Archives of Biochemistry and Biophysics 545 (2014) 63–68

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Tropomyosin movement on F-actin during muscle activation explained by energy landscapes

Marek Orzechowski [a,b], Jeffrey R. Moore [a], Stefan Fischer [b,*], William Lehman [a,*]

[a] Department of Physiology and Biophysics, Boston University School of Medicine, 72 East Concord Street, Boston, MA 02118, USA
[b] Computational Biochemistry Group, Interdisciplinary Center for Scientific Computing (IWR), University of Heidelberg, Im Neuenheimer Feld 368, Heidelberg D69120, Germany

ARTICLE INFO

Article history:
Received 27 November 2013
and in revised form 1 January 2014
Available online 8 January 2014

Keywords:
Actin
Coiled-coil
Energy landscape
Myosin
Muscle regulation
Thin filaments

ABSTRACT

Muscle contraction is regulated by tropomyosin movement across the thin filament surface, which exposes or blocks myosin-binding sites on actin. Recent atomic structures of F-actin–tropomyosin have yielded the positions of tropomyosin on myosin-free and myosin-decorated actin. Here, the repositioning of α-tropomyosin between these locations on F-actin was systematically examined by optimizing the energy of the complex for a wide range of tropomyosin positions on F-actin. The resulting energy landscape provides a full-map of the F-actin surface preferred by tropomyosin, revealing a broad energy basin associated with the tropomyosin position that blocks myosin-binding. This is consistent with previously proposed low-energy oscillations of semi-rigid tropomyosin, necessary for shifting of tropomyosin following troponin-binding. In contrast, the landscape shows much less favorable energies when tropomyosin locates near its myosin-induced "open-state" position. This indicates that spontaneous movement of tropomyosin away from its energetic "ground-state" to the open-state is unlikely in absence of myosin. Instead, myosin-binding must drive tropomyosin toward the open-state to activate the thin filament. Additional energy landscapes were computed for disease-causing actin mutants that distort the topology of the actin–tropomyosin energy landscape, explaining their phenotypes. Thus, the computation of such energy landscapes offers a sensitive way to estimate the impact of mutations.

Introduction

Tropomyosin together with the troponin complex is responsible for Ca^{2+}-dependent regulation of muscle contraction [1–3]. Tropomyosin, a 42 nm long coiled-coil, forms head-to-tail connections producing a continuous superhelical strand that wraps around the actin helix of the thin filament (Fig. 1a). Its path is defined by periodic electrostatic interactions between tropomyosin pseudo-repeat domains and each of seven successive actin subunits along the thin filament (Fig. 1b). In turn, these interactions then specify the binding of the troponin complex to the thin filament [1–7].

The azimuthal positions assumed by tropomyosin on the surface of the thin filaments are modulated by Ca^{2+}, troponin and myosin-binding [4–7]. At low Ca^{2+} concentration, troponin constrains tropomyosin in a "blocking-position" over the myosin-binding sites on actin, thereby inhibiting the acto/myosin-crossbridge cycle and consequently relaxing muscles. Rising Ca^{2+} concentration and Ca^{2+}-binding to troponin releases the constraint, and tropomyosin moves off the myosin-binding site to initiate crossbridge–thin filament interaction. However, tropomyosin is only likely to move completely away from the myosin-binding site to a wholly "open-position" on actin following myosin activation [8]. During this process, the movement of semi-rigid tropomyosin propagates to neighboring actin monomers along thin filaments and leads to a cooperative full-activation of the thin filament and contraction [1–8]. Taken together with kinetic characterization of the system, these structural insights led to formulation of a "three-state model" for thin filament regulation and the naming of the so-called low-Ca^{2+} "blocked", high-Ca^{2+} "closed", and myosin-induced "open" states, also known as the B-, C- and M-states [8–12].

