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Curvature Variation along the Tropomyosin Molecule

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Abstract

Complementarity between the tropomyosin supercoil and the helical contour of actin-filaments is required for the binding interaction of actin and tropomyosin (Li et al., 2010). Clusters of small alanine residues in place of canonical leucines along coiled-coil tropomyosin may be responsible for pre-shaping tropomyosin and promoting conformational complementarity to F-actin. A longitudinal displacement between the two chains of the tropomyosin coiled-coil induced by the alanine clusters could produce localized bending or limited flexibility along tropomyosin needed to shape tropomyosin (Brown and Cohen, 2005). To evaluate the influence of alanine clusters on tropomyosin curvature, we calculated the longitudinal displacement between amino acid residues on adjacent chains of the tropomyosin coiled-coil and related this “Z-displacement” to the position of the alanine clusters. Measurements were made on high-resolution crystal structures of tropomyosin fragments and on trajectories from Molecular Dynamics simulations of full-length $\alpha\alpha$ -tropomyosin. Unexpectedly, we found no strict spatial correlation between alanine cluster position and the Z-displacement. Neither did we find any direct correspondence between the clusters and the local curvature of tropomyosin. Rather than causing specific local structural effects, the influence of alanine clusters is more likely to be delocalized, leading to a gradually changing bending pattern along the length of tropomyosin.

Keywords

actin; tropomyosin; coiled-coils; electron microscopy; molecular dynamics

Introduction

Considered a prototypical coiled-coil displaying a simple structural design, elongated tropomyosin in fact has a unique protein sequence and distinctive shape (reviewed in Brown and Cohen, 2005). Curved into a superhelical conformation, tropomyosin is specifically tailored to bind to actin, troponin and other actin-binding proteins (Brown and Cohen, 2005). In order to facilitate actin-binding and to wrap helically around actin-filaments, tropomyosin is apt to be pre-shaped (Lorenz et al., 1995; Holmes and Lehman, 2008). Indeed, electron microscopy and Molecular Dynamics on isolated tropomyosin show that

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the coiled-coil is semi-rigid with an average curvature well-matched to its superhelical shape on F-actin (Li et al., 2010; Fig. 1). This curvature may be generated by the presence of clusters of small alanine residues replacing canonical leucine or other larger amino acid residues at the hydrophobic interface between the two α -helices of the tropomyosin coiled-coil (Brown et al., 2001; Brown and Cohen, 2005), as alanine is not abundant in straight coiled-coiled structures (Brown and Cohen, 2005; Conway & Parry, 1990). Brown and Cohen (Brown et al., 2001; Brown and Cohen, 2005) suggested that these alanine clusters induce a local staggering between α -helical chains of the coiled-coil, causing the adjacent regions of tropomyosin to bend. However, the precise molecular influence of the alanine clusters at the local level and on the global shape of tropomyosin remains uncertain. In fact, close inspection of the distinctive amino acid sequence of tropomyosin indicates additional divergences from canonical coiled-coils (McLachlan and Stewart, 1976) that are not explicitly understood. This added complexity likely involves sequence adaptations that allow tropomyosin to bind to actin and troponin precisely and move cooperatively across actin to regulate thin filaments.

The periodic features of tropomyosin are a numerologist's dream (for example, see Table 1 in McLachlan and Stewart, 1976). Like all coiled-coils, each α -helical chain of tropomyosin displays a seven amino acid long "heptad" periodicity required to build the characteristic "knobs into holes" structure at the interface between the two adjoining α -helices (Crick, 1953). A unique longer-range pseudo-repeating period generated by roughly forty amino acids (Parry, 1975; Stewart and McLachlan, 1975; McLachlan and Stewart, 1976; Stone and Smillie, 1978; Phillips et al., 1986) divides tropomyosin into seven modules, each of which is capable of binding to a successive actin monomer along seven monomer-long intervals (38.5 nm) on the F-actin helix. In their pioneering and comprehensive analysis, McLachlan and Stewart (1976) pointed out that each of these ~40 residue tropomyosin modules can be subdivided into two parts. This was concluded because of sequence considerations but also because the superhelical coiled-coiled twisting results in orthogonally alternating "broad" and "narrow" faces (the latter centered on the cross-over points of the twisting coils), which would divide a pseudo-repeat roughly in half. Thus each pair of broad and narrow faces repeats seven times per tropomyosin, viz., once per actin subunit along the thin filament (Fig. 1). In fact, we now know that actin monomers are also divided into two parts: a bulbous set of domains (subdomains 1 and 3) at the "barbed" end of the molecule and a shallower set of domains (subdomains 2 and 4) at the "pointed" end of the molecule (Holmes et al., 1990) (Fig. 1a). Thus the subdomain structure of F-actin and the respective orthogonally oriented regions of tropomyosin chains are related periodically. Whether these repeat in-phase or out-of-phase with respect to one another is not known but they are shown in-phase in figure 1, with the broad face of tropomyosin associated with actin subdomains 1 and 3 and the narrow face over subdomains 2 and 4. This arrangement is suggested by low-resolution reconstructions of F-actin-tropomyosin (Figs. 1a, b, 2), where tropomyosin appears to be wider over subdomains 1 and 3 and narrower over subdomains 2 and 4.

