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Cloning and Sequencing of a cDNA for Akazara Scallop Troponin T

Akira Inoue, Takao Ojima, and Kiyoyoshi Nishita[1]

Department of Chemistry, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido 041

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A cDNA clone encoding troponin T of Akazara scallop (*Chlamys nipponensis akazara*) striated adductor muscle has been isolated and sequenced. The complete sequence deduced consists of 314 amino acid residues with a molecular weight of 37,206. Akazara scallop troponin T contains 55 amino acid residues more and 82 residues fewer than rabbit skeletal muscle troponin T and *Drosophila melanogaster* troponin T, respectively, showing almost the lowest sequence homology with rabbit troponin T (26%) but the highest homology with *Drosophila* troponin T (33%). Further, high sequence homology was seen in the functional regions: residues 33-120 and 174-227, corresponding respectively to residues 71-158 and 197-250 of rabbit troponin T (tropomyosin-binding regions); and residues 200-204, corresponding to 223-227 of rabbit troponin T (troponin I-binding region). In residues 1-70 (tropomyosin-binding region), however, only six residues are identical with rabbit troponin T.

Key words: Ca^{2+}-regulation, invertebrates, scallop, striated muscle, troponin T.

Troponin (Tn) is a Ca^{2+}-dependent regulatory protein for muscle contraction, consisting of three distinct subunits, troponin T (TnT), troponin C (TnC), and troponin I (TnI) (*1-5*). Troponin has been found in vertebrate skeletal and cardiac muscles but not in vertebrate smooth muscle. However, it was isolated from both striated (*6-8*) and smooth (*6*) adductor muscles of Akazara scallop and Ezo-giant scallop (*Patinopecten yessoensis*), which possess two Ca^{2+}-regulatory systems, that is, Tn-Tm and myosin light chains (*9-11*).

Akazara scallop Tn subunits show some characteristic features: TnC with only one Ca^{2+}-binding site in domain IV (*12*), two types of TnI, with a large M_r of 52,000 and a small M_r of 19,000 on SDS-PAGE (*7, 13*), and TnT showing a slightly larger M_r of 40,000 than vertebrate TnT in addition to high solubility in a low salt solution (*14*). These features imply that the structural changes of scallop Tn and its subunits in regulatory action are somehow different from those of vertebrate Tn.

In this paper, we describe cloning and sequencing of a cDNA encoding TnT of Akazara scallop striated adductor muscle and sequence comparison with other TnTs in order to better understanding the relationship between structure and function of Akazara scallop TnT.

MATERIALS AND METHODS

Bacterial Strains and Vectors—Strains Y1090 and XL1-Blue of *Escherichia coli* were purchased from Amersham and Stratagene, respectively. λgt11 phage and pBluescript II KS(+) plasmid were also purchased from Amersham and Stratagene, respectively.

Preparation of mRNA—Total RNA was extracted from Akazara scallop striated adductor muscle by the guanidium isothiocyanate method described in a standard protocol (*15*). Poly(A)$^+$RNA was isolated using a oligo d(T)-cellulose column (Pharmacia).

cDNA Cloning and Sequencing—cDNA was synthesized by reverse transcription of poly(A)$^+$RNA with a random hexanucleotide primer, and λgt11 cDNA library was constructed using a commercially available system (Amersham). To select positive phage clones, immunoscreening was used with rabbit anti-Akazara scallop TnT polyclonal antibodies. The cDNAs encoding Akazara scallop TnT were obtained by the *Eco*RI digestion of cloned cDNAs in λgt11 and then subcloned to pBluescript II KS(+). Nucleotide sequence was analyzed with a dye-primer cycle sequencing kit (Perkin Elmer-ABI) using a model 373A DNA sequencer (Perkin Elmer-ABI).

Partial Amino Acid Sequencing of Akazara Scallop TnT Protein—Akazara scallop TnT was prepared as reported previously (*7, 16*). The TnT was digested with 1/200 weight of α-chymotrypsin at 25°C for 20 min in a solution containing 0.5 M KCl, 20 mM Tris-HCl (pH 7.6). Peptide fragments were separated by HPLC on a TSKgel ODS-120T column (4.6×250 mm, Tosoh). The fragments were eluted with a linear gradient of 0-50% acetonitrile at an increasing ratio of 1.0%/min. The effluents were monitored by absorbance at 214 nm. Amino acid sequence was determined by the Edman degradation method using a model 473A sequencer (Perkin Elmer-ABI).

