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Amino Acid Sequence of Short Subfragment-2 from Adult Chicken Gizzard Muscle Myosin

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The complete amino acid sequence of the short subfragment-2 (short S-2) from adult chicken gizzard muscle has been determined by direct analysis of peptides derived from digests of this portion with cyanogen bromide, lysyl endopeptidase and from hydrolysates of CNBr fragments with dilute formic acid. It contains 323 amino acid residues which span the NH_2-terminus of the subfragment-2 (S-2) to the NH_2-terminal portion of the hinge region in S-2. Although, structural differences between embryonic and adult chicken gizzard muscle myosin heavy chain (MHC) have been suggested, comparison of the amino acid sequence of the short S-2 from adult chicken gizzard muscle MHC with that from embryonic chicken gizzard MHC, deduced from its nucleotide sequence of the cDNA, showed that the sequence were identical.

The amino acid sequence of the short S-2 from adult chicken gizzard muscle MHC was compared with the corresponding sequence of chicken brain myosin, *Drosophila* non-muscle myosin, chicken skeletal muscle myosin and chicken cardiac ventricular muscle myosin. The results show relatively higher sequence identities with chicken brain myosin (76.5%) and *Drosophila* non-muscle myosin (60.0%) and lower identities with chicken skeletal muscle myosin (36.8%) and chicken cardiac ventricular muscle myosin (36.5%).

Key words : amino acid sequence, smooth muscle myosin, myosin short subfragment-2.

Introduction

Myosin is an important contractile protein in all muscle and non-muscle cells, and the myosin molecule consists of two heavy chains and two pairs of light chains. Myosin generates the force of muscle contraction by interaction with actin and ATP on their binding sites located within the head portion. By limited digestion, myosin heavy chain (MHC) is divided into the globular head (subfragment-1, S-1) and the α-helical tail region (rod). The rod is further subdivided into three substructural regions, the short subfragment-2 (short S-2), the hinge and the light meromyosin (LMM) by limited proteolysis[1)2)].

The function of the rod has been extensively studied mainly in terms of its self-assembling ability[3)], but many recent studies elucidated the important properties of the short S-2, the hinge and the hinge/LMM junction in relation to the contractile force generation and speed, or the isoform-specific function of the MHC[4)5)6)7)8)]. In striated muscle, MHC isoforms are encoded by a multigene family[9)10)], however, smooth muscle MHC isoforms are the products of alternative mRNA processing of the 3′ region[11)12)13)14)15)] or 5′ region[16)17)18)19)] from a single gene.

Sequence analysis at the protein level provides useful and important information for the protein chemistry. Differences in the amino acid sequences obtained by protein analysis and by cDNA sequencing have been reported[20)]. Tissue-specific and developmental isoforms of smooth muscle MHC between the embryonic and adult chicken were reported[17)21)]. Although the entire sequence of embryonic chicken gizzard muscle MHC has been published[22)], the entire sequence of the smooth muscle MHC from direct protein analysis has not been reported. In the present study, the sequence of the short S-2 from adult chicken gizzard muscle MHC was determined by direct protein sequencing.

Materials and Methods

Preparation of S-2

Myosin was prepared from adult Hubbered-type chicken gizzard muscle essentially according to the method described by Ebashi et al.[23)] and Ikebe et al.[24)]. The rod was obtained by digesting myosin with papain (enzyme/substrate (E/S) = 1/200. W/W) in 200 mM CH_3COONH_4 and 5 mM $MgCl_2$, pH 7.2 at 20℃ for 15 min. The reaction is stopped by the addition of iodoacetate to a final concentration of 1 mM[25)]. The rod was further subdivided into S-2 and LMM by digesting with trypsin (E/S = 1/100. W/W) in 0.6 M KCl, 20 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.5, at 20℃ for 20 min. S-2 was separated from LMM based on its solubility in lower ionic strength buffer, pH 7.5, and was reduced and S-carboxymethylated[26)]. S-carboxymethylated S-2 (CM-S-2) was further purified by DE-52 (2×18

cm, Whatman Biochemical Co.) ion-exchange column chromatography in the presence of 8 M urea. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out to examine the purity of myosin, the rod and CM-S-2[27].

Chemical Cleavages of CM-S-2

CM-S-2 (40 mg) was degraded with 200-fold excess of CNBr per Met in 2.0 ml of 70% formic acid under nitrogen at 30℃ for 24h[28]. Two larger CNBr fragments were further hydrolysed with 0.4 M formic acid at 110℃ for 2 h in evacuated sealed tubes, respectively[29].

Enzymatic Cleavages of CM-S-2

CM-S-2 (40 mg) was digested with lysyl endopeptidase (E/S = 1/100. W/W. Wako Pure Chemical Industries.) in 0.1 M Tris-HCl, pH 8.0, at 37℃ for 8 h.

