

# The Structure of the Junction between Short Subfragment-2 and the Hinge from Adult Chicken Smooth Muscle Myosin.

Bunji WATANABE<sup>1</sup> and Mihoko TANIGAWA<sup>2</sup>

**Abstract** In the preceding papers [Watanabe B (1989), *Biol. Chem. Hoppe-Seyler* 370(9): 1027-1034 and Watanabe B, Tanigawa M (1993), *Biol. Chem. Hoppe-Seyler* 374(1): 27-35] we reported primary structures of subfragment-2 (S-2) from adult chicken skeletal and cardiac muscle myosins. This paper describes the structure of the junctional region between the short subfragment -2 (short S-2) and the hinge from adult chicken gizzard muscle myosin.

The rod obtained by digesting myosin with papain was further subdivided into S-2 and light meromyosin (LMM) by limited digestion with  $\alpha$ -chymotrypsin. A 134 amino-acid-residue peptide covered with carboxy-terminal portion of the short S-2 and amino-terminal portion of the hinge was isolated by conventional method from cyanogen bromide (CNBr) digests of S-2 and sequenced. To analyse the structural differences, the sequence of this junction thus determined was compared with those of corresponding portions of embryonic chicken gizzard, chicken brain, drosophila, chicken skeletal and chicken cardiac muscle myosin.

Interestingly, results show the higher sequence identities of 100%, 81.3% and 59.7% with embryonic chicken gizzard, chicken brain and drosophila nonmuscle myosin, respectively, and lower degrees, 38.8% and 40.3% with chicken skeletal and chicken cardiac ventricular muscle myosin respectively.

Bull. Sch. Allied Med. Sci., Nagasaki Univ. 8: 1-8, 1994

**Key words** : amino-acid sequence, primary structure, smooth muscle myosin, isoform, the short subfragment-2, the hinge,

## Introduction

Myosin is an important component of the contractile system that is present in all muscle and nonmuscle cells. It is composed of two heavy chains and two pairs of light chains and shares the same basic molecular structure. The myosin heavy chains (MHC) form a dimer consisting of two globular amino(NH<sub>2</sub>)-terminal heads (subfragment-1, S-1) and carboxy(COOH)-terminal  $\alpha$ -helical coiled-coil rods.

The rod are involved in the assembly of myosin molecules into filaments, whereas the heads contain an actin-activated Mg<sup>2+</sup>-ATPase activity.<sup>1)</sup>

Thus MHCs have been extensively studied the structure, function and regulation at the molecular level in skeletal and cardiac muscle. The role of the rod has been mainly investigated in self-assembling ability, however, many recent studies have revealed the important properties of the short S-2, the

hinge, the short S-2/the hinge and the hinge/LMM junction for the contractile force generation and speed or for the isoform-specific function of the myosin.<sup>2)-6)</sup>

In striated muscle, MHC isoforms are encoded by a multigene family,<sup>7)8)</sup> however, smooth muscle MHC isoforms are the products of alternative mRNA processing from a single gene.<sup>9)-13)</sup> The characterization of the tissue specific and developmental isoform of the nonmuscle and smooth muscle MHC is not fully explored.

Furthermore, sequence analysis at the protein level is necessary for revealing the post translational processing of proteins. The amino-acid differences between sequences obtained by protein analysis and by cDNA sequencing have been reported.<sup>14)</sup>

The structural differences of the developmental isoforms of smooth muscle MHC between embryonic and adult chicken were suggested.<sup>15)16)</sup>

1 Department of General Education, The school of Allied Medical Sciences Nagasaki University

2 Department of Nursing, The school of Allied Medical Sciences Nagasaki University

From these reasons, the sequence of the junctional region of the short S-2 and the hinge from adult chicken gizzard muscle MHC was determined by direct protein sequencing.

## Materials and Methods

### preparation of S-2

Myosin was prepared from adult chicken gizzard muscle<sup>17-18</sup> and was digested with papain in 200mM CH<sub>3</sub>COONH<sub>4</sub> and 5mM MgCl<sub>2</sub>, pH 7.2 at 20°C for 15min, according to the method as described.<sup>19</sup> The generated rod was separated from S-1 by centrifugation and purified by ethanol precipitation. S-2 was obtained by digesting the rod with  $\alpha$ -chy motrypsin at a substrate to enzyme ratio of 100 : 1(w/w) in 0.5M KCl, 100mM EDTA, and 20mM Tris-phosphate, pH 7.0 at 25°C for 10min. S-2 was separated from LMM according to its solubility in lower ionic strength buffer, and was reduced and S-carboxymethylated.<sup>20</sup> S-carboxymethylated S-2 (CM-S-2) was separated from LMM by DEAE-cellulose (DE-52, 2×18cm, Whatman Biochemical Co.) ion-exchange column chromatography in the presence of 8M urea. SDS polyacrylamide gel electrophoresis

