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Biochemical and Biophysical Research Communications 369 (2008) 109–114
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Spectroscopic and ITC study of the conformational change upon Ca^{2+}-binding in TnC C-lobe and TnI peptide complex from Akazara scallop striated muscle

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Received 18 October 2007
Available online 3 December 2007

Abstract

Akazara scallop (*Chlamys nipponensis akazara*) troponin C (TnC) of striated adductor muscle binds only one Ca^{2+} ion at the C-terminal EF-hand motif (Site IV), but it works as the Ca^{2+}-dependent regulator in adductor muscle contraction. In addition, the scallop troponin (Tn) has been thought to regulate muscle contraction via activating mechanisms that involve the region spanning from the TnC C-lobe (C-lobe) binding site to the inhibitory region of the TnI, and no alternative binding of the TnI C-terminal region to TnC because of no similarity between second TnC-binding regions of vertebrate and the scallop TnIs. To clarify the Ca^{2+}-regulatory mechanism of muscle contraction by scallop Tn, we have analyzed the Ca^{2+}-binding properties of the complex of TnC C-lobe and TnI peptide, and their interaction using isothermal titration microcalorimetry, nuclear magnetic resonance, circular dichroism, and gel filtration chromatography. The results showed that single Ca^{2+}-binding to the Site IV leads to a structural transition not only in Site IV but also Site III through the structural network in the C-lobe of scallop TnC. We therefore assumed that the effect of Ca^{2+}-binding must lead to a change in the interaction mode between the C-lobe of TnC and the TnI peptide. The change should be the first event of the transmission of Ca^{2+} signal to TnI in Tn ternary complex.

Keywords: TnC; TnI; Ca^{2+}; NMR; ITC; CD

Contraction of vertebrate striated muscles is regulated by troponin (Tn) in a Ca^{2+}-dependent manner [1–3]. Tn contains three components: troponin C (TnC; Ca^{2+}-binding), troponin I (TnI; inhibitory through actin binding), and troponin T (TnT; tropomyosin binding) [4–6]. TnC is composed of two globular domains, N- and C-lobes, each of which consists of two EF-hand motifs [7]. Vertebrate cardiac and fast skeletal muscle TnCs bind three and four Ca^{2+} ions, respectively, per molecule [8–10], and act as the Ca^{2+}-sensor in the muscle contraction, which is associated with the binding of one or two Ca^{2+} ions in the N-terminal domain (N-lobe) of TnC. Thus, the N-lobe with one or two low-affinity Ca^{2+}-binding sites is called the regulatory domain [11]. In contrast, the C-terminal domain (C-lobe) with two high-affinity Ca^{2+}-binding sites is called the structural domain.

In invertebrate striated muscles, there are two regulatory mechanisms in muscle contraction, i.e., myosin-linked and

Abbreviations: Tn, troponin; TnC, troponin C; TnI, troponin I; TnT, troponin T; TnC N-lobe, TnC N-terminal lobe; TnC C-lobe, TnC C-terminal lobe; ITC, isothermal titration microcalorimetry; NMR, nuclear magnetic resonance; CD, circular dichroism; TCA, trichloroethanoic acid; EDTA, ethylenediaminetetraacetic acid; MOPS, 3-morpholinopropanesulfonic acid; PIPES, piperazine-1,4-bis(2-ethanesulfonic acid).

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doi:10.1016/j.bbrc.2007.11.124

Tn-linked regulatory mechanisms [12–17]. Although invertebrate TnCs consist of four EF-hand motifs like their vertebrate counterparts, they can bind only one or two Ca^{2+} ions per molecule due to the lack of critical amino acids for chelation to Ca^{2+} [16]. In Akazara scallop (*Chlamys nipponensis akazara*), the TnC of striated adductor muscle binds only one Ca^{2+} ion at the C-terminal EF-hand motif (Site IV) [16], but it still works as the Ca^{2+}-dependent regulator in striated adductor muscle contraction [18,19]. TnI, which acts as the regulatory component in striated adductor muscle of Akazara scallop, also has a unique feature. The TnI consists of 292 amino acid residues, and the length of the polypeptide is greater by approximately 110 residues than that of the vertebrate skeletal muscle TnI [20]. The homologous sequence to vertebrate TnIs is only found in the C-terminal portion. The inhibitory region of TnI, which binds actin and TnC and is essential to the inhibitory function of TnI, is highly conserved. The scallop TnI has 100–133 extra residues at the N-terminus when compared with vertebrate TnIs. This extra region, whose function has not yet been characterized, has a unique sequence and contains many Glu and Arg residues. Moreover, there is no sequence similarity between the second TnC-binding region of the vertebrate fast skeletal muscle TnI and the corresponding region of scallop TnI. To shed light on this feature, functional assays have been utilized in a comparison of vertebrate TnI and Akazara scallop TnI [18]. These results suggested that scallop Tn regulates muscle contraction via an activating mechanism that involves the region spanning from the TnC C-lobe binding site to the inhibitory region of TnI, with no alternative binding of the TnI C-terminal region to TnC.

