Structural Differences of Myosin Heavy Chains in Normal and Denervated Muscles

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The pectoral nerve of an adult chicken was ligated and cut, and six months later the resulting atrophied pectoral muscle was taken out. Subfragment-2 (S-2) of myosin prepared from the denervated and atrophied muscle was obtained by successive digestions in physiological and higher ionic strength buffers with α-chymotrypsin, and purified by gel chromatography on a Sephacryl S-300 column after S-carboxymethylation. The S-2 was digested with lysylendopeptidase, and 11 peptides were isolated by reverse-phase high performance liquid chromatography. Their sequences determined by using an automated protein sequencer were compared with the primary structure of myosin heavy chain normally expressed in the pectoral muscle. The results clarified that at least two kinds of different myosin heavy chains from normal one were expressed in the atrophied muscle.

Introduction

There are various diseases showing muscular atrophy as the principal symptom. In the orthopedic field, the atrophy results from long-term lying in bed, immobility due to gypsum fixation, damage of nerves by trauma, and so on. However, much is unknown concerning the mechanisms of the atrophy particularly at the molecular level.

Denervation of skeletal muscle usually causes macroscopic twitching and muscular atrophy. Because the atrophy involves a loss of protein, it is important to know what kinds of proteins decrease in the atrophying muscle. Jakubiec-Puka, A. et al.19 have reported that in the leg muscle (in adult rats) atrophying after denervation, the thick filaments disappeared before the thin filaments of myofibril.

Myosin, a major contractile protein within the thick filaments, is the most abundant protein in muscle. The skeletal myosin consists of two heavy chains and two pairs of light chains.27 Light chain polymorphism is characteristic of different types of skeletal muscle fibers, such as the fast- and slow-type fibers, and of different stages of development.30 It has been reported that light chains in skeletal muscle change to the fast-type after denervation.40

Heavy chain polymorphism is also known: specific isoforms depending on the types of muscles,26 the different stages of differentiation,13,14 the stimulation by thyroid hormone,19 the electrical stimulation,26 and the denervation20 have been reported. These isoforms have been analyzed electrophoretically, histochemically, immunologically, and/or genetically. However, there is few report describing the structural differences of the isoforms actually expressed in the respective muscles. In the myosin molecule, the amino-terminal half of each heavy chain with a pair of the light chains form a globular head, and the carboxyl terminal halves of the two heavy chains form a fibrous rod. The head and rod can generally be separated by limited proteolysis of myosin filaments.22,23 The rod can be subdivided into subfragment-2 (S-2) and light meromyosin by further proteolysis in higher ionic strength buffer.24,25

In order to reveal the structural differences of the heavy chains in normal and denervated muscles, the author sequenced lysylendopeptidase-peptides of S-2 from the denervated pectoral muscle of an adult chicken, because the complete sequence from the normal one has been known by protein sequencing.26

Materials and Methods

Denervation

The pectoral nerves of four adult chickens (Hubbard type), exposed by incision at the axillary region under chloroform anesthesia, were ligated and cut, respectively. Six months later the external pectoral (M. pectoralis superficialis) muscle was taken from each chicken.

Preparation of myosin, rod, and S-2

Myosin was prepared from the denervated muscle according to the method of Perry.25 The myosin was digested with α-chymotrypsin (Type VIII, Sigma Chemical Co.) at an enzyme-to-substrate ratio of 1:100 (w/w) in 120mM NaCl, 1mM EDTA, and 20mM imidazol-HCl (pH 7.0) at 20 °C for 10 min.26 The digestion was stopped by adding 0.5mM phenyl methyl sulfonyl fluoride (PMSF, Nakarai Chemical Co.). The produced rod was sedimented by centrifugation
at 105,000xg for 60 min, and was purified by ethanol precipitation. S-2 was obtained by digesting the rod with α-chymotrypsin at an enzyme-to-substrate ratio of 1:100 (w/w) in 0.5 M KCl, 10 mM EDTA, and 20 mM Tris-phosphate (pH 7.0), at 25 °C for 10 min. The S-2 was separated from light meromyosin and uncleaved rod on the basis of its solubility in the low ionic buffer, and was reduced and carboxymethylated with 10 mM 2-mercaptoethanol and 100 mM sodium iodoacetate (Nakarai Chemical Co.) in 6 M guanidine-HCl, 10 mM EDTA, and 0.5 M Tris-HCl (pH 8.5). The carboxymethylated S-2 was further purified by gel chromatography on a Sephacryl S-300 (Whatman Co.) column under conditions described in Fig. 1.

The purity and molecular size of the purified S-2 were examined by sodium dodecyl sulfate-polyacrylamido gel electrophoresis.

Isolation of lysylendopeptidase-peptides of the S-2

About 25 mg (500 nmol) of the purified S-2 was dissolved in 1 ml of 50 mM Tris-HCl (pH 8.0), and digested with 10 nmol of lysylendopeptidase (Wako Pure Chemical Industry) at 37 °C for 6 hr. The resulting peptides were separated and purified by reverse-phase high performance liquid chromatographies (HPLC) using a LC-6A system (Shimadzu Co.) with a Wakosil 5C8 column (4.6 x 250 mm, Wako Pure Chemical Industry) under conditions described in Fig. 2.
Analyses of amino acid composition and sequence

Amino acid analyses of peptides were performed with a JLC-300 amino acid analyzer (JEOL Co.) after hydrolyses with constant-boiling HCl at 110 °C for 22 hr. in evacuated and sealed tubes. The automated Edman degradation for sequence analysis was carried out by using a Model 477A protein sequencer (Applied Biosystem Co.), and PTH-amino acids were identified and determined by HPLC with an on-line Applied Biosystems Model 120A analyzer.