Separation of Peptides

CNBr fragments and lysyl endopeptidase peptides of CM-S-2 were first subjected to chromatography on columns of Sephadex G-100 (1.8×90 cm, Pharmacia Co.) and Sephadex G-50 (2.0×180 cm, Pharmacia Co.) equilibrated and eluted with 0.05 M NH_4HCO_3, pH 8.6.

Peptides in each pool of the above columns were further purified by reverse-phase high-performance liquid chromatography (RP-HPLC) with acetonitrile gradient elution in 0.1% trifluoroacetic acid (TFA) on columns of μ Bondas phere C18 (0.4×15 cm, Millipore Co.), Puresil C18 (0.4×15 cm, Millipore Co.) or TSK 120T (0.4×25 cm, Toyosoda Co.). Hydrolysed peptides with dilute formic acid were purified directly by RP-HPLC under the same conditions as above.

Amino acid analysis and sequence determination

The amino acid compositions of purified peptides hydrolysed with 5.7 M HCl at 110℃ for 22 h in evacuated sealed tubes were analysed using a JLC-300 automatic amino acid analyser (JEOL Co.) by a method previously described[30]. Sequences of peptides were determined in a 477A protein sequencer (Applied Biosystems Instrument Co.) with a 120 A PTH analyser (Applied Biosystems Instrument Co.) or 476 A microsequence analysis system (Perkin-Elmer Cetus.) by the pulsed liquid method[31].

Results

CM-S-2 was purified further by ion-exchange chromatography on DE-52 (Fig. 3).

The 323 amino acid sequence of CM-S-2 was determined by structural analysis of CNBr peptides, hydrolysis of peptides of larger CNBr fragments by formic acid and lysyl endopeptidase peptides of CM-S-2, as presented in Fig. 1.

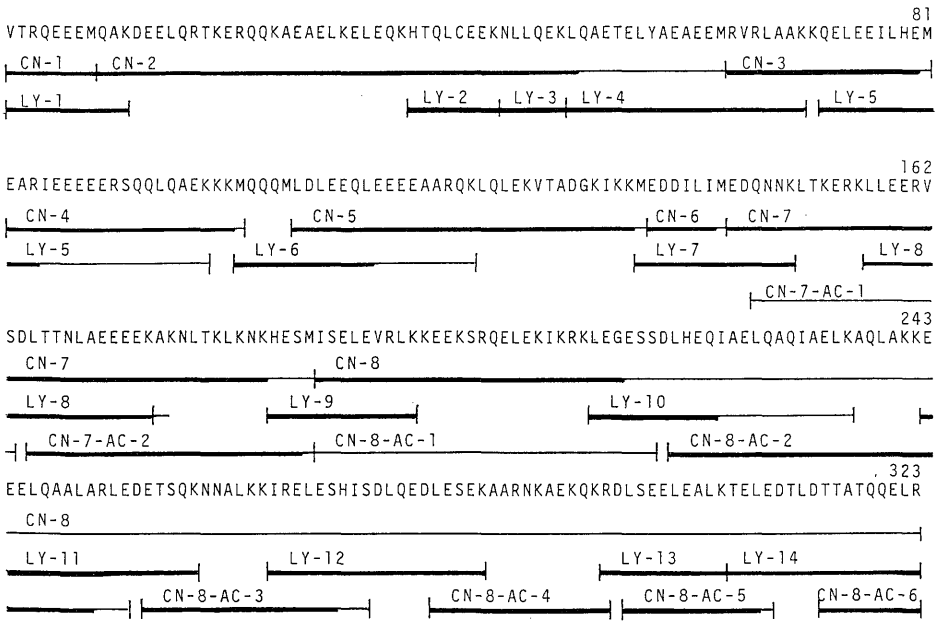

Fig.1 Summary of sequence studies of the short S-2 from adult chicken gizzard muscle myosin. Thick lines show the amino acids determined by automated Edman degradation. CN, AC and LY stand for peptides cleaved with CNBr, formic acid and lysyl endopeptidase, respectively.

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                                                                                   60
(1)chicken gizzard     VTRQEEEMQAKDEELQRTKERQQKAEAELKELEQKHTQLCEEKNLLQEKLQAETELYAEA
(2)chicken brain       +++++++L+++++++MKV++K+T+V++++E+M+R++Q++L++++I+A+Q+++++++F+++
(3)drosophila          ++K+++KLVQ+ED++KQVR+KLDTLAKNTQ+Y+R+YQ+ALV++TT+A+Q++++I++C+++
(4)chicken skeletal    SAES+K++ANMK++FEK+++ELA+S++KR++++E+MVV+LQ+++D++LQV+++ADSL+D+
(5)chicken cardiac     SAET+K++ANMK++FLKL++ALE+S++RR++++E+QVS+VQ+++D+LLQ++++QDTL+D+

