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The cMyBP-C HCM variant L348P enhances thin filament activation through an increased shift in tropomyosin position

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Abstract

Mutations in cardiac myosin binding protein C (cMyBP-C), a thick filament protein that modulates contraction of the heart, are a leading cause of hypertrophic cardiomyopathy (HCM). Electron microscopy and 3D reconstruction of thin filaments decorated with cMyBP-C N-terminal fragments suggest that one mechanism of this modulation involves the interaction of cMyBP-C's N-terminal domains with thin filaments to enhance their Ca^{2+} -sensitivity by displacement of tropomyosin from its blocked (low Ca^{2+}) to its closed (high Ca^{2+}) position. The extent of this tropomyosin shift is reduced when cMyBP-C N-terminal domains are phosphorylated. In the current study, we have examined L348P, a sequence variant of cMyBP-C first identified in a screen of patients with HCM. In L348P, leucine 348 is replaced by proline in cMyBP-C's regulatory M-domain, resulting in an increase in cMyBP-C's ability to enhance thin filament Ca^{2+} -sensitization. Our goal here was to determine the structural basis for this enhancement by carrying out 3D reconstruction of thin filaments decorated with L348P-mutant cMyBP-C. When thin filaments were decorated with wild type N-terminal domains at low Ca^{2+} , tropomyosin moved from the blocked to the closed position, as found previously. In contrast, the L348P mutant caused a significantly larger tropomyosin shift, to approximately the open position, consistent with its enhancement of Ca^{2+} -sensitization. Phosphorylated wild type fragments showed a smaller shift than unphosphorylated fragments, whereas the shift induced by the L348P mutant was not affected by phosphorylation. We conclude that the L348P mutation causes a gain of function by enhancing tropomyosin displacement on the thin filament in a phosphorylation-independent way.

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Keywords

cardiac muscle; hypertrophic cardiomyopathy; myosin binding protein C; cMyBP-C; thin filament; electron microscopy

1. Introduction

Myosin-binding protein C (MyBP-C) is an accessory protein of striated muscle thick filaments. Its cardiac isoform, cMyBP-C, plays a key role in modulating contraction in the normal beating heart¹. cMyBP-C comprises eleven immunoglobulin-like and fibronectin type III-like domains, numbered C0–C10 from the N-terminus, and a regulatory M-domain between C1 and C2^{1; 2} (Fig. 1). The M-domain is an important motif for binding to both actin and myosin^{1; 3; 4; 5; 6; 7; 8}, and as such is thought to play a key role in cMyBP-C function. It contains a highly conserved sequence in its C-terminal region, forming a compact triple α -helix bundle, while its N-terminal sequence is largely disordered^{9; 10} and contains several phosphorylation sites that modulate cMyBP-C function¹¹. Both regions of the M-domain play crucial roles in the binding of cMyBP-C to actin^{12; 13}.

One mechanism by which cMyBP-C is thought to modulate cardiac muscle contraction is through interaction of its N-terminal domains with neighboring thin filaments, which increases thin filament Ca^{2+} -sensitivity *in vitro*. The binding of N-terminal cMyBP-C domains to thin filaments lowers the Ca^{2+} concentration needed to activate myosin ATPase activity¹⁴, lowers the threshold for Ca^{2+} activation of thin filament movement in the motility assay^{15; 16} and reduces the level of Ca^{2+} needed to produce force in permeabilized cardiac myocytes¹⁷. A possible mechanism by which cMyBP-C enhances thin filament sensitivity was suggested by electron microscopy (EM) studies¹⁶. It is known from 3D reconstruction of thin filaments that Ca^{2+} binding to troponin causes thin filament activation by inducing movement of tropomyosin (Tm) from its low Ca^{2+} (blocked) position, where myosin heads are inhibited from binding to actin (by physical blocking of their binding sites on actin by Tm), to its high Ca^{2+} (closed) position, where initial head binding becomes possible (due to partial uncovering of these sites); myosin-induced movement of Tm to a third “open” position then fully opens the myosin binding site^{18; 19}; these three positions correlate with three states of activation of the thin filament that were first proposed on the basis of equilibrium and kinetic biochemical studies^{18; 20}. 3D reconstruction of actin filaments decorated with the cMyBP-C N-terminal fragment C0C2 (Fig. 1) shows that the fragment binds to actin subdomain 1 at a position that overlaps the blocked position of Tm^{21; 22}. This suggests that cMyBP-C might compete with Tm for binding to this site. 3D reconstruction of native thin filaments decorated with C0C2 have supported this possibility, showing that under low Ca^{2+} conditions, the binding of C0C2 causes movement of Tm from its blocked position, where it normally inhibits myosin binding to actin, towards its high Ca^{2+} (closed) position, where myosin binding is allowed¹⁶. This displacement would be expected to activate the filament, consistent with the Ca^{2+} -sensitization observed in functional assays^{14; 15; 16}. Similar results have also been obtained with a longer fragment, C0C3, including all the N-terminal domains of cMyBP-C²³.

