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Interaction of cardiac troponin with cardiotonic drugs: A structural perspective ☆

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Abstract

Over the 40 years since its discovery, many studies have focused on understanding the role of troponin as a myofilament based molecular switch in regulating the Ca^{2+}-dependent activation of striated muscle contraction. Recently, studies have explored the role of cardiac troponin as a target for cardiotonic agents. These drugs are clinically useful for treating heart failure, a condition in which the heart is no longer able to pump enough blood to other organs. These agents act via a mechanism that modulates the Ca^{2+}-sensitivity of troponin; such a mode of action is therapeutically desirable because intracellular Ca^{2+} concentration is not perturbed, preserving the regulation of other Ca^{2+}-based signaling pathways. This review describes molecular details of the interaction of cardiac troponin with a variety of cardiotonic drugs. We present recent structural work that has identified the docking sites of several cardiotonic drugs in the troponin C–troponin I interface and discuss their relevance in the design of troponin based drugs for the treatment of heart disease.

Keywords: Cardiac muscle contraction; Myofilament; Troponin; Cardiotonic drugs; Calcium sensitizers

The Ca^{2+}-dependent regulation of striated muscle contraction by troponin has been a fascinating area for biochemical and biophysical study since its discovery ~40 years ago by Professor S. Ebashi. Troponin is a heterotrimeric complex that is comprised of the Ca^{2+}-binding subunit troponin C (TnC), inhibitory subunit troponin I (TnI), and an elongated molecule troponin T (TnT) that binds both TnC and TnI and also to tropomyosin. At sub-micromolecular Ca^{2+} concentrations, the troponin–tropomyosin complex sterically hinders strong, force-producing cross bridges between actin and myosin. At micromolecular concentrations, Ca^{2+} binds to the regulatory domain of TnC, resulting in a cascade of structural changes in troponin and the movement of tropomyosin deeper into the groove of the actin strand, thus revealing actin binding sites for myosin attachment and releasing inhibition. Thus, the interaction of troponin and tropomyosin propagates the regulatory signal along the thin filament and as such acts as a Ca^{2+}-sensitive molecular switch of the thin filament (for reviews on the thin filament regulatory system, see [1–3]).

Structural studies have contributed a great deal to the understanding of the molecular details of the role of troponin in regulating striated muscle contraction. The first three-dimensional structure of skeletal TnC (sTnC) was solved by X-ray crystallography in 1985 [4,5]. In this structure, the two N-domain (sNTnC) Ca^{2+}-binding sites were unoccupied, while the C-domain (sCTnC) was in two Ca^{2+}-bound state. By comparing the crystal structure of the apo N-domain with its homologous Ca^{2+}-bound C-domain, Herzberg et al. [6] proposed a model for the Ca^{2+}-induced conformational change in sNTnC that involved a 'closed' to 'open' transition accompanied with the exposure of the hydrophobic pocket. The first direct structural evidence describing the Ca^{2+}-induced 'closed' to 'open' conformational change in sNTnC came in 1995