                                                                                   140
(1)EEMRVRLAAKKQELEEILHEMEARIEEEEERSQQLQAEKKKMQQQMLDLEEQLEEEEAARQKLQLEKVTADGKIKKMEDD
(2)++++A++++++++++++++DL+S+V++++++N+I++N++++++GHIQ++++++D+++G++++++++++++EA++++++EE
(3)++S+S++M+R+++++DMMQ+L+T++++++++VLA+GG++++LELNIQ++++++++++++++++++++QL+A++++Y+E+
(4)++RQDQ+IKT+IQ++AKIK+VTE+A+D+++INAE+T+K+R+LEDECSE+KKDIDDL+LTLA+VEK++HATEN+V+NLTEE
(5)++RCDL+IKS+IQ++AKVK+LTE+V+D+++MNSE+TSK+R+LEDECSE+KKDIDDL+ITLA+VEK++HATEN+V+NLTEE

                                                                                   220
(1)ILIMEDQNNKLTKERKLLEERVSDLTTNLAEEEEKAKNLTKLKNKHESMISELEVRLKKEEKSRQELEKIKRKLEGESSD
(2)++LL++++S+FL++K++M+D+IAEC+SQ+++++++++++A+++++Q+M++TD++E+++++++T++++++A++++D++TT+
(3)LALTD+++Q++L++K++++++AN++SQT+++++++++H+A+++A+++AT+T+++E++H+DQQQ+++SDRS+++I+T+VA+
(4)MAVLDETIA+++++K+A+Q+AHQQTLDD+QV++D+VNT+++A+T+L+QQVDD++GS+EQ+K+L+MD++RA++++++DLKL
(5)MATLDENIS+++++K+S+Q+AHQQVLDD+QA++D+VNT+S+A+V+L+QQVDD++GS+EQ+K+V+MD++RA++++++DLKL

                                                                                   300
(1)LHEQIAELQAQIAELKAQLAKKEEELQAALARLEDETSQKNNALKKIRELESHISDLQEDLESEKAARNKAEKQKRDLSE
(2)+QD+++++++++E+++I++++++++++++++GDE+AV+++++++V++++QAQ+AE++++++++++S+++++++++++++
(3)+K++LN+RRV+VD+MQ+++++R++++TQT+L+IDE+SAT+AT+Q+AQ+++++QLAEI+++++A+++++A++++VR+++++
(4)A+DS+MD+ENDKQQ+DEK+K++DF+ISQIQSKI+++QALGMQLQ+++K++QAR+EE+E+EI+A+RTS+A++++HRA+++R
(5)TQ+SVMD+ENDKLQMEEK+K+++F+MSQLNSKI+++QAIVMQLQ+++K++QAR+EE+E+E++A+R+++A+V+++RS++AR

                    323
(1)ELEALKTELEDTLDTTATQQELR
(2)+++++++++++++++++A+++++
(3)++++++N++L+S+++++A+++++
(4)+++EISER++EAGGA++A+I+MN
(5)+++E+SER++EAGGA++A+L+MN
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Fig. 2 Comparison of the short S-2 of known myosins. From the top to bottom : (1) adult chicken gizzard muscle (present study) and embryonic chicken gizzard muscle[22], (2) chicken brain[32], (3) *Drosophila* non-muscle[33], (4) adult chicken skeletal muscle[34] and (5) adult chicken cardiac muscle[20] myosin heavy chains. Identical residues among chicken gizzard (1) and others are indicated by '+'.

Table 1. Amino acid composition of cyanogen bromide peptides of CM-S-2.

	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6	CN-7	CN-8
CM-Cys		0.8 (1)						
Asp		2.2 (2)			2.2 (2)	2.0 (2)	6.9 (7)	10.4 (10)
Thr	1.0 (1)	2.7 (3)			0.9 (1)		3.8 (4)	5.9 (6)
Ser				0.9 (1)			2.1 (2)	8.1 (9)
Glu	4.4 (4)	24.4 (23)	5.0 (5)	10.5 (10)	10.6 (10)	1.2 (1)	10.9 (10)	40.2 (39)
Gly					1.0 (1)			1.0 (1)
Ala		5.8 (6)	2.1 (2)	1.9 (2)	3.0 (3)		1.8 (2)	13.5 (14)
Val	0.9 (1)		1.1 (1)		1.2 (1)		1.0 (1)	1.2 (1)
Met	0.8 (1)	0.8 (1)	1.0 (1)	0.9 (1)	0.9 (1)	0.9 (1)	0.9 (1)	
Ile			1.1 (1)	1.0 (1)	0.9 (1)	2.0 (2)		5.6 (6)
Leu		8.2 (8)	3.0 (3)	1.0 (1)	4.7 (5)	0.9 (1)	6.7 (7)	21.2 (21)
Tyr		0.7 (1)						
Phe								
His		0.8 (1)	1.1 (1)				1.2 (1)	2.2 (2)
Lys		6.9 (7)	1.9 (2)	2.9 (3)	4.7 (5)		7.8 (8)	17.6 (18)
Arg	0.9 (1)	1.7 (2)	1.8 (2)	2.0 (2)	0.9 (1)		1.9 (2)	7.1 (7)
Total	8	55	18	21	31	7	45	134
Position	1/8	9/63	64/81	82/102	107/137	138/144	145/189	190/323

Cysteine and methionines were determined as carboxymethyl cysteine (CM-Cys) and homoserines, respectively. Values in parentheses were taken from the sequence data.