Electron microscopy[1] and 3D reconstruction provide the average azimuthal position of tropomyosin near to the myosin-blocking site on troponin- and myosin-free F-actin [6,10,13]. This "A-state" position [7] of tropomyosin on actin–tropomyosin filaments is virtually the same as that of B-state tropomyosin in troponin-regulated filaments except that tropomyosin's positional variance on actin is greater when troponin and myosin are absent [7,13]. Nevertheless, the average A-state tropomyosin position near to the outer edge of actin could be determined without confounding contributions from TnT running alongside tropomyosin. However, the low resolution of the reconstructions precluded determination of the longitudinal

* Corresponding authors. Fax: +1 (617) 638 4273 (W. Lehman).
E-mail addresses: stefan.fischer@iwr.uni-heidelberg.de (S. Fischer), wlehman@bu.edu (W. Lehman).

[1] *Abbreviations used:* EM, electron microscopy; GBSW, Generalized Born with a simple switching; MD, molecular dynamics; myosin S1, myosin subfragment 1.

0003-9861/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2014.01.001

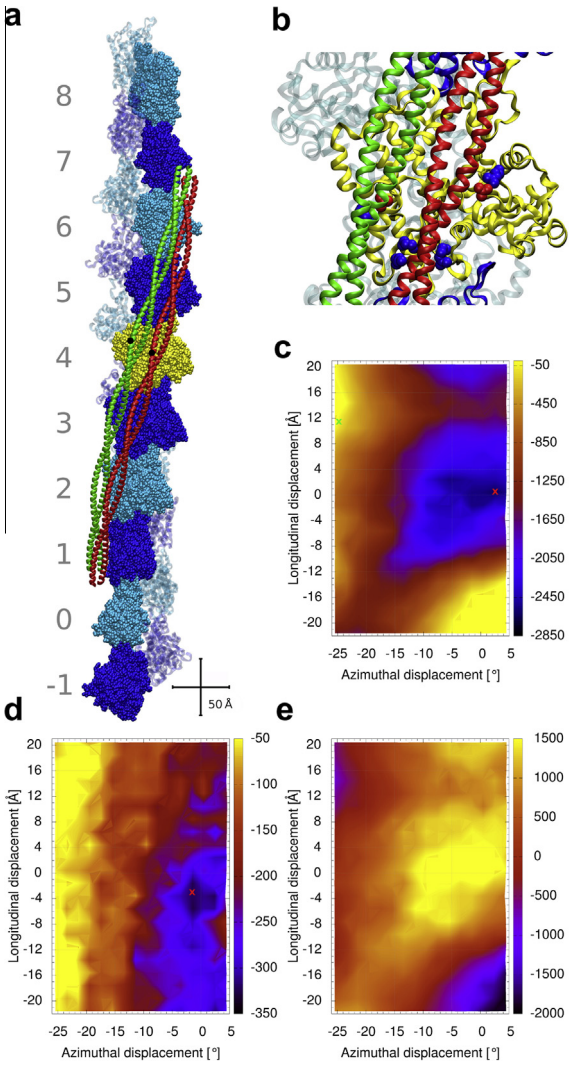

Fig. 1. Tropomyosin positions on the surface of F-actin. (a) Structure of F-actin complexed with tropomyosin in either the troponin and myosin-free A-position (colored red) [6], or in the S1-decorated M-position (green) [12]. Ten actin-pairs (each pair shown in space-filling and ribbon representations) are colored alternatively in dark-blue and cyan. The pairs are numbered from −1 to 8. The actin filament is oriented with its pointed end facing up; hence, the N-terminus of tropomyosin also faces up. Centrally located tropomyosin residue 125 is highlighted in black as a reference point to indicate the relative sliding of tropomyosin between positions. Crisscrossing scale bars – 50 Å. (b) Enlarged view of the central yellow actin subunit in (a), showing some of the acidic and basic actin residues of actin which form attractive interactions with tropomyosin when it is in the myosin-free A-position (red and blue spheres, respectively, which highlight residues D25 and R28 (top right pair), K147, K326, K328 (bottom cluster)). D311 and K315 (left) interact with tropomyosin in the M-position (green) [7,12]. (c–e) Energy landscapes, with the energy levels (in kcal/mol) shown by the color scales on their right. At each point of the landscape, tropomyosin was repositioned longitudinally and azimuthally (see Methods) and the energy of the complex was optimized. The [0, 0] position corresponds to the tropomyosin position described by the Li et al. [6] (red ribbon location in panel (a)). (c) Contributions from the Coulombic interaction energy. The energy minimum is indicated by a red cross. The open M-state position of tropomyosin [12] is indicated by a green cross. (d) Corresponding total potential energy and (e) the contribution from the solvation energy (see Methods).

