig. 1a). Each head comprises a motor domain and a regulatory domain, containing one RLC and one ELC. In the 10S structure, inhibition occurs through: interaction of the actin-binding region of one head (the blocked head (BH)) with the ATP-binding region of the other (the free head (FH))[6,14], forming an 'interacting-heads motif' (IHM); and head interactions with the three segments of the tail (seg1, seg2 and seg3) and of the tail with itself[4,7]. A similar IHM structure occurs in thick filaments, contributing to the relaxed state of striated muscle[7,15], but without the intramolecular interactions with seg2 and seg3, as the molecules are extended. The 10S structure is in equilibrium with thick filaments in smooth muscle and nonmuscle cells, regulated by phosphorylation of its RLCs. Phosphorylation promotes unfolding to the extended (6S) structure[5,10,16], which forms thick filaments that interact with actin to produce contractility and regulate actin dynamics. Filaments depolymerize to the 10S form when the RLCs are dephosphorylated[13]. Mutations in the heads and tail impair function and cause diseases of muscle and other organs[17]. The 10S conformation has been conserved throughout animal evolution[18], indicating its fundamental importance to cell function. Previous studies of 10S myosin have been limited to 20 Å resolution, leaving many unknowns concerning its structure and function[4,6,7,13]. Our cryo-electron microscopy (cryo-EM) reconstruction provides insights into the structure of the 10S molecule, the molecular basis of inhibition and activation, and the mechanism of disease.

Cryo-EM structure of 10S myosin II

Class averages of cryo-imaged molecules showed multiple views of the 10S conformation, with evidence of secondary structure in the heads and clear density for all three tail segments (Extended Data Fig. 1; Methods). The refined reconstruction (EMD-22145; resolution range approximately 4–9 Å) confirmed this appearance, revealing secondary structure in the motor domains (including side-chain detail), the light chains and the individual α-helices of the tail (Fig. 1b–d, Extended Data Figs. 1b, 2, Extended Data Table 1). To our knowledge, these features, observed here in intact myosin II, have previously been seen only in X-ray structures of the separate components. The two heads interact with each other through their motor domains. Seg1 of the tail (also known as subfragment 2[1] or S2) exits the heads at the junction of the two regulatory domains, crosses the BH, reverses direction at hinge 1 (not seen in the map owing to flexibility), where it becomes seg2. Seg2 passes around the edge of the BH and reverses direction at hinge 2, becoming seg3, which crosses the BH parallel to, but resolved from, seg1 (Fig. 1).

We interpreted the structure by rigidly fitting the motor and regulatory domains of a two-headed myosin fragment (Protein Data Bank (PDB) 1I84[14]) independently into the electron microscopy map, and then refining the fit (Fig. 2a; Methods). We fitted and refined the tail

[1]Division of Cell Biology and Imaging, Department of Radiology, University of Massachusetts Medical School, Worcester, MA, USA. [2]Department of Cellular and Molecular Biology, University of Texas Health Science Center at Tyler, Tyler, TX, USA. [3]Present address: Cryo-EM Shared Resources, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA. [4]Present address: Massachusetts Facility for High-Resolution Electron Cryo-microscopy, University of Massachusetts Medical School, Worcester, MA, USA. [5]These authors contributed equally to this work: Shixin Yang, Prince Tiwari. ✉e-mail: roger.craig@umassmed.edu

RESEARCH ARTICLE

Dilated cardiomyopathy mutation E525K in human beta-cardiac myosin stabilizes the interacting-heads motif and super-relaxed state of myosin

David V Rasicci[1], Prince Tiwari[2], Skylar ML Bodt[1], Rohini Desetty[1], Fredrik R Sadler[3], Sivaraj Sivaramakrishnan[3], Roger Craig[2], Christopher M Yengo[1]*

[1]Department of Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, United States; [2]Department of Radiology, Division of Cell Biology and Imaging, UMass Chan Medical School, Worcester, United States; [3]Department of Genetics, Cell Biology, and Development, University of Minnesota Twin Cities, Minneapolis, United States

Abstract The auto-inhibited, super-relaxed (SRX) state of cardiac myosin is thought to be crucial for regulating contraction, relaxation, and energy conservation in the heart. We used single ATP turnover experiments to demonstrate that a dilated cardiomyopathy (DCM) mutation (E525K) in human beta-cardiac myosin increases the fraction of myosin heads in the SRX state (with slow ATP turnover), especially in physiological ionic strength conditions. We also utilized FRET between a C-terminal GFP tag on the myosin tail and Cy3ATP bound to the active site of the motor domain to estimate the fraction of heads in the closed, interacting-heads motif (IHM); we found a strong correlation between the IHM and SRX state. Negative stain electron microscopy and 2D class averaging of the construct demonstrated that the E525K mutation increased the fraction of molecules adopting the IHM. Overall, our results demonstrate that the E525K DCM mutation may reduce muscle force and power by stabilizing the auto-inhibited SRX state. Our studies also provide direct evidence for a correlation between the SRX biochemical state and the IHM structural state in cardiac muscle myosin. Furthermore, the E525 residue may be implicated in crucial electrostatic interactions that modulate this conserved, auto-inhibited conformation of myosin.

Editor's evaluation

This fundamental study demonstrates that a point mutation resulting in dilated cardiomyopathy in human cardiac myosin increases the fraction of molecules that adopt the auto-inhibited super-relaxed conformation. This provides a mechanism for the lower force output observed in the hearts of affected individuals. The data supporting this, utilizing kinetic methods, a FRET-biosensor to detect conformational changes, and electron microscopy are convincing.

***For correspondence:** cmy11@psu.edu

Competing interest: The authors declare that no competing interests exist.

Funding: See page 21

Preprinted: 19 February 2022
Received: 28 January 2022
Accepted: 08 November 2022
Published: 24 November 2022

Reviewing Editor: James R Sellers, National Heart, Lung and Blood Institute, National Institutes of Health, United States

Introduction

Muscle contraction is driven by the sliding of thick and thin filaments in the muscle sarcomere. In striated muscle, contraction is regulated by both thin and thick filament mechanisms. It is well established that a rise in intracellular calcium concentration changes the conformation of the actin-containing thin filaments (thin filament regulation), allowing myosin heads in the thick filament to bind to actin and power filament sliding (***Kobayashi and Solaro, 2005***). Recently, thick filament regulation has