J. Biochem. 124, 304-310 (1998)

Amino Acid Sequence of Troponin-I from Akazara Scallop Striated Adductor Muscle[1]

Hiroyuki Tanaka, Takao Ojima, and Kiyoyoshi Nishita[2]

Department of Marine Bioresources Chemistry, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido 041-8611

Received for publication, February 9, 1998

The complete amino acid sequence of Akazara scallop, *Chlamys nipponensis akazara*, troponin-I was determined by automated Edman degradation. It is composed of 292 amino acid residues with a blocked N-terminus. The M_r is calculated to be 34,678, about 14,000 larger than that of vertebrate skeletal troponin-I but significantly smaller than the 52,000 that had been estimated by SDS-polyacrylamide gel electrophoresis. The homologous sequence to vertebrate and *arthropoda* troponin-Is is found in the C-terminal region. In particular, the sequence of the regions essential for binding to actin and troponin-C is highly conserved. On the other hand, Akazara scallop troponin-I has 100-133 extra residues at the N-terminus compared with vertebrate troponin-I. This extra region is rich in Glu and Arg and has a unique sequence, that shows in part a high sequence homology with the tropomyosin-binding site of troponin-T and caldesmon.

Key words: amino acid sequence, Ca^{2+}-regulation, Edman degradation, scallop, troponin I.

Troponin is a Ca^{2+}-dependent regulatory protein of muscle contraction (*1*). It is a complex of three distinct subunits: a Ca^{2+}-binding subunit, troponin C (TnC); a tropomyosin-binding subunit, troponin T (TnT); and an inhibitory subunit of actin-myosin interaction, troponin I (TnI). In the early 1970s, molluscan muscles were believed to be regulated only by a myosin-linked regulatory system (*2-4*). However, TnI- and TnC-like proteins were isolated from the striated adductor muscle of scallop (*5, 6*), suggesting the presence of troponin. Subsequently, we have isolated troponin from Akazara scallop and Ezo giant scallop striated and smooth adductor muscles (*7-10*). These scallop troponins and their subunits revealed somewhat different biochemical properties from vertebrate troponins. For instance, the troponin plays a regulatory role by enhancing the actomyosin Mg-ATPase activity in the presence of Ca^{2+} (*7*). In addition, the TnC binds less Ca^{2+} (1 mol Ca^{2+} per mol) (*7, 11*) than vertebrate fast skeletal TnCs (4 mol Ca^{2+} per mol) (*12*). Thus, the hybridized troponin of Akazara scallop TnC with vertebrate TnI and TnT shows no ability to regulate the actomyosin Mg-ATPase activity together with tropomyosin (*13*). Moreover, two types of scallop TnI showing M_r of 52,000 and 19,000 on SDS-PAGE are present (*7, 14, 15*). It is noteworthy that the former is more than twice as large as vertebrate TnIs. It is therefore important to clarify the amino acid sequence of scallop troponin subunits for better understanding of the molecular mechanisms of regulation.

Recently, we determined the amino acid sequence of Akazara scallop and Ezo giant scallop TnCs (*16, 17*). The sequences show that these TnCs bind only one Ca^{2+}, at the site IV located in the C-terminal region, in contrast to the four Ca^{2+} ions bound at sites I-IV in vertebrate fast skeletal TnC (*12*), where Ca^{2+}-binding to sites I and II in the N-terminal half of the molecule has been considered to be essential for the regulation (*18-20*). Subsequently, Akazara scallop and Ezo giant scallop TnTs were sequenced (*21, 22*). Both have shorter N-termini by 27 residues and longer C-termini by 78 and 83 residues, respectively, than the rabbit skeletal counterpart.

We previously reported that digestion of Akazara scallop M_r 52,000 TnI with CNBr yielded two major fragments, the N-terminal 35-kDa fragment (CN35K) and the C-terminal 17-kDa fragment (CN17K), and two minor fragments, CN47K and CN5K. CN17K inhibited the actomyosin Mg-ATPase activity along with tropomyosin, while CN35K did not (*23*). We also determined the amino acid sequence of the CN17K and found that it has a considerably high sequence homology to the vertebrate TnI (*24*).

Here, we describe the determination of the amino acid sequence of the CN35K and the complete amino acid sequence of Akazara scallop TnI.

MATERIALS AND METHODS

Preparation of the CNBr Fragments of Akazara Scallop TnI—TnI was prepared from the striated adductor muscle of Akazara scallop according to the method of Ojima and Nishita (*7, 25*), and subsequently carboxymethylated. The modified TnI was digested with 10,000-fold molar excess of CNBr in 70% formic acid at 25°C for 24 h. The digests were separated into CN17K, CN35K, CN47K, and CN5K fragments by chromatography on DEAE-Toyopearl 650M column (22 mm × 150 mm) as reported previously (*23*).

[1] This study was financially supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.
[2] To whom correspondence should be addressed.
Abbreviations: TnC, troponin-C; TnT, troponin-T; TnI, troponin-I.

Enzymatic Digestion of CN35K—CN35K was digested with either 1:120 (w/w) lysylendopeptidase (Wako Pure Chem.) in 10 mM Tris-HCl (pH 9.0) at 37°C for 2 h, 1:60 (w/w) *Staphylococcus aureus* V8 protease (Pierce Chem.) in 0.1 M NH_4HCO_3 (pH 8.0) at 30°C for 1.5 h, or 1 : 400 (w/w) α-chymotrypsin (Sigma Chem.) in 0.1 M NH_4HCO_3 (pH 8.0) at 37°C for 1 h.