Table 2. Amino acid composition of peptides from CN-7 and CN-8 hydrolysed with dilute formic acid, respectively.

	CN-7-AC-1	CN-7-AC-2	CN-8-AC-1	CN-8-AC-2	CN-8-AC-3	CN-8-AC-4	CN-8-AC-5	CN-8-AC-6
CM-Cys								
Asp	2.0 (2)	3.0 (3)			2.2 (2)	1.1 (1)		
Thr	0.9 (1)	2.5 (3)			0.9 (1)		0.9 (1)	3.1 (3)
Ser	0.8 (1)	0.9 (1)	3.5 (4)		2.5 (3)	0.8 (1)	0.8 (1)	
Glu	4.3 (4)	5.3 (5)	9.4 (9)	12.4(12)	4.4 (4)	4.5 (4)	5.2 (5)	3.2 (3)
Gly			0.8 (1)					
Ala		1.9 (2)		7.7 (8)	1.0 (1)	2.7 (3)		
Val	0.9 (1)		0.7 (1)				0.9 (1)	1.0 (1)
Met		0.9 (1)						
Ile			2.1 (2)	1.8 (2)	2.0 (2)			
Leu	3.0 (3)	4.3 (4)	4.2 (4)	7.2 (7)	2.2 (2)	1.0 (1)	4.2 (4)	0.9 (1)
Tyr								
Phe								
His		1.0 (1)		0.8 (1)	0.8 (1)			
Lys	3.2 (3)	5.2 (5)	6.2 (6)	3.1 (3)	3.1 (3)	4.1 (4)	1.0 (1)	
Arg	1.9 (2)		3.1 (3)	1.0 (1)	0.9 (1)	1.8 (2)		0.8 (1)
Total	17	25	30	34	20	16	13	9
Position	147/163	165/189	190/219	221/254	256/275	281/296	298/310	315/323

Values in parentheses were taken from the sequence data.

Table 3. Amino acid composition of lysyl endopeptidase peptides of CM-S-2.

	LY-1	LY-2	LY-3	LY-4	LY-5	LY-6	LY-7	LY-8	LY-9	LY-10
CM-Cys		0.9 (1)								
Asp			1.0 (1)			1.1 (1)	4.9 (5)	2.1 (2)		
Thr	0.9 (1)	0.8 (1)		1.0 (1)				1.8 (2)		
Ser					0.8 (1)			0.8 (1)	1.8 (2)	2.6 (3)
Glu	5.2 (5)	3.4 (3)	2.1 (2)	6.5 (6)	15.6 (15)	11.4 (11)	3.4 (3)	6.4 (6)	3.3 (3)	8.4 (8)
Gly										1.1 (1)
Ala	1.2 (1)			5.1 (5)	2.1 (2)	2.1 (2)		1.1 (1)		2.9 (3)
Val	1.0 (1)			1.0 (1)				1.0 (1)	1.0 (1)	
Met	0.8 (1)			0.8 (1)	0.8 (1)	1.7 (2)	1.8 (2)		0.8 (1)	
Ile					1.8 (2)		1.9 (2)		0.9 (1)	1.9 (2)
Leu		1.0 (1)	1.9 (2)	2.8 (3)	2.9 (3)	2.7 (3)	1.0 (1)	3.9 (4)	2.0 (2)	4.2 (4)
Tyr				0.9 (1)						
Phe										
His		0.9 (1)			1.0 (1)				1.1 (1)	0.8 (1)
Lys	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.1 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.1 (1)	1.0 (1)
Arg	0.9 (1)			1.9 (2)	1.9 (2)	1.0 (1)		0.9 (1)	1.0 (1)	
Total	11	8	6	21	28	21	14	19	13	23
Position	1/11	36/43	44/49	50/70	72/99	102/122	137/150	157/175	186/198	214/236

Purification and sequence analysis of the CNBr fragments

CM-S-2 was cleaved with CNBr. Sephadex G-100 (1.8×90 cm) column chromatography of the CNBr fragments gave five pools (Fig. 4). Peptides in each pool were separated further by RP-HPLC on a column of μ Bondasphere (0.4×15 cm) or Puresil (0.4×15 cm) (Figs. 5-8) followed by TSK-ODS 120 T column (0.4×15 cm) chromatography (data not shown).