RESULTS AND DISCUSSION

Cloning and Sequencing of a cDNA Encoding TnT—From approximately 10,000 recombinant phage plaques, 21 positive clones were obtained. The sizes of these cDNAs were estimated to be 600-2,300 bp by 1% agarose gel electrophoresis. These cDNAs were subcloned to pBlue-

[1] To whom correspondence should be addressed. e-mail: nishita@fish.hokudai.ac.jp

Abbreviations: TnT, troponin-T; TnI, troponin-I; TnC, troponin-C; Tm, tropomyosin.

script II KS(+), and the nucleotide sequences of 5′- and 3′-terminal regions were analyzed to allow selection of clones containing the complete open reading frame. As a result, one clone comprising 1,500 bp, named AT-3 clone, was selected. The restriction endonuclease map of this clone is shown in Fig. 1. The clone has a nucleotide sequence of 1,467 bp with an open reading frame of 942 bp at positions 70–1011, but no polyadenylation signal or poly-(A) tail in 3′-untranslated region. From this nucleotide sequence, a 314 amino acid sequence was deduced with M_r 37,206 (Fig. 2). As shown in Table I, the amino acid composition obtained from the deduced amino acid sequence is closely consistent with that from TnT protein analysis (*13*).

To examine whether the AT-3 clone is indeed the clone for Akazara scallop TnT, its partial amino acid sequence was determined as follows: TnT protein was digested with α-chymotrypsin, and the resultant six major fragments of 36, 28, 26, 16, 14, and 5 kDa were subjected to separation with HPLC. The 16 kDa fragment alone was obtained in a

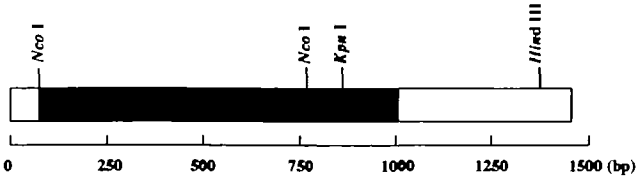

Fig. 1. **Restriction map of Akazara scallop TnT cDNA from clone AT-3.** The stippled and open flanking boxes represent the open reading frame and untranslated regions, respectively. The locations of several restriction sites are shown.

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GGCTAAACTCTCTCTGGCGGCCATTTTTGTTTTCGACATTACTAGTTTGTGTAACATTTA   60
ACTCACACCATGGACTACGACGATGAACCAAGAACTGGAGATGGAAATGAAGCAAGACTA  120
          M  D  Y  D  D  E  P  R  T  G  D  G  M  E  A  R  L     17

GCAATGGAGGAGGCCGCCAGGAAGAAGAAGGAGAAGGTGGAAAGTGAGATCGCAGAGTAC  180
 A  M  E  E  A  A  R  K  K  K  E  K  V  E  S  E  I  A  E  Y     37

GAAGAGATGAGACGCGAACAACGTGAGAAGGAGGCTGAAGACCTGGAACAACTTCGCCTG  240
 E  E  M  R  R  E  Q  R  E  K  E  A  E  D  L  E  Q  L  R  L     57

AAGAGGGAACAAAGGAAGCAGGAGCGGATAGAGGAAGATAGAAGACTGTTGGAGATTAGG  300
 K  R  E  Q  R  K  Q  E  R  I  E  E  D  R  R  L  L  E  I  R     77

AAGGAAGAGGATAAACGCCGCAAGGCAGAGGAGGAGGAGAGGAAGAGGAAGAAACAGGAG  360
 K  E  E  D  K  R  R  K  A  E  E  E  E  R  K  R  K  K  Q  E     97

GACGAAAGGAAGAGAATTGAGGCTAAGAAAGCTAAGCTAAAGGAATTGGAGGAGAGAAAA  420
 D  E  R  K  R  I  E  A  K  K  A  K  L  K  E  L  E  E  R  K    117

AAGATGTCCAAGACACCGAACTTTGTCATCACAAAGAAGGGAGCCTCCAACTTGGAGGAG  480
 K  M  S  K  T  P  N  F  V  I  T  K  K  G  A  S  N  L  E  E    137