Phenylthiocarbamylation and Subsequent Tryptic Digestion—To restrict the tryptic digestion to the Arg-X peptide bond, free amino groups of TnI were phenylthiocarbamylated. Akazara scallop TnI (20 nmol) was dissolved in 100 μl of 60% pyridine and 0.4 M dimethylallylamine (pH 9.5), then 5 μl of phenylisothiocyanate was added. The mixture was incubated at 55°C for 20 min, and excess reagents were removed by extraction three times with 500 μl of benzene. The aqueous phase containing phenylthiocarbamylated TnI was lyophilized, then digested with 1 : 50 (w/w) trypsin (Sigma Chem.) in 1.6 M urea and 0.1 M NH_4HCO_3 (pH 8.0) at 37°C for 17 h.

Separation of Peptides by HPLC—Peptide fragments were separated by HPLC with a TSK-gel ODS-120T column (4.6 mm × 250 mm, Tosoh). Elution was performed with a linear acetonitrile gradient from 0 to 60% in 0.1% trifluoroacetic acid in a total volume of 60 or 100 ml at a flow rate of 1.0 ml/min. The effluents were monitored by absorbance at 214 nm.

Analysis of Amino Acid Composition and Sequence—Akazara scallop TnI and peptide fragments (0.2 to 6.6 nmol) were hydrolyzed with 6 N HCl at 110°C for 24 h *in vacuo*, and amino acids produced were phenylthiocarbamylated using a PICO-TAG work station (Waters). The phenylthiocarbamylated amino acids were analyzed by HPLC on a PICO-TAG column (3.9 mm × 150 mm, Waters). The amino acid sequence was determined using a model 473A protein sequencer (Perkin Elmer).

RESULTS

The sequencing data for Akazara scallop TnI is summarized in Fig. 1. The chromatographic separations of peptides and amino acid compositions are shown in Fig. 2 and Table I, respectively.

Determination of the Amino Acid Sequence of CN35K—As reported previously, CN35K, the N-terminal CNBr-fragment of Akazara scallop TnI, has a blocked N-terminus (*23*). Thus, it was digested with lysylendopeptidase to yield two fragments, CN35K-L1 and CN35K-L2 (Fig. 2A). Of these, CN35K-L1 has the blocked N-terminus, and thus the deblocking method of Wellner *et al.* (*26*) was applied, which is known to remove the acetyl group from an *N*-acetylated peptide. As a result, a sequence consisting of Ser and 12 subsequent residues was determined, suggesting that the blocking group is an acetyl group. On the other hand, the N-terminal 34 residues of CN35K-L2 were directly sequenced.

CN35K was also digested limitedly with V8 protease to yield 12 fragments, named CN35K-V1 to CN35K-V12 (Fig. 2B). Based on the sequencing data of these fragments, CN35K-V3 was found to overlap CN35K-L1 and CN35K-L2, while CN35K-L2 overlaps the N-terminal region of CN35K-V7 and CN35K-V12, which possess identical N-termini. The fragments other than CN35K-V11 were sequenced as summarized in Fig. 1. CN35K-V11 appeared to be a mixture of two fragments whose N-terminal 10 residues were the same as those of the CN35K-V12 and CN35K-V10. However, the separation of the mixture was unsuccessful.

Next, CN35K was digested with α-chymotrypsin to yield nine fragments, CN35K-CT1 to CN35K-CT9 (Fig. 2C). CN35K-CT7 possesses overlapping regions with CN35K-V7, CN35K-V12, and CN35K-V9. In addition, CN35K-CT5, containing homoserine at the C-terminus, overlapped CN35K-V9. Thus, the complete sequence of the CN35K

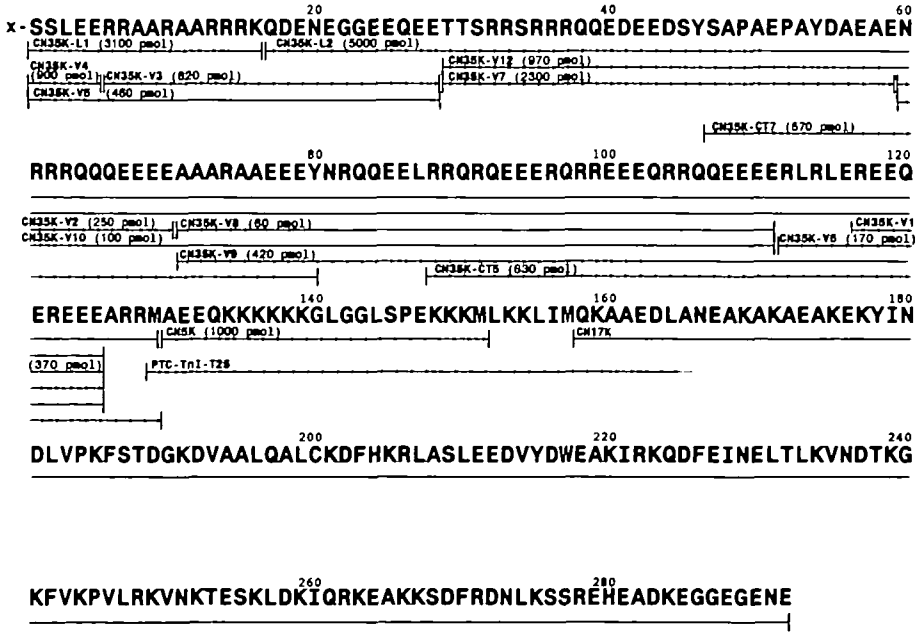


Fig. 1. **Summary of sequencing data of Akazara scallop TnI.** Sequences were determined by an automated sequencer as indicated by arrows (→). Each fragment is named in the text. The amounts of peptides applied on sequencer are indicated in parentheses. The sequences of CN35K-L1, CN35K-V4, and CN35K-V5 were analyzed after N-terminal deblocking treatment by the method of Wellner *et al.* (*26*). The detailed sequencing data of CN17K were described previously (*24*).

was determined, and its M_r was calculated to be 16,034.