To elucidate the Ca^{2+}-dependent regulatory mechanism of striated muscle, crystal and NMR structures of TnC, both of its lobes, and the core regions of Tn ternary complexes from vertebrate striated muscle have been reported [21–28]. In contrast, there have been a limited number of structural studies of invertebrate striated muscle Tns [29–31], although the solution structure of TnC from insect flight muscle has recently been reported [32].

To clarify the molecular mechanism of the Ca^{2+}-dependent regulation of muscle contraction by scallop Tn, we have analyzed the interaction between TnC C-lobe and TnI peptide (TnIpep), which corresponds to the TnC C-lobe-binding region of vertebrate skeletal and cardiac muscle TnIs, and their Ca^{2+}-dependent conformational changes using isothermal titration microcalorimetry (ITC), nuclear magnetic resonance (NMR), circular dichroism (CD), and gel filtration chromatography.

Materials and methods

Sample preparation. Akazara scallop TnC C-terminal lobe (TnC C-lobe) was expressed and purified as described previously [29,30]. Ca^{2+}-free TnC C-lobe was prepared by TCA treatment [33]. The chemically synthesized TnIpep was purchased from Operon Biotechnologies (Tokyo, Japan). ^{15}N-labeled TnC C-lobe was obtained by the same expression system using the modified minimum medium [34] containing 1 g ^{15}N-NH_4Cl per liter culture (for ^{15}N-labeled protein) or 1 g ^{15}N-NH_4Cl and 1 g ^{13}C glucose per liter culture (for $^{13}C^{15}N$-labeled protein). Each NMR sample was prepared by mixing the concentrated 0.5 mM Ca^{2+}-free ^{15}N-labeled TnC C-lobe solution, 2.5 mM nonlabeled TnIpep solution, and 100× Ca^{2+}-stock solutions to give ratios of 0, 1, 2, 5, 6.25, 7.5, 8.25, 10, and 20 [Ca^{2+}]/[complex] in the solution of 10 mM Mops–KOH (pH 7.0), 5 mM DTT, and 100 mM KCl in 90% H_2O/10% D_2O. pH was adjusted with diluted HCl or NaOH.

NMR spectroscopy. NMR experiments were performed at 600 MHz with a Varian Inova spectrometer equipped with a *z*-axis pulse field gradient and a cold-probe. All spectra were processed using NMRPipe and drawn using NMRDraw [35]. Assignments of the NH pair in the ^{15}N–^{1}H HSQC spectrum were completed using ^{15}N–^{1}H HSQC, HNCACB, and CBCACONH spectra with Sparky (T.D. Goddard and D.G. Kneller, SPARKY 3, University of California, San Francisco).

Circular dichroism. Circular dichroism (CD) spectra were acquired on a Jasco J-720 spectropolarimeter at room temperature. All experiments were carried out using the following conditions: step resolution, 0.2 nm; speed, 50 nm min^{-1}; response time, 2 s; bandwidth, 1 nm; and number of scans, 8. The sample consisted of 20 μM TnC C-lobe complexed with TnIpep, 100 mM KCl, 0.05 mM EDTA, and 10 mM Mops–KOH (pH 7.0) for the apo state, 20 μM TnC C-lobe complexed with TnIpep, 100 mM KCl, 0.05 mM EGTA, 2 mM $MgCl_2$, and 10 mM Mops–KOH (pH 7.0) for the Mg^{2+}-bound state, and 20 μM TnC C-lobe complexed with TnIpep, 100 mM KCl, 2 mM $CaCl_2$, and 10 mM Mops–KOH (pH 7.0) for the Ca^{2+}-bound state. The background derived from the buffer was subtracted from each spectrum.

Gel filtration. Gel filtration experiments were performed on a Superdex 75 HR 10/30 (GE healthcare) column at room temperature and at a flow rate of 0.5 mL/min. The elution profile was monitored with a UV detector at 280 nm using AKTA explorer 10S (GE healthcare). The sample concentrations used were 20 μM complex in the solution containing 120 mM KCl, 0.05 mM EDTA, and 20 mM Mops–KOH (pH 7.0) for the apo form; 20 μM complex in the solution containing 120 mM KCl, 0.05 mM EGTA, 10 mM $MgCl_2$, and 20 mM Mops–KOH (pH 7.0) for the Mg^{2+}-bound form; and 20 μM complex in the solution containing 120 mM KCl, 10 mM $CaCl_2$, and 20 mM Mops–KOH (pH 7.0) for the Ca^{2+}-bound form.

