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Computational Characterization of Mutations in Cardiac Troponin T Known to Cause Familial Hypertrophic Cardiomyopathy

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Abstract

Cardiac Troponin T (cTnT) is a central modulator of thin filament regulation of myofilament activation. The lack of structural data for the TNT1 tail domain, a proposed α -helical region, makes the functional implications of the FHC mutations difficult to determine. Studies have suggested that flexibility of TNT1 is important in normal protein-protein interactions within the thin filament. Our groups have previously shown through Molecular Dynamics (MD) simulations that some FHC mutations, Arg92Leu(R92L) and Arg92Trp(R92W), result in increased flexibility at a critical hinge region 12 residues distant from the mutation. To explain this distant effect and its implications for FHC mutations, we characterized the dynamics of wild type and mutational segments of cTnT using MD. Our data shows an opening of the helix between residues 105–110 in mutants. Consequently, the dihedral angles of these residues correspond to non- α -helical regions on Ramachandran plots. We hypothesize the removal of a charged residue decreases electrostatic repulsion between the point mutation and surrounding residues resulting in local helical compaction. Constrained ends of the helix and localized compaction results in expansion within the nearest non-polar helical turn from the mutation site, residues 105–109.

Keywords

protein function; flexibility; molecular dynamics

I. INTRODUCTION

In the cardiac muscle, the thin filament plays an important regulatory function in contraction. Each functional unit of the regulatory thin filament is comprised of seven actin monomers, one troponomyosin (TM) dimer, and a single troponin complex composed of three independent subunits: TnC, TnI, and TnT. cTnT is a central modulator in myofilament activation considering its interactions with all the components of the thin filament. In addition to binding the TnI-TnC complex, TnT interacts with TM along extended regions spanning the head-to-tail overlap of the contiguous array of TMs within the thin filament. TnT is also thought to promote the ordered assembly of the Tn-TM complex onto the actin filament¹.

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Thus, alterations in cTnT structure or function and its implications for the cardiac sarcomere and contractility would be poorly tolerated.

The lack of structural data for the N-terminal TNT1 tail domain, a proposed α -helical region where 65% of the known cTnT mutations can be found, makes the functional implications of FHC mutations difficult to determine^{7,8}. Recent biochemical and biophysical studies have shown that this tail domain is flexible and thus critical in modulating the protein-protein interactions between the thin filament and myosin during contraction^{9,10}. Several FHC mutations have been shown to alter cTnT flexibility and also differentially affect the stability of the TnT-tropomyosin interaction^{11–13}. More recently, MD simulations of a 101-amino acid sequence of mouse cTnT corresponding to TNT1 (residues 70–170) demonstrated that independent amino acid substitutions at residue 92 of cTnT, specifically R92W and R92L, were sufficient to alter flexibility of the N-terminal tail domain at a critical “hinge” region¹⁴. These observed changes in flexibility, both *in vitro* and *in silico*, suggest that even a single amino acid mutation in the TNT1 domain is sufficient to cause distinct alterations in TnT structure and function.

The flexible hinge region identified from the MD simulations of cTnT FHC mutations is approximately 18 Å from the point mutation and lies in the vicinity of residues 104–108¹⁴, a puzzling mutational effect. To address the question of how a single amino acid substitution in cTnT results in such a distant effect we have characterized the dynamics of wild type and mutant cTnT segments using MD. Our results demonstrate that the removal of a charged residue in R92L and R92W mutants alters the dynamic properties of these cTnT segments. This provides a valuable clue to the elusive link between cTnT structure and function.