position of tropomyosin along F-actin and hence a complete atomic model needed for landscape determinations. Li et al. [6] overcame this obstacle by developing an atomic model of the A-state by using a local computational search that optimizes the electrostatic interaction energy between actin and tropomyosin, and then showed that this structural model was consistent with the density profile of EM reconstructions. The high-resolution cryo-EM reconstructions by Behrmann et al. [12] later gave an all-atom structure of the M-state position of tropomyosin [7,8], which described "rigor-bonded" myosin at the end of the cross-bridge cycle inducing tropomyosin movement to the extreme inner edge of the actin surface. Hence, high resolution structural models are available that place tropomyosin on actin at or near to its end-states during regulatory switching.

Low-energy barriers are thought to separate the different structural states of tropomyosin on F-actin, with the underlying energy landscape possibly partitioned so that distinct energy minima would correspond to each regulatory-state position [10,11]. The absence of steric obstructions on actin limiting tropomyosin movement would be consistent with such low-energy transitions [4,7]. However, despite the recent advances in developing atomic models characterizing the actin–tropomyosin complex [6,12], the pathway(s) taken by tropomyosin during the regulatory transition remain unknown, leaving uncertainty about the switching mechanisms needed to operate the three-state model. To address this issue, we have now extended and refined the previous computational search over all regions of actin likely to be explored by tropomyosin between the A- and the M-positions. An "all-atom" model has been used to compute the energy landscape of tropomyosin on the F-actin surface and to determine the positions of energy minima in this landscape. Given that the two atomic structures available locate tropomyosin at or near to what likely are its end point positions on the F-actin surface (Fig. 1a,b) [7], low energy pathways between these minima should then specify the motions of tropomyosin between the different regulatory-states.

The energy landscape was obtained by first generating all combinations of longitudinal displacements (i.e. parallel to the central axis of F-actin) and azimuthal rotations (around the central axis of F-actin) of tropomyosin, and then optimizing the energy of each of these configurations of the tropomyosin/F-actin complex. Contrary to expectation, only a single significant energy minimum is found on the energy landscape. It is located within a broad but shallow energy basin centered on the A-position. Thus, unconstrained by troponin or myosin, tropomyosin presumably can explore the surrounds of the energy minima at relatively low-energy cost. The breadth and flatness of this basin explains how troponin can shift tropomyosin from the low-Ca^{2+} B-state at one edge of the basin towards the other edge of the basin to yield the C-state at high Ca^{2+}. In contrast, the myosin-induced M-state position for tropomyosin is well outside of the energy basin in an unfavorable high-energy region of the landscape. Thus, tropomyosin is only likely to move to the M-position following strong myosin-binding to F-actin. The energy landscape has no deep valleys, which means that distinct minima and connecting pathways for the tropomyosin transitions between A-, B-, C-, and M-positions are not well defined in absence of troponin and myosin. Therefore, both troponin and myosin must change the contours of the actin–tropomyosin energy landscape for muscle regulation to operate properly. Troponin is likely to reshape the landscape in relaxed muscle to trap tropomyosin in the B-state position and bias tropomyosin to the C-state position in Ca^{2+}-activated filaments. In addition, the strong binding of myosin to actin must completely reconfigure the actin–tropomyosin energy landscape to fully activate the thin filament.

Materials and methods

All-atom model

The energy landscapes were computed for a model system consisting of a single tropomyosin molecule bound to one of the helical strands of a 20 subunit-long model of F-actin (Fig. 1a). The Oda et al. [14] structure of F-actin was used. A canonical model was used for