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Functional importance of Ca^{2+}-deficient N-terminal lobe of molluscan troponin C in troponin regulation

Teppei Doi[a], Ai Satoh[a], Hiroyuki Tanaka[a], Akira Inoue[a], Fumiaki Yumoto[b,c], Masaru Tanokura[c], Iwao Ohtsuki[b], Kiyoyoshi Nishita[a], Takao Ojima[a,*]

[a] *Laboratory of Biochemistry and Biotechnology, Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan*
[b] *Laboratory of Physiology, The Jikei University School of Medicine, Tokyo 105-8461, Japan*
[c] *Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan*

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Abstract

Ca^{2+}-binding sites I and II in the N-terminal lobe of molluscan troponin C (TnC) have lost the ability to bind Ca^{2+} due to substitutions of the amino acid residues responsible for Ca^{2+} liganding. To evaluate the functional importance of the Ca^{2+}-deficient N-terminal lobe in the Ca^{2+}-regulatory function of molluscan troponin, we constructed chimeric TnCs comprising the N-terminal lobes from rabbit fast muscle and squid mantle muscle TnCs and the C-terminal lobe from akazara scallop TnC, TnC_{RA}, and TnC_{SA}, respectively. We characterized their biochemical properties as compared with those of akazara scallop wild-type TnC (TnC_{AA}). According to equilibrium dialysis using $^{45}Ca^{2+}$, TnC_{RA}, and TnC_{SA} bound stoichiometrically 3 mol Ca^{2+}/mol and 1 mol Ca^{2+}/mol, respectively, as expected from their primary structures. All the chimeric TnCs exhibited difference-UV-absorption spectra at around 280–290 nm upon Ca^{2+} binding and formed stable complexes with akazara scallop troponin I, even in the presence of 6 M urea, if Ca^{2+} was present. However, when the troponin complexes were constructed from chimeric TnCs and akazara scallop troponin T and troponin I, they showed different Ca^{2+}-regulation abilities from each other depending on the TnC species. Thus, the troponin containing TnC_{SA} conferred as high a Ca^{2+} sensitivity to Mg-ATPase activity of rabbit actomyosin–akazara scallop tropomyosin as did the troponin containing TnC_{AA}, whereas the troponin containing TnC_{RA} conferred virtually no Ca^{2+} sensitivity. Our findings indicate that the N-terminal lobe of molluscan TnC plays important roles in molluscan troponin regulation, despite its inability to bind Ca^{2+}.

Keywords: Ca^{2+} regulation; Chimeric TnC; Mg-ATPase activity; Mollusks; Muscle contraction; Rabbit; Scallop; Squid; Tropomyosin; Troponin

Troponin is a Ca^{2+}-dependent regulatory protein in striated muscle, consisting of three distinct subunits, TnC, TnI, and TnT [1–5]. TnC is the Ca^{2+}-receptor subunit that binds the intracellular Ca^{2+} released from the sarcoplasmic reticulum by neural stimulation; TnI is the subunit that inhibits actin–myosin interactions; and TnT is the tropomyosin-binding subunit that anchors the troponin complex to tropomyosin–actin filaments. The primary event in the initiation of muscle contraction is the binding of Ca^{2+} to TnC. This Ca^{2+} binding causes conformational changes in the TnC molecule and neutralizes TnI inhibition, which allows the actin–tropomyosin filament to react with myosin.

X-ray crystallography and nuclear magnetic resonance studies on vertebrate TnC have shown that the molecule is shaped like a dumbbell [6–8]. Each N- and C-terminal lobe contains two EF-hand or helix–loop–helix Ca^{2+}-binding motifs, which are called sites I and II and sites III and IV, respectively [3,4,9–11]. Sites I and II in the N-terminal lobe, the regulatory sites, specifically bind Ca^{2+} ($K_{app}[Ca^{2+}] = 5 \times 10^5\,M^{-1}$) at physiological

* Corresponding author. Fax: +81 138 40 8800.
E-mail address: ojima@fish.hokudai.ac.jp (T. Ojima).