Sequence Analysis of CN5K and CN47K—CN5K, consisting of 22 residues, was purified by HPLC and sequenced completely. It possesses an unblocked N-terminus and a homoserine residue at the C-terminus. Therefore, CN5K seemed to originate from the region between CN35K and the C-terminal CN17K. The sequence of CN17K was reported previously (*24*).

Another fragment, CN47K, was also purified by HPLC and subjected to sequence analysis. Like CN35K, however, its N-terminus was blocked, suggesting that CN47K originated from the N-terminus of TnI. CN47K showed similar contents of Glx and Arg and higher contents of Lys and Gly compared with CN35K. In addition, CN47K contained a small amount of Met (Table I). CN47K gave three fragments, CN47K-L1, CN47K-L2, and CN47K-L3, on digestion with lysylendopeptidase (data not shown). The N-terminal sequence of CN47K-L1 was determined to be SSLEE after deblocking treatment, which agreed with that of CN35K-L1. CN47K-L3 showed the N-terminal sequence

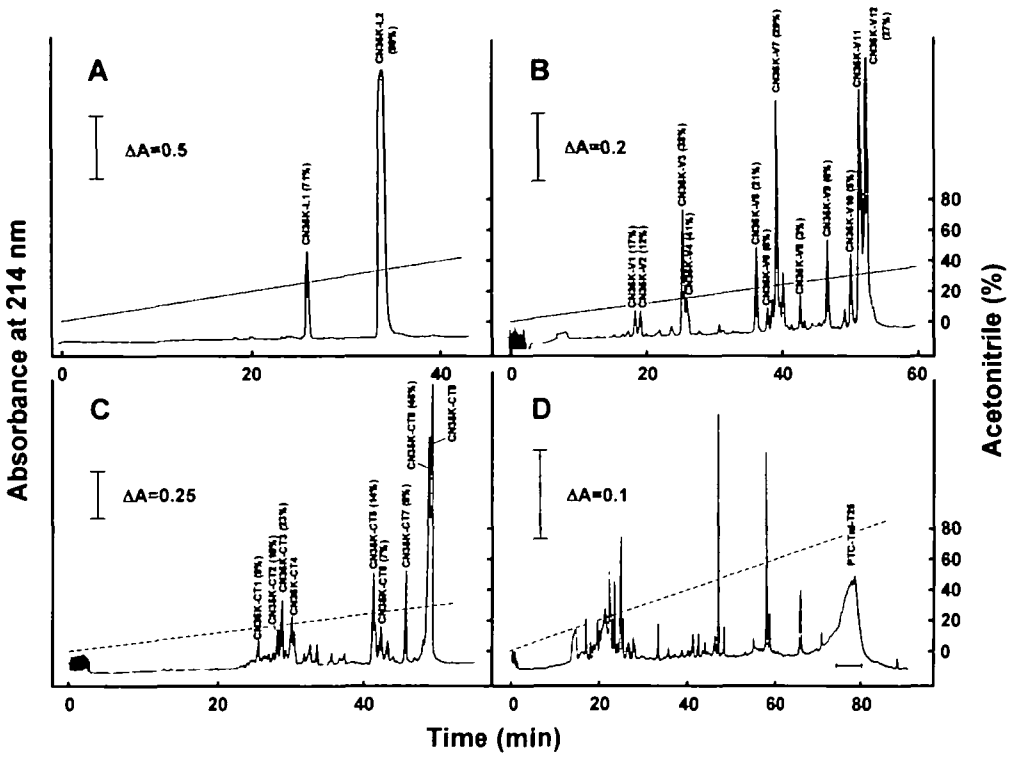


Fig. 2. **Separation of protease digests of TnI by HPLC.** A, lysylendopeptidase digests of CN35K (42 nmol); B, V8 protease digests of CN35K (22 nmol); C, α-chymotryptic digests of CN35K (22 nmol); D, tryptic digests of the phenylthiocarbamylated Akazara scallop TnI (6.9 nmol). The fraction of PTC-TnI-T25 is indicated by a bar (⊢⊣). The recoveries (mol %) of peptides estimated by amino acid analysis are shown in parentheses. The values for CN35KV-11, CN35K-CT4, and CN35K-CT9 could not be determined because of the heterogeneity of these fractions. Dashed lines indicate the linear gradient of acetonitrile concentration.

TABLE I. **Amino acid compositions of Akazara scallop TnI and its fragments.**[a]

