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Actin Affinities of Recombinant α-Tropomyosins That Residues 276 or 277 in the Carboxyl Terminal Region are Individually Substituted to a Cysteine Residue

Don-Kyu Kim and Young-Joon Cho*

Department of Molecular Biology, Daegu University, Gyeongsan, 712-714

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It has been previously reported that the carboxyl terminal residues 276 and 277 of α-tropomyosin are important for actin affinity. In order to investigate actin affinities of these two residues of skeletal (HA) and smooth (QT) muscle α-tropomyosins, a series of mutant tropomyosins were constructed in which residues at either 276 or 277 were individually replaced with a cysteine residue for chemical modification. These mutants were overexpressed in E. coli as unacetylated and Ala-Ser (AS) dipeptide fusion forms. While actin affinities of unacetylated tropomyosins were considerably low, those of AS/TMs were remarkably higher than those of corresponding unacetylated tropomyosins. However, actin affinities of AS/TM24 (QC) and AS/TM29 (HC) were dramatically lower than those of other AS/TMs and were close to those of unacetylated tropomyosins. In addition, actin affinities of unacetylated TM24 (QC) and TM29 (HC) failed to be restored in the presence of troponin, unlike unacetylated TM10 (HA) and TM23 (CA). These results indicated that the presence of a cysteine residue at 277 caused a drastic decrease in actin affinity, and also that the residue 277 is important for actin affinity of α-tropomyosin. Since TM23 (CA) showed high actin affinity, it may serve as a valuable tool for chemical modification studies for investigating the interaction of the carboxyl terminal residues of α-tropomyosin with actin and/or troponin.

Key words : Actin affinity, recombinant tropomyosin, C-terminal, cysteine

Introduction

Tropomyosin (TM), an important actin binding protein, is involved in regulations of muscle contraction and actin cytoskeleton that play essential roles in cellular movement and maintenance of cell shape. Tropomyosin is a family of proteins that are highly conserved and present in all types of muscle tissue and in most of nonmuscle cells [2,31, for reviews]. Tropomyosins are encoded by a multigene family and are expressed in most eukaryotes in cell- and tissue-specific manners. In mammals there are four, α, β, γ, and δ gene of tropomyosin. Additional isoforms are generated by alternative exon splicing, by the use of alternate promoters, and by formation of heterodimers [16]. The alternate promoters result in two tropomyosin classes; high molecular weight tropomyosins that are 284 residues long and expressed in both muscle and nonmuscle cells, and low molecular weight tropomyosin are 247 residues long and found in cytoskeletons of nonmuscle cells.

Muscle α-tropomyosins, which are most widely studied, are fibrous molecules composed of two polypeptide chains of 33,000 (284 amino acid residues) each in a two-stranded coiled coil configuration and have high α-helix content (over 90%). Amino acid sequence analysis revealed that 40 heptapeptide repeats are present in muscle tropomyosin molecule [26]. The heptapeptide (heptad) repeats, characteristics of coiled coil structure, has 7 amino acid residues (*abcdefg*) altering polar and nonpolar residues. Nonpolar residues occupy at *a* and *d* positions and these residues interface with nonpolar residues at *a* and *d* in the other strand of dimeric tropomyosin molecule. Hydrophobic interactions among the interfacing nonpolar residues stabilize coiled coil configuration [14]. The function of tropomyosin in skeletal and cardiac muscle is primarily in association with the troponin complex to regulate interaction of actin and myosin in a calcium sensitive manner.

All tropomyosins (isoforms) bind cooperatively to F-actin. Muscle tropomyosin spans the length of 7 actin monomers and binds to actin along the long pitch grooves of the helical actin filament [33]. Despite of periodic nature of 7 actin binding sites, the importance of the ends of tropomyosin for actin binding and regulatory function is well established [4,13, 20,30]. Both ends, the amino (N) and carboxyl (C) terminal regions, are encoded by alternatively expressed exons and

*Corresponding author
Tel : +82-53-850-6463, Fax : +82-53-850-6469
E-mail : yjcho@daegu.ac.kr

are the binding sites for other proteins including tropomodulin at the N-terminus and troponin T at the C-terminus in striated muscle isoforms [2]. It has been indicated that local changes at the amino terminus greatly influence the actin affinity [6,7,32]. It has been also reported that carboxyl terminal 9 amino acid residues define actin affinity of tropomyosin [4,17] and among 9 residues the residues 276 and 277 of the carboxyl terminal region are important for actin affinity [5,22].

