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Modulation of the picosecond dynamics of troponin by the cardiomyopathy-causing mutation K247R of troponin T observed by quasielastic neutron scattering

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ABSTRACT

Troponin (Tn), consisting of three subunits (TnC, TnI, and TnT), regulates cardiac muscle contraction in a Ca^{2+}-dependent manner. Various point mutations of human cardiac Tn are known to cause familial hypertrophic cardiomyopathy due to aberration of the regulatory function. In this study, we investigated the effects of one of these mutations, K247R of TnT, on the picosecond dynamics of the Tn core domain (Tn-CD), consisting of TnC, TnI and TnT2 (183–288 residues of TnT), by carrying out the quasielastic neutron scattering measurements on the reconstituted Tn-CD containing either the wild-type TnT2 (wtTn-CD) or the mutant TnT2 (K247R-Tn-CD) in the absence and presence of Ca^{2+}. It was found that Ca^{2+}-binding to the wtTn-CD decreases the residence time of atomic motions in the Tn-CD with slight changes in amplitudes, suggesting that the regulatory function mainly requires modulation of frequency of atomic motions. On the other hand, the K247R-Tn-CD shows different dynamic behavior from that of the wtTn-CD both in the absence and presence of Ca^{2+}. In particular, the K247R-Tn-CD exhibits a larger amplitude than the wtTn-CD in the presence of Ca^{2+}, suggesting that the mutant can explore larger conformational space than the wild-type. This increased flexibility should be relevant to the functional aberration of this mutant.

1. Introduction

Troponin (Tn) plays a central role in the regulation of skeletal and cardiac muscle contraction depending on the intracellular Ca^{2+} concentration [1]. Tn consists of three subunits TnC, TnI, and TnT. Together with another regulatory protein tropomyosin (Tm), Tn forms muscle thin filaments by periodically binding to the F-actin backbone. Upon Ca^{2+}-binding to TnC, a series of structural changes takes place in Tn, which in turn affects the structure of Tm [2]. These structural changes of the thin filaments release inhibition of the interaction between myosin on the thick filaments and actin on the thin filaments, and accelerate the myosin ATPase activity leading to the tension development [3].

Various mutations in human cardiac Tn have been shown to cause familial hypertrophic cardiomyopathy [4–6]. Many physiological or biochemical studies have shown that these mutations modulate the myosin ATPase activity and/or its Ca^{2+}-sensitivity [7–11]. Recent studies by molecular dynamics (MD) simulations on several mutants of the Tn core domain (Tn-CD, consisting of TnC, TnI and TnT2, the domain containing the residues 183–288 of TnT) [12–15], those on the Tn-Tm system containing the mutants of Tn [16,17], and those on the whole thin filament [18] have suggested that the dynamic nature of the Tn molecules is important to elucidate not only the regulatory mechanism but also the mechanism of the functional aberrations caused by the mutations. Investigating how the cardiomyopathy-causing mutations affect the dynamic properties of the Tn molecules is thus essential to understand the mechanism of these aberrations, and this should ultimately lead to elucidation of the molecular mechanism of the pathogenesis of inherited cardiomyopathy.

While most of the cardiomyopathy-causing mutations are found in the regions directly involved with the Ca^{2+}-regulation, such as the Tm-binding regions, the actin binding regions, the inhibitory region of TnI, and the regions where the interaction between TnI and TnC occurs in a Ca^{2+}-dependent manner, mutations are also found in the region not directly involved with the Ca^{2+}-regulation. These mutations are E244D and K247R of TnT [21]. They are located in the middle of the IT-arm (a

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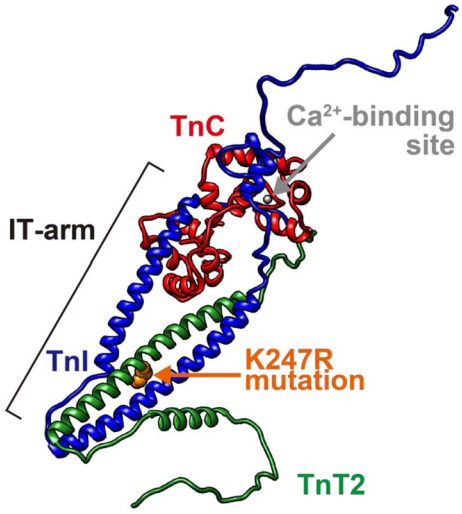

Fig. 1. Structure of the human cardiac Tn-CD. TnC, TnI, and TnT2 are colored in red, blue, and green, respectively. The 247 residue of TnT2 (the mutation site) is shown by spheres in orange. A Ca^{2+} ion bound to the N-lobe of TnC is shown by a grey sphere. Note that TnT1, which is the N-terminal tail domain and the Tm-binding region of TnT, is truncated in the Tn-CD. This model is based on the crystal structure of Tn-CD (PDB ID: 4Y99) with its missing regions complemented by random coils as described previously [19]. The diagram is drawn using UCSF Chimera [20].

coiled-coil region formed by TnI and TnT) [22], which is the pathway of transmitting the Ca^{2+}-binding signal from TnC to TnT (see Fig. 1 for the structure of the Tn-CD and the location of the mutation, K247R). Investigation of these mutations should thus be important in terms of the regulatory mechanism itself as well as the mechanism of the pathogenesis.

