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The primary structure of the myosin head

(amino acid sequence/subfragment 1/limited tryptic fragments)

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ABSTRACT **The sequence of the NH_2-terminal 808 amino acid residues of chicken *pectoralis* muscle myosin head was determined. Three characteristic 20-, 23-, and 50-kDa fragments were isolated from a digest of myosin subfragment 1 (S1) by gel filtration on a Sephadex G-100 column in the presence of 5 M guanidine hydrochloride, followed by anion-exchange chromatography on a QAE-Sephadex A-50 column in the presence of 8 M urea. The fragments were sequenced completely by conventional methods. Peptides overlapping the 23- and 50-kDa fragments and also overlapping the 50- and 20-kDa fragments were obtained by cleaving S1 with cyanogen bromide. Comparison of the 23-kDa and 50-kDa sequences with that of the overlapping peptide indicated that no additional amino acid exists between the 23- and 50-kDa fragments and that 5 amino acids exist between the 50- and 20-kDa fragments of S1. Methylated amino acid residues were found at four positions: ε-*N*-monomethyllysine at position 35, ε-*N*-trimethyllysine residues at 130 and 550, and 3-*N*-methylhistidine at 754.**

The myosin molecule, a major component of the contractile apparatus, consists of two heavy chains and two pairs of light chains. The NH_2-terminal portion of each heavy chain and two different light chains form a globular head. The head is generally prepared by limited proteolysis of myosin; it bears both the ATPase and actin-binding functions of myosin (1) and is often called subfragment 1 (S1). Limited tryptic digestion of S1 yields three major fragments of 23, 50, and 20 kDa, which are aligned in that order from the NH_2 terminus (2–4). Many studies have been mounted to elucidate the role of myosin in contraction at the molecular level (5), but, to date, only parts of the sequence of rabbit myosin heavy chain have been published (6, 7). The entire sequence, of course, is necessary for interpretative studies. Here we report the complete sequence of the NH_2-terminal 808 residues of heavy chain from the head region of chicken *pectoralis* myosin. Together with our previously reported light chain sequences (8–10), this work completes the primary structure of the myosin head from chicken—the same myosin head that has been crystallized recently (11, 12).

MATERIALS AND METHODS

Myosin was prepared from adult Hubberd-type chicken *pectoralis* muscle (13). S1 was prepared by digestion of myosin filaments with α-chymotrypsin (Sigma) as described (14). S1 was digested with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington) in 0.12 M NaCl/1 mM EDTA/20 mM imidazole hydrochloride, pH 7.0, at 15°C for 30 min. The digest was denatured in 5 M guanidine hydrochloride/0.5 M Tris·HCl, pH 8.1, reduced with 2-mercaptoethanol, and S-carboxymethylated with iodoacetic acid (15). The tryptic fragments were isolated by gel filtration on a Sephadex G-100 column and chromatographed on a QAE-Sephadex A-50 column under conditions as described in Fig. 2. The heavy chain of S1 was separated from the light chain as follows: S1 was denatured, reduced, and S-carboxymethylated, as above, and the reaction mixture was dialyzed against water. The heavy chain, which precipitated in the dialyzing tube, was collected by centrifugation at 2700 × *g* for 15 min.

The experimental procedures used for further fragmentation of peptides with cyanogen bromide, trypsin, chymotrypsin, *Staphylococcus aureus* V8 protease (Sigma), and thermolysin (Sigma) and for isolation of peptide fragments by chromatography on Chromo Beads P (Technicon), DEAE-cellulose, and Sephadex G-70 and G-50 have been described (16, 17). Some peptides were purified by reverse-phase HPLC with a Waters Associates Bondapak C_{18} semipreparative column (0.8 × 25 cm) using a gradient of 0.1% trifluoroacetic acid and 80% acetonitrile.

For amino acid analysis, peptides were hydrolyzed at 110°C for 22 hr in evacuated tubes with distilled 5.7 M HCl and were analyzed in a JEOL JCL-200A analyzer. ε-*N*-Monomethyllysine, ε-*N*-trimethyllysine, and 3-*N*-methylhistidine were identified and determined in the analyzer with a lithium citrate buffer system. Manual Edman degradation, subtractive Edman degradation, and digestion with carboxypeptidases A, B, and Y for sequence analysis were carried out as described (16, 17). Analysis of the NH_2-terminal acylpeptide was performed by digestion with acylamino acid-releasing enzyme [(EC 3.4.19.3); a generous gift from S. Tsunasawa of Osaka University] as described (18).