The amino terminus of majority of muscle proteins including tropomyosin is acetylated. Unlike N-acetylated muscle tropomyosin, bacterially produced recombinant tropomyosin is unacetylated at the amino terminus. Unacetylated striated α-tropomyosin hardly bound to actin, whereas the N-acetylated muscle tropomyosin bound well to actin [18]. Monteiro *et al.* [28] previously demonstrated that recombinant tropomyosin with Ala-Ser dipeptide extension at the amino terminus restored muscle tropomyosin functions.

The amino acid residue cysteine has a sulfhydryl group and has been an ideal target for chemical modification studies owing to its high reactivity with various chemicals and its sole and unique presence (a single cysteine residue at 190) in skeletal muscle α-tropomyosin. A mutant tropomyosin that Cys190 was replaced with Ser residue was constructed previously for the purpose of chemical modification studies. The mutant, designated as TM/C190S, was shown that the substitution of cysteine residue at 190 to serine had little or no effect on tropomyosin functions [21].

In order to understand molecular interaction between actin and residues in the carboxyl terminal region of α-tropomyosin by chemical modification, we constructed a series of mutant tropomyosins that the residues 276 or 277 of skeletal (His276Ala277) and smooth (Gln276Thr277) muscle α-tropomyosin were individually substituted to a cysteine residue. The mutants, termed TM22(CT), TM23(CA), TM24(QC), TM29(HC) were overexpressed in *E. coli* as unacetylated and Ala-Ser (AS) fusion forms. The amino acid sequences of mutant tropomyosins were listed in Table 1. After purification of mutant tropomyosins, actin affinities of these tropomyosins were examined and compared to each other.

Table 1. Amino acid sequences in the carboxyl terminal region of TM10(HA), TM19(QA), TM22(CT), TM23(CA), TM24(QC), TM29(HC), and TM31(HS)

	276	277	278	279	280
TM10(HA)	H	A	L	N	D
TM19(QA)	Q	A	L	N	D
TM22(CT)	C	T	L	N	D
TM23(CA)	C	A	L	N	D
TM24(QC)	Q	C	L	N	D
TM29(HC)	H	C	L	N	D
TM31(HS)	H	S	L	N	D
heptad repeat	*c*	*d*	*e*	*f*	*g*

Either residue 276 or 277 was individually replaced with ^{276}Q for TM19, ^{276}CT^{277} for TM22, ^{276}C for TM23, ^{276}QC^{277} for TM24, C^{277} for TM29, and S^{277} for TM31 and rest of amino acid sequences were identical to the striated TM (284 residues) except for a substitution of cysteine 190 to serine.

Materials and Methods

Construction of mutant tropomyosins Recombinant mutant α-tropomyosins were constructed by polymerase chain reaction (PCR) with mutagenic oligonucleotide primers. Rat striated C190S tropomyosin cDNA (pET11d/C190S) was used as a template. The C190S tropomyosin was previously constructed that a cysteine residue at 190 was replaced with Ser residue [21]. The PCR was carried out in a Hybaid PCR Express thermal cycler using *Pfu* DNA polymerase (Stratagene) which possesses 3′ to 5′ exonuclease activity. The PCR was performed with 5′-sense primer whose sequence was 5′-TAATACGACTCACTATAGGGGAATTGTGAGC-3′ (primer C; T7 promoter primer) and 3′ mutagenic antisense primer whose sequences were shown in Table 1. The conditions for PCR were as follows; the template was denatured at 94°C for 2 min and 30 cycles of amplification at 94°C for 45 sec, 65°C for 60 sec and 72°C for 2 min followed by 6 min at 72°C for a final extension. Ala-Ser (AS) dipeptide fusion tropomyosins were constructed with 5′ mutagenic primer whose sequence was CCACCGCCACC**ATG GCTAGC**ATGGACGCCATCAAG (the underlined corresponds to NheI site and mutagenic site is in bold face) and a 3′ primer (primer D; T7 terminator primer), using pET11d plasmids harboring corresponding cDNA sequences as templates. PCR conditions were as follows; 94°C for 2 min denaturation, 25 cycles of 94°C for 90 sec, 60°C for 30 sec, 72°C for 2 min followed by a final step of 72°C for 6 min. The PCR products were cleaned using Wizard Prep Kit (Promega) as suggested by manufacturer and analyzed on agarose gel electrophoresis. DNA sequencing confirmed that no incidental changes had been introduced by PCR. The PCR products were cloned into NcoI-BamH1 site in the expression plasmid pET11d and transformed into *E. coli* strain