Microcalorimetry. Isothermal titration microcalorimetry (ITC) was performed at 25 °C on a VP-ITC instrument (Microcal LLC, Northampton, MA, USA). TnC C-lobe was dialyzed against 150 mM KCl, 10 mM Pipes–KOH (pH 6.8), 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and either 0.2 mM EGTA (in the absence of Ca^{2+}) or 0.2 mM EGTA plus 0.3 mM $CaCl_2$ (in the presence of Ca^{2+}), and then clarified by centrifugation prior to use. The outer dialysate was used as a solvent for lyophilized TnIpep. The 1.4-mL sample cell was filled with 24 μM TnIpep and titrated with 260 μM TnC C-lobe. For each titration, 17 consecutive 15-mL aliquots of the C-lobe solution were injected at 220/s intervals. The heat of sample dilution was obtained from a final injection in the presence of a 2-fold molar excess of TnC C-lobe over TnIpep, and was subtracted from the C-lobe-peptide binding isotherm. Data analysis was performed using the Origin-ITC analysis package (Microcal) in "One set of sites" mode.

Results and discussion

The Ca^{2+}-dependent change of the interaction between TnC C-lobe and TnIpep was demonstrated by ITC. Fig. 1A shows a trace of the calorimetric titration of TnIpep with TnC C-lobe in the presence of Ca^{2+}. Fig. 1B shows integrated heats after subtraction of the heat of dilution and normalization with moles of TnC C-lobe injected together with single-site binding model fit. Reaction enthalpy ($\Delta H = -3.27$ kcal/mol), binding constant ($K_a = 2.93 \times 10^6\ M^{-1}$), and stoichiometry ($n = 0.907$) were

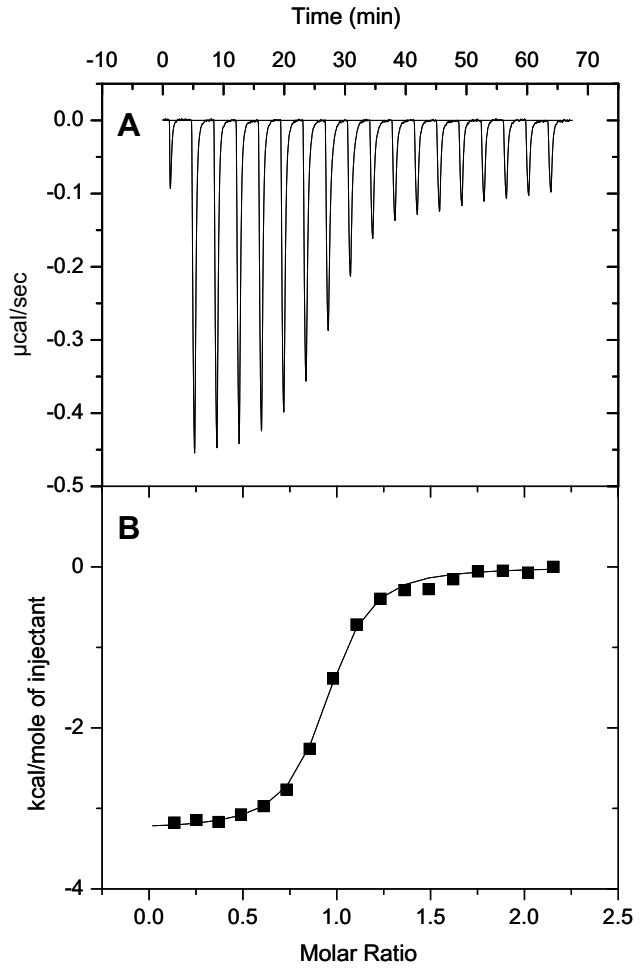

Fig. 1. Microcalorimetric titration of TnIpep with TnC C-lobe in the presence of Ca^{2+}. (A) The trace of the titration obtained by 17 injections of 260 µM TnC C-lobe into 24 M TnIpep. (B) Integrated heats for each injection versus the molar ratio of TnC C-lobe to TnIpep. The solid line indicates the curve fit of the data using the single-site binding model.

determined directly from the curve-fitting. The changes in Gibbs free energy ($\Delta G = -8.82$ kcal/mol) and entropy ($\Delta S = 18.6$ cal/mol/K) were determined by using the equation $\Delta G = -RT \ln K_a = \Delta H - T\Delta S$, where R is the universal gas constant and T is the temperature in K. In the absence of Ca^{2+}, the heat produced at the initial injection was smaller than that observed in the control injection of TnC C-lobe into buffer, indicating that the reaction is endothermic and therefore should be entropically driven. However, it was difficult to fit the data with theoretical binding curves, and the thermodynamic parameters could therefore not be determined (data not shown). These results indicate that the property of the interaction between the N-terminal TnC-binding site of Akazara scallop TnI and the C-lobe of TnC is drastically changed upon Ca^{2+}-binding to Site IV of TnC.