While frame-shift and splice-site mutations are the most common type of alteration in cMyBP-C associated with HCM in patients ²⁴, there is also a growing number of HCM-associated single amino acid missense mutations ^{25; 26}. However, because large family pedigrees are not available for many of the variants identified, it is unclear whether such single amino acid substitutions are simply benign polymorphisms or whether they in fact disrupt protein function and/or processing, leading to disease as a “poison polypeptide” ²⁷. In the absence of a large linkage study that unequivocally demonstrates the clinical significance of a given variant, functional studies are necessary to establish whether a variant disrupts protein function in some way.

Given the regulatory significance of the cMyBP-C M-domain and its role in binding to the thin filament, we previously investigated whether single amino acid missense variants found in HCM patients affected the ability of cMyBP-C N-terminal domains to bind to actin ¹³. One variant, a leucine to proline substitution at codon 352 (mouse sequence: L348P), occurs in the third helix of the tri-helix bundle in the M-domain, downstream from cMyBP-C's phosphorylation sites. This variant increases the affinity of the M-domain for actin and also the Ca²⁺-sensitizing effects of cMyBP-C's N-terminal domains observed when they are added to permeabilized rat trabeculae, where they cause force generation even in the absence of activating Ca²⁺ (i.e. at pCa 9.0) ¹³. Myocytes from transgenic mice expressing the L348P mutation also showed Ca²⁺-sensitization as demonstrated by a leftward shift in the pCa-tension relationship ²⁸.

The structural mechanism by which the L348P mutation increases thin filament Ca²⁺-sensitivity beyond that caused by wild type cMyBP-C is unknown. Here we used EM and 3D reconstruction of thin filaments decorated with N-terminal cMyBP-C fragments (Fig. 1) to test the hypothesis that the L348P mutant causes a greater displacement of Tm than the wild type.

2. Material and Methods

Proteins

F-actin purified from chicken pectoralis muscle ²⁹ was the gift of Ms. Samantha Beck-Previs. Skeletal actin was used for consistency with earlier studies ^{15; 16; 21; 30}; it differs from cardiac actin by only four conservative amino acid substitutions ³¹. Tropomyosin and troponin purified from bovine cardiac tissue ³² were the gift of Dr. Larry Tobacman. cMyBP-C fragments were bacterially expressed from murine cDNA using pQE-2 expression vector (Qiagen) ¹⁵. Site-directed mutagenesis of wild-type C1C2 (domains C1-M-C2) to L348P-C1C2 was performed using the QuikChange II Site-directed Mutagenesis kit (Stratagene) with primers designed using the Stratagene primer design software ¹³. Mouse fragments were used in preference to human to enable correlation of results with physiological and other experiments using wild type and L348P transgenic mouse tissue ^{13; 28}.

Phosphorylation

Expressed proteins were treated with recombinant protein kinase A (PKA, Sigma) as previously described ⁵. Following PKA treatment, proteins were purified using an affinity

column to remove any residual PKA. Phosphorylation was confirmed by Pro-Q Diamond staining according to the manufacturer's directions (Molecular Probes Inc.).