(SDS-page) was performed to examine the purity of myosin, the rod and CM-S-2.<sup>21</sup>

### Chemical cleavage of CM-S-2

CNBr cleavage was carried out in 70% formic acid at 30°C for 24h with 200-fold excess of CNBr per Met.<sup>22</sup> Partial hydrolysis of a peptide was performed with 0.4M formic acid at 110°C for 2h in evacuated sealed tube.<sup>23</sup>

### Enzymatic cleavage of CM-S-2

CM-S-2 was digested with lysyl endopeptidase (S/E=100/1, w/w, Wako Pure Chemical Co.) in 0.1M Tris-HCl buffer, pH 8.0 at 37°C for 6h.

### Separation of peptides

CNBr fragments and lysyl endopeptidase peptides were applied to columns of Sephadex G-100 (1.8×90 cm, Pharmacia Co.) and Sephadex G-50 (1.8×180 cm, Pharmacia Co.) respectively, and were eluted with 50mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8.5.

Peptides in each pool of above columns and hydrolysed peptides of a long CNBr fragment with formic acid were purified by reverse-phase HPLC

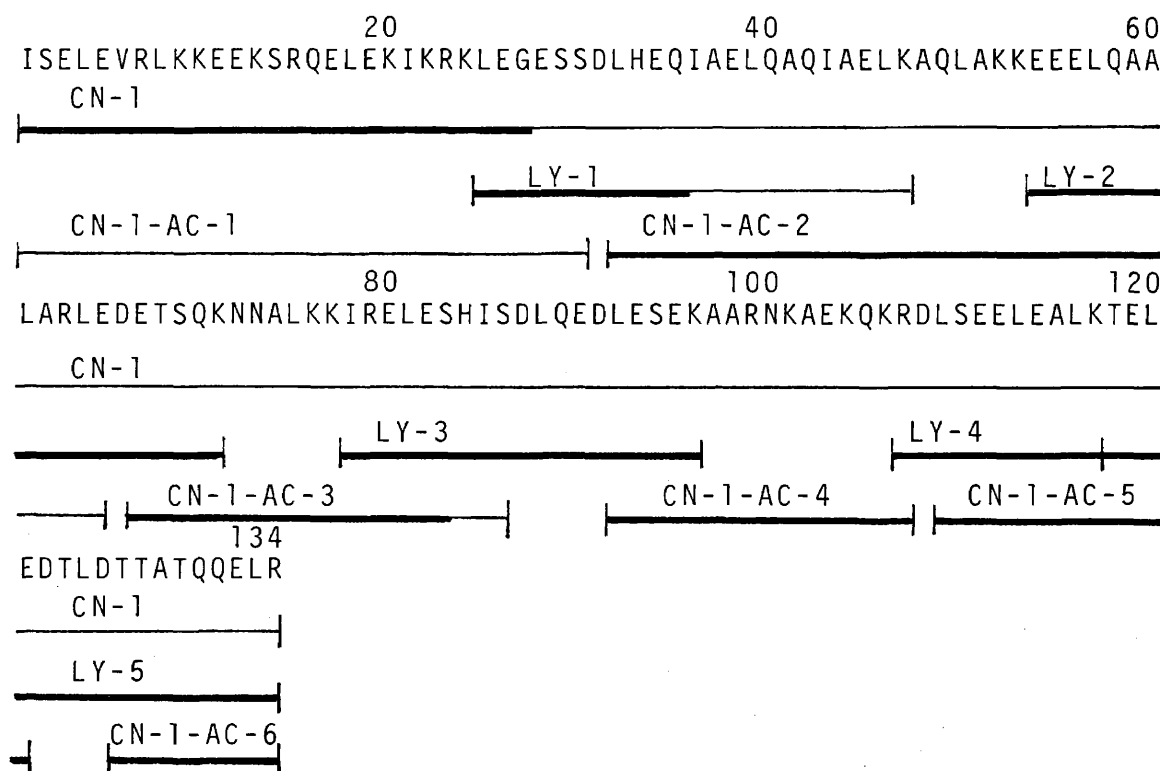


Fig. 1 Summary of sequence studies of the junctional region between the short S-2 and the hinge from adult chicken gizzard muscle myosin heavy chain. Thick lines show the amino-acids determined by automated Edman degradation. CN, AC and LY stand for peptides cleaved with CNBr, formic acid and lysyl endopeptidase, respectively.