BL21(DE3). Overexpression of the mutant tropomyosin was induced for 4 hrs by the addition of IPTG as previously described [5]. Primers were synthesized and purchased from Bionics. Autosequencing was performed at Macrogen using an ABM Prism 3700.

Protein purifications Recombinant tropomyosins were isolated and purified as described [5]. All mutant tropomyosins were soluble and were heat stable upon lysis. They were successfully overproduced and purified to near homogeneity, over 95 percent as determined by scanning densitometry. Purification steps of mutant TMs included ammonium sulfate fractionation, DE52 DEAE-cellulose ion exchange, followed by hydroxyapatite column chromatography.

Chicken pectoral muscle actin was extracted from acetone powder and purified as described [5]. Troponin isolated from chicken pectoral muscle was purchased from Sigma Chemical Company. Protein concentrations were determined by Bradford (Biorad) method [1] using bovine serum albumin as a standard or by measuring absorbance at 280 nm spectrophotometrically. The percent extinction coefficients used were 11.1 for actin, 2.8 for tropomyosins, and 3.5 for troponin (TN), respectively.

Actin binding assay Actin binding assay for tropomyosin was carried out by cosedimentation of F-actin and tropomyosins at room temperature in a Beckman Table Top Ultracentrifuge (Model TL-100) as described previously [5]. The conditions of the assays are described in the figure legends. The pellets and supernatants were run on 12% SDS-polyacrylamide gel electrophoresis [24]. Tropomyosin bands of the supernatant (free) and tropomyosin and actin bands of the pellet (bound) on the gels were quantified by densitometry using a Biorad scanning densitometer Model GS-700. Binding constants were estimated using Sigmaplot 2000 by fitting the data to the Hill equation as previously described [23]. The data were normalized because background values estimated by densitometer were slightly different from gels to gels.

Results

Actin affinities of unacetylated mutant tropomyosins

To determine actin affinities of mutant unacetylated tropomyosins quantitatively the binding isotherm experiments were performed and the results were shown in Fig. 1. Unacetylated TM19(QA) bound strongest to actin.

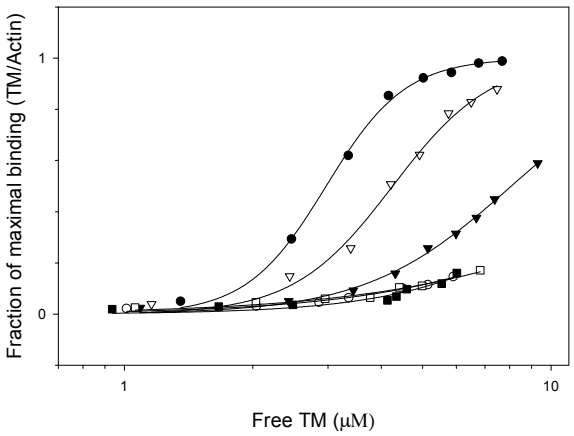

Fig. 1. Binding of unacetylated mutant tropomyosins to actin. Condition: 5 μM actin, 0 - 10 μM tropomyosin in 10 mM imidazole, pH 7.0, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT Symbols: ○, TM10(HA); ●, TM19(QA); ▼,TM22 (CT); ▽,TM23(CA); ■, TM24(QC); □, TM29(HC).