The effects of Mg^{2+}- and Ca^{2+}-binding to the C-lobe of Akazara scallop TnC–TnIpep complex were investigated by CD spectroscopy. The CD spectrum in each state showed two negative peaks around 208 and 222 nm (Fig. 2A). The results suggest that each form of the complex was folded and rich in α-helix. Spectral change upon Ca^{2+}-binding was slightly larger than that upon Mg^{2+}-binding. However, the secondary structures of the three states are relatively conserved.

The effects of Mg^{2+}- or Ca^{2+}-binding to the C-lobe of TnC in complex with the TnIpep were also examined by gel filtration chromatography (Fig. 2B). The results revealed that the elution volumes differed among the apo and metal ion-bound forms. They also indicate that the elution volumes of the apo, Mg^{2+}-bound, and Ca^{2+}-bound forms of the complex were 12.1 mL, 12.3 mL, and 12.4 mL, respectively. Therefore, the molecular size of the complexes demonstrated the following order of magnitude: apo form > Mg^{2+}-bound form ≈ Ca^{2+}-bound form. The results indicate that the metal ion-bound complexes have a more compact conformation than the apo form. A similar gel filtration analysis of only the C-lobe of TnC revealed that the elution volume of the various forms had the following order of magnitude: apo form > Mg^{2+}-bound form > Ca^{2+}-bound form (data not shown). Therefore, based on the kind of complex formation of the TnIpep, the elution volume of Mg^{2+}-bound and Ca^{2+}-bound forms

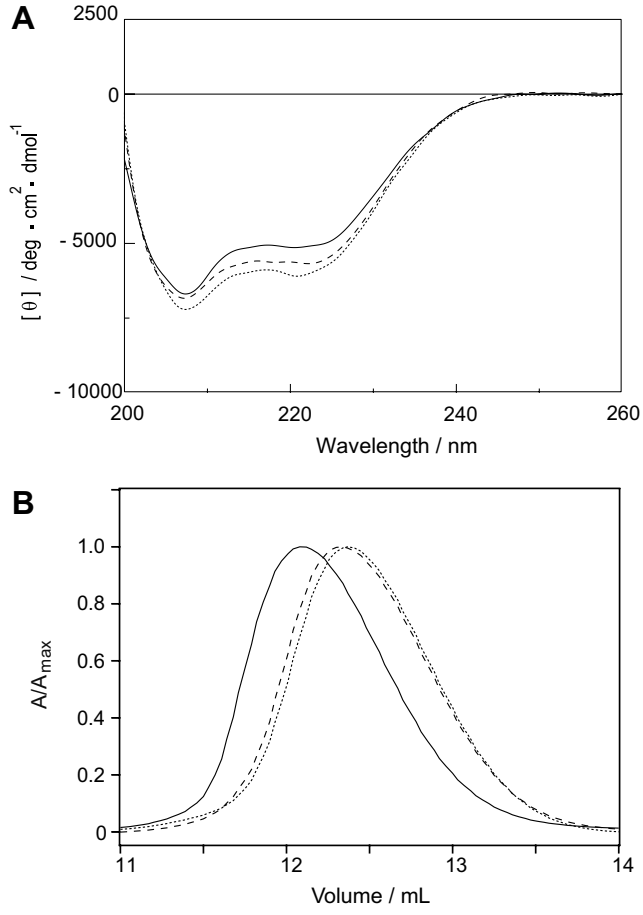

Fig. 2. (A) CD spectra of the C-lobe of TnC complexed with the TnIpep in the apo (-), Mg^{2+}-bound (- - -), and Ca^{2+}-bound (·····) forms. (B) Gel filtration chromatograms of the C-lobe of TnC in complex with the TnIpep in the apo (-), Mg^{2+}-bound (- - -), and Ca^{2+}-bound (·····) forms.