RESULTS AND DISCUSSION

Isolation of the Limited Tryptic Fragments of S1. The characteristic 23-, 50-, and 20-kDa fragments were produced by limited digestion of S1 with trypsin (Fig. 1, lane 1). After S-carboxymethylation, the three fragments were isolated by gel filtration on a Sephadex G-100 column and chromatography on a QAE-Sephadex A-50 column (Fig. 2; also Fig. 1, lanes 2–4).

Sequence of the 23-kDa Fragment (Residues 1–204). The NH_2-terminal residue of the 23-kDa fragment was not identified. From a digest of the fragment with trypsin at pH 8.5 and 37°C for 8 hr, 19 nonoverlapping peptides, three overlapping peptides, free lysine, and free arginine were obtained by chromatography on Chromo Beads P and by DEAE-cellulose and paper electrophoresis. One of them (18-residue peptide, residues 1–18 in Fig. 3) with a blocked NH_2 terminus was cleaved with cyanogen bromide, and two peptides—a 7-residue peptide (residues 1–7) and an 11-residue peptide (residues 8–18)—were obtained. The former, having the blocked NH_2 terminus, was subjected to acylamino acid-releasing enzyme digestion, and *N*-acetylalanine was identified by HPLC with a TSK-gel ODS-80 column (Toyosoda,

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Abbreviation: S1, subfragment 1.
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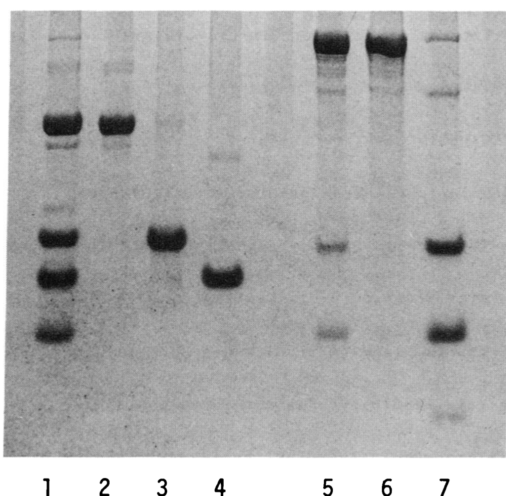

FIG. 1. Electrophoretogram of the tryptic fragments from S1 of chicken *pectoralis* muscle myosin. Polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ was carried out as described (19). The gel was stained with Coomassie brilliant blue. Lanes 1, tryptic digest of S1; 2–4, the isolated 50-, 23-, and 20-kDa fragments, respectively; 5, S1; 6, heavy chain of S1; 7, light chains of S1.

Tokyo). The other tryptic peptides were sequenced completely by conventional methods. The alignment of these tryptic peptides was deduced from partial sequences and compositions of chymotryptic peptides and/or cyanogen bromide peptides of the 23-kDa fragment, so that the complete sequence of the fragment was determined (Fig. 3, residues 1–204).

Sequence of the 50-kDa Fragment (Residues 205–635). Strategies for sequence analysis of the largest fragment are summarized in Fig. 4. In a typical experiment, 500 mg of the 50-kDa peptide were cleaved with 2 g of cyanogen bromide in 18 ml of 70% formic acid. After lyophilization, the resulting peptides were suspended in 50 ml of 50 mM NH_4HCO_3 (pH 8.5) and centrifuged. Peptides in the supernatant (soluble fraction) were isolated by chromatography first on a DE-52 column and then on a Sephadex G-50 column. Peptides in the precipitate (insoluble fraction) were separated by gel filtration on a Sephadex G-75 column with 5% acetic acid in 8 M urea and purified by CM-cellulose column chromatography in the presence of 8 M urea. Longer cyanogen bromide peptides were further cleaved with trypsin, chymotrypsin, and *Staphylococcus aureus* V8 protease, respectively, as indicated in Fig. 4. The alignment of the cyanogen bromide peptides was deduced from the sequences of tryptic peptides of the 50-kDa fragment. The complete sequence of this fragment is shown in Fig. 3, residues 205–635.

