Sequence of the 20-Kilodalton Heavy Chain Peptide from the Carboxyl-terminus of Bovine Cardiac Myosin Subfragment-1

Irwin L. Flink and Eugene Morkin
Departments of Internal Medicine and Pharmacology, University of Arizona College of Medicine, Tucson, Arizona 85724

Abstract. An almost complete amino acid sequence of the carboxyl-terminal 20-kD tryptic heavy chain peptide from bovine cardiac myosin Subfragment-1 (S-1) has been determined by automated sequential degradation of the undigested peptide and subfragments derived by chemical and enzymatic digestion. The fragment contains 169 residues, including two reactive cysteinyl residues which are located nine residues apart. At six positions in the sequence, two amino acid residues were present and two different versions of a chymotryptic peptide were isolated in ∼53 and 24% yields, suggesting that there are two cardiac myosin β-type heavy chains in this species. Analysis of the secondary structure of the 20-kD peptide predicts that there are two distinct regions within the fragment. The first region (residues 1–121) contains 12% α-helix, 25% β-sheet, 40% β-bends, and 19% coil; the second region (residues 122–169) may form an extended α-helix. Comparison of the bovine sequence with the deduced amino acid sequence of a recombinant plasmid containing DNA sequences coding for the β-heavy chain of rabbit cardiac myosin (pMHCβ174) reveals ∼86% homology.

Introduction

Subfragment-1 (S-1)[1], the globular head region of the myosin molecule which contains the ATPase and actin binding sites, can be prepared from chymotryptic or papain digests of myosin (1). These procedures result in the formation of an α-helical rod and two molecules of S-1 per myosin molecule. Further tryptic digestion of S-1 from cardiac or skeletal myosin under controlled conditions gives rise to three fragments of 27, 50, and 20 kD (2). A problem of interest in studying these tryptic fragments of myosin S-1 has been the question of whether they represent functionally important domains, since in many proteins limited proteolytic digestion has been observed to occur between, but not within, domains of substructure with specific biological function (e.g., references 3 and 4). For example, the structural integrity of the carboxyl-terminal region of the heavy chain at the junction of the 20- and 50-kD peptides may be essential for the actin-stimulated ATPase of S-1 (5). Furthermore, the 20-kD fragment contains two reactive thiols, SH_1 and SH_2, and in skeletal myosin, there is a unique N^r-methylhistidine residue. This part of the myosin molecule lies adjacent to the Subfragment-2 (S-2) region which may play an important role in mechanochemical transduction (6).

Cardiac myosin from different species is similar in molecular weight and overall dimensions, but differs appreciably in ATPase activity, subunit composition, and other properties which may be related to differences in contractile behavior between these muscle types (7). In the present paper, the isolation and almost complete sequence of the 20-kD COOH-terminal peptide from bovine cardiac myosin S-1 heavy chain fragment is reported and compared with a similar region of amino acid sequence deduced from a recombinant plasmid containing cDNA sequences coding for the β-heavy chain of rabbit cardiac myosin (8, 9). Part of the bovine cardiac sequence, namely, residues 52–79 which comprise the region around the two reactive sulfhydryl groups, have been reported in a preliminary communication (10).

Methods

Preparation of tryptic fragments 20, 27, and 50 kD from S-1. S-1 that was essentially devoid of contamination by actin or other proteolytic

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1. *Abbreviations used in this paper:* HPLC, high performance liquid chromatography; LMM, light meromyosin; MHC, myosin heavy chain; PTH, phenylthiohydantoin; S-1, Subfragment-1; S-2, Subfragment-2; trypsin-TPCK, trypsin-treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone to inactivate any chymotrypsin present.

fragments of myosin could be prepared from bovine left ventricle essentially as described earlier (10). Two or three hearts were used for each preparation. Freshly prepared S-1 was subjected to tryptic digestion according to the procedure of Balint et al. (11). The S-1 pellet was solubilized by dialysis against 20 mM KCl, 2 mM EDTA, 3 mM Tris-HCl, pH 8.0, and was digested with trypsin treated with L-(1-tosylamide-2-phenyl)ethyl chloromethyl ketone (trypsin-TPCK) (1:110, wt/wt) for 40 min at 20°C. The reaction was stopped by the addition of trypsin soybean inhibitor in a ratio of 2:1 (wt/wt) with trypsin. The digest then was reduced and carboxymethylated by the method of Crestfield et al. (12). In some experiments, carboxymethylation was performed using [C^{14}]iodoacetate (New England Nuclear Corp., Boston, MA) at a final concentration of 20.4 mCi/mmol. The mixture was dialyzed exhaustively against 25% acetic acid, freeze-dried, and stored at $-20°C$ until used.

Purification of tryptic fragments. After alkylation, the tryptic digest of S-1 was applied to a Sepharose CL-6B column (5 × 70 cm) and eluted at 22°C with 25% acetic acid containing 8 M urea. The peak containing the 20-kD fragment was purified further by chromatography in a column of SP-Sephadex (2.5 × 50 cm) which had been equilibrated with buffer containing 6 M urea and 0.01 M NH_4 formate, pH 2.5, and was developed with a linear gradient of 0.1–0.4 M NaCl in the same buffer. As shown in Results, this procedure resulted in the separation of three fragments of ~50, 27, and 20 kD, respectively, which migrated as single bands upon electrophoresis in sodium dodecyl sulfate (SDS)-polyacrylamide gels.