differs. To more precisely assess the effects of Ca^{2+}-binding on the tertiary structures of the C-lobe of Akazara scallop TnC, we used multidimensional NMR techniques. The ^{15}N–^{1}H heteronuclear single quantum coherence (HSQC) spectra of uniformly ^{15}N-labeled apo (black), Mg^{2+}-bound (blue), and Ca^{2+}-bound (red) forms were collected (Fig. 3). The signals in the spectra of Apo and Mg^{2+}-bound forms were similar but not identical, whereas the spectrum of the Ca^{2+}-bound form showed that almost all the signals changed upon Ca^{2+}-binding. The Ca^{2+}-binding to Site IV induced a structural change in the C-lobe at a global level. With regard to the Ca^{2+}-bound form, we assigned the backbone signals of the C-lobe of TnC complexed with the TnIpep using ^{15}N–^{1}H HSQC, HNCACB, and CBCA (CO)NH with the reference of the assignments for TnC C-lobe complexed with a longer TnI fragment [30]. In the NMR spectrum of the Ca^{2+}-bound form of the complex, two downfield-shifted signals were observed. The results show the presence of hydrogen bonds between Asp95 and Gly100, and between Asp131 and Gly136 [36]. A Ca^{2+}-titration experiment on the apo form of the complex shows that the downfield-shifted signal of G136 could first be observed at the 5-fold of Ca^{2+} to the concentration of the complex (Fig. 4). Subsequently, K99, G134, and T137 were observed at 6.25-fold of Ca^{2+}. On the other hand, the downfield-shifted signal of G100 was observed at 7.5-fold of Ca^{2+}, following which those of K103 and D139 were observed at 8.75-fold Ca^{2+}. Finally, the downfield-shifted signal of V138 was observed at 10-fold of Ca^{2+}. Therefore, it was shown that the Ca^{2+}-binding to Site IV first stabilized Site IV and then Site III as well.

A hydrogen-bond network is critical to maintaining the structural integrity of the Ca^{2+}-binding loop [37]. In the EF-hand-type Ca^{2+}-binding protein, two EF-hand motifs form a globular lobe [38]. In the Ca^{2+}-binding loop, short antiparallel β-sheets are formed and stabilized. In the C-lobe of Akazara scallop TnC, only the NH of Val138 is observed in the low field assumed as a hydrogen bond formation with carbonyl oxygen in main chain of I102. This result is similar to that observed for the N-terminal domain of vertebrate cardiac TnC. In this case, only the NH of I36 was observed in the low field [36,39].

The drastic change in the NMR spectra from both apo and Mg^{2+}-bound states due to Ca^{2+}-binding states implies that the local structures of the residues interacting with the TnIpep should be changed. It is very interested that the single Ca^{2+}-binding to the Site IV leads to structural change not only in Site IV but also in Site III through the structural network in the C-lobe of TnC. Therefore, we assumed that the effects of Ca^{2+}-binding must lead to change in the interaction mode between the C-lobe of TnC and the TnIpep, as suggested by ITC analysis. This structural transition in Ca^{2+}-binding is the first event in transmitting the Ca^{2+}-signal to the other component of Tn, namely TnI, to regulate the striated adductor muscle contraction in Akazara scallop.

