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Familial hypertrophic cardiomyopathy related E180G mutation increases flexibility of human cardiac α-tropomyosin

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ABSTRACT

α-Tropomyosin (αTm) is central to Ca^{2+}-regulation of cardiac muscle contraction. The familial hypertrophic cardiomyopathy mutation αTm E180G enhances Ca^{2+}-sensitivity in functional assays. To investigate the molecular basis, we imaged single molecules of human cardiac αTm E180G by direct probe atomic force microscopy. Analyses of tangent angles along molecular contours yielded persistence length corresponding to ~35% increase in flexibility compared to wild-type. Increased flexibility of the mutant was confirmed by fitting end-to-end length distributions to the worm-like chain model. This marked increase in flexibility can significantly impact systolic and possibly diastolic phases of cardiac contraction, ultimately leading to hypertrophy.

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1. Introduction

α-Tropomyosin (αTm) is a dimeric, α-helical coiled-coil protein that binds actin and is an integral component of thin filaments for Ca^{2+}-regulated contraction of striated muscle. One troponin complex (Tn) and one αTm molecule form a structural regulatory unit with seven adjacent actin monomers in the thin filament, where αTm binds to and spans all seven actins. The Ca^{2+} regulation mechanism of cardiac muscle contraction can be described by a three-state model of regulatory units [1]. In the 'blocked' state, when Ca^{2+} is effectively absent in the cytoplasm (cardiac diastole), αTm sterically blocks myosin-binding sites on actin, inhibiting cross-bridge formation and thus contraction. During systole, Ca^{2+} ions are released from the sarcoplasmic reticulum and bind to Tn. Subsequent conformational change of Tn leads to the 'closed' state, in which azimuthal movement of αTm on the thin filament uncovers part of myosin-binding sites on actin and allows formation of weak cross-bridges. In the 'open' state, strong cross-bridge formation between myosin and actin is associated with further αTm movement away from myosin binding sites, and full activation of regulatory units. Activation of one regulatory unit may cooperatively influence activation of adjacent regulatory units through end-to-end interactions between adjacent αTm molecules. Taken together, this suggests that mechanical flexibility of αTm is likely to be an essential parameter in this regulatory process within and between regulatory units, and thus could influence normal function of the human heart [2,3].

Familial hypertrophic cardiomyopathy (FHC) is an inherited disease that affects ~0.2% of the population [4]. It is typically characterized by thickening of the myocardium and may be relatively benign, or can lead to heart failure or sudden cardiac death [4]. Genetic linkage studies have demonstrated that FHC is associated with any of a large number of mutations, primarily in cardiac cytoskeletal proteins including thick filament proteins β-myosin heavy chain, myosin essential and regulatory light chains, and cardiac myosin-binding protein C, and thin filament proteins including all three cardiac Tn subunits (cTnT, cTnI, and cTnC) and αTm [5–11]. The E180G mutation in αTm is close to the primary cTnT binding site in the C-terminal end of the molecule. This missense mutation leads to severe cardiac hypertrophy and early death in transgenic mice [12]. At the molecular level, the mutant protein

Abbreviations: AFM, atomic force microscopy; αTm, α-tropomyosin; cTnC, cardiac troponin C subunit; cTnI, cardiac troponin I subunit; cTnT, cardiac troponin T subunit; F-actin, filamentous actin; FHC, familial hypertrophic cardiomyopathy; L_c, contour length; l_{e-e}, end-to-end length normalized to contour length; L_{e-e}, end-to-end length; l_p, persistence length normalized to contour length; L_p, persistence length; p-Lys, poly-lysine; s, segment length; Tn, troponin complex; WLC, worm-like chain; WT, wild type

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has a lower binding affinity for actin [5,13–15] and decreased thermal stability [13,14,16] compared to wild-type (WT) protein. In vitro studies with mutant αTm using myofibrillar ATPase activity, motility assays and permeabilized cardiac cell mechanics showed markedly enhanced Ca^{2+}-sensitivity [6,17–19] and reduced functional cooperativity [19]. A comparable increase in Ca^{2+}-sensitivity of actomyosin binding kinetics has also been reported with this FHC-associated mutation of αTm [20].

A mechanistic relationship between the E180G mutation, the above observations, and cardiac hypertrophy has not yet been established, and more fundamentally there is no direct experimental information about the mutation's effects on the structure and mechanical properties of single αTm molecules. Modeling studies suggest that the presence of, and variations in, myofilament compliance could alter myocyte function at all levels of Ca^{2+}-activation [21–24] and some aspects of muscle cell mechanics are most simply explained by Ca^{2+}-dependent changes in sarcomere compliance [25]. Estimates of thin filament flexibility suggest that Tn and αTm modulate compliance in a Ca^{2+}-dependent manner [26] and this could be directly influenced by flexibility of αTm. We therefore hypothesized that the E180G mutation alters the mechanical flexibility of αTm, which could contribute to functional differences between thin filaments containing the E180G mutant versus WT as suggested by modeling studies on other FHC-related, thin filament mutations [23].