A larger peptide (CN-8, residues 190-323) was purified from pool 1 in Fig. 4 (Fig. 5). CN-2, CN-5 and CN-7, and CN-1, CN-3, CN-4 and CN-6 were obtained from pool 2, pool 3 and pool 4 in Fig. 4, respectively (Figs, 6-8) by RP-HPLC. These 8 CNBr peptides (CN-1 to CN-8) purified covered 319 out of 323 amino acid residues. Small CNBr peptides (residues 103-106) could not be isolated.

The amino acid composition of the CNBr fragments were analysed (Table 1).

Complete sequences of CN-1, CN-3, CN-4, CN-5 and CN-6, and partial sequences of CN-2, CN-7 and CN-8 were determined by automated Edman degradation (Fig. 1).

Table 3 (continued).

	LY-11	LY-12	LY-13	LY-14
CM-Cys				
Asp	1.0 (1)	2.3 (2)	0.9 (1)	2.0 (2)
Thr	0.9 (1)			4.7 (5)
Ser	0.8 (1)	2.6 (3)	0.9 (1)	
Glu	7.4 (7)	6.3 (6)	3.2 (3)	5.4 (5)
Gly				
Ala	2.7 (3)		1.0 (1)	1.0 (1)
Val				
Met				
Ile		2.0 (2)		
Leu	2.9 (3)	3.3 (3)	3.0 (3)	2.9 (3)
Tyr				
Phe				
His		0.7 (1)		
Lys	1.2 (1)	0.9 (1)	0.9 (1)	
Arg	1.1 (1)	0.9 (1)	1.1 (1)	0.9 (1)
Total	18	19	11	17
Position	243/260	267/285	293/306	307/323

Cysteine was determined as carboxymethyl cysteine (CM-Cys).
Values in parentheses were taken from the sequence data.

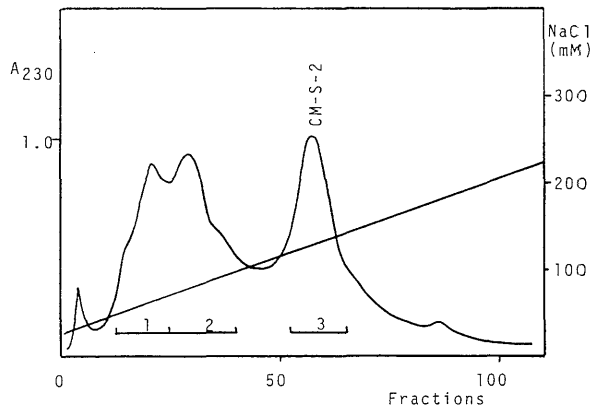

Fig. 3 Purification of S-2 on DEAE-cellulose (2×20 cm) equilibrated with 25 mM Tris-HCl, pH 8.4, in 8 M urea and eluted with a linear gradient of NaCl (25→250 mM, 500+500 ml). Flow rate was 60 ml/h and 10-ml fractions were collected. The effluent was pooled as indicated by the bars (pools 1-3).

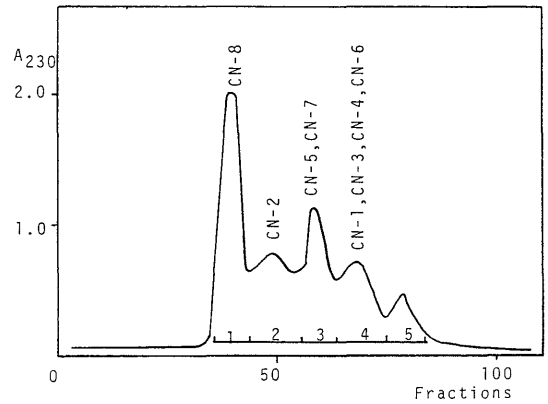

Fig. 4 Separation of CNBr degraded CM-S-2 on Sephadex G-100 (1.8×90 cm) equilibrated and eluted with 0.05 M NH_4HCO_3, pH 8.6 at a flow rate of 20 ml/h. 2.6 ml fractions were collected. The effluent was pooled as indicated by the bars (pools 1-5).

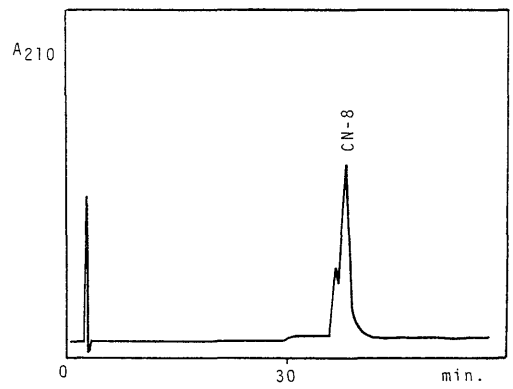

Fig. 5 Separation of peptides in pool 1 in Fig. 4 by RP-HPLC on a column of μ Bondasphere (0.4×15 cm). Elution was performed with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid from 20% to 80% in 100 min. at a flow rate of 0.7 ml/min.