Mutational analysis demonstrates that alanine clusters are necessary for the pre-shaping of tropomyosin (Li et al., 2010) and the binding of tropomyosin to F-actin (Singh & Hitchcock-DeGregori, 2003; Singh & Hitchcock-DeGregori, 2006). Less certain, however, is whether the seven repeating sets of alanine clusters are localized to discrete flexible links or kinks along tropomyosin specifically needed to generate the tropomyosin supercoil (Brown et al., 2001; Brown and Cohen, 2005) or alternatively promote a more delocalized and continuous effect on curvature along the tropomyosin molecule (Lorenz et al., 1995; Holmes and Lehman, 2008). Our analysis presented here indicates that the actual positions of alanine clusters do not correlate strictly with the staggering of coiled-coil chains or the longitudinal changes in curvature along tropomyosin; hence alanine clusters appear to affect supercoiling in a more indirect delocalized way. In fact, we find a roughly 20 amino acid long periodic

variation in tropomyosin curvature, which lacks a direct connection to alanine clusters (occurring at closer to ~40 residue intervals). This curvature modulation that we have observed may allow tropomyosin to mold itself to better correspond to the actin surface or to actin-tropomyosin binding proteins.

Results and Discussion

Analysis of crystal structures

As mentioned, a longitudinal displacement (Z-displacement) between adjacent amino acid residues on the two parallel α -helical chains of tropomyosin may be augmented by the relative staggering of adjoining small alanine residues along tropomyosin's hydrophobic stripe (Brown et al., 2001; Brown and Cohen, 2005). In turn, this extra displacement might cause a local bend in the coiled-coil. These structural features have not been correlated over the length of tropomyosin as high resolution crystal structures of full-length tropomyosin are not available, but tropomyosin fragments have been solved at high resolution. We therefore assessed the Z-displacement between respective coiled-coiled chains in the crystal structures of several of these tropomyosin fragments (PDB IDs: 1IC2 (Brown et al., 2001), 2B9C (Brown et al, 2005), 2D3E (Nitanai et al, 2007); these structures were analyzed since they were the components used to build the starting model for the MD simulation of full length tropomyosin (see below)). No obvious pattern of periodic Z-displacement appears to occur along the length of these tropomyosin fragments (Fig. 3). Despite the expected narrowing of coiled-coiled radius associated with the location of alanine clusters (Fig. 3) (due to the small size of alanine compared to canonical leucines, isoleucine and valine residues), the correlation between Z-displacement and coiled-coil radius is weak (Table 1).

Conspicuously, there is no discernible correspondence between the magnitude of Z-displacement and the local curvature of the coiled-coils (measured over 11 residue stretches) (Fig. 3). The change in Z-displacement per unit distance (i.e. the derivative of the Z-displacement, $\partial/\partial Z$) could reflect variation in local bending behavior, but there also is no noticeable correlation between the position of alanine clusters and this parameter, although plots of the latter are very noisy (Fig. 4, Table 1). However, we do find a recurrent oscillation in the curvature of these tropomyosin fragments that is roughly 20 amino acids long. Again, this pattern of curvature change is not related spatially to the positions of alanine clusters or the Z-displacement between chains. The parameters mentioned above were compared qualitatively by direct inspection of the graphs (Table 1); however, it should be noted that, because of the complexity of the data and its inherent noise, the values of these parameters relate extremely weakly to each other in plots to determine correlation coefficients.