CNBr digestion. Freeze-dried S-1 was cleaved with CNBr (10) and the reaction was terminated by the addition of 9 vol of H_2O. The solution was dialyzed exhaustively against 25% acetic acid, freeze-dried, and stored at $-20°C$ until used.

Chymotrypsin digestion. Cleavage of the 20-kD peptide with chymotrypsin (Worthington Biochemical Corp., Freehold, NJ) was carried out in 0.2 M NH_4HCO_3, pH 8.0, at 3–5 mg/ml at room temperature (22–25°C) for 18 h. An enzyme to substrate ratio of 1:100 (wt/wt) was used. At the end of the reaction time, the digest was lyophilized.

Trypsin digestion. Tryptic (trypsin-TPCK) digestion of peptides was carried out in 0.1 M NH_4HCO_3, pH 8.0, using an enzyme to substrate ratio of 1:50 (wt/wt) at room temperature (22–24°C) for 18 h. In some cases, the peptides had been modified with citraconic anhydride (13) prior to digestion. The reaction was terminated by acidification with glacial acetic acid to pH 2.5 and the digest was lyophilized.

Digestion with carboxypeptidase A and B. Intact 20-kD fragment was digested with carboxypeptidase A and B (Worthington Biochemical Corp.) at 0.5 mg/ml in 0.1 M NH_4HCO_3, pH 8.0, using 0.5 mg of enzyme to 1 mmol of peptide. Aliquots were removed at each of four time points (0, 1, 2, and 3.5 h). After acidification with 0.1 N HCl, the digest was centrifuged, and amino acids released into the supernatant were detected by amino acid analysis.

Gel chromatography. Lyophilized CNBr digests of S-1 were applied to Biogel P-10 (200–400 mesh) columns (2.0 × 70 cm) in 25% acetic acid. Peptides were detected by either absorbance at 280 nm or by the fluorescamine reaction after alkaline hydrolysis.

Gel electrophoresis. Polyacrylamide gel electrophoresis was performed on 7.5% gels (0.5 × 10 cm) by the method of Weber and Osborn (14) using a 1:19.9 ratio of N,N′-methylenebisacrylamide to acrylamide. Samples were dissolved in 0.01 M Na phosphate buffer, pH 7.0, containing 1% SDS and 1% β-mercaptoethanol, and heated at 75°C for 3 min before electrophoresis.

Peptide separation by high performance liquid chromatography (HPLC). Lyophilized fractions from Biogel P-10 chromatography or chymotryptic digests of S-1 were dissolved in 25% acetic acid and applied

to either Biosil C18 columns (Bio-Rad Laboratories, Richmond, CA) or to μBondapak C18 columns (Waters Associates, Milford, MA) equilibrated with either 0.05% trifluoroacetic acid or 0.01 M H_3PO_4, pH 2.5, for purification by reverse-phase HPLC (15, 16). A Spectraphysics 8000 system equipped with a variable wavelength detector was used for all separations. Both column systems were eluted with either 0.05% trifluoroacetic acid-acetonitrile or 0.01 M H_3PO_4-acetonitrile gradients and the effluent was monitored at 220 and 280 nm.

Amino acid analysis. Peptides were hydrolyzed in vacuo in 0.2 ml 6 N HCl at 110°C for 22 h. Tryptophan was determined after hydrolysis in methanesulfonic acid (17). Hydrolysates were analyzed on either a Beckman 118 (Beckman Instruments, Inc., Fullerton, CA) or a LKB 4150 amino acid analyzer (LKB Instruments, Inc., Gaithersburg, MD) using Na citrate buffers and single column methodology.

Liquid-phase sequencing. Automated sequence analysis was performed with a Beckman 890C sequencer (Beckman Instruments, Inc.) using program no. 121078 which employs 0.1 M Quadrol. Polybrene (3 mg) was placed in the cup and one complete cycle was run prior to adding peptides (18). Phenylthiohydantoin (PTH) norleucine (1–2 nmol) was added to each tube in the fraction collector prior to sequencing to serve as an internal standard.

Conversion of sequence steps to the PTH derivative was performed in 0.2 ml of 1 N HCl at 80°C for 10 min under nitrogen. Samples were extracted twice with ethyl acetate and the combined extracts evaporated to dryness under nitrogen. Residues were dissolved in a mixture (85:15) of 0.01 M Na acetate, pH 4.9, and acetonitrile for analysis by HPLC. Generally, 10–40% of the sample was analyzed as above. In some cases, the remainder of the organic or aqueous phase was hydrolyzed with hydriodic acid to convert the PTH derivative to the parent amino acid (19). Amino acid analysis was performed either on a Beckman 118 (Beckman Instruments, Inc.) or an LKB 4150 (LKB Instruments, Inc.) using Na citrate buffers and single column methodology.

Analysis of PTH amino acids. A Spectraphysics 8000 HPLC system with a Dupont Zorbax octodecylsilyl column (Dupont Co., Wilmington, DE) was employed to detect PTH amino acids as described by Zimmerman et al. (20).

Results

Tryptic peptides of S-1. Limited tryptic digestion of cardiac myosin S-1 yielded three major peptides of 50, 27, and 20 kD corresponding closely to the fragments obtained from skeletal myosin S-1 (2). As shown in Fig. 1, during the course of tryptic digestion, the 70- and 27-kD fragments were released first, followed by the 50- and 20-kD fragments (11). For batch preparation of the tryptic fragments, a 40 min digestion time was chosen.