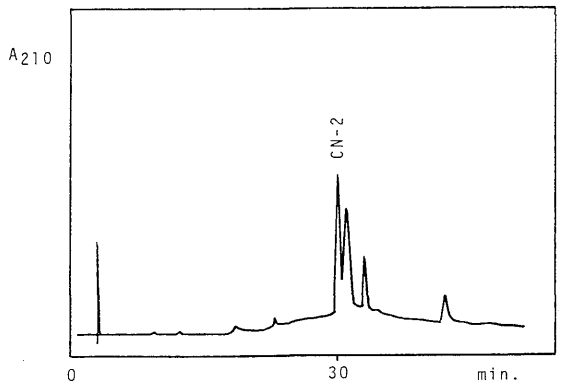

Fig. 6 Separation of peptides in pool 2 in Fig. 4 by RP-HPLC on a column of μ Bondasphere (0.4×15 cm). Conditions were as in Fig. 5.

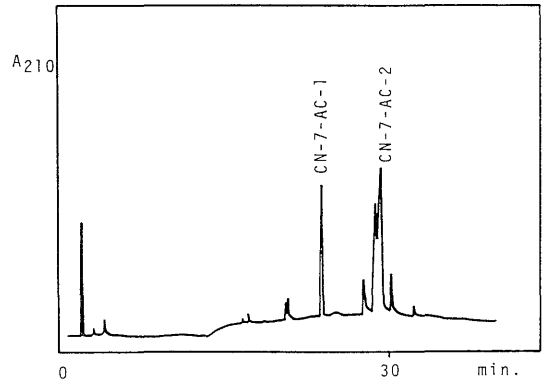

Fig. 9 Separation of hydrolysed peptides of CN-7 with formic acid by RP-HPLC on a column of Puresil (0.4×15 cm). Conditions were as in Fig. 8.

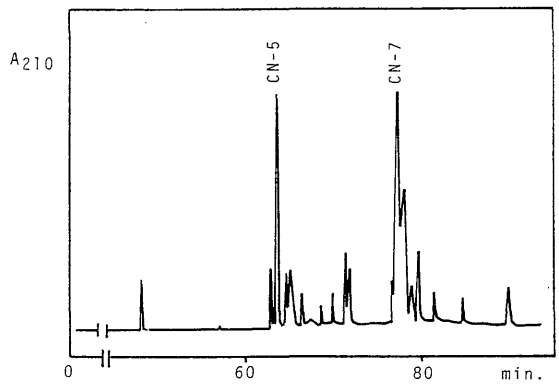

Fig. 7 Separation of peptides in pool 3 in Fig. 4 by RP-HPLC on a column of μ Bondasphere (0.4×15 cm). Elution was performed with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid from 0 to 60% in 120 min. Other conditions were as in Fig. 5.

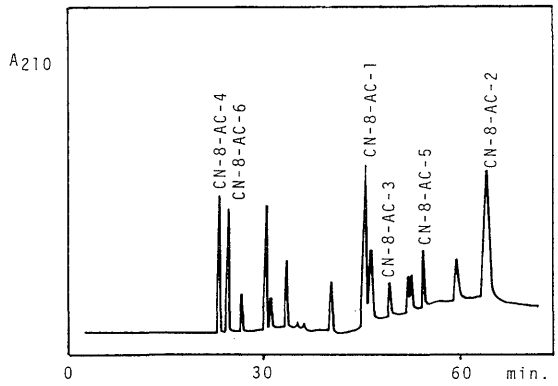

Fig. 10 Separation of hydrolysed peptides of CN-8 with formic acid by RP-HPLC on a column of Puresil (0.4×15 cm). Elution was performed with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid from 0 to 80% in 120 min. at a flow rate of 0.7 ml/min.

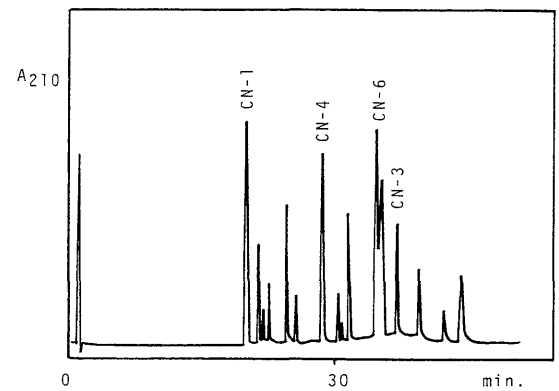

Fig. 8 Separation of peptides in pool 4 in Fig. 4 by RP-HPLC on a column of Puresil (0.4×15 cm). Elution was performed with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid from 0 to 60% in 60 min. at a flow rate of 0.5 ml/min.