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Mg^{2+} concentrations and interact with the putative regulatory region of TnI (i.e., residues 117–126 in the rabbit skeletal sequence) to remove the TnI inhibition of actin–myosin interactions [3,4,12,13]. Sites III and IV in the C-terminal lobe, the structural sites, bind both Ca^{2+} and Mg^{2+} ($K_{app}[Ca^{2+}] = 2 \times 10^7\,M^{-1}$ and $K_{app}[Mg^{2+}] = 5 \times 10^3\,M^{-1}$) and maintain the integrity of the troponin complex. The number and location of active Ca^{2+}-binding sites in TnCs vary depending on the animal species and the muscle tissue. For example, site I of both vertebrate slow skeletal TnC and cardiac TnC have lost the Ca^{2+}-binding ability because of substitution of amino acids essential for Ca^{2+} coordination [14,15]. These TnCs bind at most 3 mol Ca^{2+}/mol at sites II, III, and IV. Arthropod TnCs lack the Ca^{2+}-binding ability at sites I and III; hence, these TnCs bind 2 mol Ca^{2+}/mol at sites II and IV [16–19]. In both vertebrate and arthropod TnCs site II is regarded as the regulatory site, whereas site IV is as the structural site. TnCs of mollusks, such as scallop and squid, have lost the Ca^{2+}-binding ability at sites I to III, and thus these TnCs bind only one Ca^{2+} at site IV [20–22]. Therefore, unlike vertebrate and arthropod TnCs, molluscan TnCs employ site IV as the regulatory site. Recently, site IV of akazara scallop TnC was shown to bind Ca^{2+} in the bidendate coordination of the Glu–COO^- group, whereas it bound Mg^{2+} through the pseudo-bridging mode with low affinity ($K_{app}[Mg^{2+}] = 1.67 \times 10^2\,M^{-1}$) [23–25]. This implies that site IV of akazara scallop TnC acts as both a regulatory and structural site through the exchange of Mg^{2+} and Ca^{2+}.

Molecular mechanism underlying vertebrate troponin function has been investigated on the basis of three-dimensional structures of TnC and troponin complexes [12,26]. Recently, Takeda et al. [26] elucidated the crystal structures of the core domain of human cardiac troponin. The core domain is composed of two structurally distinct sub-domains: (1) a regulatory head consisting of TnC residues 3–84 and TnI residues 150–159; and (2) an IT-arm consisting of TnC residues 93–161, TnI residues 42–136, and TnT residues 203–271. Because the two sub-domains are connected by the flexible D/E-linker of TnC, their relative orientation is considered to vary greatly in some regulatory steps. In fact, crystal structures with different orientations between the two sub-domains, in which the IT-arm is rotated by 20° relative to the regulatory head, have been determined. On the basis of this structural feature, Takeda et al. [26] proposed a regulatory mechanism involving large changes in the relative orientation of the regulatory head to the IT-arm, mediated by Ca^{2+} binding to the regulatory sites of TnC.

Although this mechanism explains vertebrate troponin action fairly well, it appears to be unsuitable for explaining molluscan troponin function. Namely, if the primary structure of molluscan TnC is considered based on the core domain model, the single Ca^{2+}-binding site of molluscan TnC (site IV) would be involved in the IT-arm, the structural sub-domain; this cannot easily account for the regulatory actions of site IV. Thus, it appears that molluscan troponin functions through Ca^{2+} binding to the IT-arm, implying that the molecular mechanism underlying molluscan troponin regulation is substantially different from that of vertebrate troponin. In support of this, a ternary complex constructed from akazara scallop TnC and rabbit fast skeletal TnI and TnT did not confer Ca^{2+} sensitivity to Mg-ATPase activity of rabbit actomyosin–akazara scallop tropomyosin [27].

Previously, we investigated the functional roles of the N- and C-terminal lobes of akazara scallop TnC by using half-molecular mutants and a site IV-abolished mutant; we found that the major functional properties of the TnC, namely Ca^{2+} and TnI binding, are in the C-terminal lobe [28]. However, a ternary complex consisting of the C-terminal lobe of akazara scallop TnC and akazara scallop TnI and TnT hardly conferred Ca^{2+} sensitivity to the Mg-ATPase activity of rabbit actomyosin–akazara scallop tropomyosin. This finding suggests that the N-terminal lobe of akazara scallop TnC also has essential functions in Ca^{2+} regulation in akazara scallop troponin. In addition, we questioned whether the C-terminal lobe mutant lacking a large mass, corresponding to the N-terminal lobe, could form stable ternary complexes with TnT and TnI.

In the present study, we constructed chimeric TnCs consisting of the N-terminal lobes from rabbit and squid TnCs and the C-terminal lobe from akazara scallop TnC, and analyzed their biochemical properties to examine the functional roles of the N-terminal lobe of molluscan TnC. In light of our findings, the N-terminal lobes of molluscan TnCs are essential for the regulatory function of molluscan troponin, despite lacking the ability to bind Ca^{2+}.

Materials and methods

Proteins

Akazara scallop troponin, troponin subunits, and tropomyosin were prepared as reported previously [29,30]. Rabbit myosin and actin were prepared by the methods of Perry [31] and Spudich and Watt [32], respectively.

Preparation of chimeric TnCs

The schematic structures of TnCs prepared in the present study are shown in Fig. 1. The cDNAs for the chimeric TnCs were constructed from akazara scallop and squid TnC-cDNAs cloned previously [20,22]