# Primary structure of chicken smooth muscle myosin

(RP-HPLC) on columns of  $\mu$ Bondasphere C18 (0.4  $\times$  15cm, Millipore Co.), Puresil C18 (0.4  $\times$  15cm, Millipore Co.) or TSK 120T (0.4  $\times$  25cm, Toyosoda Co.) with acetonitrile gradient elution in 0.1% trifluoroacetic acid (TFA).

## Amino-acid analysis and sequence determination

Amino-acid compositions of isolated peptides were analysed using a JLC-300 automatic amino-acid analyser (JEOL Co.) by the method described in literature after hydrolysis with 5.7M HCl at 110°C for 22h in evacuated sealed tubes.<sup>20</sup>

Sequence determination was carried out in a 476A

		40
(1)chicken gizzard	ISELEVRLKKEEKSQRQELEKIKRKLEGESSDLHEQIAELQ	
(2)chicken brain	+TD++E+++++T+++++A+++D++TT++QD+++++	
(3)drosophila	+T+++E++H+DQQQ+++SDRS+++I+T+VA++K++LN+RR	
(4)chicken skeletal	VDD++GS+EQ+K+L+MD++RA+++++DLKLA+DS+MD+E	
(5)chicken cardiac	VDD++GS+EQ+K+V+MD++RA+++++DLKLTQ+SVMD+E	
		100
(1)AQIAELKAQLAKKEEELQAALARLEDETSQKNNALKKIRELESHISDLQEDLESEKAARN		
(2)+++E+++I+++++GDE+AV+++++V++++QAQ+AE+++++S++		
(3)V+VD+MQ+++++R++++TQT+L+IDE+SAT+AT+Q+AQ+++++QLAEI+++++A++++A		
(4)NDKQQ+DEK+K++DF+ISQIQSKI+++QALGMQLQ+++K++QAR+EE+E+EI+A+RTS+A		
(5)NDKLQMEEK+K+++F+MSQLNSKI+++QAIVMQLQ+++K++QAR+EE+E+E++A+R+++A		
		134
(1)KAEKQKRDLSEELEALKTELEDTLDTTATQQELR		
(2)+++++A+++++		
(3)++++VR+++++N++L+S++++A+++++		
(4)++++HRA+++R+++EISER++EAGGA++A+I+MN		
(5)+V+++RS++AR+++E+SER++EAGGA++A+L+MN		

Fig. 2 Comparison of the junctional regions of known myosins. From the top, (1) adult chicken gizzard muscle (present study) and embryonic chicken gizzard muscle,<sup>20</sup> (2) chicken brain,<sup>20</sup> (3) drosophila nonmuscle,<sup>30</sup> (4) adult chicken skeletal muscle,<sup>30</sup> (5) adult chicken cardiac muscle,<sup>30</sup> myosin heavy chain, respectively. Identical residues among chicken gizzard (1) and others are indicated by '+'.

Table. 1 Amino-acid compositions of CN-1, hydrolysed peptides (AC) of CN-1 with dilute formic acid and lysyl endopeptidase peptides (LY) of CN-1.

Amino acids	CN-1	CN-1 AC-1	CN-1 AC-2	CN-1 AC-3	CN-1 AC-4	CN-1 AC-5	CN-1 AC-6	LY-1	LY-2	LY-3	LY-4	LY-5
Asp	10.4(10)			2.2(2)	1.1(1)				1.0(1)	2.3(2)	0.9(1)	2.0(2)
Thr	5.9(6)			0.9(1)		0.9(1)	3.1(3)		0.9(1)			4.7(5)
Ser	8.1(9)	3.5(4)		2.5(3)	0.8(1)	0.8(1)		2.6(3)	0.8(1)	2.6(3)	0.9(1)	
Glu	40.2(39)	9.4(9)	12.4(12)	4.4(4)	4.5(4)	5.2(5)	3.2(3)	8.4(8)	7.4(7)	6.3(6)	3.2(3)	5.4(5)
Gly	1.0(1)	0.8(1)						1.1(1)				
Ala	13.5(14)		7.7(8)	1.0(1)	2.7(3)	0.9(1)	1.0(1)	2.9(3)	2.7(3)		1.0(1)	1.0(1)
Val	1.2(1)	0.7(1)										
Ile	5.6(6)	2.1(2)	1.8(2)	2.0(2)				1.9(2)		2.0(2)		
Leu	21.2(21)	4.2(4)	7.2(7)	2.2(2)	1.0(1)	4.2(4)	0.9(1)	4.2(4)	2.9(3)	3.3(3)	3.0(3)	2.9(3)
His	2.2(2)		0.8(1)	0.8(1)				0.8(1)		0.7(1)		
Lys	17.6(18)	6.2(6)	3.1(3)	3.1(3)	4.1(4)	1.0(1)		1.1(1)	1.2(1)	0.9(1)	0.9(1)	
Arg	7.1(7)	3.1(3)	1.0(1)	0.9(1)	1.8(2)		0.8(1)		1.1(1)	0.9(1)	1.1(1)	0.9(1)
Total	134	30	34	20	16	13	9	23	18	19	11	17
Position	1/134	1/30	32/65	67/86	92/107	109/121	126/134	25/47	54/71	78/96	107/117	118/134