Unacetylated TM23(CA) bound to actin less strongly than TM19(QA) and stronger than, TM22(CT), while unacetylatd TM10(HA), TM24(QC), TM29(HC) bound hardly to actin. The apparent binding constants of mutant tropomyosins were summarized in Table 3. Actin affinity of TM19(QA) was approximately 1.5 times and 6 times higher than that of TM23(CA) and TM10(HA), respectively. The fact that TM19(QA) was much higher than TM10(HA) was consistent with previous report that Gln(Q) at 276 in smooth muscle α-tropomyosin was primarily responsible for higher actin affinity, implying the importance of residue 276 on actin affinities [22]. The result that unacetylated TM24(QC) showed approximately 5 times lower actin affinity than unacetylated TM19(QA) suggested that the presence of Cys at 277 was attributed to lower actin affinity of unacetylated TM24(QC). Unacetylated TM29(HC) also showed lower actin affinity comparable to that of unacetylated TM24(QC). It was, however, plausible that the presence of His at 276 as well as Cys at 277 might cause the lower actin affinity since both unacetylated TM29(HC) and unacetylated TM10(HA) exhibited lower actin affinities.

It has been known that troponin enhances actin affinity of tropomyosin [19]. To examine effect of troponin on actin affinities of recombinant mutant tropomyosins, qualitative actin binding assays was carried out in the presence of troponin. As shown in Fig. 2, actin affinities of unacetylated TM10(HA) and TM23(CA) were increased in the presence of troponin (lane 1 and 2) as compared to those of TM10(HA) and TM23(CA) in the absence of troponin (lane 5 and 6),

Table 2. Primer sequences used for construction of mutant tropomyosins

Name	Primer sequences
19 (H276Q)	5′-TGGGAGGAGggatccAAAGAAACTTATATGGAAGTCA TATCGTTGAGAGCTTGGTCCAGCTCC-3′
22 (H276C/A277T)	5′-TGGGAGGAGggatccAAAGAAACTTATATGGAAGTCA TATCGTTGAGAGTACAGTCCAGCTCC-3′
23 (H276C)	5′-TGGGAGGAGggatccAAAGAAACTTATATGGAAGTCA TATCGTTGAGAGCGCAGTCCAGCTCC-3′
24 (H276Q/A277C)	5′-TGGGAGGAGggatccAAAGAAACTTATATGGAAGTCA TATCGTTGAGACACTGGTCCAGCTCC-3′
29 (A277C)	5′-TGGGAGGAGggatccAAAGAAACTTATATGGAAGTCA TATCGTTGAGGCAGTGGTCCAGCTCC-3′
31 (A277S)	5′-TGGGAGGAGggatccAAAGAAACTTATATGGAAGTCA TATCGTTGAGGCTGTGGTCCAGCTCC-3′

The boldface letters represent a mutagenic site and the lower case letters indicate the BamHI restriction site, which was introduced for removal of a long 3′-untranslated region of rat striated cDNA.

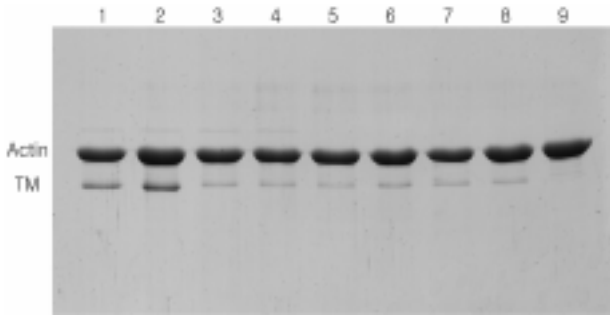

Fig. 2. SDS-Polyacrylamide gel electrophoresis analysis of pellets of actin binding assay with unacetylated tropomyosins in the presence and absence of troponin. Condition: 5 μM actin, 1.5 μM tropomyosin, 1.5 μM troponin in 10 mM imidazole buffer, pH 7.0, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT *Lane 1*, actin, TM10(HA) with TN; *lane 2*, actin, TM23(CA) with TN; *lane 3*, actin, TM24(QC) with TN; *lane 4*, actin, TM29(HC) with TN; *lane 5*, actin, TM10(HA) without TN; *lane 6*, actin, TM23(CA) without TN; *lane 7*, actin, TM24(QC) without TN; *lane 8*, actin, TM29(HC) without TN; *lane 9*, actin alone.

whereas troponin hardly increased actin affinities of TM24(QC) and TM29(HC) even in the presence of troponin (lane 3 and 4). This result suggested that introduction of a cysteine residue at 277 of α-tropomyosin may cause the loss of crucial functions of tropomyosin such as actin binding and interaction with troponin.

