

STRUCTURE NOTE

Conformational Variation of Calcium-Bound Troponin C

Jayashree Soman,¹ Terence Tao,² and George N. Phillips, Jr.^{1*}

¹Department of Biochemistry and Cell Biology, Rice University, Houston, Texas

²Muscle Research Group, Boston Biomedical Research Institute, Boston, Massachusetts

Introduction. Troponin is the Ca²⁺-binding regulatory protein in mammalian skeletal and cardiac muscles. Together with tropomyosin, troponin regulates the interaction between myosin crossbridges and actin in response to rising and falling levels of intracellular Ca²⁺ (10⁻⁵ to 10⁻⁷ M). The troponin complex has three subunits: troponin C (TnC) which binds Ca²⁺ specifically, troponin I (TnI), and troponin T (TnT). When TnC binds 4 Ca²⁺ ions, it undergoes a conformational transition which is transmitted to TnI, causing TnI to release its inhibition of the actin-myosin interaction via TnT and tropomyosin.¹

TnC contains four Ca²⁺-binding sites: two high-affinity sites in the C-domain that also bind Mg²⁺ and are always occupied, and two low-affinity Ca²⁺-specific sites in the N-domain. Based on a wealth of studies, including the crystal structure of 2-Ca²⁺ avian TnC^{2,3} and the NMR structure of calcium-saturated TnC,⁴ it is generally accepted now that muscle contraction is initiated by Ca²⁺-binding to the N-domain, causing the domain to undergo a transition from a closed conformation to an open one and exposing a hydrophobic patch. It has been suggested that a region of TnI may then bind to this patch. The recent crystal structures of 2-Ca²⁺ chicken N-TnC⁵ and 4-Ca²⁺ rabbit TnC⁶ corroborate the conformational transition in the N-domain.

We present here the crystal structure of 4-Ca²⁺ rabbit TnC in a new crystal form. A detailed comparison with other calcium-saturated structures shows differences that help describe the dynamics of TnC and its range of conformations.

Materials and Methods. The mutant Cys98Leu rabbit skeletal TnC studied here was expressed and purified as previously described.⁷ Crystals were grown by the hanging-drop method by microseeding at 4°C from solutions containing 50–60% MPD, 50 mM sodium acetate pH 5.6 and 5 mM CaCl₂. The crystals were characterized as belonging to the space group P2₁ with a = 32.1, b = 59.4, c = 47.8 Å, β = 101.1° and one molecule per asymmetric unit based on a Matthews' coefficient of 2.4 Å³/Da. The structure was determined using anomalous selenomethionine data and MADSYS.⁸ The initial electron density maps showed a bilobed structure, and the clusters of Se positions could be correlated with the two domains in the apo TnC structure. Using the Ca²⁺-saturated average NMR struc-

ture of chicken TnC as the starting model, the structure was refined using XPLOR⁹ with several rounds of positional and restrained temperature factor refinements. 2Fo–Fc maps showed clear density for all four Ca²⁺ ions. The final results correspond to R = 25.5% and R_{free} = 36.0% for 156 residues and 162 water molecules. Coordinates and structure factors were deposited at the Protein Data Bank. (Entries 1TCF and R1TCFSF respectively.)

Results and Discussion. As shown by other workers, the overall structure of 4-Ca²⁺ rabbit TnC is similar to that seen in 2-Ca²⁺ avian TnC, an elongated α-helical protein consisting of two domains connected by a nine-turn helix. There are a total of nine helices in the structure. The N-domain contains the N (1–10); A (13–26); B (36–46); C (52–62); and D (72–83) helices. The C-domain contains the E (93–102); F (112–122); G (128–138); and H (148–156) helices. Each domain is filled with two Ca²⁺ ions in the two helix-loop-helix calcium-binding motifs.