Sequence of the 20-kDa Fragment (Residues 641–808). Strategies for sequence analysis of the fragment were almost the same as those used for the 23-kDa peptide—i.e., the sequences of all of the tryptic peptides of the 20-kDa peptide were determined, and the alignment of the peptides was deduced from the sequences of the chymotryptic and peptic peptides of the 20-kDa fragment. The results are shown in Fig. 3, residues 641–808.

Linkages of the 23- and 50-kDa Fragments and of the 50- and 20-kDa Fragments. A peptide overlapping the 23- and 50-kDa fragments and one overlapping the 50- and 20-kDa fragments were isolated by cleavage of the heavy chain (Fig. 1, lane 6) of S1 with cyanogen bromide by procedures shown in Fig. 5. The first overlapping peptide was further cleaved with thermolysin, and a 14-residue peptide composed of the COOH-terminal 5-residue peptide of the 23-kDa fragment and the NH_2-terminal 9-residue peptide of the 50-kDa fragment was obtained. The sequence of this peptide indicated that no additional amino acid existed between the 23-kDa and 50-kDa fragments. From the peptide overlapping the 20- and 50-kDa fragments, a 24-residue peptide was obtained by *S. aureus* V8 protease digestion. Its sequence (Fig. 5) indicated that another 5 amino acid residues existed between the 50-kDa and 20-kDa peptides.

Structure of the Myosin Head. The sequence of the NH_2-terminal 808-amino acid residues of the heavy chain from chicken *pectoralis* muscle myosin is shown in Fig. 3. The NH_2-terminal residue of the chain is acetylated. Methylated amino acids were recognized at four positions: ε-*N*-monomethyllysine at position 35, ε-*N*-trimethyllysine at 130 and 550, and 3-*N*-methylhistidine at 754. Among these, position 35 was occupied by both ε-*N*-monomethyllysine and lysine in an approximate 7:3 ratio (calculated from the yields of heterogeneous peptides), while the others were fully occupied by the methylated amino acids. This distribution closely resembles that of rabbit skeletal muscle myosin (7). This may suggest that post-translational modifications are essential for function of skeletal muscle myosin. ε-*N*-Trimethyllysine at position 130 may provide part of the binding site for the triphosphate portion of ATP (20).

Several microheterogeneities have been reported in the 23-kDa fragment from rabbit skeletal muscle myosin (7). In our early experiments, some heterogeneous residues were observed—for example, alanine at 43, tyrosine at 47, isoleucine at 77, tyrosine at 411, glutamine at 419, etc.—with low