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when the NMR solution structures of sTnC in $4Ca^{2+}$ state [7] and of sNTnC in apo and $2Ca^{2+}$ states [8] were determined. Subsequently, the X-ray structures of sNTnC·$2Ca^{2+}$ [9] and sTnC·$4Ca^{2+}$ [10] were reported. These structures provide detailed features of the Ca^{2+}-binding loops that were not as well defined in the NMR structures. The main difference between the X-ray and solution structures of sTnC is in the central linker that connects the N- and C-domains; while a long α-helix in the crystal form, this linker is unstructured and flexible in solution. The response of cardiac TnC (cTnC) to Ca^{2+}-binding was unknown until the determination of the NMR solution structures of cTnC·$3Ca^{2+}$ [11] and the regulatory domain of cTnC (cNTnC) in both the apo and Ca^{2+} states [12]. Strikingly, cNTnC remains essentially 'closed' in the Ca^{2+} state, unlike sNTnC, a consequence of the defunct site I. The significant reduction in the hydrophobic surface of cNTnC suggested that the mode of interaction between cTnC–cTnI maybe different than that between sTnC and sTnI. However, both the regulatory domains of sTnC and cTnC assume similar 'open' conformations when bound to switch regions of sTnI and cTnI ($sTnI_{115–131}$ and $cTnI_{147–163}$), respectively [13,14]. This indicates that the pathways involved in initiating skeletal and cardiac muscle contraction are structurally very similar; however, the kinetics and thermodynamics of the pathways must differ for the two systems to account for the different physiological behavior of the two muscle types [15]. NMR studies of TnC with various TnI peptides have yielded detailed structural information on the structure of TnC when bound to TnI [16–19], on the structure of TnI inhibitory peptide [20,21], and on the overall topology of TnC–TnI arrangement [22–32]. The high-resolution structures of TnC–TnI available are the X-ray structure of sTnC·$2Ca^{2+}$·$sTnI_{1–47}$ [33], the NMR structures of cNTnC·Ca^{2+}·$cTnI_{147–163}$ [13], sNTnC(rhodamine) ·$2Ca^{2+}$·$sTnI_{115–131}$ [14], and cCTnC·$2Ca^{2+}$·$cTnI_{128–147}$ [34], the X-ray structure of the core domain cardiac troponin complex, cTnC·$3Ca^{2+}$·$cTnI_{34–210}$·$cTnT_{182–288}$ [35], and the X-ray structures of skeletal troponin complex in both the apo and Ca^{2+}-state, sTnC·apo·$sTnI_{1–182}$·$sTnT_{156–262}$ and sTnC·$4Ca^{2+}$·$sTnI_{1–182}$·$sTnT_{156–262}$ [36]. In the structure of sTnC·$2Ca^{2+}$·$sTnI_{1–47}$, the 31-residue long sTnI α-helix (residues 3–33) stretches on the surface of the sTnC and stabilizes its compact conformation by multiple contacts with both TnC domains [33]. The corresponding region of cTnI ($cTnI_{34–71}$) was found to bind to the hydrophobic cleft of the C-domain of cTnC [19] and exhibit a similar conformation and orientation as observed in the structure of cTnC·$3Ca^{2+}$·$cTnI_{34–210}$·$cTnT_{182–288}$ [35]. In the structure of cNTnC·Ca^{2+}·$cTnI_{147–163}$, the bound $cTnI_{147–163}$ peptide adopts an α-helical conformation spanning residues 4–12 in the 17-residue peptide. The C-terminus of the peptide is unstructured and the N-terminus of the peptide interacts with the center of the hydrophobic pocket. The most important finding is that the α-helical region interacts with the AB helical interface and stabilizes the opening conformation of cNTnC·Ca^{2+}. The corresponding $sTnI_{115–131}$ peptide adopts a similar structure and a similar mode of interaction with the N-domain of sTnC as observed in the structure of sNTnC(rhodamine)·$2Ca^{2+}$·$sTnI_{115–131}$ [14]. The backbone atoms of the cNTnC·Ca^{2+}·$cTnI_{147–163}$ structure superimposes to 1.5 Å with the corresponding regions in the cardiac troponin structure. In the structure of cCTnC·$2Ca^{2+}$·$cTnI_{128–147}$, residues L134–K139 of cTnI forms a helix and residues R140–R147 adopts an extended conformation. With the helical region interacting with the E and H helices of cCTnC·$2Ca^{2+}$, the highly basic R140–R147 region containing R140, R144, R145, and R147 makes potential electrostatic contacts with the acidic residues present on the linker region (beginning of the E-helix) including E94, E95, and E96. The conformation and orientation of $cTnI_{128–147}$ in this structure are similar to those reported from an electron spin labeling work which shows that a section of the inhibitory region ($cTnI_{129–137}$) displays a helical profile, with the C-terminal residues 139–145 displaying no discernible secondary structure characteristics [37]. While most of the inhibitory region of cTnI is not visualized in the cTnC·$3Ca^{2+}$·$cTnI_{34–210}$·$cTnT_{182–288}$ structure, the conformation and orientation of the N-terminal and switch regions of cTnI are in good agreement with those observed from the binary complexes. The overall core domain structure (Fig. 1A) is dominated by α-helical elements and can be divided into structurally distinct subdomains, denoted as the regulatory head (consisting of the N-domain of cTnC and the switch region of cTnI) and the IT arm (consisting of the C-domain of cTnC, $cTnI_{42–136}$, and $cTnT_{203–271}$). The subdomains are connected by flexible linkers including the one connecting the two domains of cTnC and the inhibitory region of cTnI. The arrangement of cTnC and cTnI in this structure is antiparallel, with the N-terminal domain of TnI interacting with the C-terminal domain of TnC and *vice versa*. This agrees with the earlier proposal emerged from integrating various biochemical and biophysical data (for a review, see [1]). Two other α-helices of cTnI are observed in this structure, $cTnI_{164–188}$ and $cTnI_{90–136}$. While $cTnI_{164–188}$ is free of contact with cTnC and cTnT, $cTnI_{90–136}$ forms a coiled-coil with a portion of the T2 fragment of cTnT, $cTnT_{226–271}$, as predicted previously [38]. Upstream from the coiled-coil, $cTnT_{204–220}$ forms another α-helix (Fig. 1A). The N-terminal extension of cTnI is not present in the core domain structure. The overall orientation of the cTnI in this complex would direct the cardiac specific region upwards toward the N-domain of cTnC. Direct interaction of this region of cTnI with cNTnC has been demonstrated [28,32,39,40]. This interaction has been reported to influence conformational exchange in the regulatory domain by shifting the equilibrium to favor an open conformation that exposes the hydrophobic cleft. These contacts are disrupted by protein kinase A (PKA) phosphorylation of S22 and S23 and loss of these interactions shifts the conformational equilibrium in cNTnC towards a more closed state. A recent study has shown that bis-