II. METHODS

To explain this distant effect and its implications for FHC mutations, we characterized the dynamics of wild type and mutational segments of cTnT using MD. α -helical models corresponding to residues 70–170 of murine cTnT sequences of WT, R92L, and R92W mutants were made using the commercial software INSIGHTII (Accelrys, Inc., San Diego). To simulate the protein environment, charged residues were assigned partial charges corresponding to physiological conditions. Minimization, equilibration, and production run of MD were performed as previously described¹⁴ using CHARMM29 macromolecular simulation package with a classical potential for all atoms. The nonbond interaction cutoff was set to 12 Å and smoothed to zero between 12 Å and 14 Å. Starting structures were created by minimizing each cTnT segment using the steepest-descent method for 20,000 cycles and a step size of 200 fs or until the gradient of the energy converged¹⁵. For the remaining phases of the simulation, we used the SHAKE algorithm to constrain all bonds involving hydrogen atoms and an integration time step of one fs. We slowly increased the temperature to 300 K over 10 ps, at which point we allowed the systems to equilibrate for 50 ps prior to proceeding with the production run. Initial velocities were assigned based on a Gaussian distribution at a low temperature and rescaled every 100 steps during the heating phase. During the production run, the dynamics were simulated for an additional 300 ps with a time step of one fs. We monitored the coordinates and system properties every five fs for

the WT, R92L, and R92W models, using VMD to create movies of the production run dynamics.

Structural changes were characterized through two basic means, changes in distance between α carbons (C_α) four amino acids apart and changes in corresponding dihedral angles graphed on Ramachandran plots. The first panel of Fig. 1 depicts conventional characterization of peptide helices by the number of amino acids per helical turn, m , and the distance the helix rises along its axis per turn, p , where a standard α -helix has $m=3.6$ -aa and $p=5.4^\circ\text{\AA}$ ¹⁶. We adapted this method to track $n - (n + 4)$ distances along the helices throughout the MD production run to determine relative changes in helical structure. We deemed any shortening in $n - (n + 4)$ distance as a compaction; whereas, any lengthening in $n - (n + 4)$ distance is called an expansion, or helical opening.

We further analyzed helical changes of the region from residue 92–110 by measuring the dihedral angles of adjoining C_α . A Ramachandran plot of dihedral angles between C_{α_n} and $C_{\alpha_{n+1}}$ allows for a quick visual perspective of any changes in local helical structure¹⁶. Significant local compactations or expansions should result in points lying outside the allowable region for a right-handed α -helix. Neither proline nor glycine residues are present in the region of residues 92–110 cTnT, so proline and glycine Ramachandran plots—which would display significantly different allowable helical regions—are not necessary to map this region.

III. RESULTS

R92L and R92W mutants alter the dynamics of TNT1 segment of cTnT. We averaged structures over 300 ps and visualized mutational effects on the structure of TNT1. Panels in Fig. 1 reflect average structural characteristics of WT and changes that resulted from mutations in TNT1. It is clear that leucine and tryptophan mutations at residue 92 result in helical disruption in regions which correspond to the previously described hinge region¹⁴. A pronounced bend in the helix between residues 104–110 is observed in both R92L and R92W but not in the WT segment. MD simulations of TnT are clearly altered by point mutations R92L and R92W in comparison to WT. Visual observation of R92L and R92W dynamics exhibit increased flexibility about the hinge residues, while WT dynamics show little fluctuation through the helical segment.

In Fig. 2 both R92L and R92W display distinct helical openings between residues 105–109 and 106–110 and compactations local to the point mutation at residue 92. Average WT n -($n + 4$) distances range from $5.5^\circ\text{\AA}$ to $8^\circ\text{\AA}$; whereas, the average mutation n -($n + 4$) distances, not including 105–109 and 106–110 distances, range from $5.5^\circ\text{\AA}$ to $7^\circ\text{\AA}$. 105–109 and 106–110 distances stand out in R92L and R92W, averaging approximately $9^\circ\text{\AA}$. This observation is unique to the mutational segments and is not seen in WT.

In addition to $n - (n + 4)$ distances, the TNT1 region of residues 92–110 show a relaxation of corresponding dihedral angles. As shown in Fig. 3 this relaxation results in numerous outliers from the α -helical region of the plot. Table I shows that the percentage of dihedral angles that lie outside the allowable α -helical region for the compaction and expansion

regions are 8% and 20% for WT, 42% and 60% for R92L, and 0% and 40% for R92W, respectively.