After carboxymethylation, the tryptic digest was subjected to a combination of gel permeation chromatography in Sepharose CL-6B (Fig. 2) and ion-exchange chromatography in SP-Sephadex (Fig. 3). As shown in Fig. 4, the major fragments of 50, 27, and 20 kD migrated as single bands upon electrophoresis in SDS-polyacrylamide gels.

Amino acid analysis of the 20-kD peptide. The 20-kD peptide was isolated in ~62% yield from S-1. Its amino acid composition is shown in Table I. The fragment is comprised of 169 residues, including a large number of hydrophobic and charged residues. We were unable to detect N^r-methylhistidine, although one residue of this unusual methylated amino acid has been identified
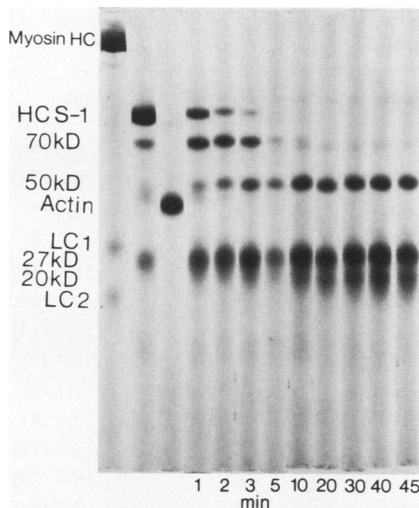
Figure 1. Time course of tryptic digestion of cardiac myosin S-1. S-1 was digested with trypsin-TPCK as described in Methods and the reaction was stopped at the times indicated with trypsin soybean inhibitor. About 75 μg of total digest was applied to SDS-polyacrylamide disk gels (0.5 × 12 cm). About 50 μg undigested myosin, S-1, and actin, respectively, were run for comparison. HC, heavy chain; LC, light chain.

in the skeletal fragment (21). Also, no tryptophan could be detected after hydrolysis in methanesulfonic acid (17).

Amino acid sequence of the 20-kD peptide. A summary of the peptides used to determine the amino acid sequence of the 20-kD fragment is shown in Fig. 5. The NH₂-terminal residues in the 20-kD sequence were determined by automated Edman degradation. Analysis of the amino acids released from the intact
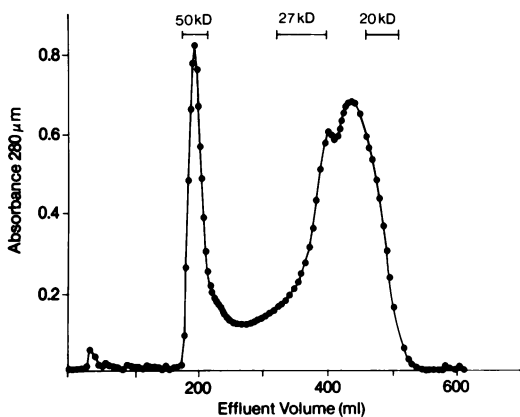

Figure 2. Fractionation of tryptic digest of cardiac myosin S-1. S-1 was digested with trypsin-TPCK as described in the text and ~300–600 mg of the digest were applied to a column of Sepharose CL-6B (5.5 × 60 cm) equilibrated and eluted with 25% acetic acid containing 8 M urea at a rate of 10 ml/h. Material in the eluent was detected by absorbance at 280 nm, pooled as indicated, and lyophilized.
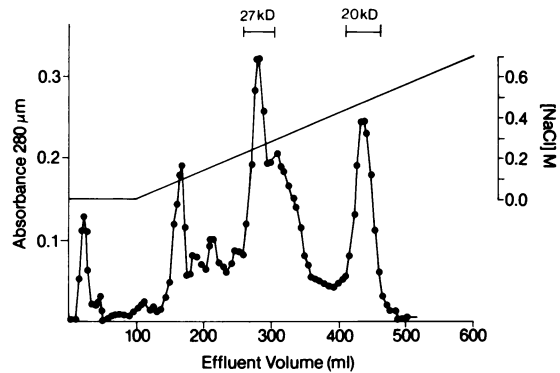

Figure 3. SP-Sephadex chromatography of pooled fractions from filtration of S-1 digest on Sepharose CL-6B (Fig. 2). About 50–100 mg of sample were applied to a column of SP-Sephadex (1 × 60 cm) equilibrated at 55°C in 0.01 M ammonium formate, pH 2.5, containing 8 M urea. A linear gradient of 0–0.6 M NaCl was applied and 3-ml fractions were collected at a flow rate of 25 ml/h. Fractions were analyzed for absorbance at 280 nm. Fractions containing the 27 and 20-kD peptides were pooled, dialyzed against 25% acetic acid, and lyophilized.

fragment by digestion with carboxypeptidase B confirmed that the carboxyl-terminal residue was arginine. The strategy used to determine the remaining amino acid sequence of the 20-kD peptide was deduced by CNBr and enzymatic cleavages of the intact peptide to produce large, overlapping fragments. A CNBr digestion was performed initially, and the resulting peptides were isolated (Fig. 6) and subjected to automated sequential Edman degradation. CNBr cleavage of the four methionine residues of the 20-kD peptide theoretically should yield five fragments. Three peptides (CB2, CB4, and CB5) could be obtained in purified form by a combination of P-10 gel filtration and reverse-phase HPLC. Peptide CB3 was purified by G-75 gel filtration and ion-exchange chromatography in SP-Sephadex as described earlier (10). Several attempts were made to isolate CB1 from CNBr digests, including gel filtration in the presence of 8 M urea followed by reverse-phase HPLC of the void volume fractions. However, these efforts proved unsuccessful and the sequence of CB1 was determined by sequential degradation of the undigested 20-kD fragment. Peptide CB5 was sequenced to the carboxyl-terminus. Partial sequences were obtained of CB2, CB3, and CB4. To complete these sequences, the CNBr peptides were chemically modified with citraconic anhydride and subjected to tryptic digestion, or were enzymatically cleaved without prior modification.