Actin affinities of the N-terminal Ala-Ser fusion tropomyosins

The addition of Ala-Ser (AS) dipeptide to the N-terminus of unacetylated tropomyosin restored functions of N-acetylated muscle tropomyosin and the addition of AS was apparently sufficient for role of the acetyl group [28]. The result of the binding isotherm experiment with AS tropomyosins was shown in Fig. 3, and the apparent binding constants were summarized in Table 3. The actin affinities of AS/TM10(HA) as well as AS/TM19(QA) were drastically increased, at least an order of magnitude, as compared to those of corresponding unacetylated tropomyosins. While AS/TM10(HA) binds to actin strongly, AS/TM29(HC) failed to bind significantly to actin. In addition, actin affinity of AS/TM24(QC) was slightly higher than that of unacetylated TM24(QC), it remained much lower, about a hundred fold

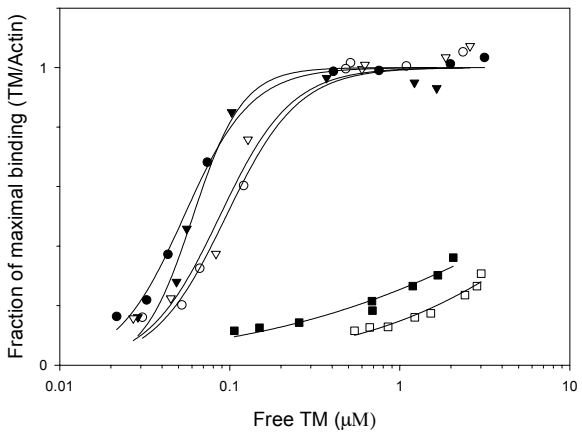

Fig. 3. Binding of mutant tropomyosins with Ala-Ser dipeptide fusion tropomyosins to actin. Condition: 5 μM actin, 0 - 4 μM tropomyosin in 10 mM imidazole, pH 7.0, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT Symbols: ○, AS/TM10(HA); ●, AS/TM19(QA); ▼, AS/TM22(CT); ▽, AS/TM23(CA); ■, AS/TM24(QC); □, AS/TM29 (HC)

Table 3. The apparent actin binding constants (Kapp) of recombinant mutant tropomyosins

	Unacetylated	AS dipeptide fusion
	K_{app} (10^6M^{-1})	K_{app} (10^6M^{-1})
TM10(HA)	0.05	10.5
TM19(QA)	0.33	18.6
TM22(CT)	0.12	16.5
TM23(CA)	0.23	11.5
TM24(QC)	0.07	0.13
TM29(HC)	0.04	0.09
TM31(HS)	N. D.	1.6

The data from the binding isotherms shown in Fig. 1, 3, and 4 were fitted to the Hill equation as described in "Materials and Methods". N. D.: not determined

lower than that of AS/TM19(QA). These results demonstrated that lower actin affinity of TM24(QC) and TM29(HC) were attributed to the presence of Cys at 277 of α-tropomyosin.

Drastic loss of TM24(QC) or TM 29(HC) in tropomyosin functions such as actin binding and troponin interaction led to examine further the effect of the residue 277 on actin binding. TM31(HS) that residue 277 was substituted from Ala to Ser residue was constructed and actin affinity of TM31(HS) was compared to TM20(HA) and TM29(HA). As shown in Fig. 4, actin affinity of AS/TM31(HS) was approximately 10 times higher than that of AS31(HS), whereas it was 10 times lower than that of AS/TM10(HA). This result indicated that a remarkable difference in actin affinity was