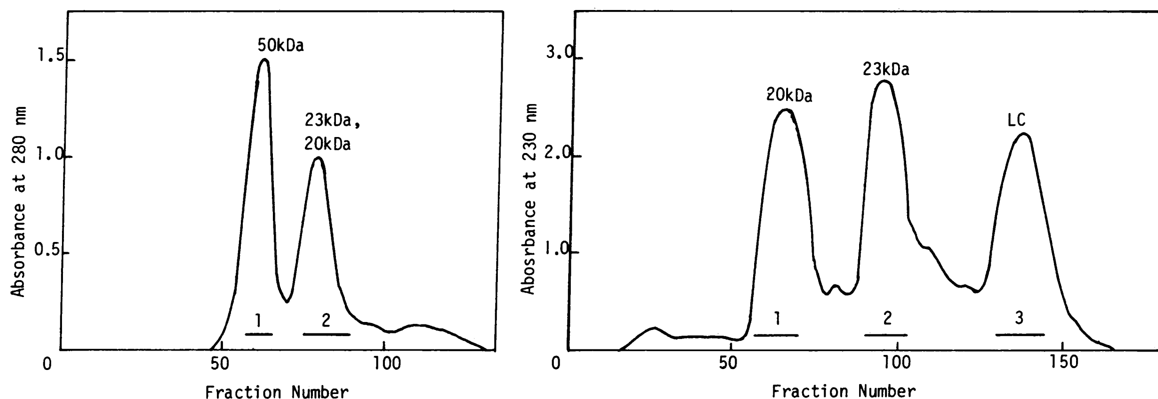

FIG. 2. Isolation of the tryptic fragments from S1. (*Left*) About 660 mg of the denatured, S-carboxymethylated tryptic digest of S1 were applied to a Sephadex G-100 column (4.2 × 130 cm). Peptides were eluted in two peaks with 5 M guanidine hydrochloride/5 mM EDTA/0.2 M Tris·HCl, pH 7.5, at a flow rate of 50 ml/hr. The fraction volume was 10 ml. (*Right*) Peptide sample involved in peak 2 in *Left* was applied to a QAE-Sephadex A-50 column (3.6 × 45 cm) equilibrated with 50 mM $NaHCO_3$, pH 9.0/8 M urea. Elution was with a linear gradient of that buffer relative to 50 mM $NaHCO_3$, pH 9.0/8 M urea/0.4 M NaCl. The fraction volume was 12 ml.

10 20
Ac-Ala-Ser-Pro-Asp-Ala-Glu-Met-Ala-Ala-Phe-Gly-Glu-Ala-Ala-Pro-Tyr-Leu-Arg-Lys-Ser-Glu-Lys-Glu-Arg-Ile-

30 40 50
Glu-Ala-Gln-Asn-Lys-Pro-Phe-Asp-Ala-$\overset{\text{MML}}{\text{Lys}}$-Ser-Ser-Val-Phe-Val-Val-His-Pro-Lys-Glu-Ser-Phe-Val-Lys-Gly-

60 70
Thr-Ile-Gln-Ser-Lys-Glu-Gly-Gly-Lys-Val-Thr-Val-Lys-Thr-Glu-Gly-Gly-Glu-Thr-Leu-Thr-Val-Lys-Glu-Asp-

80 90 100
Gln-val-Phe-Ser-Met-Asn-Pro-Pro-Lys-Tyr-Asp-Lys-Ile-Glu-Asp-Met-Ala-Met-Met-Thr-His-Leu-His-Glu-Pro-

110 120
Ala-Val-Leu-Tyr-Asn-Leu-Lys-Glu-Arg-Tyr-Ala-Ala-Trp-Met-Ile-Tyr-Thr-Tyr-Ser-Gly-Leu-Phe-Cys-Val-Thr-

130 140 150
Val-Asn-Pro-Tyr-TML-Trp-Leu-Pro-Val-Tyr-Asn-Pro-Glu-Val-Leu-Ala-Tyr-Arg-Gly-Lys-Lys-Arg-Gln-Glu-Ala-

160 170
Pro-Pro-His-Ile-Phe-Ser-Ile-Ser-Asp-Asn-Ala-Tyr-Gln-Phe-Met-Leu-Thr-Asp-Arg-Glu-Asn-Gln-Ser-Ile-Leu-

180 190 200
Ile-Thr-Gly-Glu-Ser-Gly-Ala-Gly-Lys-Thr-Val-Asn-Thr-Lys-Arg-Val-Ile-Gln-Tyr-Phe-Ala-Thr-Ile-Ala-Ala-

210 220
Ser-Gly-Glu-Lys-Lys-Lys-Glu-Glu-Gln-Ser-Gly-Lys-Met-Gln-Gly-Thr-Leu-Glu-Asp-Gln-Ile-Ile-Ser-Ala-Asn-

230 240 250
Pro-Leu-Leu-Glu-Ala-Phe-Gly-Asn-Ala-Lys-Thr-Val-Arg-Asn-Asp-Asn-Ser-Ser-Arg-Phe-Gly-Lys-Phe-Ile-Arg-

260 270
Ile-His-Phe-Gly-Ala-Thr-Gly-Lys-Leu-Ala-Ser-Ala-Asp-Ile-Glu-Thr-Tyr-Leu-Leu-Glu-Lys-Ser-Arg-Val-Thr-

280 290 300
Phe-Gln-Leu-Pro-Ala-Glu-Arg-Ser-Tyr-His-Ile-Phe-Tyr-Gln-Ile-Met-Ser-Asn-Lys-Lys-Pro-Glu-Leu-Ile-Asp-