A chymotryptic digest of S-1 also was performed and the resulting peptides were separated by reverse-phase HPLC. Six chymotryptic peptides were purified and used to extend and overlap the sequences of CNBr peptides (Fig. 5).

From this data we were able to unambiguously assign 153 of 169 positions in the bovine cardiac 20-kD peptide. Five of the remaining residues (positions 47 to 51) were located at the carboxyl-terminus of a cyanogen bromide peptide which could
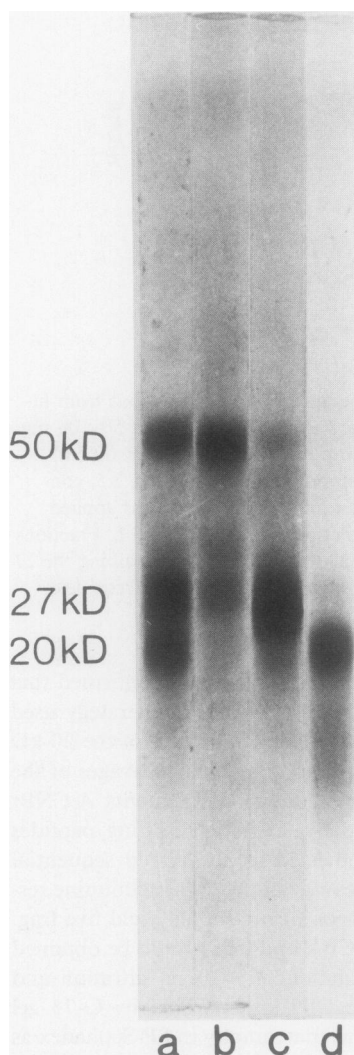
Figure 4. SDS-polyacrylamide gel electrophoresis of isolated tryptic fragments of cardiac myosin S-1. About 75 µg of total digest or 50 µg of fragments prepared as described in Methods were applied to SDS-polyacrylamide disk gels (0.5 × 12 cm). (*a*) Tryptic digest of S-1; (*b*) 50-kD fragment; (*c*) 27-kD fragment; (*d*) 20-kD fragment.

Table I. Amino Acid Composition of the 50-, 27-, and 20-kD Tryptic Fragments of Cardiac Myosin S-1 Heavy Chain*

Amino acid	50 kD	27 kD	20 kD
Cys‡	5.2	2.4	3.0 (3)
Asp	54.6	32.0	16.4 (15)
Thr	20.6	14.3	6.3 (7)
Ser	13.4	6.8	7.3 (8)
Glu	58.1	38.4	18.9 (18)
Pro	13.7	15.7	7.0 (6)
Gly	30.4	17.4	13.8 (14)
Ala	30.7	15.1	8.4 (7)
Val	18.9	13.5	7.1 (8)
Met	11.8	6.5	3.9 (4)
Ile	32.4	10.7	12.8 (11)
Leu	42.8	20.0	19.7 (20)
Tyr	13.7	3.8	2.8 (3)
Phe	29.1	10.9	8.5 (10)
His	11.8	4.1	6.7 (6)
Tri-CH₃-Lys§	1.2	0.7	<0.1 (0)
Lys	33.3	15.1	9.9 (11)
Arg	12.9	6.8	15.9 (18)
Total residues	435	235	(169)

* Results are expressed as residues per molecule. Values for the 50- and 27-kD fragments have been normalized to their apparent molecular weights as determined by gel electrophoresis. For the 20-kD fragment values were normalized to 169 residues as deduced from the sequence. Integral values in parentheses for the 20-kD peptide were obtained from sequence analysis.
‡ The cysteine content was determined as S-carboxymethylcysteine.
§ ε-N-Trimethyllysine.

not be sequenced to the end. The residues shown were deduced by subtraction of the partial sequence from the amino acid composition of the peptide. A tryptic decapeptide (positions 128–137) did not yield a sequence, possibly because the NH₂-terminus had become blocked during the lengthy isolation procedure. However, the amino acid composition agreed precisely with the amino acid sequence deduced from the cDNA clone. A lysine residue was assigned at position 118 by analogy with the rabbit cardiac myosin sequence.

At six positions in the sequence of the 20-kD fragment, there was evidence of "microheterogeneity," that is, two amino acid residues in approximately equal yields were identified at a single sequence position. Additional evidence of heterogeneity was provided by the isolation of two peptides (CH3 and CH4) by reverse-phase HPLC from a chymotryptic digest of the 20-kD peptide which contained Histidine 114 in slightly different sequences: Leu-Gly-Ser-Leu-Asp-Ile-Asp-His-Asn-Gln-Tyr; Gly-Phe-Met-Glu-Ile-Asp-His-Asn-Gln-Tyr.

The longer version of this peptide was isolated in higher yield (53%) than the shorter one (24%) and was assumed to represent the major component. The sequence around Histidine 114 in bovine cardiac myosin was reported earlier by Huszar and Elzinga (21) to be Leu-Leu-Gly-Ser-Leu-Asp-Ile-Asp-His-Gln-Asn-Tyr-Lys, which differs only from the sequence reported here (Fig. 5) in that the order of the Gln and Asn residues are reversed. In the earlier work, however, these residues were not sequenced directly, but the positions were deduced from the results of a partial acid hydrolysis.