Amino acid	TnI	CN35K	CN17K	CN5K	CN47K	CN35K-L1	CN35K-L2	CN35K-V3	CN35K-V9	CN35K-V12	CN35K-CT5	CN35K-CT7
Asx	27.1 (27)	6.8 (7)	18.3 (20)		7.1 (7)		6.9 (7)	1.9 (2)	1.0 (1)	4.8 (5)		1.2 (2)
Glx	81.5 (81)	55.3 (56)	21.9 (21)	3.7 (4)	59.8 (60)	2.2 (2)	53.6 (54)	8.4 (8)	31.1 (31)	46.1 (46)	24.5 (24)	13.0 (13)
Cmc[b]	+ (1)		+ (1)									
Ser	12.5 (13)	5.3 (6)	5.9 (6)	0.9 (1)	6.0 (7)	1.7 (2)	3.7 (4)			3.3 (4)		0.7 (1)
Gly	10.7 (10)	2.1 (2)	5.6 (5)	3.1 (3)	4.8 (5)		2.1 (2)	2.1 (2)				
His	1.9 (2)		2.0 (2)									
Hse[c]		+ (1)		+ (1)	+ (1)		+ (1)				+ (1)	
Arg	36.5 (36)	31.4 (30)	5.8 (6)		31.3 (30)	6.0 (6)	24.9 (24)	5.5 (6)	13.6 (14)	22.3 (22)	13.1 (14)	3.6 (4)
Thr	5.9 (6)	1.1 (2)	3.7 (4)		0.9 (2)		1.0 (2)			1.2 (2)		
Ala	29.6 (30)	15.1 (15)	14.1 (14)	1.1 (1)	15.3 (16)	4.0 (4)	11.4 (11)	3.9 (4)	5.5 (5)	9.8 (10)	1.3 (1)	10.4 (10)
Pro	5.1 (5)	1.9 (2)	2.4 (2)	0.7 (1)	2.7 (3)		1.9 (2)			1.8 (2)		2.0 (2)
Tyr	5.2 (5)	3.1 (3)	2.0 (2)		3.0 (3)		3.1 (3)		0.9 (1)	2.4 (3)		1.6 (2)
Val	6.8 (7)		7.1 (7)									
Met	2.8 (3)				0.5 (1)							
Ile	4.9 (5)		4.2 (4)									
Leu	19.1 (19)	3.7 (4)	11.1 (11)	2.3 (2)	5.4 (6)	0.9 (1)	2.8 (3)		2.6 (3)	2.7 (3)	2.1 (2)	
Phe	5.0 (5)		4.9 (5)									
Trp[d]	(1)		(1)									
Lys	34.5 (36)	1.1 (1)	22.9 (24)	9.0 (9)	9.4 (10)	1.0 (1)		1.0 (1)				
Total	292	129	135	22	151	16	113	23	55	97	42	34
Position	1-292	1-129	158-292	130-151	1-151	1-16	17-129	6-28	71-125	29-125	88-129	47-80

[a]For the protease fragments of CN35K, only the data needed to establish the sequence are shown. The values in parentheses are the number of residues determined by sequencing. [b]Carboxymethylcysteine. [c]Homoserine. [d]Decomposed during hydrolysis with 6 N HCl.

QDENEGGEEQEETT, identical with that of CN35K-L2. CN47K-L2 was sequenced as GLGGLSPEK, which is identical with the sequence of residues 11-19 of CN5K. Thus, it was concluded that CN47K corresponds to the peptide CN35K-CN5K and arose through incomplete CNBr cleavage.

Amino Acid Sequence of Akazara Scallop TnI—To determine the sequence of the region connecting CN47K and CN17K, Akazara scallop TnI was phenylthiocarbamylated and digested with trypsin. An overlapping peptide named PTC-TnI-T25 was isolated from the digests by HPLC (Fig. 2D) and its N-terminal 36 residues were sequenced. The sequence corresponds to that of the region from the C-terminus of CN35K to the N-terminal seventh residue of CN17K. Accordingly, the CNBr fragments were aligned as CN35K-CN5K-LKKLIM-CN17K. Moreover, the small peptide showing the sequence LKKLIM was obtained when the whole CNBr digest of the TnI was directly applied to HPLC (data not shown). It should be noted that only one overlapping residue, Met-129, between CN35K and CN5K was obtained from PTC-TnI-T25 (Fig. 1). However, the linkage of CN35K and CN5K was confirmed by the sequence of another fragment of residues 114-134, which was prepared by digestion of TnI with lysylendopeptidase and α-chymotrypsin (data not shown).

Accordingly, complete amino acid sequence of Akazara scallop TnI was determined as in Fig. 1.

DISCUSSION

Akazara scallop TnI is composed of 292 amino acid residues and has a blocked N-terminus, probably by an acetyl group. As reported previously (*24*), Akazara scallop TnI possesses sequence homology to various species of TnI in the C-terminal region of residues 134-292. On the other hand, the N-terminal residues 1-133 are not found in vertebrate skeletal TnI. This extra region is extremely rich in Glu and Arg and shows almost no similarity with other TnIs (Fig. 3). The molecular weight of Akazara scallop TnI is calculated to be 34,678, which is approximately 6,000-14,000 larger than those of other species of TnI. However, it is considerably smaller than the value of 52,000 that was estimated previously by SDS-PAGE (*7, 14*). This slower mobility may be attributable to the presence of many acidic and basic residues in the N-terminal region, as seen in the case of caldesmon (*33, 34*), calpastatin (*35*), and some species of virus coat protein (*36*).

The sequence of the C-terminal region of residues 134-292 (all residue numbers hereafter are based on the Akazara scallop TnI sequence) of Akazara scallop TnI exhibits 39% homology with *arthropoda* TnIs and 26-30% with vertebrate ones. Residues 138-179, 211-231, 235-250, and 269-285 of the Akazara scallop TnI are highly conserved in many TnIs (Fig. 3). In particular, residues 235-250 show the highest homology, with significant overlapping with residues 240-251 of rabbit skeletal TnI,