310 320
Met-Leu-Leu-Ile-Thr-Thr-Asn-Pro-Tyr-Asp-Tyr-His-Tyr-Val-Ser-Gln-Gly-Glu-Ile-Thr-Val-Pro-Ser-Ile-Asp-

330 340 350
Asp-Gln-Glu-Glu-Leu-Met-Ala-Thr-Asp-Ser-Ala-Ile-Asp-Ile-Leu-Gly-Phe-Ser-Ala-Asp-Glu-Lys-Thr-Ala-Ile-

360 370
Tyr-Lys-Leu-Thr-Gly-Ala-Val-Met-His-Tyr-Gly-Asn-Leu-Lys-Phe-Lys-Gln-Lys-Gln-Arg-Glu-Glu-Gln-Ala-Glu-

380 390 400
Pro-Asp-Gly-Thr-Glu-Val-Ala-Asp-Lys-Ala-Ala-Tyr-Leu-Met-Gly-Leu-Asn-Ser-Ala-Glu-Leu-Leu-Lys-Ala-Leu-

410 420
Cys-Tyr-Pro-Arg-Val-Lys-Val-Gly-Asn-Glu-Phe-Val-Thr-Lys-Gly-Gln-Thr-Val-Ser-Gln-Val-His-Asn-Ser-Val-

430 440 450
Gly-Ala-Leu-Ala-Lys-Ala-Val-Tyr-Glu-Lys-Met-Phe-Leu-Trp-Met-Val-Ile-Arg-Ile-Asn-Gln-Gln-Leu-Asp-Thr-

460 470
Lys-Gln-Pro-Arg-Gln-Tyr-Phe-Ile-Gly-Val-Leu-Asp-Ile-Ala-Gly-Phe-Glu-Ile-Phe-Asp-Phe-Asn-Ser-Phe-Glu-

480 490 500
Gln-Leu-Cys-Ile-Asn-Phe-Thr-Asn-Glu-Lys-Leu-Gln-Gln-Phe-Phe-Asn-His-His-Met-Phe-val-Leu-Glu-Gln-Glu-

510 520
Glu-Tyr-Lys-Lys-Glu-Gly-Ile-Glu-Trp-Glu-Phe-Ile-Asp-Phe-Gly-Met-Asp-Leu-Ala-Ala-Cys-Ile-Glu-Leu-Ile-

530 540 550
Glu-Lys-Pro-Met-Gly-Ile-Phe-Ser-Ile-Leu-Glu-Glu-Glu-Cys-Met-Phe-Pro-Lys-Ala-Thr-Asp-Thr-Ser-Phe-TML-

560 570
Asn-Lys-Leu-Tyr-Asp-Gln-His-Leu-Gly-Lys-Ser-Asn-Asn-Phe-Gln-Lys-Pro-Lys-Pro-Ala-Lys-Gly-Lys-Ala-Glu-

580 590 600
Ala-His-Phe-Ser-Leu-Val-His-Tyr-Ala-Gly-Thr-Val-Asp-Tyr-Asn-Ile-Ser-Gly-Trp-Leu-Glu-Lys-Asn-Lys-Asp-

610 620
Pro-Leu-Asn-Glu-Thr-Val-Ile-Gly-Leu-Tyr-Gln-Lys-Ser-Ser-Val-Lys-Thr-Leu-Ala-Leu-Leu-Phe-Ala-Thr-Tyr-

630 640 650
Gly-Gly-Glu-Ala-Glu-Gly-Gly-Gly-Gly-Lys-Lys-Gly-Gly-Lys-Lys-Lys-Gly-Ser-Ser-Phe-Gln-Thr-Val-Ser-Ala-

660 670
Leu-Phe-Arg-Glu-Asn-Leu-Asn-Lys-Leu-Met-Ala-Asn-Leu-Arg-Ser-Thr-His-Pro-His-Phe-Val-Arg-Cys-Ile-Ile-

680 690 700
Pro-Asn-Glu-Thr-Lys-Thr-Pro-Gly-Ala-Met-Glu-His-Glu-Leu-Val-Leu-His-Gln-Leu-Arg-Cys-Asn-Gly-Val-Leu-