Comparison of the 20-kD peptide and pMHCβ174. Fig. 7 shows the amino acid sequence of the 20-kD fragment from bovine cardiac myosin together with the amino acid sequence deduced from pMHCβ174 (8, 9). The sequences, which have been numbered based upon the sequence deduced from the cDNA clone in this region, reveals two points of interest. First, 144 of 169 residues in the bovine cardiac sequence are identical with the sequence deduced from the rabbit cardiac myosin cDNA. Secondly, the NH₂-terminus of the 20-kD fragment is formed by cleavage between two lysine residues while the COOH-

Figure 5 (sequence diagram)

Position markers: 5   10   15   20   25   30

β
β'
1 Lys-Gly-Gly-Lys-Phe- Phe-Val-Val- Leu-Ala-Ser-His- Arg-Glu-Asn-Leu- Ser-Asn-Leu- Met-Thr-Asn-Leu-Arg- Ser-Thr- His-Pro- His-Phe
                                                    Arg

CB1   CB2 (SH₂)

β
β'
31 Val-Arg- Cys-Ile- Ile- Pro-Asn- Glu-Thr-Lys-Ser- Pro-Gly- Val-Leu-Ala- (Asp-Glu-Gly-Val-Met) -His-Gln-Leu-Arg- Cys-Asn-Gly-Val-Leu

CB2   CB3

CB2-CT1 (SH₁)

β
β'
61 Glu-Gly- Ile-Arg- Ile-Cys-Arg-Lys-Gly- Phe- Pro-Asn- Arg-Ile- Leu- Tyr-Gly- Asp- Phe -Arg -Gln-Arg -Tyr -Arg-Ile-Leu-Asn- Pro-Ala-Ala

CB3
CB3-CT1   CB3-CT2
CH1   CB3-CH1   CB3-CH2

β
β'
91 Ile-Pro-Glu- Gly-Gln-Phe- Ile-Asp- Ser-Arg-Lys-Gly- Ala-Gln-Lys-Leu- Leu-Gly- Ser-Leu -Asp- Ile- Asp(His)Asn-Gln-Tyr-(Lys)-Ser-Gly
                                                                        Phe-Met-Glu-

CB3
CB3-CT2   CH4   CH5
CB3-CH2   CH2   CH3

β
β'
121 His-Thr-Lys- Val-Phe- Phe-Lys- (Ala-Gly-Leu-Leu-Gly-Leu-Leu-Glu-Glu-Met) -Thr- Asp-Gln-Arg-Leu- Thr-Arg- Ile- Ile-Thr- Arg-Ile- Gln
                                                                          Leu   Ile

CB3   CB4
CB3-CT3   CB3-CT4
CH5

β
β'
151 Ala-Gln-Phe-Val-Glu-Gln-Leu-Arg-Arg-Met-Glu-Phe-Lys-Lys-Leu-Leu-Glu-Arg-Arg
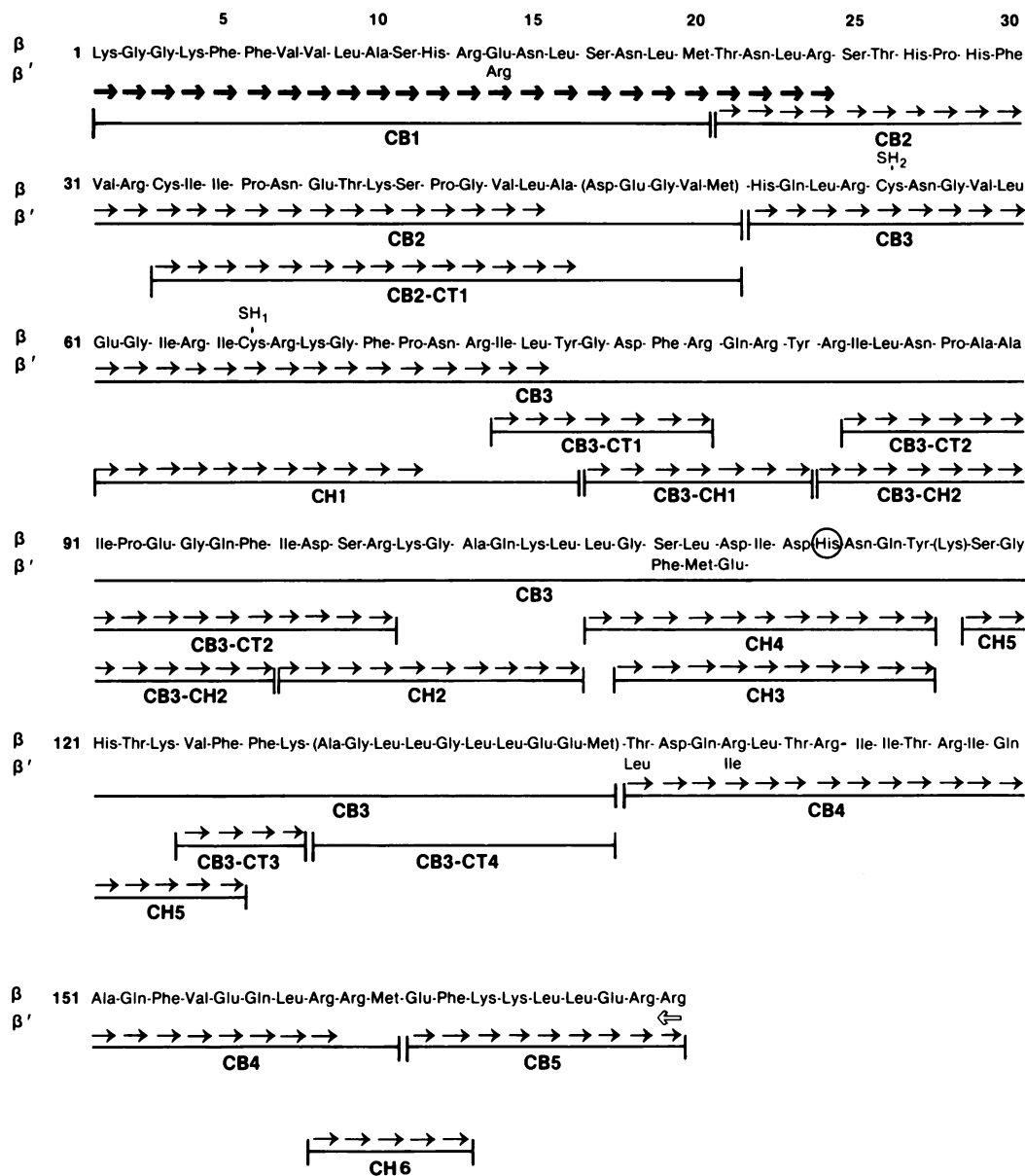
CB4   CB5