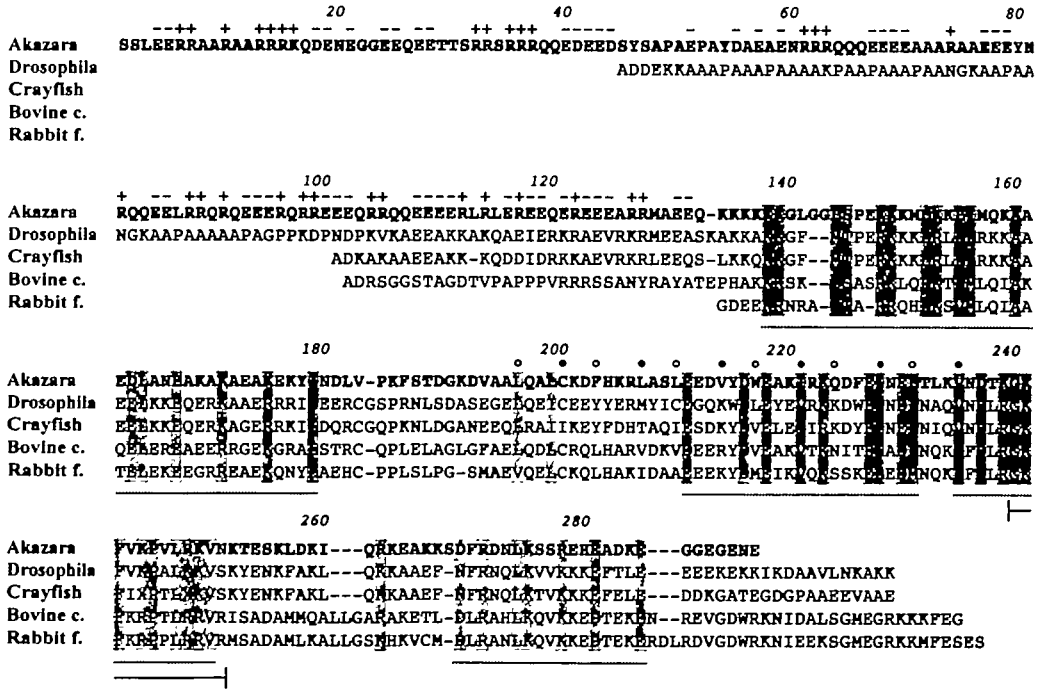


Fig. 3. **Alignment of the amino acid sequences of various TnIs.** Akazara, Akazara scallop striated adductor muscle TnI (present paper); Drosophila, *Drosophila melanogaster* TnI (sequencing of cDNA clone A16) (*27*); Crayfish, crayfish tail muscle TnI (*28*); Bovine c., bovine cardiac muscle TnI (*29*); Rabbit f., rabbit fast skeletal muscle TnI (*30-32*). Each sequence was aligned based on the similarity in the region corresponding to residues 134-292 of the Akazara scallop TnI. The letter X in the crayfish sequence represents a trimethyllysine. Residues conserved in all these five TnIs are boxed and shaded. The conserved repetitive pattern of hydrophobic residues is indicated by open and closed circles. The positions of acidic and basic residues in the N-terminal region (residues 1-133) of Akazara scallop TnI are indicated by − and +, respectively. Dotted lines indicate the highly conserved regions. A predicted actin-binding region is indicated by a solid bar (⊢⊣).

Fig. 4. **Sequence comparison of residues 76-115 of Akazara scallop TnI with chicken gizzard caldesmon and rabbit skeletal TnT.** Residues 76-115 of Akazara scallop TnI were compared with those of corresponding regions of the caldesmon and TnT. Identical amino acids are marked by double dots and conservative substitutions by single dots. The alternating charge motif is emphasized by outlining the acidic and basic amino acid blocks.

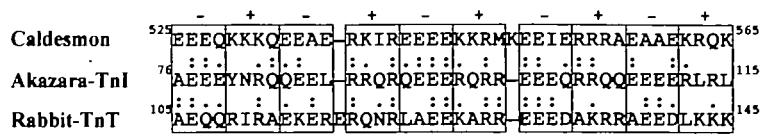
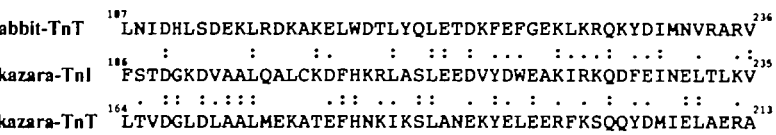


Fig. 5. **Sequence similarity of residues 186-235 of Akazara scallop TnI with TnTs.** Residues 186-235 of Akazara scallop TnI were compared with a segment of rabbit skeletal TnT (*47*) and Akazara scallop TnT (*21*). Identical amino acids are marked by double dots and conservative substitutions by single dots. Calculated sequence identities for rabbit skeletal and Akazara scallop TnTs are 27 and 30%, respectively.



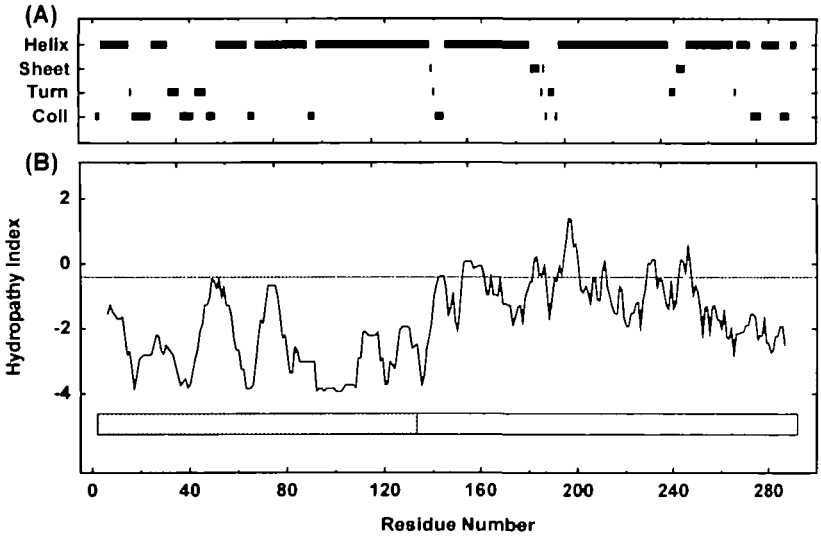


Fig. 6. **Predicted secondary structure and hydropathy plot of Akazara scallop TnI.** (A) Secondary structure prediction by the method of Garnier *et al.* (*53*). The predicted conformations are indicated by bars. (B) Hydropathy plot prepared by the method of Kyte and Doolittle (*54*) with a span setting of nine residues. The additional N-terminal region (residues 1-133) and the homologous region to other species of TnI (residues 134-292) are indicated by hatched and open boxes, respectively.