710 720
Glu-Gly-Ile-Arg-Ile-Cys-Arg-Lys-Gly-Phe-Pro-Ser-Arg-Val-Leu-Tyr-Ala-Asp-Phe-Lys-Gln-Arg-Tyr-Arg-Val-

730 740 750
Leu-Asn-Ala-Ser-Ala-Ile-Pro-Glu-Gly-Gln-Phe-Met-Asp-Ser-Lys-Lys-Ala-Ser-Glu-Lys-Leu-Leu-Gly-Ser-Ile-

760 770
Asp-Val-Asp-MMH-Thr-Gln-Tyr-Arg-Phe-Gly-His-Thr-Lys-Val-Phe-Phe-Lys-Ala-Gly-Leu-Leu-Gly-Leu-Leu-Glu-

780 790 800
Glu-Met-Arg-Asp-Asp-Lys-Leu-Ala-Gln-Leu-Ile-Thr-Arg-Thr-Gln-Ala-Arg-Cys-Arg-Gly-Phe-Leu-Met-Arg-Val-

808
Glu-Tyr-Arg-Met-Val-Glu-Arg-Arg

FIG. 3. Amino acid sequence of the S1 heavy chain from chicken *pectoralis* muscle myosin. MML, TML, and MMH represent ε-*N*-monomethyllysine, ε-*N*-trimethyllysine, and 3-*N*-methylhistidine, respectively.

yields (<20%). But samples from carefully prepared *pectoralis* muscle gave no detectable heterogeneity in later work. Recently, Robbins and co-workers reported the nucleotide sequences encoding the 5′ ends of five chicken myosin