Results

About 287 g (wet weight) of the external pectoral muscle was obtained from four denervated chickens, and 1.1 g of myosin was prepared from the muscle. Chymotryptic S-2 from the myosin was purified by gel chromatography on a Sephacryl S-300 column as shown in Fig. 1. About 40 mg of purified S-2 was obtained from the fractions indicated by the bar in the figure. A mobility of the S-2 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis coincided with that of a long S-2 of the pectoral myosin from normal adult chicken reported by Maita, T. et al.24

The purified S-2 was digested with lysylendopeptidase at 37 °C for 6 hr., and the resulting peptides were separated by reversephase HPLC with a WakoSIL 5C8 column as shown in Fig. 2. Peptides in each peak in the figure were rechromatographed on the column under a more moderate gradient of acetonitrile concentration. Thus eleven peptides, Peptide A to K could be obtained with no detectable contamination from the respective peaks in Fig. 2. The amino acid compositions of the peptides are shown in Table 1.

The eleven peptides were sequenced completely except for the carboxyl terminal three residues of Peptide J, by using an automated protein sequencer. The results are given in Table 2. Out of the eleven peptides, the sequences of Peptide A, B, D, F, and H were identical with those of 1046-Leu to 1054-Lys, 1000-Ala to 1018-Lys, 1096-Ile to

Table 1. Amino acid composition of the lysylendopeptidase-peptides of the S-2 from the denervated muscle myosin.

<table>
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<tr>
<th>Peptide</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
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<td>2.09(2)</td>
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<tr>
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Table 2. Amino acid sequences of the lysylendopeptidase-peptides of the S-2 from the denervated muscle myosin.

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<tr>
<th>Peptide No.</th>
<th>Sequence</th>
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<tr>
<td>A</td>
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<td>E</td>
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<tr>
<td>G</td>
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<td>Asp-Ile-Asp-Asp-Leu-Glu-Leu-Thr-Leu-Ala-Lys</td>
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<tr>
<td>I</td>
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<tr>
<td>K</td>
<td>Asn-Leu-Glu-Glu-Ile-Asp-Leu-Thr-Thr-Leu-Ala-Ala-Glu-Gly-Glu-Asp-Arg-Thr-Ser-Arg-Ala-Lys</td>
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Fig. 3. Comparison of the amino acid sequences of S-2 from the normal and denervated muscle myosins. The upper sequence shows the Res. 920 to Res. 1169 of the myosin heavy chain normally expressed in pectoral muscle. The sequences of Peptide A to K obtained from the S-2 of myosin in the denervated muscle are placed at homologous positions to the upper one, respectively. The carboxyl terminal three residues could not be identified. The different residues from the normal sequence are squared.

1108-Lys, 1029-Leu to 1044-Lys, and 955-Asp to 965-Lys of the myosin heavy chain normally expressed in the pectoral muscle of chicken, respectively. On the other hand, the sequences of the other peptides differed: in Peptide C clearly corresponding to 1063-Leu to 1075-Lys of the normal heavy chain, amino acid exchanges of 1065-His -> Thr, 1067-Ser -> Ala, 1070-Asp -> Ala, and 1074-Asp -> Val were recognized. Substitutions in Peptide E 927-Arg -> Leu, 932-Glu -> Leu and 938-Leu -> Ala, in Peptide G 981-Thr -> Ala, in Peptide K 981-Thr -> Leu, in Peptide J 1111-Lys -> Glu, and in Peptide K 981-Thr -> Glu, 984-Met -> Ile, 986-Val -> Asp, 988-Asp -> Thr, 993-Lys -> Glu, 994-Leu -> Gly and 995-Thr -> Gly were also recognized. These substitutions are summarized in Fig. 3.

Discussion

The pectoral muscle of adult chicken was remarkably atrophied by cutting the pectoral nerve as might have been expected. The chicken pectoral muscle can be distinguishable to the external one (M. pectoralis superficialis) and the internal one (M. pectoralis profundus). It is suggested that the two muscles contain structurally different myosin heavy chains, respectively, and that the primary structure reported by Maita, T. et al. is for a major heavy chain in the external muscle. Therefore, in the present studies myosin was prepared from only the external muscle.

The eleven lysyelendopeptidase-peptides of S-2 from the denervated muscle myosin could be isolated, and sequenced. Among them, ten peptides, Peptide A to J were obtained with considerable amount (36-78% yields calculated from the results of amino acid analyses of samples before the rechromatography). By comparing their sequences with the reported primary structure (Fig. 3.), it was clarified that the denervated muscle produced at least one major heavy chain structurally different from the normal one. Further, the sequences of the major and minor heavy chains in the denervated external pectoral muscle were also different from that in the normal internal one reported by Matsuzono, K.31

Schiaffino, S. et al.20 have reported that the embryonic and neonatal myosins could be detected by the immunohistochemical method in the denervated adult rat skeletal
muscle. Several cloned cDNAs for the embryonic and neonatal heavy chains from chicken have been reported. Among them, an entire amino acid sequence was predicted from the nucleotide sequence of the cDNA from 14-day embryonic pectoral muscle. The embryonic sequence were clearly different from those of the above described major and minor components. Therefore, it was suggested that the embryonic one did not express in the denervated muscle at least as a major component. Unfortunately, no sequence around S-2 is known for the other embryonic or neonatal cDNAs.

Robbins, J. et al. have suggested that more than 30 genes for the skeletal myosin heavy chain exist in the genome. It might be very important to know what kinds of the genes express by what mechanisms after denervation. We are going to obtain cDNA for the major heavy chain detected in this study.

Acknowledgement

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References