GCGAGTAAGGACATGGCTAAGTCTAAAGAACAGTTGGAGGAGGAGAAACGGGCCATCCTT  540
 A  S  K  D  M  A  K  S  K  E  Q  L  E  E  E  K  R  A  I  L    157

GCTCAGCGTATCCAGCCACTGACAGTGGATGGACTCGACTTGGCCGCCCTCATGGAGAAG  600
 A  Q  R  I  Q  P  L  T  V  D  G  L  D  L  A  A  L  M  E  K    177

GCTACTGAGTTTCACAATAAGATCAAGAGCCTCGCCAACGAGAAGTATGAGCTGGAGGAG  660
 A  T  E  F  H  N  K  I  K  S  L  A  N  E  K  Y  E  L  E  E    197

CGCTTCAAGAGCCAGCAATATGATATGATTGAACTGGCTGAGAGAGCTAGACAGATGAAC  720
 R  F  K  S  Q  Q  Y  D  M  I  E  L  A  E  R  A  R  Q  M  N    217

AAGGGAAAGAAGAGAGCTGTCCAGGTCGATGACTCATATGATCCCATGGCAGAAAAATAT  780
 K  G  K  K  R  A  V  Q  V  D  D  S  Y  D  P  M  A  E  K  Y    237

GGCAGTTGTCCTCCCAAAGTTCAGATGTACAGCAAATATGAGAGACACACTGACCTCAGA  840
 G  S  C  P  P  K  V  Q  M  Y  S  K  Y  E  R  H  T  D  L  R    257

ACATACGGTACCAGGGTAGATTATTTCGAAACAAAAGCCAAAAAAATAGAAGCCGAAATG  900
 T  Y  G  T  R  V  D  Y  F  E  T  K  A  K  K  I  E  A  E  M    277

GCAATCGGTAGGAAAAAGGAAGAAGATAACCTCTTGAAGACAATGGAAGAAACGGAGGAA  960
 A  I  G  R  K  K  E  E  D  N  L  L  K  T  M  E  E  T  E  E    297

ACTTCAGAAGCCGCACCTGCACCTGAGGCAGAAGTCGCCGCAGAGGAGGAATAGAGTGTC 1020
 T  S  E  A  A  P  A  P  E  A  E  V  A  A  E  E  E Stop        314

ATCAGGTCAAGGTCATAGCCTAGGAGGTCAACAGATTGAGTCATAGATGTCGGTAACACC 1080
TGTAATCTTTGCCAGAGGGTGTGTGTCATGGGCGTACTTACGACGACAAATGTGTGTGAT 1140
GTCATCCTAGTCACCATAGTAACGACAACTGTTGTCGTTAGCAACAGTGAAACATTTGTT 1200
GTCAGTAGCAACAGTGAACTCATTCATTAGTTTACCCATTATTAAGGTATTAGTTCGAAA 1260
AATACTCCATGTCAGAAAGTGAAAAGCGTCATAGCCTTACACCAGTGATCACAACCAATC 1320
GAAAACAAACCTTTTGCCCTTGACTATAAGTGTATAAAGCTTGTAATTTGTTTCCTCTAA 1380
TTTAATTGATTAAACATGTTGTGCAGTAGATAACGTTTGAAGAAGTTCTAAATCATACCT 1440
TCCCTTTTTGGAAAAATAAGGTCAGCG                                   1467
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Fig. 2. **Nucleotide sequence and its deduced amino acid sequence of Akazara scallop TnT.** The initiation site for translation and the termination codon are boxed. The deduced amino acid sequence is shown in bold letters. The underlined amino acid sequence has been confirmed with the 16 kDa peptide fragment obtained by α-chymotryptic digestion of the TnT.

pure state, and its sequence was analyzed. The sequence of 17 amino acids, SKYERHTDLRTYGTRVD, was determined and found to be consistent with residues 248-264 of the deduced sequence. Therefore, the AT-3 is identified as an Akazara scallop TnT clone.

Sequence Comparison of Various TnTs—Amino acid sequences of Akazara scallop, *Drosophila* (*17, 18*), and rabbit TnTs (*19*) are aligned to maximize sequence homology in Fig. 3. According to a computer search for the data base NBRF-PIR (release 42.0), Akazara scallop TnT showed the highest homology (33%) with *Drosophila* TnT but almost the lowest homology (26%) with rabbit TnT.