Electron microscopy

F-actin and thin filaments were decorated with the N-terminal cMyBP-C fragment C1C2 to determine the position of fragment binding on actin and its impact on Tm position. C1C2 was used (rather than full length cMyBP-C) because it has been shown consistently to activate the thin filament. Using this short fragment makes possible clean decoration and correspondingly clear reconstructions in which Tm is plainly visualized. By contrast, decoration with full length cMyBP-C results in bundling of filaments and noisy images due to untethered C-terminal domains, which obscure actin and Tm detail. The binding of recombinant cMyBP-C N-terminal fragments to actin and thin filaments was modified from previously described procedures^{8; 21}. 2 μ M F-actin and 6 μ M expressed C1C2 were mixed together in 100 mM KAcetate, 2 mM MgCl₂, 0.2 mM EGTA, 10 mM MOPS, pH 7.0. For thin filament studies, 2 μ M F-actin was pre-incubated with tropomyosin and troponin (7:2:2 molar ratio)³³ and 6 μ M expressed fragment (1:3 ratio)¹⁶ was then added in the same buffer. Samples were incubated at room temperature for 30 min and 5 μ l aliquots were applied to EM grids coated with thin carbon supported by a holey carbon film. Grids were negatively stained with 1% uranyl acetate³⁴ and observed in a Philips CM120 electron microscope at 80 KV under low dose conditions. Images were acquired at a pixel size of 0.35 nm using a 2K x 2K CCD camera (F224HD, TVIPS GmbH, Gauting, Germany).

Measurement of filament diameter

Filaments were straightened using Image J, and selected regions projected along the filament axis in SPIDER (v11.2, Wadsworth Center, Albany, NY) to generate a one-dimensional density distribution. The two minima in the density distribution correspond to the boundary of the filament, and the distance between them was taken as the diameter¹⁶.

3D reconstruction

Iterative helical real space reconstruction (IHRSR)³⁵ was carried out in SPIDER³⁶ (v11.2, Wadsworth Center, Albany, NY) using F-actin as the initial reference model¹⁶. UCSF Chimera was used for visualization, analysis, and atomic fitting of 3D volumes³⁷.

Comparison and Atomic Fitting of Reconstructions

Matching of reconstructions to each other was carried out using an F-actin reconstruction as a reference to which all other reconstructions were fitted. This matching was done using the highest-density region of actin in the different filament structures as a guide to the superposition. After adjusting the two reconstructions to a high-contour cutoff (showing only the highest density region of actin), an approximate fit was carried out manually; this was then optimized using the "fit in map" tool of Chimera. The contour level was then lowered to reveal the full volume of actin¹⁶ and associated Tm. The reconstructions were fitted with atomic models of F-actin and/or F-actin-Tm determined by X-ray diffraction and cryo-EM^{38; 39}, with Tm in the open, closed, or blocked positions, as described previously¹⁶.

3. Results

3.1 EM and 3D reconstruction of F-actin decorated with C1C2

To investigate whether the L348P mutation in the M-domain affected actin binding, images of negatively stained control F-actin filaments (Fig. 2A) and filaments decorated with the N-terminal fragment C1C2 and L348P-C1C2 were compared (Figs. 2B,C). The C1C2 fragment (lacking the N-terminal C0 domain) was used for consistency with previous studies¹³. Filaments decorated with both wild type or mutant C1C2 (Fig. 2B and C) showed a wider diameter than control F-actin alone (Fig. 2A; 8.9 ± 0.5 nm; Suppl. Table 1)²¹; the width was greater for the L348P mutant than for wild type C1C2 (13.9 ± 1.0 nm vs. 12.7 ± 1.3 nm; Suppl. Table 1), although this was not statistically significant. Bundling of F-actin filaments commonly observed with N-terminal cMyBP-C decoration^{5; 8; 21} was more frequent with L348P-C1C2. Single filaments showing obvious decoration (from their wider diameter) were selected for 3D reconstruction. Reconstructions of filaments decorated with wild type or mutant C1C2 showed additional density on the surface of actin (Fig. 3B, C, E, and F) compared with control filaments (Fig. 3A and D). By docking the atomic structure of actin into the reconstructions (Fig. 3D, E, and F), this extra density was seen to be on subdomain 1 and we interpret it as the ordered region of C1C2. The extra density was more prominent with L348P-C1C2 (Fig. 3C) than with wild type C1C2 (Fig. 3B).