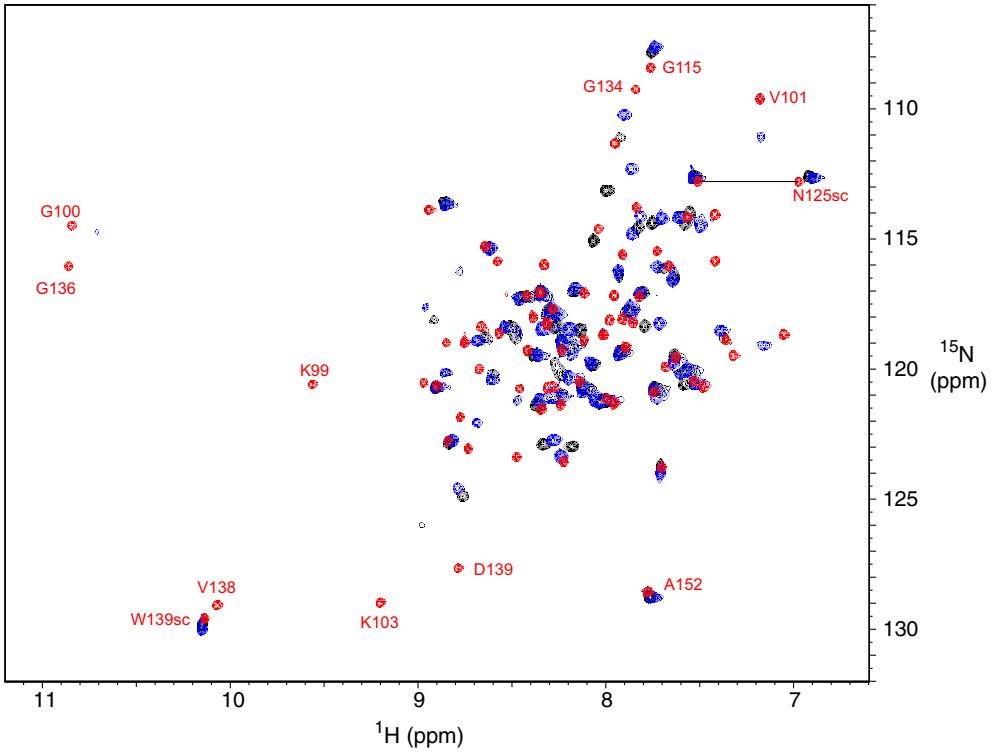

Fig. 3. Superimposed ^{15}N–^{1}H HSQC spectra of metal-free (black), Mg^{2+}-bound (blue), Ca^{2+}-bound (red) TnC C-lobe complexed with TnIpep.

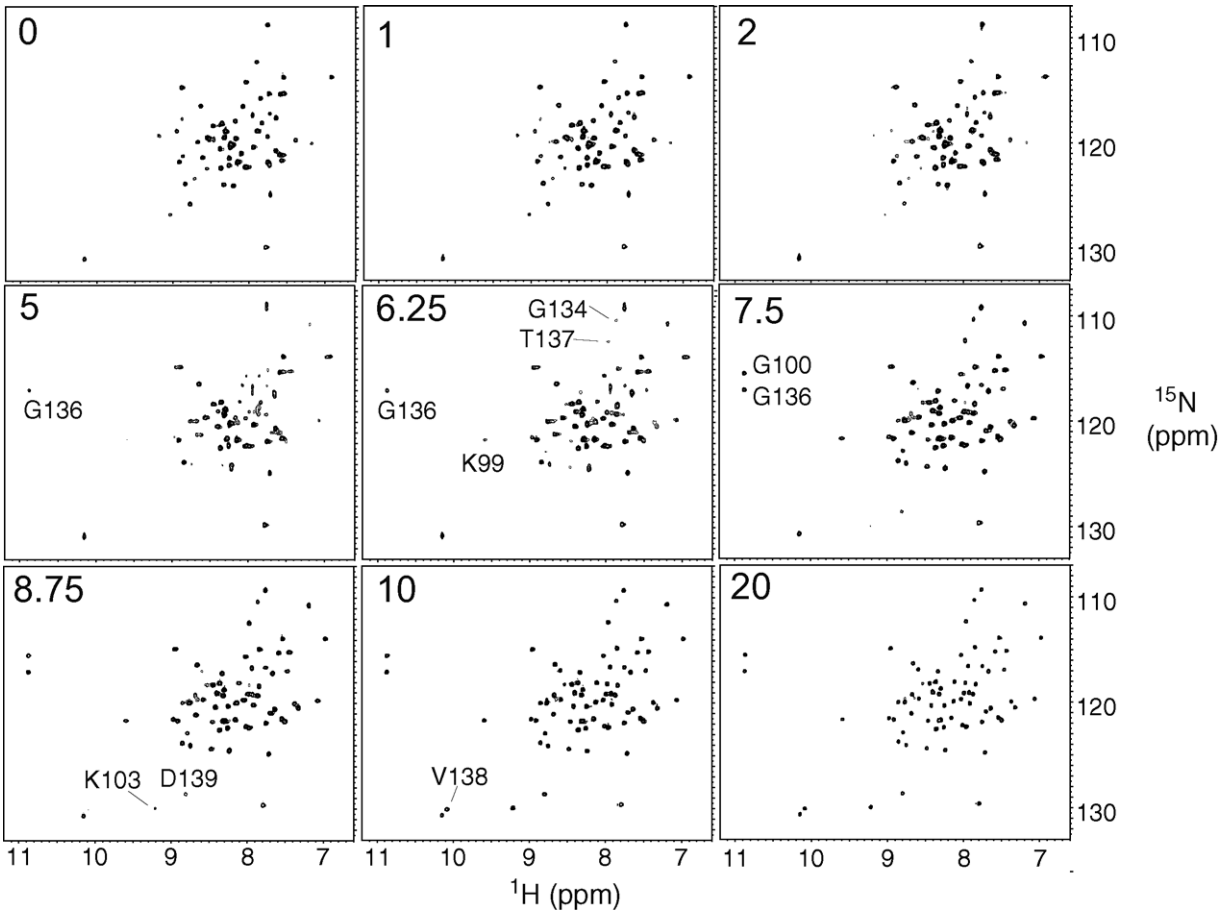

Fig. 4. ^{15}N–^{1}H HSQC spectra of 50 μM uniformly ^{15}N-labeled TnC C-lobe complexed with TnIpep. Each [Ca^{2+}]/[TnC C-lobe complexed with TnIpep] ratio is marked at the top left of the panel. The resonances of some residues are as marked on the spectra.