which is regarded as an actin- and TnC-binding site (*37-39*). Among these residues, Lys-243, Arg-244, Pro-246, Arg-249, and Arg-251 of rabbit skeletal TnI are replaced by Val, Lys, Val, Lys, and Asn, respectively, in Akazara scallop TnI. These replacements lead to a decrease in positive charges in this region. Since the ionic interaction of this positively charged region and negatively charged residues of actin is thought to participate in the binding of TnI and actin (*40*), these substitutions may be responsible in part for the weaker inhibition of the Mg-ATPase activity of rabbit actomyosin-tropomyosin by Akazara scallop TnI than by rabbit TnI (*14*). Another conserved region comprises residues 138-179, which is comparable to the region required for interaction with TnC irrespective of the presence or absence of Ca^{2+} and for the structural integrity of the troponin complex (*32, 37, 41*). In addition, residues 211-231 showed overlapping with residues 176-226 of rabbit skeletal TnI, a region assumed to interact with TnT (*42, 43*). Interestingly, the heptad hydrophobic repeat typical of the α-helical coiled coil is conserved around residues 211-231 (Fig. 3), suggesting that the coiled coil structure is involved in the interaction between TnI and TnT. In many TnIs, the sequence of residues 269-285 is highly conserved (*28*), though its function is still obscure. Farah *et al.* indicated that the C-terminal 67 residues including this region of chicken fast skeletal TnI are important for Mg-ATPase inhibition (*41*). Thus, the regions required for TnI function are highly conserved in the C-terminal portion of Akazara scallop TnI.

On the contrary, residues 1-133 showed an unusual sequence with alternating Glu-rich and Arg-rich blocks (Fig. 3). Vertebrate cardiac and *arthropoda* TnIs possess regions longer by 20 to 83 residues at the N-terminus than vertebrate skeletal TnI (*27-29*). Phosphorylation of Ser in this region of vertebrate cardiac TnI is involved in modulation of troponin function (*44-46*), although the role of these residues of *arthropoda* TnI is unknown. Scanning the sequence of Akazara scallop TnI for patterns stored in the PROSITE database revealed the existence of three potential phosphorylation sites (Thr-30, Ser-31, and Ser-34) for protein kinase C, two sites (Ser-1 and Ser-2) for casein kinase II, and one site (Tyr-80) for tyrosine kinase in the N-terminal region, though it is uncertain whether the residues are phosphorylated or not. At the same time, the N-terminal region of Akazara scallop TnI is considerably larger than those of the cardiac and *arthropoda* TnIs, with which it has sequence homology as low as 20%.

On the other hand, a search of the SWISS-PROT database revealed that residues 76-115 of Akazara scallop TnI have high homology to residues 525-565 of chicken gizzard caldesmon (*33*) and residues 105-145 of rabbit skeletal TnT (*47*) (Fig. 4). Although the sequence identity is not so

high (37-39%), these three proteins have a characteristic charge distribution, *i.e.*, an arrangement of alternating acidic and basic blocks of four residues. The region of rabbit skeletal TnT is a part of residues 71-151, the so-called CB2 fragment, which has been considered to bind to tropomyosin (*48, 49*). Chicken gizzard caldesmon is also assumed to possess a tropomyosin-binding region at residues 508-565, judging from the sequence similarity with the CB2 fragment (*33*). Huber *et al.* suggested that this region has little affinity for smooth muscle tropomyosin but high affinity for the skeletal counterpart (*50*). Consequently, residues 76-115 of Akazara scallop TnI may play a role in its binding to tropomyosin. However, we could not prove the interaction between CN35K (residues 1-129) and actin-tropomyosin complex by means of cosedimentation (data not shown).

The sequence similarity among the above regions of Akazara scallop TnI, rabbit TnT, and chicken gizzard caldesmon may reflect their molecular evolutionary relationship. This is supported by the functional consideration that caldesmon is an actin-linked regulatory protein of smooth muscle and possesses both TnI- and TnT-like functions in that it has the abilities to inhibit strongly actomyosin Mg-ATPase activity, to neutralize this inhibition together with Ca^{2+}-binding protein, and to bind tropomyosin (*51, 52*). Moreover, Akazara scallop TnI shows sequence similarity to Akazara scallop and rabbit TnTs at not only residues 76-115 but also residues 186-235 of the C-terminal region, where it shows high sequence homology to various TnIs (Fig. 5).

According to the method of secondary structure prediction of Garnier *et al.* (*53*), the sequence of the N-terminal region, especially residues 51-133, of Akazara scallop TnI shows high α-helix content as seen in Fig. 6. The arrangement of alternating acidic and basic blocks in the region of residues 76-115 mentioned above might stabilize an α-helix through intrachain ionic interactions between oppositely charged residues four positions apart. On the other hand, the hydropathy plot prepared by the method of Kyte and Doolittle (*54*) indicates that the N-terminal region is very hydrophilic compared with the C-terminal 19 K region, which has considerably high homology to other TnIs (Fig. 6).

We are now interested in the functions of the N-terminal region, in particular whether its unique amino acid sequence is related to the dual regulatory mechanisms specific to molluscan muscle.