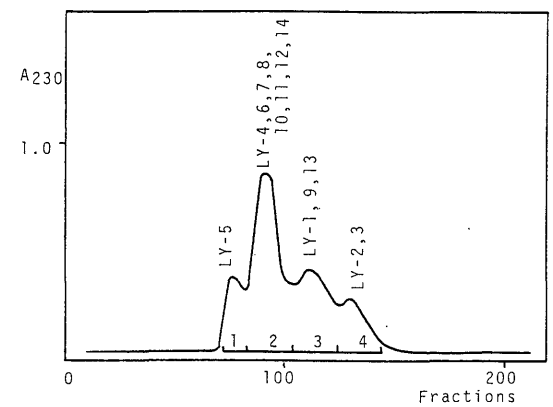

Fig. 11 Separation of lysyl endopeptidase peptides of CM-S-2 on Sephadex G-50 (2.0×120 cm) equilibrated and eluted with 0.05 M NH_4HCO_3, pH 8.6, at a flow rate of 18 ml/h. 3-ml fractions were collected. The effluent was pooled as indicated by the bars (pools 1-4).

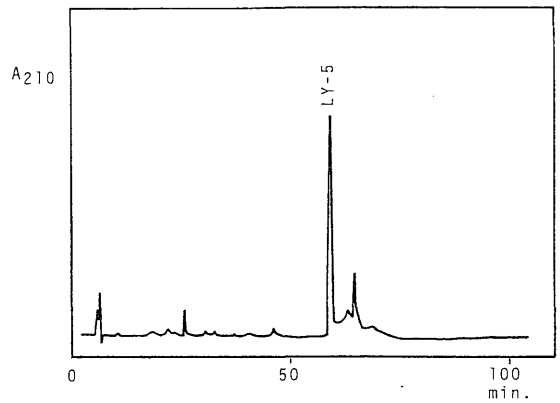

Fig. 12 Separation of peptides in pool 1 in Fig. 11 by RP-HPLC on a column of Puresil (0.4×15 cm) with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid from 0 to 60% in 100 min. at a flow rate of 0.7 ml/min.

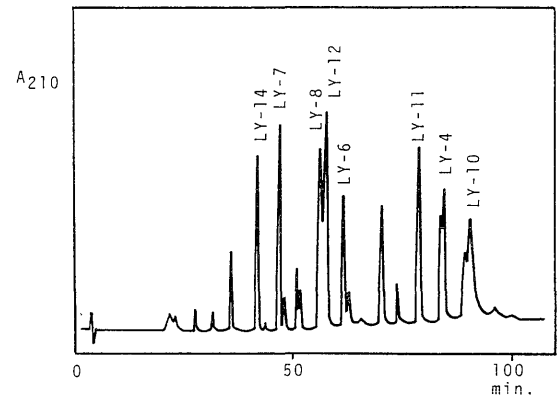

Fig. 13 Separation of peptides in pool 2 in Fig. 11 by RP-HPLC on a column of Puresil (0.4×15 cm). Conditions were as in Fig. 12.

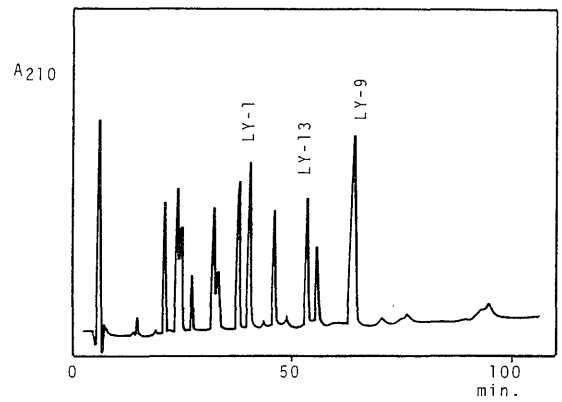

Fig. 14 Separation of peptides in pool 3 in Fig. 11 by RP-HPLC on a column of Puresil (0.4×15 cm). Conditions were as in Fig. 12.

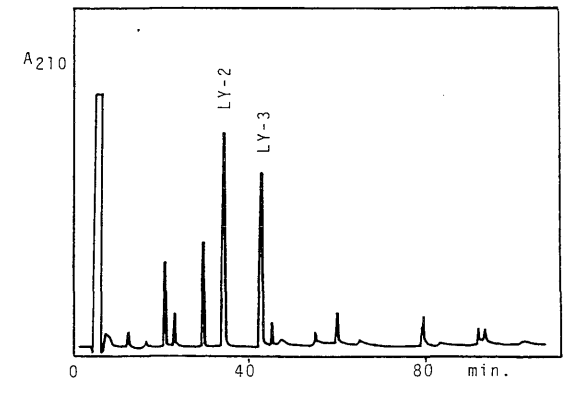

Fig. 15 Separation of peptides in pool 4 in Fig. 11 by RP-HPLC on a column of Puresil (0.4×15 cm). Conditions were as in Fig. 12.