Cysteine, methionine, tyrosine and phenylalanine were not detected in this region. Values in parentheses were taken from the sequence data.

micro sequence analysis system (Parkin Elmer Co.) using a pulsed liquid method.<sup>25)</sup>

## Results

Typically approximately 250mg of crude CM-S-2 was obtained from 2Kg of gizzard muscle. By ion-exchange column chromatography on DE-52 (Fig. 3), 80mg of purified CM-S-2 was prepared.

### *Purification and sequence analysis of the junctional region*

CM-S-2 (40mg) was cleaved with CNBr in 70% formic acid under nitrogen at 30 °C for 24h. Resulting fragments were fractionated by Sephadex G-100 (1.8 × 90cm) column chromatography into five pools (Fig. 4). A large peptide of the junctional region (CN-1) between the short S-2 and the hinge was isolated from pool 1 in Fig. 4 by following RP-HPLC on a column of  $\mu$ Bondasphere (0.4 × 15cm) (Fig. 5). The amino-acid composition of this CN-1 are analysed (Table. 1) and the partial sequence of the NH<sub>2</sub>-terminal portion was determined up to 27th residue by automated Edman degradation (Fig. 1).

### *Subfragmentation, purification and sequence analysis of CNBr peptides, CN-1*

CN-1 was subfragmented with 0.4M diluted formic acid at 110°C for 2h, and generated peptides were directly subjected to RP-HPLC on a column of Puresil C18 (0.4 × 15cm) (Fig. 6).

Six hydrolysed peptides (CN-1-AC-1 to CN-1-AC-6) were purified and amino-acid composition of each peptide was analysed (Table. 1). These peptides were sequenced completely or partially except for CN-1-AC-1 (Fig. 1).

To obtain overlapping fragments of hydrolysed peptides, CM-S-2 (40mg) was digested with lysyl endopeptidase (S/E = 100/1, w/w) at 37°C for 6h and the digest was separated into 4 pools by Sephadex- G50 (1.8 × 180cm) column chromatography (Fig. 7). Five lysyl endopeptidase peptides were purified from pool 2 and 3 in Fig. 7 by following RP-HPLC on a column of Puresil (0.4 × 15cm).

LY-1, LY-2, LY-3 and LY-5 were isolated from pool 2 in Fig. 7 (Fig. 8) and LY-4 was from pool 3 in Fig. 7 (Fig. 9), respectively. Amino-acid compositions of these five peptides were summarized in Table.1 These lysyl endopeptidase peptides were sequenced completely (LY-2 to LY-5)

or partially (LY-1) by automated Edman degradation. From above results, the complete amino-acid sequence of the junctional portion between the short S-2 and the hinge from adult chicken gizzard muscle MHC was established (Fig. 1).

## Discussion

In striated muscles including cardiac muscle, many myosin heavy chain (MHC) isoforms have been reported to be formed a large multigene family. Their expression is regulated by various physiological or pathophysiological states.

This isoform diversity is necessary to enable muscles to modulate its contractile properties in response to internal or external stimuli.

Although, the rod is involved in the assembly of myosin molecules into filaments, recently many studies have been demonstrated the important roles of the short S-2, the short S-2/the hinge junction, the hinge and the hinge/LMM junctional portions on the modulation of ATPase activity and muscle contraction force and speed.<sup>2)-6)</sup>

Moreover, it has been known that isoform specific regions are located in S-2.<sup>3)</sup> Therefore, it may be possible to thought that S-2 affects the binding of Mg<sup>2+</sup>-ATP and actin to S-1. In smooth muscles, MHC isoform diversity has been shown to be occurred at NH<sub>2</sub>-terminal 25KDa-50KDa junction and COOH-terminal portion in the myosin molecule by their cDNA analysis.<sup>15), 26)-28)</sup> These isoforms are likely to be encoded by a single gene, and above four isoforms are produced by alternative mRNA splicing.<sup>29)</sup> The structure, function, regulation of enzymatic activity and expression of distinct isoforms of smooth muscle MHC are relatively unexplored compared with striated muscle MHC.