3.2 EM and 3D reconstruction of thin filaments decorated with C1C2

It is known that binding of N-terminal domains of cMyBP-C to thin filaments at low $[Ca^{2+}]$ causes displacement of Tm from its low Ca^{2+} (blocked) position towards its high Ca^{2+} (closed) position, and that this is associated with thin filament activation^{14; 15; 16; 40}. We therefore tested the possibility that the L348P mutation might alter this Tm shift. Images of negatively stained control thin filaments (containing actin, Tm and Tn; Fig. 4A) were compared with filaments decorated with C1C2 (Fig. 4B) and L348P-C1C2 (Fig. 4C). Both types of decorated thin filaments showed a similar increase in diameter (C1C2, 13.6 ± 1.4 nm; L348P-C1C2, 14.2 ± 1.6 ; Suppl. Table 1) compared with the thin filament control (11.0 ± 1.3 nm; Suppl. Table 1). Filaments showing the highest level of decoration, based on their increased diameter, were straightened and used for 3D reconstruction. As found previously for the C0C2 fragment¹⁶, C1C2 caused Tm to move from the blocked to the closed position (Fig. 5B). This was demonstrated by fitting atomic models of actin together with Tm in the blocked (red ribbon) and closed positions (green ribbon) to the 3D reconstructions (Fig. 5A, B). The similarity in Tm position with C0C2 and C1C2 shows that the C0 domain is not required for Tm movement. However, filaments decorated with L348P-mutant C1C2 showed a substantially larger movement of Tm (Fig. 5C) compared with wild type C1C2 (Fig. 5B); this difference was clearly demonstrated by superimposing control, C1C2 and L348P-C1C2 reconstructions (Fig. 5D, E, and F). The position of Tm with L348P-C1C2 no longer coincided with the closed state, but instead matched the open position (Fig. 5C, yellow ribbon). This enhanced movement of Tm would be consistent with increased activation of the thin filament by L348P-C1C2²⁸.

The enhanced ability of L348P-C1C2 to displace Tm was further demonstrated in experiments using lower amounts of N-terminal domains to decorate thin filaments. The

above experiments used a 3:1 molar ratio of C1C2 to actin subunits to maximize binding, as in our previous work¹⁶ (see Methods). Even when the ratio was reduced to 1:7, Tm still moved a considerable distance (to approximately the closed position) with L348P-C1C2; in contrast, movement with wild type C1C2 at this ratio was about half as far, as found previously with the C0C2 fragment (Suppl. Fig. 1)¹⁶.

3.3 The effect of M-domain phosphorylation on Tm shift

In previous work using the C0C3 fragment, it was shown that phosphorylation of the M-domain reduced C0C3-induced movement of Tm¹⁴. To study the effect of phosphorylation on the ability of L348P-mutant C1C2 to move Tm, phosphorylated wild type and L348P-C1C2 were used to decorate thin filaments. Phosphorylation was confirmed by ProQ-Diamond staining of SDS-PAGE gels¹³. Both phosphorylated fragments caused an increase in thin filament diameter (Fig. 6A and B; C1C2, 13.8 ± 0.9 nm; L348P-C1C2, 14.3 ± 1.0 nm; Suppl. Table 1), demonstrating that phosphorylation did not abolish binding to the thin filament, in agreement with previous observations^{5; 8}. However, with phosphorylated wild type C1C2, the number of filaments showing the increase was smaller than with unphosphorylated C1C2. Filaments showing an increased diameter with the two phosphorylated fragments were selected for 3D reconstruction; the filaments chosen had similar diameters to those decorated with the respective unphosphorylated fragments. Reconstructions of thin filaments decorated with either phosphorylated C1C2 or phosphorylated L348P-C1C2 showed clear Tm density. The positions of Tm were compared by fitting atomic structures of Tm in the blocked, closed, and open positions into the 3D structures (Fig. 7A and B). In filaments decorated with phosphorylated wild type C1C2, Tm showed a smaller movement away from the blocked position than with unphosphorylated C1C2 (Fig. 7A, D). In contrast, with phosphorylated L348P-C1C2, Tm still shifted to the open position (Fig. 7B, E). Thus, while phosphorylation reduced Tm shift caused by wild type C1C2, it did not reduce the ability of L348P-C1C2 to shift Tm all the way to the open position.

4. Discussion

Together our results suggest that the L348P mutation in the tri-helix bundle of the M-domain of cMyBP-C enhances cMyBP-C's ability to sensitize thin filaments to Ca^{2+} ¹³ by increasing the tropomyosin shift normally caused by binding of cMyBP-C N-terminal domains to actin. The observed correlation between Ca^{2+} -sensitization and degree of Tm shift strengthens earlier conclusions that Ca^{2+} -sensitization by wild type cMyBP-C results, at least in part, from Tm movement on the thin filament^{16; 40}. Our results provide support for the idea that the L348P variant (human L352P) is a pathogenic mutation and that increased Ca^{2+} -sensitivity of fibers can potentially cause incomplete relaxation leading to diastolic dysfunction, a common phenotype in HCM patients⁴¹. Our results support the suggestion that this occurs through a shift in the equilibrium in Tm position from the blocked towards the closed or even open position⁴².