The Akazara scallop TnT contains 55 residues more and 82 residues fewer than rabbit and *Drosophila* TnTs, respectively. It is shorter by 27 residues at the N-terminus and longer by 78 residues at the C-terminus compared with rabbit skeletal TnT; and shorter by 5 residues at the N-terminus and by 65 residues at the C-terminus compared with *Drosophila* TnT.

A hydropathy plot (*20*) and the predicted secondary structure (*21*) of Akazara scallop TnT are shown in Fig. 4. These features are similar to those of TnTs so far investigated. Thus, Akazara scallop TnT was assumed to contain 74% α-helix, 8% β-sheet, and 10% β-turn. Such a high content of α-helix is also in common with TnTs.

Sequence Comparison of Functional Regions—Residues 1-70 of rabbit TnT have been reported to bind to both the C-terminal region and the head-to-tail junction of Tm (*22*). However, in view of the low sequence homology, it is questionable whether the corresponding residues of Akazara scallop TnT possess the same functions.

Residues 71-158 of rabbit TnT are thought to bind by ionic bonds with the middle portion of Tm (*22*), where many acidic and basic amino acid residues exist (*23*). The

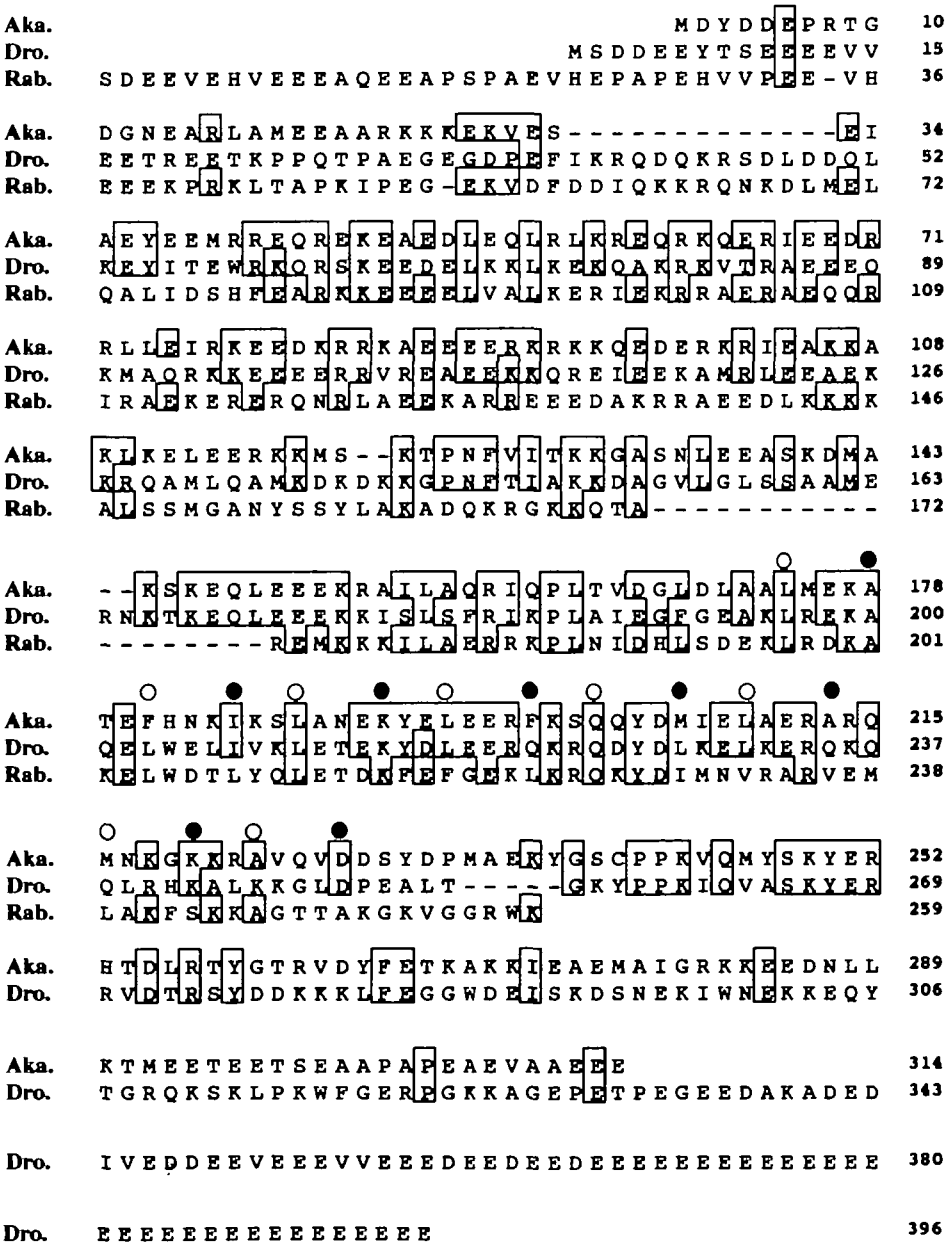

Fig. 3. **Comparison of amino acid sequences of several TnTs.** *Aka*, Akazara scallop TnT (present paper); *Dro*, *Drosophila melanogaster* TnT (*17, 18*); *Rab*, rabbit skeletal muscle TnT (*19*). Residues identical to Akazara scallop TnT are indicated by boxes. Open and closed circles represent two patterns of heptapeptide-repeating units of hydrophobic residues.

TABLE I. **Comparison of the amino acid compositions of the Akazara scallop TnT.**

Amino acid	TnT	