There are two dominant features in a delta-distance-plot of 4-Ca²⁺ TnC vs. apo TnC. First, there is a large movement of helix B and the linker away from helices N/A. Second, the stretch of residues 35–57 moves away from 70–83. This corresponds to the B/C pair of helices and the linker between them moving away from the D-helix. Therefore, a significant feature of the transition from the apo state of the N-domain to the Ca²⁺-saturated state is the large, relative movement of helices B/C with respect to A/D, causing it to adopt an open conformation. There are no prominent features involving just the C-domains, suggesting that their conformations in the two structures are essentially identical.

Comparison with other Ca²⁺-saturated structures. The NMR structures of 2-Ca²⁺ N-TnC and 4-Ca²⁺ TnC were the first to directly confirm the opening up of the N-domain upon binding calcium. But the N-domain in the crystal structure of 4-Ca²⁺ TnC is very different from that seen in the NMR structures. A delta-distance-plot compar-

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*Correspondence to: Dr. George N. Phillips, Jr., Department of Biochemistry and Cell Biology, Rice University, 6100 S. Main, Houston, TX 77005. E-mail: georgep@bioc.rice.edu

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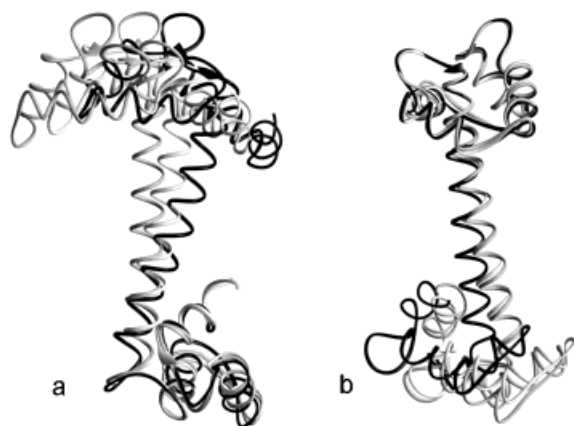


Fig. 1. Diagram illustrating the flexibility of the linker in the central helix of TnC. When the C-domains of the three 4-Ca²⁺ crystal structures are superposed, the variation in the N-domains is much larger (a) than the variation in the C-domains when the N-domains are superposed (b). This suggests the presence of a hinge region in the central helix where the D-helix enters the C-domain.

ing the two N-domains shows several large areas of contraction involving helices C/D, suggesting that the N-domain in the crystal structure is much less open than in the NMR structure. Whether the differences are due to the methods or the protein environments has yet to be determined.

A comparison of the current structure with other Ca²⁺-saturated crystal structures also provides interesting details. Delta-distance plots involving the relevant N-domains show that the N-domain in the current structure is a little less open than in 2-Ca²⁺ N-TnC, but is more open than those in the other 4-Ca²⁺ rabbit TnC structures. The C-domain in the current structure is quite different from that seen in the monoclinic crystal in the Houdusse⁶ study, but closely resembles the C-domain in their orthorhombic structure.

The existence of three different crystal structures of 4-Ca²⁺ TnC also allows a comparison of the flexibility of the linker between the N and C domains. When the C-domains are superposed the variation in the N-domains is quite large, confirming the existence of a hinge region where the D-helix enters the C-domain (Fig. 1a). A similar plot with the N-domains superposed shows less hinge-bending at

the intersection of the central D-helix with the N-domain (Fig. 1b). A structure of TnC with a helical segment of TnI bound has also recently appeared.¹⁰ Unlike other TnC structures, the central D/E helix connecting the two domains in the complex is unwound at the center, resulting in a 90° inclination between the D/E helices.

Conclusions. Comparisons of NMR and crystallographic structures of TnC, with and without calcium in the N-domain, reveal a range of structures, consistent with current models of calmodulin/TnC mechanisms of action. The set of structures allows a finite range of conformations to be described in different environments. In view of the multiple crystal forms all with an extended shape it seems unlikely that TnC (and by extension calmodulin) would undergo collapse from the general dumbbell to a compact form to any appreciable extent in the absence of other protein partners.

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