Our interest has been in the structure-function relationship of S-2 in the myosin and tissue specific expression mechanism in the physiological or pathophysiological diversity of muscle tissues. The sequence determination by direct protein analysis is necessary to elucidate the molecular basis of functions or post translational modifications in distinct isoforms. In the present paper, the structure of the short S-2/the hinge junctional portion in S-2 from adult chicken gizzard muscle MHC is described.

The results demonstrated here (Fig.1) are completely identical with that of cDNA sequence from embryonic chicken gizzard muscle MHC,<sup>30)</sup> and any

amino-acid difference could not be observed in these portions. Any heterogeneity was not recognized. The periodicity of hydrophobic and charged residues in the 7- and 28-residue units and frequency values of hydrophobic residues at "a" plus "d" in the 7-residue repeat unit pattern<sup>1)</sup> in this junctional portion are well conserved. This sequence was compared with corresponding regions of chicken brain MHC,<sup>28)</sup> drosophila nonmuscle MHC,<sup>30)</sup> chicken skeletal muscle MHC<sup>31)</sup> and chicken cardiac muscle MHC<sup>32)</sup> as presented in Fig. 2. The results showed 81.3%, 59.7%, 38.8%, and 40.3% sequence identities, respectively, suggesting that the degrees of sequence homology of MHC are depended on tissues rather than species.

At present, it is unclear whether the entire structure of two MHCs from embryonic and adult chicken gizzard muscle are completely identical or not. In this regard, further evidence is necessary to elucidate the developmental diversity of chicken smooth muscle MHC. It will be of interest to analyse gizzard MHC isoforms among various physiological changes. Studies such as these should lead to a more complete understanding of the role of each portion and isoforms of smooth muscle MHC in normal and pathological states.

## References

- 1) McLachlan AD and Karn J: Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacing in muscle. *Nature* (London) 1982, 299: 226-231.
- 2) Lovell S, Karr T and Harrington WF: Suppression of contractile force in muscle fibers by antibody to myosin subfragment-2. *Proc. Natl. Acad. Sci. USA*. 1988, 85: 1849-1853.
- 3) McNally EM, Kraft R, Bravo-Zehnder M, Tayler DA and Leinwand LA: Full-length rat alpha and beta cardiac myosin heavy chain sequence. *J. Mol. Biol.* 1989, 210: 665-671.
- 4) Harrington WF, Karr T, Busa WB and Lovell S J: Contraction of myofibrils in the presence of antibodies to myosin subfragment 2. *Proc. Natl. Acad. Sci. USA*. 1990, 87: 7453-7456.
- 5) Collier VK, Kronert WK, O'Donnell PT, Edwards KA and Bernstein SI: Alternative myosin hinge region are utilized in a tissue-specific fashion that correlates with muscle contraction speed. *Genes and Dev.* 1990, 4: 885-895.
- 6) Margossian SS, Krueger JW, Sellar JR, Cuda G, Gaulfield JB, Norton P and Slayter HS: Influence of the cardiac myosin hinge region on contractile activity. *Proc. Natl. Acad. Sci. USA*. 1991, 88: 4941-4945.
- 7) Kavinsky C, Umeda P, Sinha A, Elzinga M, Tong S, Zak R, Jakovich S and Robinowitz M: Cloned mRNA sequences for two types of embryonic myosin heavy chains from chick skeletal muscle. *J. Biol. Chem.* 1983, 258: 5196-5202.
- 8) Periasamy M, Wieczorek D and Nadel-Ginard B: Characterization of a developmentally regulated perinatal myosin heavy chain gene expressed in skeletal muscle. *J. Biol. Chem.* 1984, 259: 13573-13578.
- 9) Rovner AS, Thompson MM and Murphy RA: Two different heavy chains are found in smooth muscle myosin. *Am. J. Physiol.* 1986, 250: C861-C870.
- 10) Kawamoto S and Adelstein RS: Characterization of myosin heavy chains in cultured aorta smooth muscle cells. *J. Biol. Chem.* 1987, 262: 7282-7288.
- 11) Eddinger TJ and Murphy RA: Two smooth muscle myosin heavy chains differ in their light meromyosin fragment. *Biochemistry*. 1988, 27: 3807-3811.
- 12) Nagai R, Kuro-o M, Babij P and Periasamy M: Identification of two types smooth muscle myosin heavy chain isoform by cDNA cloning and immunoblot analysis. *J. Biol. Chem.* 1989, 264: 9734-9737.
- 13) Sartore S, Demarzo N, Borriore AC, Zanellato AMC, Saggin L, Fabbri L and Schiaffino S: Myosin heavy-chain isoforms in human smooth muscle. *Eur. J. Biochem.* 1989, 179: 79-85.
- 14) Watanabe B, Tanigawa M: Primary structure of subfragment-2 from adult chicken cardiac ventricular muscle myosin. *Biol. Chem. Hoppe-Seyler*. 1993, 374: 27-35.
- 15) Kelley CA, Takahashi M, Yu JH and Adelstein S: An insert of seven amino acids confers functional differences between smooth muscle myosins from the intestines and vasculature. *J. Biol. Chem.* 1993, 268: 12848-12854.
- 16) Kuro-o M, Nagai R, Nakahara K, Katoh H, Tsai RC, Tsuchimochi H, Yazaki Y, Ohkubo, A and Takaku F: cDNA cloning of a myosin heavy chain isoform in embryonic smooth muscle and its expression during vascular development and in arteriosclerosis. *J. Biol. Chem.* 1991, 266: 3768-3773.
- 17) Ebashi S: A simple method of preparing actin-