The L348P mutation occurs in the third helix of a structurally ordered triple α -helical bundle in the C-terminal half of the M-domain¹⁰. The tri-helix bundle was originally proposed as an actin-binding site in cMyBP-C on the basis of structural similarity to other actin-binding

proteins¹⁰. This proposal received support from functional studies, which demonstrated that the tri-helix bundle, especially helix 3, played an important role in actin binding and thin filament Ca^{2+} -sensitization¹³. Our observation that a single point mutation in helix 3 of the bundle has a major effect on the degree of Tm shift further supports the view that the tri-helix bundle contributes to the functional effects of the M-domain via its binding to actin.

The tri-helix bundle is highly conserved in all MyBP-C isoforms, suggesting that it plays a critical (and probably similar) role in all striated muscle types. Its importance in the functioning of cardiac MyBP-C *in vivo* is suggested by the occurrence of several sequence variants in this part of the molecule identified in HCM patients¹³. Recent evidence from skeletal muscle strengthens this view. In addition to MyBP-C's well-established role in cardiac disease, two MyBP-C missense mutations have recently been linked to skeletal myopathies. One of these is a single amino acid substitution (tryptophan → arginine) at residue 236, in the M-domain of slow skeletal muscle MyBP-C, which leads to distal arthrogryposis type 1⁴³. Sequence alignment shows that the equivalent residue of the cardiac isoform is in helix 1¹⁰, suggesting that this mutation may impact actin binding. This was recently confirmed in binding and motility studies showing that W236R is a loss of function mutation that abolishes binding of the N-terminal domains of slow skeletal MyBP-C to actin⁴⁴, with consequent effects on its regulation of actomyosin interactions⁴⁰.

The replacement of leucine by proline in the L348P mutant was predicted to cause destabilization of the tri-helix bundle¹⁰, which could account for the mutant's enhanced binding to actin. This could occur, for example, if the bundle becomes more open or extended, enlarging its binding site on actin, which might in turn induce the larger movement of Tm that we observe¹³. This enhanced movement would be expected to make the thin filaments more sensitive to Ca^{2+} , consistent with the observed increased Ca^{2+} -sensitization of motility and enhanced Ca^{2+} -sensitivity of tension in cardiac myocytes¹³.

Phosphorylation of serines in the N-terminal half of the M-domain, immediately up-stream of the tri-helix bundle, strongly modulates cMyBP-C's N-terminal interactions with actin^{5; 8; 12} and myosin subfragment 2^{3; 4; 45} *in vitro*. Whereas binding to S2 is abolished, binding to actin is weakened, and Ca^{2+} -sensitization of thin filaments is reduced following phosphorylation. This phosphorylation-based fine-tuning of cMyBP-C's influence on filament interactions may be responsible, at least in part, for the changes in contractility that occur when cMyBP-C is phosphorylated in response to β -adrenergic stimulation of the heart (fight or flight response). The importance of cMyBP-C phosphorylation in cardiac contractility is demonstrated by the substantial levels of phosphorylation (> 50%) in the normal heart^{46; 47}, and by the greatly reduced levels that are associated with heart failure^{45; 46}. Previous 3D reconstructions of thin filaments decorated with dephosphorylated and phosphorylated cMyBP-C suggest that the reduction in thin filament Ca^{2+} -sensitization due to phosphorylation is caused by a smaller cMyBP-C-induced Tm shift¹⁶; a similar result was obtained here (Fig. 7A). Biochemical studies suggest that phosphorylation of L348P-mutant cMyBP-C causes a much smaller decrease in thin filament Ca^{2+} -sensitization than phosphorylation of wild type¹³. Consistent with this, we find that phosphorylation of the L348P mutant has minimal impact on the degree of displacement of Tm; i.e. structurally Tm still lies in the open position. However because PKA phosphorylates troponin I as well

as cMyBP-C in the living heart, TnI phosphorylation could potentially influence the ability of cMyBP-C to displace Tm. It will be interesting to study this possibility in future 3D reconstructions of thin filaments containing phosphorylated cTnI. Regardless, our finding that the L348P mutation sensitizes thin filaments to a greater degree than wild type cMyBP-C suggests that proper relaxation (e.g., during diastole) may be compromised in hearts bearing this mutation; i.e. thin filaments remain too sensitive to Ca^{2+} due to the greater Tm shift. In the case of L348P-mutant cMyBP-C, this would be exacerbated by the fact that phosphorylation does little to modulate the level of Ca^{2+} -sensitization or Tm shift.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- cMyBP-C binding to thin filaments shifts tropomyosin, increasing Ca^{2+} -sensitivity
- A mutation in cMyBP-C's M-domain (L348P) causes hypertrophic cardiomyopathy
- The mutation enhances cMyBP-C/actin binding, further increasing Ca^{2+} -sensitivity
- We use EM to show that L348P-mutant cMyBP-C causes a larger tropomyosin shift
- This could explain the greater Ca^{2+} -sensitivity, which may cause defective diastole

Figure 1.