REFERENCES

1. Ebashi, S. and Kodama, A. (1965) A new protein factor promoting aggregation of tropomyosin. *J. Biochem.* **58**, 107-108
2. Kendrick-Jones, J., Lehman, W., and Szent-Györgyi, A.G. (1970) Regulation in molluscan muscles. *J. Mol. Biol.* **54**, 313-326
3. Szent-Györgyi, A.G., Szentkiralyi, E.M., and Kendrick-Jones, J. (1973) The light chains of scallop myosin as regulatory subunits. *J. Mol. Biol.* **74**, 179-203
4. Lehman, W. and Szent-Györgyi, A.G. (1975) Regulation of muscular contraction. Distribution of actin control and myosin control in the animal kingdom. *J. Gen. Physiol.* **66**, 1-30
5. Goldberg, A. and Lehman, W. (1978) Troponin-like proteins from muscles of the scallop, *Aequipecten irradians*. *Biochem. J.* **171**, 413-418
6. Lehman, W., Head, J.F., and Grant, P.W. (1980) The stoichiometry and location of troponin I- and troponin C-like proteins in the myofibril of the bay scallop, *Aequipecten irradians*. *Biochem. J.* **187**, 447-456
7. Ojima, T. and Nishita, K. (1986) Troponin from Akazara scallop striated adductor muscles. *J. Biol. Chem.* **261**, 16749-16754
8. Ojima, T. and Nishita, K. (1986) Isolation of troponins from striated and smooth adductor muscles of Akazara scallop. *J. Biochem.* **100**, 821-824
9. Nishita, K., Ojima, T., Takahashi, A., and Inoue, A. (1997) Troponin from smooth adductor muscle of Ezo-giant scallop. *J. Biochem.* **121**, 419-424
10. Ojima, T. and Nishita, K. (1992) Comparative studies on biochemical characteristics of troponins from Ezo-giant scallop (*Patinopecten yessoensis*) and Akazara scallop (*Chlamys nipponensis akazara*). *Comp. Biochem. Physiol.* **103B**, 727-732
11. Shima, Y., Tsuchiya, T., Lehman, W., and Matsumoto, J.J. (1984) The characterization of invertebrate troponin C. *Comp. Biochem. Physiol.* **79B**, 525-529
12. Potter, J.D. and Gergely, J. (1975) The calcium and magnesium binding sites on troponin and their role in the regulation of myofibrillar adenosine triphosphatase. *J. Biol. Chem.* **250**, 4628-4633
13. Ojima, T. and Nishita, K. (1992) Akazara scallop troponin C: Ca^{2+}-induced conformational change and interaction with rabbit troponin subunits. *Arch. Biochem. Biophys.* **299**, 344-349
14. Ojima, T. and Nishita, K. (1988) Biochemical characteristics of the M_r 52,000 component of Akazara scallop troponin. *J. Biochem.* **104**, 207-210
15. Ojima, T. and Nishita, K. (1991) A binary complex of troponin-I and troponin-T from Akazara scallop striated adductor muscle. *J. Biochem.* **110**, 847-850
16. Ojima, T., Tanaka, H., and Nishita, K. (1994) Cloning and sequence of a cDNA encoding Akazara scallop troponin C. *Arch. Biochem. Biophys.* **311**, 272-276
17. Nishita, K., Tanaka, H., and Ojima, T. (1994) Amino acid sequence of troponin C from scallop striated adductor muscle. *J. Biol. Chem.* **269**, 3464-3468
18. Johnson, J.D., Charlton, S.C., and Potter, J.D. (1979) A fluorescence stopped flow analysis of Ca^{2+} exchange with troponin C. *J. Biol. Chem.* **254**, 3497-3502
19. Robertson, S.P., Johnson, J.D., and Potter, J.D. (1981) The time-course of Ca^{2+} exchange with calmodulin, troponin, parvalbumin, and myosin in response to transient increases in Ca^{2+}. *Biophys. J.* **34**, 559-569
20. Sheng, Z., Strauss, W.L., Francois, J.-M., and Potter, J.D. (1990) Evidence that both Ca^{2+}-specific sites of skeletal muscle TnC are required for full activity. *J. Biol. Chem.* **265**, 21554-21560
21. Inoue, A., Ojima, T., and Nishita, K. (1996) Cloning and sequencing of a cDNA for Akazara scallop troponin T. *J. Biochem.* **120**, 834-837
22. Inoue, A., Ojima, T., and Nishita, K. (1998) Cloning and sequence of a cDNA for troponin-T of Ezo-giant scallop striated muscle. *Fisheries Sci.* **64**, 459-463
23. Ojima, T., Tanaka, H., and Nishita, K. (1990) Cyanogen bromide fragments of Akazara scallop M_r 52,000 troponin-I. *J. Biochem.* **108**, 519-521
24. Ojima, T., Tanaka, H., and Nishita, K. (1995) Amino acid sequence of C-terminal 17 kDa CNBr-fragment of Akazara scallop troponin-I. *J. Biochem.* **117**, 158-162
25. Ojima, T. and Nishita, K. (1988) Separation of Akazara scallop and rabbit troponin components by a single-step chromatography on CM-Toyopearl. *J. Biochem.* **104**, 9-11
26. Wellner, D., Panneerselvam, C., and Horecker, B.L. (1990) Sequencing of peptides and proteins with blocked N-terminal amino acids: *N*-Acetylserine or *N*-acetylthreonine. *Proc. Natl. Acad. Sci. USA* **87**, 1947-1949
27. Barbas, J.A., Galceran, J., Krah-Jentgens, I., De La Pompa, J.L., Canal, I., Pongs, O., and Ferrús, A. (1991) Troponin I is encoded in the haplolethal region of the Shaker gene complex of *Drosophila*. *Genes Dev.* **5**, 132-140
28. Kobayashi, T., Takagi, T., Konishi, K., and Cox, J.A. (1989) Amino acid sequence of crayfish troponin I. *J. Biol. Chem.* **264**,