CH6

Figure 5. Amino acid sequence of the 20-kD tryptic S-1 peptide from bovine cardiac myosin and a schematic outline of the peptides used to establish the primary structure. The peptides were designated according to the cleavage method used as follows: CB, cyanogen bromide; CT, trypsin-TPCK (trypsinization of citraconylated fragments); CH, chymotrypsin. Some peptides have been assigned a combination of these letters in chronological order of the reagent and the proteases employed. As indicated in the text, the alignment of several peptides and individual residues (indicated in brackets) has been made by analogy with the amino acid sequence derived from the DNA sequence of pMHCβ174. The following symbols were used to indicate the method of sequential degradation: ▶, automatic Edman degradation of the intact 20-kD peptide; →, automatic Edman degradation of purified peptides from the 20-kD fragment; ◊, residues positioned by carboxypeptidase B digestion of the intact 20-kD peptide.

terminus is the result of cleavage after the second of two arginyls. The amino acid sequences that form the cleavage points for the 20-kD fragment in bovine cardiac myosin occur in the same locations in the rabbit sequence, suggesting that tryptic digestion would give rise to a similar sized fragment from rabbit cardiac myosin.

Discussion

The protein sequence data indicate that two different but closely related versions of myosin β-type heavy chain normally exist in bovine left ventricular muscle. Isoenzymes of ventricular myosin have been identified in several animal species, and des-

ignated as V_1, V_2, and V_3 in order of decreasing electrophoretic mobility and ATPase activity (7). All three myosin forms seem to contain the same light chains, but differ in their heavy chain compositions. The slowest migrating form, V_3, has been thought to contain two β-heavy chains; the fastest migrating component, V_1, two α-heavy chains; and the intermediate form, V_2, a heavy chain of each type. The proportions of these isoenzymes have been shown to vary with thyroid status, age, and other factors (7).

Bovine ventricular myosin migrates as a single slow migrating band under nondenaturing electrophoretic conditions (22), corresponding to the V_3 form in other species, and has been thought to contain two identical β-heavy chains in each molecule. The
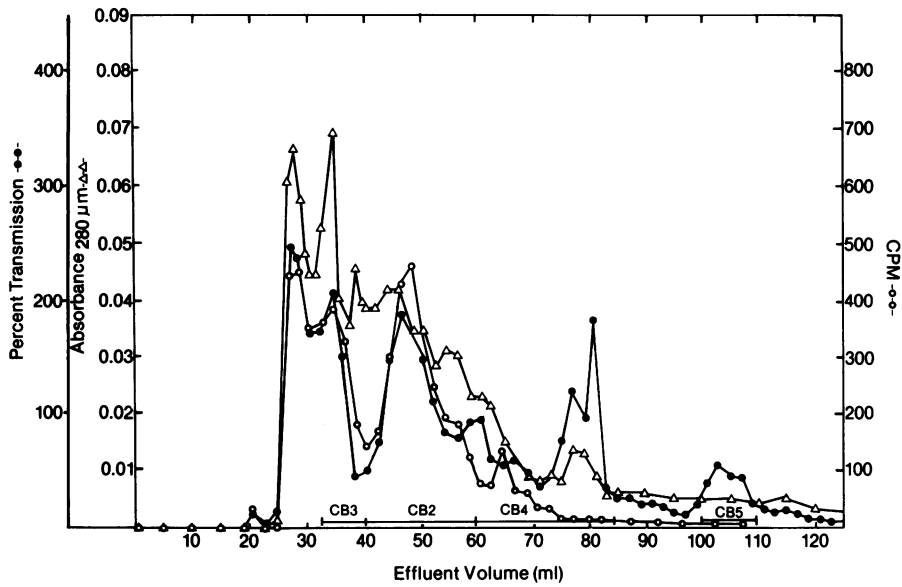
Figure 6. Fractionation of CNBr digest of the 20-kD fragment. About 500 nmol of [C¹⁴]carboxymethylated peptide was cleaved and chromatographed in Biogel P-10 as described in Methods. Material in eluent fractions was detected by absorbance at 280 nm and sampled for liquid scintillation counting and for the fluorescamine reaction. Effluent fractions were pooled as indicated by bars and lyophilized.

presence of a single band upon electrophoresis of bovine cardiac myosin probably is not the result of failure to separate V_3 from faster migrating α-heavy chain-containing forms (V_1 and V_2),

since these forms can be demonstrated in cardiac myosin from this species after treatment with thyroid hormone (7).