Schematic of cMyBP-C and expressed N-terminal domains used in this study. cMyBP-C consists of 8 immunoglobulin-like domains (yellow), 3 fibronectin-like domains (cyan), and a phosphorylatable MyBP-C-specific sequence, the M-domain (blue). C1C2 contains the C1, C2 and M-domains. The amino acid sequence of the conserved C-terminal portion of the M-domain shows the mutated site (Leu348) in the third of the three conserved α -helices (indicated by underlines).

Figure 2.

Electron microscopy of C1C2 bound to F-actin compared with control F-actin. (A) Actin only; (B) Actin with C1C2; (C) Actin with L348P-C1C2. Upper panels show raw images and bottom panels are selected and straightened F-actin and decorated F-actin filaments. The binding of C1C2 was obvious through the greater width of the decorated filaments. Scale bar = 50 nm.

Figure 3.

3D reconstruction and atomic fitting of F-actin decorated with C1C2. (A,D) F-actin; numbers show actin subdomains (SD). (B,E) F-actin decorated with wild type C1C2. (C,F) F-actin decorated with L348P-C1C2. (D, E, F) Reconstructions fitted with F-actin atomic structure (Ivory). In (B,C) arrows point to additional density originating on SD1, thought to represent the ordered region of C1C2. This density was somewhat larger with L348P-C1C2 than with wild type C1C2. Additional density on the rear side of actin subunits in (C,E) may represent additional binding of L348P. This is distant from the location of Tm on the thin filament, and so appears unlikely to be involved in Tm displacement (see Fig. 5).

Figure 4.

Negative staining of thin filaments (A.Tm.Tn) decorated with C1C2. (A) Thin filaments only; (B) Thin filaments with C1C2; (C) Thin filaments with L348P-C1C2. Upper panels show raw images and bottom panels are selected and straightened thin filaments and decorated thin filaments. The binding of C1C2 was obvious from the larger diameter of the decorated filaments. Scale bar = 50 nm.

Figure 5.

Reconstructions fitted with A.Tm atomic models; F-actin model (ivory); Tm shown in the blocked (red), closed (green) or open (yellow) position. (A) Low Ca control filament shows Tm in blocked position. (B) Tm moves to closed position when decorated with wild type C1C2. (C) Tm moves to open position when decorated with L348P-C1C2. (D–F) comparison of Tm movements by superposing reconstructions. (D) C1C2 displaces Tm from blocked (grey, A) to closed position (purple, B). (E) L348P-C1C2 causes a larger movement (brown, C) compared with control filament (grey). (F) Superimposed wild type C1C2-decorated filament (purple) and L348P-C1C2 decorated filament (brown) clearly demonstrates the greater Tm shift caused by the L348P variant.

Figure 6.

Negative staining of thin filaments decorated with phosphorylated C1C2. (A) Thin filaments with phosphorylated C1C2; (B) Thin filaments with phosphorylated L348P-C1C2. Upper panels show raw images and bottom panels are selected and straightened decorated thin filaments. The binding of phosphorylated C1C2 was obvious from the larger diameter of the decorated filaments. Scale bar = 50 nm.

Figure 7.

3D reconstruction and atomic fitting of thin filaments decorated with phosphorylated C1C2 and L348P-C1C2. (A, D) With phosphorylated C1C2, Tm moves less than with unphosphorylated C1C2 (Fig. 5B) and is between the blocked and closed position. (B, E) With phosphorylated L348P-C1C2, Tm still moves to the open position, as with the unphosphorylated variant (Fig. 5C). (C) Superimposed 3D structures of phosphorylated C1C2 (A) and phosphorylated L348P-C1C2 (B) decorated thin filament. The phosphorylated mutant (L348P-C1C2) moves Tm more than phosphorylated WT C1C2