1551-1557
29. Leszyk, J., Dumaswala, R., Potter, J.D., and Collins, J.H. (1988) Amino acid sequence of bovine cardiac troponin I. *Biochemistry* **27**, 2821-2827
30. Wilkinson, J.M. and Grand, R.J.A. (1975) The amino acid sequence of troponin I from rabbit skeletal muscle. *Biochem. J.* **149**, 493-496
31. Wilkinson, J.M. and Grand, R.J.A. (1978) Comparison of amino acid sequence of troponin I from different striated muscles. *Nature* **271**, 31-35
32. Sheng, Z., Pan, B.S., Miller, T.E., and Potter, J.D. (1992) Isolation, expression, and mutation of a rabbit skeletal muscle cDNA clone for troponin I. The role of the NH_2-terminus of fast skeletal muscle troponin I in its biological activity. *J. Biol. Chem.* **267**, 25407-25413
33. Bryan, J., Imai, M., Lee, R., Moore, P., Cook, R.G., and Lin, W.G. (1989) Cloning and expression of a smooth muscle caldesmon. *J. Biol. Chem.* **264**, 13873-13879
34. Graceffa, P. and Jancso, A. (1990) Electrophoresis of caldesmon. *J. Muscle Res. Cell Motil.* **11**, 440
35. Takano, E., Maki, M., Mori, H., Hatanaka, M., Marti, T., Titani, K., Kannagi, R., Ooi, T., and Murachi, T. (1988) Pig heart calpastatin: identification of repetitive domain structures and anomalous behavior in polyacrylamide gel electrophoresis. *Biochemistry* **27**, 1964-1972
36. Hu, C.C. and Ghabrial, S.A. (1995) The conserved, hydrophilic and arginine-rich N-terminal domain of cucumovirus coat proteins contributes to their anomalous electrophoretic mobilities in sodium dodecylsufate-polyacrylamide gels. *J. Virol. Methods* **55**, 367-379
37. Syska, H., Wilkinson, J.M., Grand, R.J.A., and Perry, S.V. (1976) The relationship between biological activity and primary structure of troponin I from white skeletal muscle of the rabbit. *Biochem. J.* **153**, 375-387
38. Talbot, J.A. and Hodges, R.S. (1981) Synthetic studies on the inhibitory region of rabbit skeletal troponin I. Relationship of amino acid sequence to biological activity. *J. Biol. Chem.* **256**, 2798-2802
39. Van Eyk, J.E. and Hodges, R.S. (1988) The biological importance of each amino acid residue of the troponin I inhibitory sequence 104-115 in the interaction with troponin C and tropomyosin-actin. *J. Biol. Chem.* **263**, 1726-1732
40. Levine, B.A., Moir, A.J.G., and Perry, S.V. (1988) The interaction of troponin-I with the N-terminal region of actin. *Eur. J. Biochem.* **172**, 389-397
41. Farah, C.S., Miyamoto, C.A., Ramos, C.H.I., da Silva, A.C.R., Quaggio, R.B., Fujimori, K., Smillie, L.B., and Reinach, F.C. (1994) Structural and regulatory functions of the NH_2- and COOH-terminal regions of skeletal muscle troponin I. *J. Biol. Chem.* **269**, 5230-5240
42. Hitchcock-DeGregori, S.E. (1982) Study of the structure of troponin-I by measuring the relative reactivities of lysines with acetic anhydride. *J. Biol. Chem.* **257**, 7372-7380
43. Chong, P.C.S. and Hodges, R.S. (1982) Proximity of sulfhydryl groups to the sites of interaction between components of the troponin complex from rabbit skeletal muscle. *J. Biol. Chem.* **257**, 2549-2555
44. Moir, A.J.G., Solaro, R.J., and Perry, S.V. (1980) The site of phosphorylation of troponin I in the perfused rabbit heart. The effect of adrenaline. *Biochem. J.* **185**, 505-513
45. Noland, T.A., Jr., Guo, X., Raynor, R.L., Jideama, N.M., Averyhart-Fullard, V., Solaro, R.J., and Kuo, J.F. (1995) Cardiac troponin I mutants. Phosphorylation by protein kinase C and A and regulation of Ca^{2+}-stimulated MgATPase of reconstituted actomyosin S-1. *J. Biol. Chem.* **270**, 25445-25454
46. Zhang, R., Zhao, J., and Potter, J.D. (1995) Phosphorylation of both serine residues in cardiac troponin I is required to decrease the Ca^{2+} affinity of cardiac troponin C. *J. Biol. Chem.* **270**, 30773-30780
47. Pearlstone, J.R., Johnson, P., Carpenter, M.R., and Smillie, L.B. (1977) Primary structure of rabbit skeletal muscle troponin-T. Sequence determination of the NH_2-terminal fragment CB3 and the complete sequence of troponin-T. *J. Biol. Chem.* **252**, 983-989
48. Jackson, P., Amphlett, G.W., and Perry, S.V. (1975) The primary structure of troponin T and the interaction with tropomyosin. *Biochem. J.* **151**, 85-97
49. Pearlstone, J.R. and Smilie, L.B. (1977) The binding site of skeletal alpha-tropomyosin on troponin-T. *Can. J. Biochem.* **55**, 1032-1038
50. Huber, P.A.J., Fraser, I.D.C., and Marston, S.B. (1995) Location of smooth-muscle myosin and tropomyosin binding sites in the C-terminal 288 residues of human caldesmon. *Biochem. J.* **312**, 617-625
51. Smith, C.W., Pritchard, K., and Marston, S.B. (1987) The mechanism of Ca^{2+} regulation of vascular smooth muscle thin filaments by caldesmon and calmodulin. *J. Biol. Chem.* **262**, 116-122
52. Marston, S.B. and Redwood, C.S. (1993) The essential role of tropomyosin in cooperative regulation of smooth muscle thin filament activity by caldesmon. *J. Biol. Chem.* **268**, 12317-12320
53. Garnier, J., Osguthorpe, D.J., and Robson, B. (1978) Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* **120**, 97-120
54. Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132