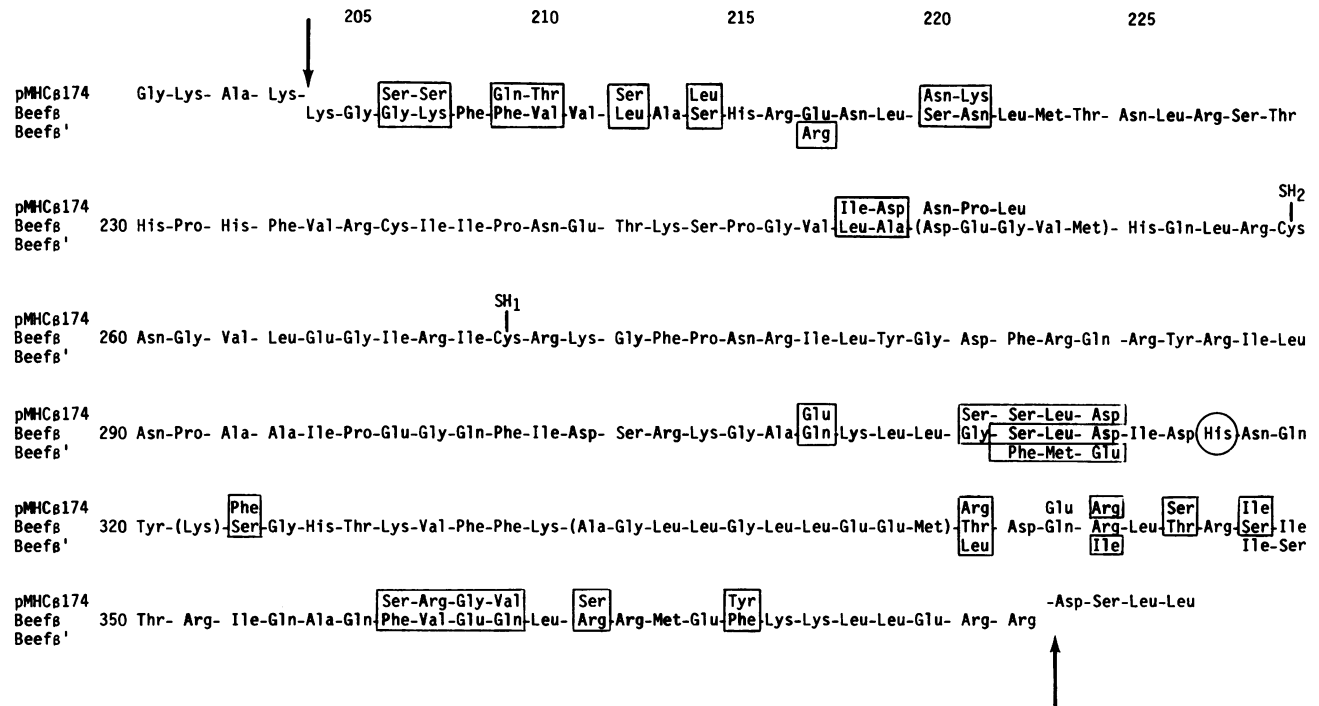The findings reported here indicate that despite the apparent

Figure 7. Comparison of the sequence of the 20-kD tryptic S-1 peptide from bovine cardiac myosin with the amino acid sequence derived from a similar region of pMCHβ174 (8). Residues are numbered according to the amino acid sequence derived from

pMHCβ174. Sequence differences are indicated by boxes. Residue numbers 259 and 270 are the SH_2 and SH_1 thiols, respectively. In skeletal myosin, Histidine 318 (circled) is N^r-methylated.

electrophoretic homogeneity of bovine ventricular myosin at least two β-type heavy chain forms are present. Since sequence heterogeneity was found in several myosin preparations, it seems likely that individual animals synthesize both β-heavy chain types. Thus, bovine cardiac myosin molecules may be comprised of either two populations of heavy chain dimers, ββ and β'β', or hybrid heavy chain dimers, ββ', which are not separated electrophoretically under nondenaturing conditions.

An alternative explanation for the occurrence of heterogeneity within the 20-kD sequence might be that the β'-heavy chain gene occurs as an allelic variant in some individuals. Inclusion of ventricular tissue from such individuals would cause sequence heterogeneity within the myosin heavy chain preparation. Furthermore, if β- and β'-heavy chain genotypes occur with approximately equal frequency, then most myosin preparations would contain both of the gene products.

Prediction of the secondary structure of the bovine cardiac 20-kD fragment according to the rules of Chou and Fasman (23) is shown in Fig. 8. There appear to be two distinct regions. The first region (positions 1–121 in the bovine cardiac sequence) contains 12% α-helix, 29% β-sheet, 40% β-bends, and 19% coil; the second region (positions 122–169) consists entirely of a long α-helix.