- free myosin from smooth muscle. *J. Biochem.* 1976, 79: 229-231.
- 18) Ikebe M, Aiba T, Onishi H and Watanabe S: Calcium sensitivity of contractile proteins from chicken gizzard muscle. *J. Biochem.* 1978, 83: 1643-1655.
  - 19) Margossian SS, Lowey S and Barshop B: Effect of DTNB light chain on the interaction of vertebrate skeletal myosin with actin. *Nature.* 1975, 258: 163-166.
  - 20) Crestfield AM, Moore S and Stein WH: The preparation and enzymatic hydrolysis of reduced and S-carboxymethylated proteins. *J. Biol. Chem.* 1963, 238: 622-627.
  - 21) Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London).* 1970, 227: 680-685.
  - 22) Gross E and Witkop B: Nonenzymatic cleavage of peptide bonds: The methionine residues in bovine pancreas ribonuclease. *J. Biol. Chem.* 1962, 237: 1856-1860.
  - 23) Inglis AS: Cleavage at aspartic acid. *Methods Enzymol.* 1983, 91: 324-332.
  - 24) Spackman DH, Stein WH and Moore S: Automatic recording apparatus for use in the chromatography of amino-acids. *Anal. Chem.* 1958, 30: 1190-1206.
  - 25) Hewick RM, Hunkapiller MW, Hood LE and Dreyer W. J: A gas-liquid solid phase peptide and protein sequenator. *J. Biol. Chem.* 1981, 256: 7990-7997.
  - 26) Hamada Y, Yanagisawa M, Katsuragawa Y, Coleman J, Nagata S, Matsuda G and Masaki T: Distinct vascular and intestinal smooth muscle myosin heavy chain mRNAs are encoded by a single-copy gene in the chicken. *Biochem. Biophys. Res. Commun.* 1990, 170: 53-58.
  - 27) Babij P, Kelley C and Periasamy M: Characterization of a mammalian smooth muscle myosin heavy chain gene: Complete nucleotide and protein coding sequence and analysis of the 5' end of the gene. *Proc. Natl. Acad. Sci. USA.* 1991, 88: 10676-10680.
  - 28) Takahashi M, Kawamoto S and Adelstein R: Evidence for inserted sequences in the head region on nonmuscle myosin specific to the nervous system. *J. Biol. Chem.* 1992, 267: 17864-17871.
  - 29) Yanagisawa M, Hamada Y, Katsuragawa Y, Imamura M, Mikawa T and Masaki T: Complete primary structure of vertebrate smooth muscle myosin heavy chain deduced from its complementary DNA sequence. *J. Mol. Biol.* 1987, 198: 143-157.
  - 30) Ketchum AS, Stewart CT, Stewart M and Kiehart DP: Complete sequence of the drosophila nonmuscle myosin heavy chain transcript : Conserved sequences in the myosin tail and differential splicing in the 5' untranslated sequence. *Proc. Natl. Acad. Sci. USA.* 1990, 87: 6316-6320.
  - 31) Watanabe B: Complete amino-acid sequence of subfragment-2 in adult chicken skeletal muscle myosin. *Biol. Chem. Hoppe-Seyler.* 1989, 370: 1027-1034.
  - 32) Watanabe B: Primary structure of subfragment-2 from adult chicken cardiac ventricular muscle myosin. *Biol. Chem. Hoppe-Seyler.* 1993, 374: 445-454.