Purification and sequence analysis of subfragments of CNBr peptides

Two larger peptides, CN-7 and CN-8, were subfragmented with 0.4 M dilute formic acid and the resulting peptides were directly separated by RP-HPLC on a column of Puresil (Figs. 9, 10).

Two (CN-7-AC-1 and CN-7-AC-2, Fig. 9) and six (CN-8-AC-1 to CN-8-AC-6, Fig. 10) acid-hydrolysed peptides from CN-7 and CN-8 were purified and the amino acid composition of each peptide was analysed (Table 2). These peptides were sequenced completely or partially except for CN-7-AC-1 and CN-8-AC-1 (Fig. 1).

Purification and sequence analysis of lysyl endopeptidase peptides of CM-S-2

To obtain overlapping peptides of CNBr fragments, CM-S-2 was digested with lysyl endopeptidase, and the digest was fractionated into 4 pools by Sephadex G-50 (2×180 cm) column chromatography (Fig. 11).

Of many lysyl endopeptidase peptides separated by RP-HPLC on a column of Puresil, the amino acid compositions of 14 peptides (LY-1 to LY-14) were analysed and the results are summarized in Table 3. LY-5 was isolated from pool 1 in Fig. 11 (Fig. 12), 8 peptides of LY-4, LY-6 to LY-8, LY-10 to LY-12 and LY-14 from pool 2 in Fig. 11 (Fig. 13), 3 peptides of LY-1, LY-9 and LY-13 from pool 3 in Fig. 11 (Fig. 14), and 2 peptides of LY-2 and LY-3 from pool 4 in Fig.11 (Fig.15).

From the above results, the complete amino acid sequence of the short S-2 of adult chicken gizzard muscle MHC was established.

Discussion

It has been reported that MHC of the chicken smooth muscle is encoded by a single copy gene rather than by a multigene family[22]. The MHC isoforms of the smooth muscle are generated by alternative mRNA processing from a single gene at the 3′ end region, and have different molecular weights[11)12)13)14)15)].

Many recent studies revealed that alternative splicing of the 5′ end region of mRNA from a single gene of the smooth muscle MHC produced two isoforms, one containing an insert nucleotides near the ATP-binding region in subfragment-1 (S-1) and the other lacking this insert, are produced and expressed in different tissue[16)17)19)32)]. MHC isoforms expressed in only embryonic smooth muscle but not in adult tissue were reported[21].

The specific functions of these distinct smooth muscle MHC isoforms in each tissue remains unclear at present. In striated muscle, distinct MHC isoforms are produced by the expression of a multigene family with different contractile force generation and speed.

These functions are modulated in part by the S-2 portion of the rod, and isoform-specific sites are located within S-2[5)6)8)]. The amino acid sequence determination of the functional proteins in tissue by direct protein analysis is necessary to elucidate the molecular basis of the relationship between structure and function or post-translational modifications.

Although sequences of the smooth muscle MHCs have been established from various tissues of many animals, these were deduced from nucleotide sequences of their cDNAs rather than being analyzed at the protein level. In the present study, for the above reasons and to clarify the structure of the short S-2 of the adult chicken gizzard muscle MHC isoform, the primary structure was analysed by direct protein sequencing as demonstrated in Fig. 1. This region encompasses from NH_2-terminal region of the short S-2 to NH_2-terminal portion of the hinge and consists of 323 amino acid residues. Comparing the sequence of the short S-2 thus determined with the corresponding region of embryonic chicken gizzard muscle MHC[22], no amino acid difference could be observed and those two sequences were concluded to be identical.

No heterogeneity was detected. The periodicity of the 7-and 28-residue units of hydrophobic and charged residues and the frequency values of the hydrophobic residues at 'a' plus 'd' in the 7-residue repeat in this short S-2 were well conserved, as had been discussed[22].

The entire sequence of the short S-2 from adult chicken gizzard muscle MHC was compared with corresponding regions of chicken brain MHC[32], *Drosophila* non-muscle MHC[33], chicken skeletal muscle MHC[34] and chicken cardiac muscle MHC[20], as shown in Fig. 2. The results showed 76.5 %, 60.0%, 36.8% and 36.5% sequence identities, respectively, suggesting that the degree of sequence identity among MHCs is more tissues-specific than species-specific.

To elucidate whether the entire sequence of two MHCs from embryonic and adult chicken gizzard muscle is completely identical, it is necessary to determine the whole sequences of these two by protein sequencing.

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