In the first region, the SH₂ thiol is located at the beginning of a β-turn. The SH₁ thiol, which is located nine residues away in the sequence, is part of a tetrapeptide Ile-Arg-Ile-Cys (residues 63–66) with high potential for β-pleat formation and may be located in a short β-pleat structure. Although it has been thought that these two sulfhydryls are essential for ATPase activity, Weiel et al. (24) have demonstrated recently that the SH₁ thiol is likely to be at least 32–35 Å from the active site, which would make direct participation in ATP binding or cleavage very unlikely.

The distal portion of the first region of the cardiac 20-kD peptide contains a histidine at position 114, which is N^r-methylated in skeletal myosin. Since N^r-methylation occurs post-translationally, this region is thought to be located on the surface of myosin. Interestingly, there also are charged residues nearby which would support the idea that this region is not buried within the molecule.

The second or carboxyl-terminal portion of the 20-kD fragment appears to be a long stretch of α-helix, extending from positions 122 to 169. It contains 17 charged residues, 11 of which are basic amino acids. The position of most of these charged residues is conserved in the beef and rabbit cardiac myosin sequences.

Difference in amino acid sequence between the β and β' forms of the bovine 20-kD fragment, which are indicated by an insert in Fig. 8, are predicted to cause only one difference in secondary structure. The substitution of Ser-Leu-Asp for Phe-Met-Glu at positions 109-111 may extend the α-helix from positions 108 to 112. Similarly, amino acid substitutions found in the amino acid sequence deduced from the cDNA clone pMHCβ174 would cause alterations in the secondary structure in the 20-kD region (Fig. 8).
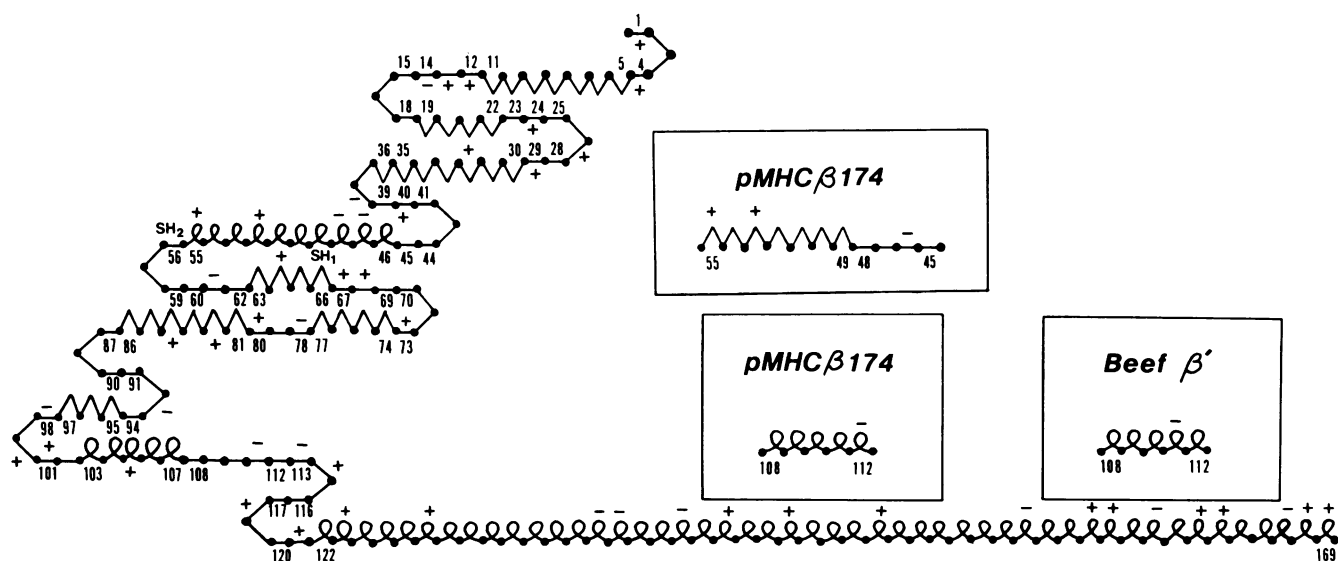

Figure 8. Predicted secondary structure of cardiac 20-kD peptide. Coil, α-helix; sawtooth pattern, β-sheet; arches, β-bends. Amino acid residues are numbered according to the cardiac sequence. Positions 1–121 is comprised of seven regions of β-sheet, two α-helical regions, twelve β-bends, and eleven segments of coil. This part of the 20-kD peptide contains the SH₁ and SH₂ thiols, as indicated, and a histidine residue at position 114 which is N^r-methylated in skeletal myosin.

The region represented by positions 122–169 is predicted to be entirely α-helical. Positively and negatively charged residues are indicated by + and −, respectively. Differences in secondary structure among the β- and β'-forms of bovine cardiac myosin and a similar region of rabbit cardiac myosin heavy chain sequence deduced from the cDNA clone pMHCβ174 are shown in the inserts.

The 20-kD peptide is contiguous with S-2, which with light meromyosin (LMM), forms the rod-like region of myosin. The latter regions are comprised of double-stranded α-helix forming a coiled-coil structure. Both LMM and S-2 contain a heptapeptide-type repeating sequence of the form (a-b-c-d-e-f-g)n, like that found in tropomyosin, where a and d are most commonly apolar residues and e and g are charged residues (25). This quasi-repeating sequence of LMM and S-2 is not present in the 20-kD regions of cardiac S-1, suggesting that it is not part of the coiled-coil structure.

Acknowledgments

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