# Supplemental Materials

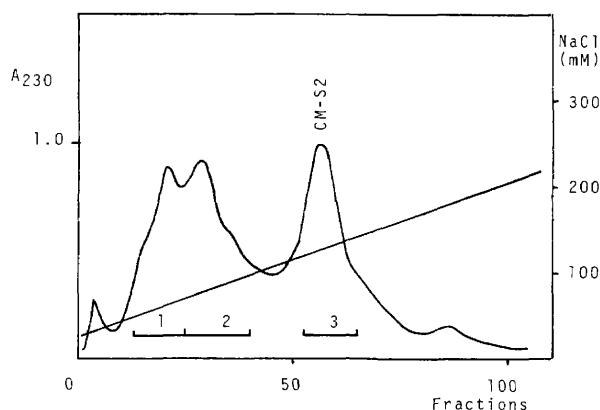


Fig. 3 Purification of CM-S-2 on DEAE-cellulose (DE-52, 2×18cm) equilibrated with 25mM Tris-HCl buffer, pH8.4 in 8M urea and eluted with a linear gradient of NaCl (25 → 250mM, 500+500ml). Flow rate was 60 ml/h and 10ml fractions were collected. The effluent was pooled as indicated by the bars (pools 1-3).

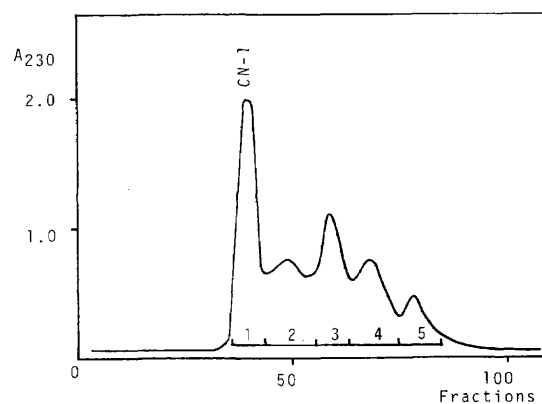


Fig. 4 Separation of CNBr degraded CM-S-2 on Sephadex G-100 (1.8×90cm) equilibrated and eluted with 50mM NH<sub>4</sub>HCO<sub>3</sub>, pH8.5 at a flow rate of 20ml/h. 2.6ml fractions were collected. The effluent was pooled as indicated by the bars (pools 1-5).

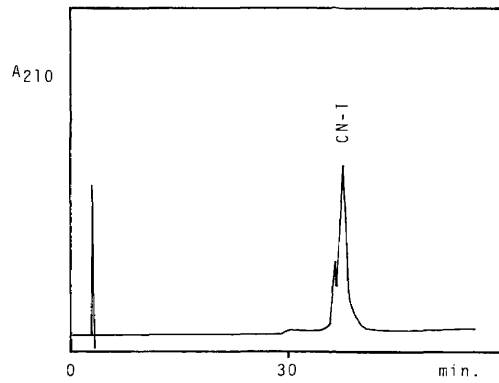


Fig. 5 Separation of peptides in pool 1 in Fig.4 by RP-HPLC on a column of  $\mu$  Bondasphere ( $0.4 \times 15$ cm). Elution was performed with a liner gradient of acetonitrile in 0.1% trifluoroacetic acid from 20% to 80% in 100min. at a flow rate of 0.7ml/min.

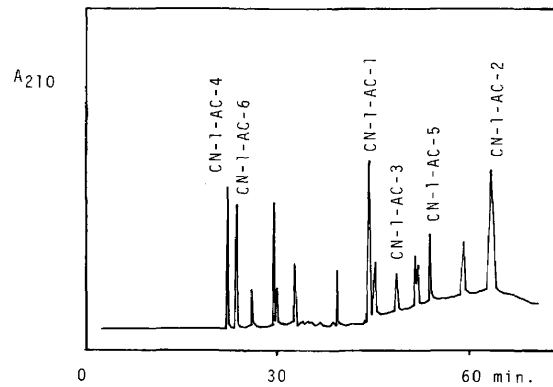


Fig. 6 Separation of hydrolysed peptides of CN-1 with formic acid by RP-HPLC on a column of Puresil ( $0.4 \times 15$ cm). Elution was performed with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid from 0 to 80% in 120min. at a flow rate of 0.7ml/min.