These mutations are known to increase the myosin ATPase activity without changing its Ca^{2+}-sensitivity [7,11]. These mutations have been shown to disrupt the hydrogen bond network around the mutation sites [11], suggesting that mobility of the residues around the mutation sites is affected by the mutations. Furthermore, a small-angle X-ray scattering study on the Tn-CD containing the wild-type proteins (wtTn-CD) and that containing these mutants (mtTn-CD) has shown that the difference between the wtTn-CD and the mtTn-CD lies in the distribution of the conformations rather than distinct structural changes [19]. These findings indicate importance of the dynamic properties of the Tn-CD for understanding the molecular mechanism of the functional aberration caused by these mutations. The protein dynamics spans over a wide range of time and spatial scales from local fluctuations of atoms in the side and main chains in the proteins at the picosecond time scale, through loop motions and the domain motions, to the structural changes occurring at the millisecond or slower time scales [23]. Since the local atomic motions within proteins at the picosecond timescale are essential for the structural changes that are required for protein functions [24], it is crucial to investigate how the internal dynamics of the Tn-CD at the picosecond timescale is affected by these mutations. Here, we focus on the mutation K247R of TnT because both E244D and K247R show similar functional aberration but the effects are somewhat more significant for K247R than E244D [11,19].

We employed incoherent quasielastic neutron scattering (QENS) to investigate how the mutation affects the picosecond dynamics of the Tn-CD. QENS provides a unique tool to measure directly the picosecond dynamics of proteins [25]. Because of much larger incoherent neutron scattering cross-section of hydrogen than any other atoms found in proteins and the isotope deuterium [26], the QENS spectra are dominated by the signals from hydrogen atoms in the sample. Since about half of the atoms in protein molecules are hydrogens and they are uniformly distributed in the molecules, the QENS measurements on the proteins in D_2O-solutions provide information on the average motions of the protein molecules [24]. In this study, we carried out the QENS measurements on the D_2O-solution samples of the Tn-CD containing the wild-type TnT2 (wtTn-CD) and the Tn-CD containing the K247R mutant of TnT2 (K247R-Tn-CD) in the absence and presence of Ca^{2+}. Our results show that the mutation modulates the internal dynamics of the Tn-CD, and thereby changing the dynamics of the Tn-CD corresponding to Ca^{2+}-binding.

2. Materials and methods

2.1. Sample preparations

All subunits of the human cardiac Tn-CD, including the K247R mutant of TnT, were cysteinless and expressed in *E. coli* BL21(DE3)-pLys-S. Each subunit was purified separately as described previously [27]. The purified subunits were reconstituted into the Tn-CD (the molecular mass of 52 kDa) by dialysis, and lyophilized. For the preparation of the D_2O-solution samples, the lyophilized proteins were dissolved in the D_2O-buffer containing 50 mM HEPES (pD 8.0), 0.5 M NaCl, and 5 mM $MgCl_2$. The samples in the presence of Ca^{2+} were prepared by adding $CaCl_2$ so that the final $CaCl_2$ concentration is 5 mM. The samples in the absence of Ca^{2+} was prepared by adding ethylene glycol tetraacetic acid (EGTA) so that the final EGTA concentration is 5 mM. The protein concentration was measured spectrophotometrically using the extinction coefficients $E_{280}^{1\%}$ of 5.33, calculated using the ProtParam tool (http://web.expasy.org/protparam/). The final concentrations of the Tn-CD solution samples were 22.1 mg/ml, 22.7 mg/ml, 21.2 mg/ml, and 21.8 mg/ml for the wtTn-CD in the presence of Ca^{2+}, the K247R-Tn-CD in the presence of Ca^{2+}, the wtTn-CD in the absence of Ca^{2+}, and the K247R-Tn-CD in the absence of Ca^{2+}, respectively. For the neutron scattering experiments, each sample solution was put into a double-cylindrical aluminum cell with the thickness of 1.0 mm, and sealed with indium wire. Transmission of these samples was > 0.9, and thus contribution of multiple scattering to the spectra was neglected in the analysis.

2.2. Neutron scattering experiments

The QENS measurements were carried out using the near-backscattering spectrometer BL02 DNA [28] at the Materials and Life Science Facility at the Japan Proton Accelerator Research Complex, Tokai, Ibaraki, Japan. The measurements were carried out at 300 K at the energy resolution of 12 μeV, at which the motions faster than 55 ps are accessible. The measured QENS spectra S(Q, ω), where Q ($= 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and λ is the wavelength of the incident neutrons) is the momentum transfer and ω is the energy transfer of neutrons, were corrected for the empty cell contribution and the detector efficiency, and normalized to the vanadium standard, which was also used for defining the instrumental energy resolution. Subtraction of the spectra of the D_2O-buffer from those of the D_2O-solution samples was done using the scaling factors calculated from the scattering cross-section of the samples [29]. The spectra thus obtained were verified by the static structure factor, S(Q), calculated by integrating the S(Q, ω) along the ω-direction. S(Q) of the D_2O-solution samples show large increase in intensity at $Q > \sim 1.4\ Å^{-1}$, which arises from coherent scattering of D_2O [30,31]. On the other hand, S(Q) of the proteins do not contain such a contribution [31]. Proper subtraction of the D_2O-buffer spectra should thus provide the S(Q) curves with rather flat intensity at $Q > \sim 1.4\ Å^{-1}$. Fig. 2 shows, as an example, the S(Q) curves of the D_2O-solution of the wtTn-CD in the absence of Ca^{2+}, the D_2O buffer, and the protein, which was obtained by subtraction of the spectra of the D_2O buffer from those of the solution sample. While significant increase in intensity at $Q \geq 1.4\ Å^{-1}$ is observed in the curves of the solution sample and the buffer, such increase is not observed in the protein data. This indicates that the solvent contribution is negligible in the spectra of the protein (Another possible factor