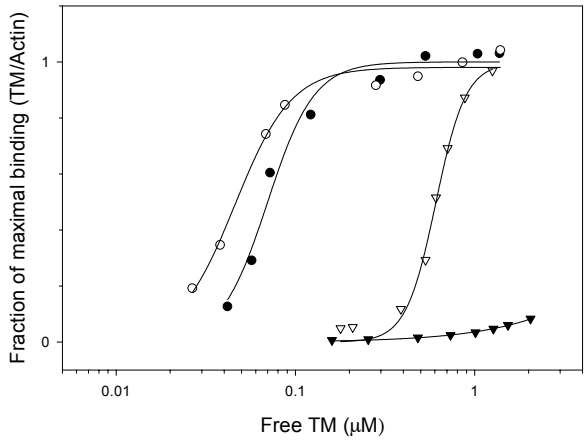

Fig. 4. Comparison of actin affinities AS dipeptide fusion mutant tropomyosins that Ala, Ser, or Cys residues is present at 277. Condition: 5 μM actin, 0 - 1.5 μM tropomyosin, in 10 mM imidazole, pH 7.0, 150 mM NaCl, 2 mM MgCl2, 1 mM DTT Symbols: ●, AS/TM10(HA); ○, AS/TM19(QA); ▼, AS/TM29(HC); ▽, AS/TM31(HS).

ascribed to amino acid residue 277 since AS/TM10(HA), AS/TM29(HC), and AS/TM31(HS) are identical to each other in amino acid sequence except for residue 277 and suggested importance of the residue 277 of α-tropomyosin on for actin affinity.

Discussion

As an initial step to investigate protein-protein interaction between tropomyosin and actin and/or troponin, we have constructed a series of mutant tropomyosins that a single cysteine residue was introduced in order to attach covalently to tropomyosin molecule chemical probes for cross-linking and fluorescence and luminescence resonance energy transfer studies [3,10,27,29]. Skeletal muscle α-tropomyosin contains a single cysteine residue at 190. Substitution of residue 276 or 277 to cysteine residue generates two cysteine residues in α-tropomyosin molecule. This may cause undesirable problems such as multiple and unequal modifications during the processes of modification and crosslinking. As a consequence it was imperative to remove Cys190 prior to introduction of a cysteine residue to the carboxyl terminal region to avoid these problems. A mutant tropomyosin, termed as TM/C190S originally and designated as TM10(HA) in the paper, was constructed in previous experiment and it was shown that replacement of cysteine 190 with serine residue had little or no effect on actin binding since it was indistinguishable to a recombinant tropomyosin with Cys residue at 190 [21]. Consequently cDNA of TM10(HA) was employed for site-directed mutagenesis using polymerase chain reaction to construct of mutant tropomyosins that contained a single cysteine residue in the carboxyl terminal region of α-tropomyosin so that a single cysteine residue was present in entire tropomyosin molecule.

Substitution of Ala residue to Ser and Cys at 277 of skeletal α-tropomyosin dramatically reduced actin affinities. Amino acid residues of alanine, serine, cysteine are relatively small residues and the van der Waals volumes ($Å^3$) of are 67, 73, 86, respectively. It appeared that actin affinity was progressively lowered as the volume of amino acid residues present at 277 were increased, implying that to some extent steric hindrance may be involved. Lower actin affinity of mutant tropomyosin of TM29(HC) or TM24(QC) may be interpreted that the residue 277 was directly involved in an interaction between tropomyosin and actin molecules.

Alternatively it may be interpreted that the residue 277 confers a decreased stability or flexibility to tropomyosin molecule, in turn, resulted in lower actin affinity.

It has been long known that tropomyosin molecules undergo head-to-tail polymerization, that is, the carboxyl terminal region of α-tropomyosin interacts with the amino terminal region of α-tropomyosin and forms a complex in head-to-tail fashion. Although tropomyosin molecule is a coiled coil structure and showed heptapeptide repeat (heptad) throughout the molecule, the crystal structure of the complex revealed that two polypeptide chains in the carboxyl terminal region of dimeric tropomyosin molecule were splay apart and had noncanonical coiled coil structure, whereas the amino terminal region maintained canonical coiled coil structure [25]. According to a solution NMR that was solved recently, the carboxyl terminal region of the head-to-tail complex was flexible and the structure showed a symmetric interleaved packing interaction in which the helical chains of the carboxyl terminal region spread apart to allow the insertion of the amino terminal coiled-coil into the resulting cleft [15]. This complex is stabilized primarily by hydrophobic interactions involving the amino terminal residues Met1, Ile4, Met8, and Leu11, and the carboxyl terminal residues Ile274, Ala277, Met281, and Ile284. In particular, it was suggested that Ala277 be involved in hydrophobic interaction and van der Waals interaction with Met1 and Lys5, respectively [8,15]. Thus the substitution of Ala277 to polar Ser and Cys residues may interfere with hydrophobic interaction with Met1, which subsequently reduced stability of the head-to-tail complex and this resulted in lower actin affinities of TM24(QC) and TM29(HC).