	Protein[a]	Clone AT-3
Ala	9.8	9.9
Arg	9.5	9.6
Asx[b]	8.0	8.0
Cys	—	0.3
Glx[c]	25.9	24.2
Gly	2.6	2.6
His	0.6	0.6
Ile	3.4	3.5
Leu	6.8	6.7
Lys	13.6	14.0
Met	2.8	3.8
Phe	1.3	1.3
Pro	2.6	2.5
Ser	3.1	3.5
Thr	4.2	3.8
Trp	—	0.0
Tyr	3.2	3.2
Val	2.6	2.5
Total	100.0	100.0

[a]Ojima and Nishita (*13*). [b]Asp and Asn. [c]Glu and Gln. Values are expressed as mol%.

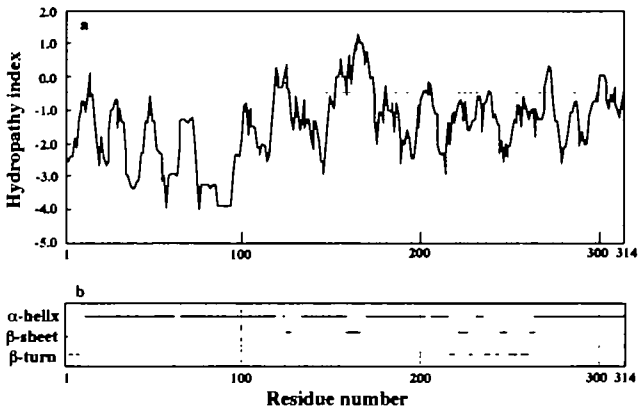

Fig. 4. **Hydropathy plot and predicted secondary structure of Akazara scallop TnT.** *a*, hydropathy plot by the method of Kyte and Doolittle (*20*). *b*, predicted secondary structure by the method of Garnier *et al.* (*21*).

corresponding region of Akazara scallop TnT, residues 33-120, contains 30 acidic and 33 basic residues, with similar features of hydropathy index and predicted α-helix (Fig. 4).

Further, rabbit TnT is also thought to bind to Tm at residues 197-250 (*24*), where two patterns of heptapeptide-repeating units of hydrophobic residues were observed. The repeating units are thought to be important for formation of a coiled-coil structure required for Tm-binding (Fig. 4). Akazara scallop TnT showed similar patterns at residues 174-227.

In addition, rabbit TnT showed a sequence, KRQKY, at residues 223-227 that is important for TnI-binding (*5*). A similar sequence, KSQQY, was seen in Akazara scallop TnT at residues 200-204.

Finally, it was found that the sequences of residues 123-153 and 241-259 of Akazara scallop TnT showed high homology with corresponding residues of *Drosophila* TnT. The roles of these regions are obscure, but it can be considered that these regions of invertebrate TnT possess important functions.

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