IV. DISCUSSION

There is a distinct opening of the helix between residues 105–110 in R92L and R92W cTnT segments. Consequently, the dihedral angles of these residues correspond to non- α -helical regions on Ramachandran plots. The disparate proportion of outliers in the compaction and expansion regions calculated from Table I can be interpreted as a counterbalance of similar degrees of helical disruption distributed over unequal spans of residues. Helical compaction is spread over thirteen residues, 92–104, while helical expansion is spread over six residues, 105–110. Uniform expansion over relatively few residues causes a pronounced opening in this predominantly non-polar region, whereas uniform compaction over a greater number of residues in the compaction region—an area rich with charged residues—is less distinct. This may account for the higher proportion of outliers on Ramachandran plots for the expansion region than the compaction region.

From this we recognize the importance of electrostatic interactions in the dynamics of this segment, which appear to minimally affect the compaction region and significantly affect the expansion region approximately 18°Å away. We hypothesize the removal of a charged residue decreases electrostatic repulsion between the point mutation site and surrounding charged residues resulting in local helical compaction. A helix constrained at its ends would accommodate local compaction by expanding in a region of weak side chain interactions, such as a complete non-polar helical turn. Regions 105–109 and 106–110 in Fig. 2 correspond directly to residues 105–110, the nearest non-polar helical turn from the mutation. Thus, a helical opening in region 105–110, within the hinge region, accounts for increased flexibility of mutant TNT1.

TNT1 is a key player in cTnT-TM interactions⁸. Alterations in charge interactions, which are clearly important in TNT1, may have significant effects on thin filament dynamics as well. Examination of the human cTnT 70–170 sequence revealed a coiled-coil complex with a heptad repeat spanning residues 92–110 with Arg92 (R92) capable of hydrogen-bonding with TMGln263(Q263)¹³. This leads us to believe that hydrophobic mutations such as R92L and R92W, in addition to altering electrostatic interactions within TNT1, would disrupt known cTnT R92-TM Q263 hydrogen bonding and side-chain packing between the cTnT and TM interface¹³. In addition, the lower dielectric constant in the hydrophobic interface between cTnT and TM would amplify any electrostatic changes. Further, it is possible that disordered assembly of the Tn-TM complex onto the actin filament would impair thin filament function within the cardiac sarcomere.

The difference between the computational characterization of R92L and R92W are also important to point out. The average structure and dynamics of R92L show a more distinct bend and greater flexibility about the hinge region in comparison to R92W. In addition, despite that the $n - (n + 4)$ distances for the two mutations are similar, Ramachandran plots reveal more compaction and expansion residues outside of the alpha helical regions for R92L. Thus, the electrostatic and functional consequences of such differences in dynamics

and structure may account for the variable phenotypes associated with these distinct FHC mutations.

Future experiments will include simulations and characterizations of substitutional FHC mutations at residue 92. Additional simulations comparing wild type cTnT will address the relative importance of electrostatic and structural contributions to mutational effects.

V. CONCLUSION

We showed through MD that structural changes, namely local helical compaction and remote helical opening, result from R92L and R92W mutations. The effects of the point mutations at mutational hotspot residue 92 appeared as distinct helical openings from residues 105–110, approximately 18° Å away. These mutations suggest an increased flexibility of TNT1 and may have farther-reaching implications on cTnT-TM interactions. A clearer understanding of the mechanisms of mutational effects on TNT1 will shed light on the cause of and possible treatments for FHC.

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FIG. 1. Models of residues 70–170 of murine cTnT, where hotspot mutation site residue 92 is shown in red and the hinge region, residues 104–110, are shown in purple. Top left, illustration of pitch, p , and rise, m as defined in the text; top right, WT; bottom left, R92L; bottom right, R92W.

FIG. 2. cTnT mutational effects on n-(n+4) distances as a function of time for residues 92–110 WT, R92L, and R92W for 300ps.

FIG. 3.
Ramachandran plots for residues 92–110 of average structures for WT, R92L, and R92W cTnT.

TABLE I

Tabulation of Ramachandran plot TNT1 outliers per region of cTnT variants (residue numbers identified in parantheses).

TNT1 Region	cTnT Variant		
	WT	R92L	R92W
Compaction (92–104)	1 (98)	5 (93, 94, 95, 96, 103)	0
Expansion (105–110)	1 (108)	3 (105, 108, 109)	2 (108, 109)