The introduction of sulfhydryl group in cysteine residue into dimeric tropomyosin molecule potentially forms disulfide bond between two cysteine residues and this might alter local conformation of the carboxyl terminal region of tropomyosin molecule. In fact there was a report that Cys190 of skeletal α-tropomyosin tended to form disulfide bond even with exposure to air without the addition of oxidizing agent and the crosslinking of dimeric tropomyosin by air oxidation caused lower actin affinity [12]. However, this was highly unlikely to occur since all experiments were performed under the reducing condition in the presence of dithiothreitol (DTT), at least a hundred times excess in molar ratio. Nevertheless we may not positively exclude a possibility of local conformational change due to the participation of a fraction of sulfhydryl groups in disulfide formation.

According to the solution NMR structure, His276 was exposed to solvent and did not participate in intermolecular contact in the head-to-tail complex structure. Unacetylated TM10(HA) drastically increased actin affinity in the presence of troponin, suggesting that His276 may be involved in troponin binding. Skeletal muscle β-tropomyosin has 70 percent identity to skeletal muscle α-tropomyosin and both α- and β- tropomyosin have His residue at 276. As matter of fact it was recently reported that His276 in β-tropomyosin was involved in troponin (troponin T1 fragment) binding [9]. In spite of the presence of His at 276 unacetylated TM29(HC) showed no enhancement of actin affinity in the presence of troponin, unlike unacetylated TM10(HA). This might imply that the effect of substitution to Cys at 227 was not confined locally to the residue 277 but presumably extended to residue 276 or beyond, to wider range in the carboxyl terminal region.

Even though substitution of residue 277 to cysteine caused detrimental loss of tropomyosin function, the substitutions of residue 276 to cysteine managed to maintain tropomyosin functions. Thus both TM22(CT) for smooth muscle and TM23(CA) for skeletal muscle α-tropomyosin may be used for as a tool for further detailed analysis of tropomyosin functions. The results presented in the paper, together with previous results, supported that residues 276 and 277 in the carboxyl terminal region of muscle α-tropomyosin are crucial for tropomyosin functions.

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초록 : α-트로포마이오신의 276 또는 277 아미노산 잔기가 단일 시스테인 잔기로 치환된 돌연변이 트로포마이오신의 액틴친화력

김돈규 · 조영준*
(대구대학교 자연과학대학 분자생물학과)

화학적 변형 방식에 의한 트로포마이오신과 액틴의 상호작용을 규명하기 위하여 액틴결합에 중요한 역할을 하는 C-말단부위의 아미노산 잔기 276 또는 277을 단일 시스테인 잔기로 치환한 돌연변이 트로포마이오신을 제조하여 대장균에서 대량 발현시킨 후 액틴 결합력을 측정하였다. 잔기277을 시스테인 잔기로 치환시킨 TM24(QC) 및 TM29(HC)는 액틴 결합 성질을 잃어버렸을 뿐만 아니라 트로포닌 존재 하에서도 액틴결합력이 증가하지 않았다. 이 결과는 잔기 277이 트로포마이신 기능에 중요한 역할을 한다는 것을 제시한다. 반면 잔기 276을 시스테인 잔기로 치환한 TM22(CT) 및 TM23(CA)는 액틴과 비교적 잘 결합하였을 뿐만 아니라 트로포닌 존재 하에서 액틴결합력이 증가하였다. 따라서 TM23(CA)는 시스테인 잔기를 도입하여도 트로포마이오신의 기능을 유지하였으며 향후 화학적 변형 연구를 위한 도구로 중요하게 사용될 수 있을 것이다.