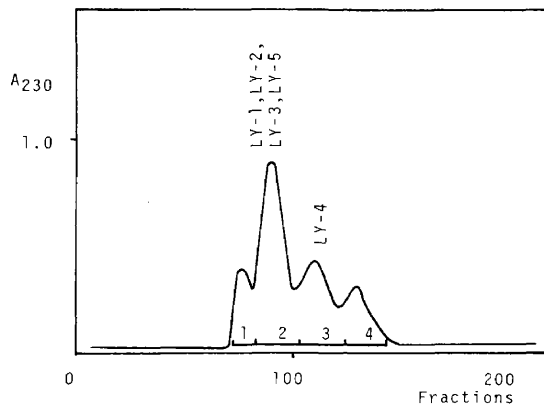


Fig. 7 Separation of lysyl endopeptidase peptides of CM-S-2 on Sephadex G-50 ( $1.8 \times 180$ cm) equilibrated and eluted with 50mM  $\text{NH}_4\text{HCO}_3$ , pH8.5 at a flow rate of 18ml/h. 3ml of fractions were collected. The effluent was pooled as indicated by the bars (pools 1-4).

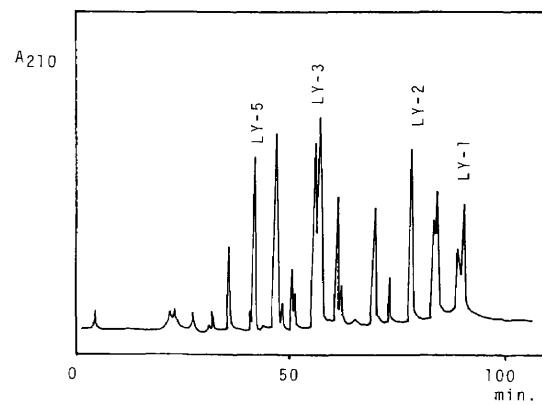


Fig. 8 Separation of peptides in pool 2 in Fig.7 by RP-HPLC on a column of Puresil ( $0.4 \times 15$  cm) with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid from 0 to 60% in 100min, at a flow rate of 0.7ml/min.

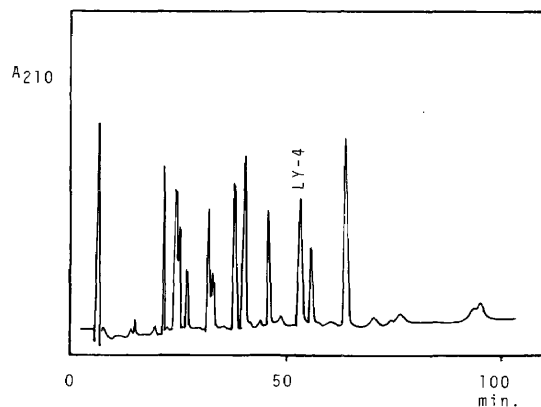


Fig. 9 Separation of peptides in pool 3 in Fig.7 by RP-HPLC on a column of Puresil ( $0.4 \times 15$  cm). conditions were as in Fig.8.

# 成鶏平滑筋ショート・サブフラグメント-2, ヒンジ連結領域の一次構造

渡辺 文治<sup>1)</sup> 谷川美保子<sup>2)</sup>

1 長崎大学医療技術短期大学部一般教育

2 長崎大学医療技術短期大学部看護学科

**要 旨** われわれは [Watanabe B (1989), Biol. Chem. Hoppe-Seyler 370(9): 1027-1034 and Watanabe B, Tanigawa M (1993), Biol. Chem. Hoppe-Seyler 374(1): 27-35] において成鶏の骨格筋, 心室筋ミオシンのサブフラグメント-2の構造と機能の関連性について発表した, 今回の論文は成鶏砂のう筋ミオシン(平滑筋)のショート・サブフラグメント-2とヒンジとの連結部領域の構造を明らかにしたものである。

ミオシンをパパインで消化して得られた尾部(ロッド)をさらに $\alpha$ -キモトリプシンで限定消化を行い, サブフラグメント-2(S-2)とライトメロミオシン(LMM)に分離した。次いでサブフラグメント-2のプロムシアン分解ペプチドの中から, ショート・サブフラグメント-2のカルボキシ末端領域とヒンジのアミノ末端領域を含む134個のアミノ酸からなるペプチドを精製単離し, 一次構造を決定した。

さらに構造的相異を分析するために上記のようにして決定した連結部分の構造を胎鶏の砂のう筋(平滑筋), 成鶏の脳, ドロソフィラ, 成鶏骨格筋, 成鶏心室筋ミオシンの相同部分の構造と比較した。その結果, 興味あることに胎鶏の砂のう筋, 成鶏の脳, ドロソフィラの非筋ミオシンとはそれぞれ100%, 81.3%, 59.7%の高いアミノ酸の同一性が認められ, これに対し成鶏骨格筋と成鶏心室筋ミオシンとはそれぞれ38.8%, 40.3%と低いアミノ酸の同一性が認められた。

長崎大医療技短大紀: 8: 1-8, 1994