Recently we reported the persistence length (L_p), which is a measure of the rigidity of the molecule, of WT human cardiac αTm [3]. Here we present the first mechanical flexibility measurements of the E180G mutant using direct probe, atomic force microscopy (AFM). The mutant is more flexible than WT, with ~35% shorter L_p. We propose that, corresponding to the increased flexibility, a lesser extent of Ca^{2+}-induced conformational change of Tn is required to perturb αTm to initiate thin filament activation during systole, leading to enhanced Ca^{2+}-sensitivity [2]. Hypersensitivity to Ca^{2+} could overwork cardiac muscle, resulting in FHC. Preliminary data were presented in an abstract [27].

2. Materials and methods

Experimental techniques were essentially as described in a previous publication [3] and are briefly summarized below.

2.1. Protein preparation

WT human cardiac αTm cDNA was cloned previously into a bacterial expression vector [28] and the E180G mutation was introduced using site directed mutagenesis (Stratagene QuickChange Kit, La Jolla, CA) [19]. Bacterial expression and purification of recombinant mutant αTm was carried out as described.

2.2. Atomic force microscopy

Single molecules of E180G mutant αTm were imaged with a MFP-3D (Asylum Research, Santa Barbara, CA) atomic force microscope on poly-lysine (p-Lys) coated mica substrate. A 200 μl aliquot of 1 nM αTm E180G was deposited on the substrate and incubated for 600 s; this incubation time was demonstrated to be sufficient for obtaining consistent estimates of L_p for WT αTm under the same experimental conditions [3]. The sample was then rinsed, and dried with compressed nitrogen gas. AFM images were acquired at 0.5 nm/pixel in AC mode.

2.3. Image processing and data analysis

Images of individual αTm molecules were processed with a custom routine developed in MATLAB (The MathWorks, Inc., Natick, MA) and the data were analyzed to yield L_p. Three separate methods of data analysis were applied to populations of αTm molecules from independently prepared samples: second moment of tangent angles, tangent angle correlation, and end-to-end length distribution.

Briefly, the shape of each αTm molecule was traced with subpixel precision to obtain a polynomial skeleton representation of the molecular contour. Angles, $\theta(s)$, between the tangents of the molecular contour at two points separated by segment length s were computed in 0.5 nm steps. End-to-end length (L_{e-e}) and contour length (L_c) of each αTm molecule were calculated, respectively, as the linear distance between the two ends of the molecule and the integrated length along the polynomial fit.

Equilibration of αTm molecules on the substrate was verified by the ratio between tangent angle fourth moment ($\langle\theta^4(s)\rangle$) and squared second moment $\langle\theta^2(s)\rangle^2$ [29] (Supplementary Materials). L_p estimate was obtained by fitting the dependence of $\langle\theta^2(s)\rangle$ on s according to the linear relation:

$$\langle\theta^2(s)\rangle = \frac{s}{L_p} \tag{1}$$

A zero intercept confirmed that the molecules were equilibrated on the substrate; the inverse of the slope gave L_p.

In tangent angle correlation analysis, the average of $\cos\theta(s)$, $\langle\cos\theta(s)\rangle$, is assumed to be an exponential function of s:

$$< \cos\theta(s) >= e^{-s/2L_p} \tag{2}$$

$\langle\cos\theta(s)\rangle$, at a given s, was computed both along the contour of each molecular skeleton and over different skeletons. L_p was obtained by weighted linear regression on the logarithmically transformed data.

A third estimate of L_p was obtained by analysis of end-to-end length distributions. Scaled end-to-end length (l_{e-e}) was obtained as the ratio between L_{e-e} and L_c for each αTm molecule. Distributions of l_{e-e} were fitted to that expected of a two-dimensional worm-like chain (WLC) [30]:

$$p(l_{e-e}) = \frac{l_p l_{e-e}}{\eta} \sum_{m=0}^{\infty} \frac{(2m-1)!!}{2^m m!} \frac{1}{[2l_p(1-l_{e-e})]^{5/4}} \times \exp\left[-\frac{(m+1/4)^2}{2l_p(1-l_{e-e})}\right] D_{3/2}\left[-\frac{2(m+1/4)}{\sqrt{2l_p(1-l_{e-e})}}\right] \tag{3}$$

where $l_p = L_p/L_c$, η is a normalization factor, and $D_{3/2}(x)$ is a parabolic cylinder function. In short, analyses of tangent angle second moment, tangent angle correlation, and end-to-end length distribution resulted in three independent estimates of L_p.

3. Results

Fig. 1A and the left-most collage in Fig. 1C show AFM scans of αTm E180G on p-Lys coated mica. Lengths of clearly distinguishable elongated structures on the substrate are consistent with that expected for single αTm molecules [31]. For comparison, p-Lys coated mica (no αTm) is shown in Fig. 1B. In general, individual molecules of αTm E180G appeared more bent in the images when compared to WT (Fig. 1C). A small subset of mutant molecules exhibits a noticeable kink along the molecular contour. As our spatial resolution was limited by tip convolution and the N- and C-termini of the molecule were indistinguishable, we were not able to quantitatively correlate the location of kinks in these molecules with that expected for the E180G mutation along the molecular contour (data not shown).

Fig. 2A shows the results of tangent angle second moment analysis of images obtained from three samples independently prepared under identical experimental conditions. In this analysis, L_p

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