Acknowledgments

We express our sincere thanks to Professor S. Ebashi for his constant encouragement. We are grateful to Dr. I. Ohtsuki for his continuous encouragement of this work. This work was supported by the 21st Century Center of Excellence Program and by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References

[1] S. Ebashi, F. Ebashi, A new protein component participating in the superprecipitation of myosin B, J. Biochem. (Tokyo) 55 (1964) 604–613.
[2] S. Ebashi, A. Kodama, A new protein factor promoting aggregation of tropomyosin, J. Biochem. (Tokyo) 58 (1965) 107–108.
[3] I. Ohtsuki, K. Maruyama, S. Ebashi, Regulatory and cytoskeletal proteins of vertebrate skeletal muscle, Adv. Protein Chem. 38 (1986) 1–68.
[4] D.J. Hartshorne, S.V. Perry, M.C. Schaub, A protein factor inhibiting the magnesium-activated adenosine triphosphatase of desensitized actomyosin, Biochem. J. 104 (1967) 907–913.
[5] S. Ebashi, T. Wakabayashi, F. Ebashi, Troponin and its components, J. Biochem. 69 (1971) 441–445.
[6] M.L. Greaser, J. Gergely, Reconstitution of troponin activity from three protein components, J. Biol. Chem. 246 (1971) 4226–4233.
[7] A.S. Zot, J.D. Potter, Structural aspects of troponin–tropomyosin regulation of skeletal muscle contraction, Ann. Rev. Biophys. Chem. 16 (1987) 535–539.
[8] J.H. Collins, J.D. Potter, M.J. Horn, G. Wilshire, N. Jackman, The amino acid sequence of rabbit skeletal muscle troponin C: gene replication and homology with calcium-binding proteins from carp and hake muscle, FEBS Lett. 36 (1973) 268–272.
[9] J.M. Wilkinson, Troponin C from rabbit slow skeletal and cardiac muscle is the product of a single gene, Eur. J. Biochem. 103 (1980) 179–188.
[10] J.P. van Eerd, K. Takahashi, Determination of the complete amino acid sequence of bovine cardiac troponin C, Biochemistry 15 (1976) 1171–1180.
[11] J.D. Potter, J. Gergely, The calcium and magnesium binding sites on troponin and their role in the regulation of myofibrillar adenosine triphosphatase, J. Biol. Chem. 250 (1975) 4628–4633.
[12] J. Kendrick-Jones, W. Lehman, A.G. Szent-Györgyi, Regulation in molluscan muscles, J. Mol. Biol. 54 (1970) 313–326.
[13] A.G. Szent-Györgyi, Regulation of contraction by calcium binding myosins, Biophys. Chem. 59 (1996) 357–363.
[14] W. Lehman, J.F. Head, P.W. Grant, The stoichiometry and location of troponin I- and troponin C-like proteins in the myofibril of the bay scallop, *Aequipecten irradians*, Biochem. J. 171 (1980) 413–418.
[15] T. Ojima, K. Nishita, Troponin from Akazara scallop striated adductor muscles, J. Biol. Chem. 261 (1986) 16749–16754.
[16] K. Nishita, H. Tanaka, T. Ojima, Amino acid sequence of troponin C from scallop striated adductor muscle, J. Biol. Chem. 269 (1994) 3464–3468.