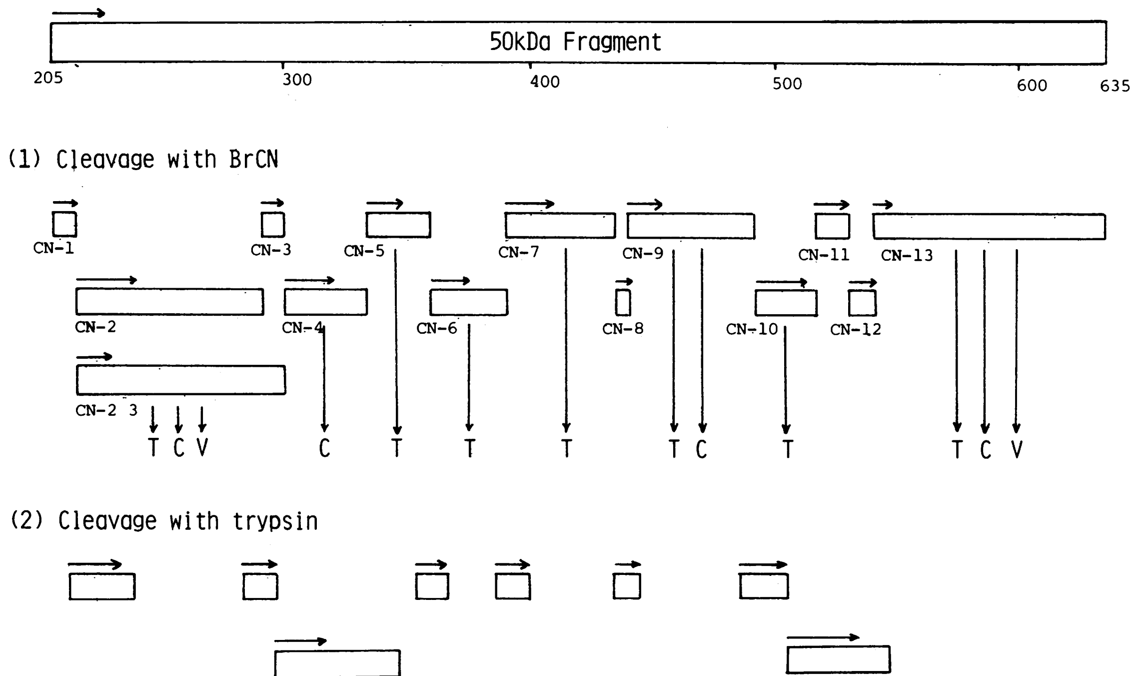

FIG. 4. Strategies for sequence analysis of the 50-kDa fragment (residues 205–635 in Fig. 3). The length of each box is proportional to the number of amino acid residues in the peptides. The arrow above each box represents residues sequenced by the Edman method. Among the peptide formed by cyanogen bromide cleavage, CN-2, CN-2 3, CN-7, CN-9, and CN-13 were obtained from the insoluble fraction, and the others were obtained from the soluble one (see the text). Longer peptides were subjected to further fragmentation with trypsin (T), chymotrypsin (C), and/or *S. aureus* V8 protease (V) to complete the sequences of the respective peptides. Digestion of the 50-kDa fragment was carried out at pH 8.5 and 37°C for 8 hr. The peptides were separated by gel filtration on a Sephadex G-50 column and purified by chromatography on DEAE-cellulose or Chromo Beads P. Only useful peptides for the alignment of cyanogen bromide peptides are shown by the boxes.

heavy chain genes and deduced the NH_2-terminal 169-amino acid sequences (21, 22). Among them, the sequence of clone N116 is identical with the sequence shown in Fig. 3. The heterogeneous amino acids observed in our early experiments are recognized in clones N118, N124, and N125. Further studies should be carried out concerning myosin isoenzymes or isoforms.

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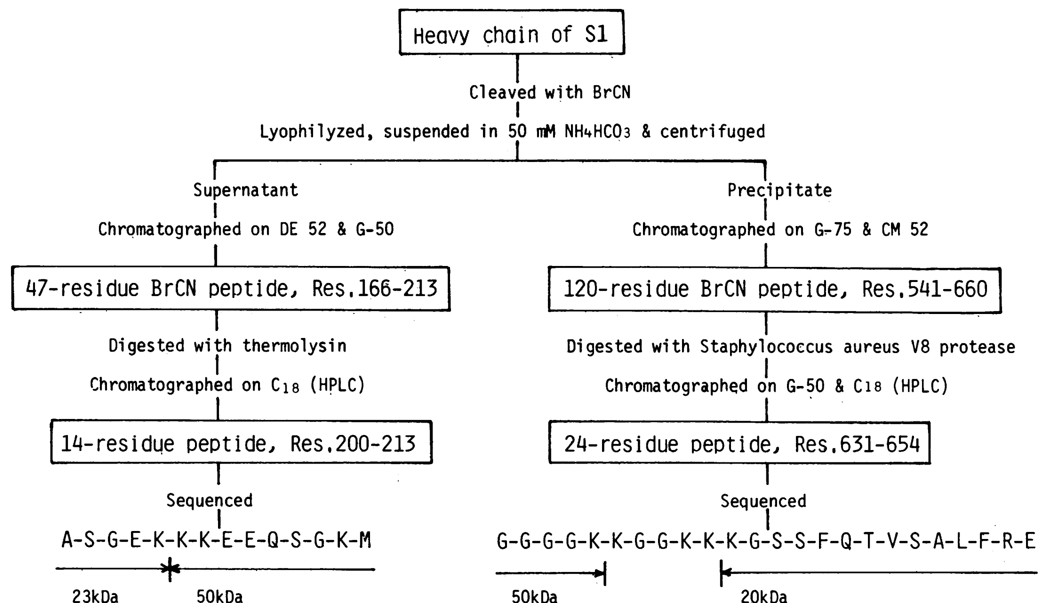

FIG. 5. Diagram showing the isolation of peptides overlapping the 23- and 50-kDa fragments and overlapping the 50- and 20-kDa fragments. Conditions of the chromatographies were: DE-52 column (3.2 × 20 cm), elution with a gradient of 50–500 mM NH_4HCO_3 (pH 8.5); G-50 column (3.8 × 140 cm), elution with 50 mM NH_4HCO_3; G-75 column (3.8 × 142 cm), elution with 5% acetic acid/8 M urea; CM-52 column (2.8 × 15 cm), elution with a gradient of 20–120 mM sodium phosphate, pH 6.8/8 M urea; Waters Associates Bondapak C_{18} column (0.8 × 25 cm), elution with a linear gradient of 0.1% trifluoroacetic acid toward 80% acetonitrile. Sequencing was carried out by the manual Edman method.

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