Analysis of Molecular Dynamics structures

Protein modifications or GCN4 adducts used in order to stabilize tropomyosin constructs during crystallography (Brown et al., 2001; Brown et al., 2005; Nitanaï et al., 2007) may alter the influence of alanine clusters on the Z-displacement between tropomyosin chains or otherwise affect tropomyosin curvature. In addition, crystal packing forces may have an impact on alanine cluster effects, as evident from the different Z-displacement and curvature profiles for two (AB and CD) solutions of the 1IC2 structure (the 1IC2 unit cell contains two molecules, denoted AB and CD (Brown et al., 2001)) (Figs. 3, 4). Moreover, the effects of normal coordinate errors in the crystal structure maps cannot be underestimated. Thus it is difficult to distinguish between wobble and curvature, let alone estimate the accuracy of local Z-displacement and coiled-coil radius measurements. Analysis of MD structures is not confounded by these limitations. We therefore examined the relationship between Z-displacement, alanine clusters and curvature for our recently elucidated MD structure of full-length tropomyosin (Li et al., 2010). Hence we thereby assessed the above trends, but using

a better defined model system to correlate the same variables. Again, there may be a weak and inconsistent correlation between the position of the alanine clusters and the Z-displacement, but there is no apparent spatial correlation of these amino acid clusters to tropomyosin curvature in the average MD structure (Fig. 5A, Table 1), nor any such correlation detected when isolated time points during MD simulation were evaluated separately (not shown) or a correlation when the $\partial/\partial Z$ derivative of Z-displacement was compared to alanine cluster positioning (Fig. 5B, Table 1). As is the case for the crystal structures, approximately twenty amino acid long increases and decreases in tropomyosin curvature are again observed in the average MD structure, but the pattern is more complex than that for the static crystal structures.

Conclusions

Our current and previous results (Li et al., 2010) indicate that the bending of tropomyosin is not spatially restricted to the sites of alanine clusters along the molecule. However, mutational analysis has produced strong experimental support for the involvement of alanine clusters in the generation of curvature and possibly even the bending behavior of tropomyosin, since substitution of canonical hydrophobic residues for alanine leads to straightening of tropomyosin (Li et al., 2010) and a failure of tropomyosin to bind to F-actin (Singh and Hitchcock-DeGregori, 2003; Singh and Hitchcock-DeGregori, 2006). These seemingly discordant observations can best be rationalized if alanine clusters actually cause a generalized overall effect on curvature, as concluded previously (Li et al., 2010), instead of locally restricted bending (Brown et al., 2001; Brown and Cohen, 2005; Singh and Hitchcock-DeGregori, 2003, 2006). Alanine clusters thus appear to induce and/or amplify a spreading curvature signal, acting like broadcast relay stations would.

The notion that delocalized collective forces combine to define the curvature of tropomyosin and are uninterrupted along tropomyosin is supported by EM of isolated tropomyosin molecules that display smooth and continuous curvature profiles (Li et al., 2010). In addition, mutational studies show that point mutations at one site along tropomyosin result in delocalized changes in curvature (and flexibility) at a significant distance (Li et al., 2010; Nirody et al., 2010). How alanine clusters and other non-canonical residues along the length of tropomyosin promote and propagate the structural information responsible for the gradual tropomyosin curvature remains uncertain, but consider, for example, a model of tropomyosin represented by two ropes wrapped around each other in the form of a coiled-coil. Pulling on one of the ropes but not the other (as if by alanine clusters), will cause a local displacement between the two, which will spread distally. Depending on how tightly the two ropes are wrapped together and their lateral connectivity (e.g. elastic or viscoelastic), a longitudinal displacement on one versus the other will cause the cord to curve and/or bend in a delocalized manner at a distance. As the displacement dissipates, a secondary distal pull will relay the effect further. We anticipate the same principles that define $\alpha\alpha$ -tropomyosin shape (*cf.* Holmes and Lehman, 2008, Li et al., 2010) will apply to other tropomyosin isoforms, but expect that the semi-rigidity of different isoforms may vary depending on individual requirements for positional specificity on actin and interactions with other accessory actin-binding proteins (Lehman et al., 2000).

As mentioned, our analysis reveals approximately 20 amino acid long changes in the amplitude of tropomyosin curvature. These changes may be patterned on the half turns of the tropomyosin coiled-coil seen in figure 1. As also mentioned, the length of these intervals may also correspond to the changing configuration of actin subdomains that is evident along thin filaments. Future studies will address whether or not tropomyosin bound to F-actin is more curved over the articulated parts of subdomains 1 and 3 of actin and less curved over the shallow subdomains 2 and 4. Such studies should also determine whether or not the variation in the curvature of tropomyosin involves in-plane or out-of-plane bending relative

to the contours of F-actin, which, at present, cannot be predicted from studies on isolated tropomyosin.