[17] T. Ojima, T. Maita, A. Inoue, K. Nishita, Bacterial expression, purification, and characterization of Akazara scallop troponin C, Fish. Sci. 63 (1997) 137–141.
[18] H. Tanaka, Y. Takeya, T. Doi, F. Yumoto, M. Tanokura, I. Ohtsuki, K. Nishita, T. Ojima, Comparative studies on the functional roles of N- and C-terminal regions of molluskan and vertebrate troponin-I, FEBS J. 17 (2005) 4475–4486.
[19] T. Ojima, N. Koizumi, K. Ueyama, A. Inoue, K. Nishita, Functional role of Ca^{2+}-binding site IV of scallop troponin C, J. Biochem. 128 (2000) 803–809.
[20] H. Tanaka, T. Ojima, K. Nishita, Amino acid sequence of troponin-I from Akazara scallop striated adductor muscle, J. Biochem. 124 (1998) 304–310.
[21] O. Herzberg, M.N.G. James, Structure of the calcium regulatory muscle protein troponin-C at 2.8 Å resolution, Nature 313 (1985) 653–659.
[22] M. Sundaralingam, R. Bergstrom, G. Strasburg, S.T. Rao, P. Roychowdhury, M. Greaser, B.C. Wang, Molecular structure of troponin C from chicken skeletal muscle at 3-angstrom resolution, Science 227 (1985) 945–948.
[23] S.M. Gagné, S. Tsuda, M.X. Li, L.B. Smillie, B.D. Sykes, Structures of the troponin C regulatory domains in the apo and calcium-saturated states, Nat. Struct. Biol. 9 (1995) 784–789.
[24] D.G. Vassylyev, S. Takeda, S. Wakatsuki, K. Maeda, Y. Maéda, Crystal structure of troponin C in complex with troponin I fragment at 2.3 Å resolution, Proc. Natl. Acad. Sci. USA 95 (1998) 4847–4852.
[25] S. Takeda, A. Yamashita, K. Maeda, Y. Maéda, Structure of the core domain of human cardiac troponin in the Ca^{2+}-saturated form, Nature 424 (2003) 35–41.
[26] N.L. Finley, J.W. Howarth, P.R. Rosevear, Structure of the Mg^{2+}-loaded C-lobe of cardiac troponin C bound to the N-domain of cardiac troponin I: comparison with the Ca^{2+}-loaded structure, Biochemistry 43 (2004) 11371–11379.
[27] M.V. Vinogradova, D.B. Stone, G.G. Malanina, C. Karatzaferi, R. Cooke, R.A. Mendelson, R.J. Fletterick, Ca^{2+}-regulated structural changes in troponin, Proc. Natl. Acad. Sci. USA 102 (2005) 5038–5043.
[28] K. Murakami, F. Yumoto, S.-y. Ohki, T. Yasunaga, M. Tanokura, T. Wakabayashi, Structural basis for Ca^{2+}-regulated muscle relaxation at interaction sites of troponin with actin and tropomyosin, J. Mol. Biol. 362 (2005) 178–201.
[29] F. Yumoto, M. Nara, H. Kagi, W. Iwasaki, T. Ojima, K. Nishita, K. Nagata, M. Tanokura, Coordination structures of Ca^{2+} and Mg^{2+} in Akazara scallop troponin C in solution. FTIR spectroscopy of side-chain COO-groups, Eur. J. Biochem. 268 (2001) 6284–6290.
[30] F. Yumoto, K. Nagata, K. Adachi, N. Nemoto, T. Ojima, K. Nishita, I. Ohtsuki, M. Tanokura, NMR structural study of troponin C C-terminal domain complexed with troponin I fragment from Akazara scallop, Adv. Exp. Med. Biol. 538 (2003) 195–201.
[31] F. Yumoto, K. Nagata, Y. Miyauchi, T. Ojima, H. Tanaka, K. Nishita, I. Ohtsuki, M. Tanokura, Crystallization and preliminary X-ray analysis of the Ca^{2+}-bound C-terminal lobe of troponin C in complex with a troponin I-derived peptide fragment from Akazara scallop, Acta Crystallogr. F 63 (2007) 535–537.
[32] G. De Nicola, C. Burkart, F. Qiu, B. Agianian, S. Labeit, S. Martin, B. Bullard, A. Pastore, The structure of Lethocerus troponin C: insights into the mechanism of stretch activation in muscles, Structure 7 (2007) 813–824.
[33] M. Tanokura, K. Yamada, Heat capacity and entropy changes of calmodulin induced by calcium binding, J. Biochem. 95 (1984) 643–649.
[34] M. Cai, Y. Huang, K. Sakaguchi, G.M. Clore, A.M. Gronenborn, R. Craigie, An efficient and cost-effective isotope labeling protocol for proteins expressed in Escherichia coli, J. Biomol. NMR 11 (1998) 97–102.
[35] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A. Bax, NMRPipe: a multidimensional spectral processing system based on UNIX pipes, J. Biomol. NMR 6 (1995) 277–293.
[36] G.A. Krudy, R.M. Brito, J.A. Putkey, P.R. Rosevear, Conformational changes in the metal-binding sites of cardiac troponin C induced by calcium binding, Biochemistry 31 (1992) 1595–1602.
[37] N.C. Strynadka, M.N. James, Crystal structures of the helix–loop–helix calcium-binding proteins, Annu. Rev. Biochem. 58 (1989) 951–998.
[38] R.H. Kretsinger, C.E. Nockolds, Carp muscle calcium-binding protein. II. Structure determination and general description, J. Biol. Chem. 248 (1973) 3313–3326.
[39] L. Spyracopoulos, M.X. Li, S.K. Sia, S.M. Gagné, M. Chandra, R.J. Solaro, B.D. Sykes, Calcium-induced structural transition in the regulatory domain of human cardiac troponin C, Biochemistry 36 (1997) 12138–12146.