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Figure 1.

Fitting atomic structures of F-actin and tropomyosin to three-dimensional reconstructions of negatively stained F-actin-tropomyosin. (a) surface view of F-actin-tropomyosin reconstruction ($\alpha\alpha$ -cardiac tropomyosin, no troponin; data from Lehman et al. (2009) with permission), actin subdomains numbered on one actin monomer, tropomyosin strand indicated by arrow. Note that tropomyosin is wider over actin subdomains 1 and 3 and narrower over subdomains 2 and 4. (b) model of F-actin (Oda et al., 2009) (actin monomers colored yellow, red, orange) and the average MD model of tropomyosin (Li et al., 2010) (tropomyosin colored magenta) fitted into the volume of the reconstructions (translucent) using alignment tools provided in the program Chimera (Pettersen et al., 2004). (c) The α -carbon chains of the fitted F-actin-tropomyosin model in (b), actin subdomains again numbered on one actin monomer and tropomyosin coiled-coil marked by arrow. Note that the broad face of tropomyosin is over subdomains 1 and 3 and the narrow face of tropomyosin over subdomains 2 and 4.

Figure 2.

Tropomyosin orientation in sections of the reconstruction of F-actin-tropomyosin. Sections made at 7 Å intervals through a distance of ~56 Å (i.e. through a distance of two actin monomers on each side of the filament shown in Figure 1); atomic model tropomyosin fitted on one side of the filament (method as in figure 1b). Note that the broad face of tropomyosin is closest to actin subdomains 1 and 3 (for example, in the sections at 21 Å and 28 Å) and narrowest over subdomains 2 and 4 (for example, in the sections at 0 Å, 42 Å, 49 Å and 56 Å); actin subdomains labeled.

Figure 3.

Comparison of Z-displacement, radius and curvature of the tropomyosin coiled-coil at each residue along the length of crystal structures of $\alpha\alpha$ -striated muscle tropomyosin. (a, b) over N-terminal residues (PDB IDs: 11C2, (a, b) AB and CD structures), (c) over mid-region residues (PDB ID: 2B9C), (d) over C-terminal residues (PDB ID: 2D3E). Residues numbers and respective amino acids at d position of the heptad repeats noted on the x-axis (the d position is the most frequent location of alanine residues), residues in alanine clusters highlighted in magenta above each graph; values for Z-displacement (black), curvature (red) and radius (green) plotted together for comparison. Radius and curvature were determined as in Li et al. (2010). Z-displacement between coiled-coiled chains (at the level of each amino

acid along tropomyosin) was calculated by first aligning the tropomyosin coiled-coil center vertically to the z-axis and then calculating the difference in position between respective residues on the A and B chain. A window size of 10 amino acids on either side of the residues being measured was used for alignment of tropomyosin to the z-axis.

Figure 4.

Comparison of the change in coiled-coiled Z-displacement at each residue along the length of crystal structures of α -tropomyosin. (a, b) over N-terminal residues (PDB IDs: 1IC2, AB and CD structures), (c) over mid-region residues (PDB ID: 2B9C), (d) over C-terminal residues (PDB ID: 2D3E) plotted as in figure 2, except that tropomyosin curvature (red) is compared to the derivative of Z-displacement (black). The derivative of Z-displacement for each residue was calculated by dividing the absolute value of the differences of Z-displacement between neighboring residues (i.e. residues $i-1$, i) by the arc length between the two residues.

Figure 5. Coiled-coil Z-displacement and curvature change along the average MD structure of full-length $\alpha\alpha$ -tropomyosin (Li et al., 2010). (A) Comparison of coiled-coil Z-displacement and curvature. (B) Comparison of the change in coiled-coil Z-displacement and curvature. Color coding as in Figure 3 and 4; same analysis used as above.

Table 1

Scoring the correspondence along tropomyosin between different parameters studied.

	narrowing of coiled-coil radius vs. alanine cluster position	patches of increased Z- displacement vs. alanine cluster position	patches of increased Δ Z- displacement vs. alanine cluster position	patches of increased Δ Z- displacement vs. patches of increased curvature
Crystal Structures	6/6	4/6	2/6	7/8
MD Structure	6/6	3–4/6	2/6	11/14

Simple inspection of plots in figures 3, 4, and 5 was used to relate the coincidence of sets of two parameters along the length of tropomyosin.