THE L-2 LIGHT CHAIN OF CHICKEN SKELETAL MUSCLE MYOSIN

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1. Introduction

As with rabbit skeletal muscle myosin, four kinds of light chains may be separated from chicken skeletal muscle myosin by cellogel electrophoresis at pH 8.3. They are designated L-1, L-2, L-3 and L-4 in the order of their increasing anionic mobilities. L-1 is also known as alkali light chain 1 (A1) and L-4 as alkali light chain 2 (A2). L-2 and L-3 are also called DTNB light chain. L-3 is a phosphorylated L-2 light chain. Interest in the role of these myosin light chains in the mechanism of muscle contraction has been marked. The primary structures of L-1 and L-4 light chains of rabbit skeletal muscle myosin have been determined by Frank and Weeds [1]; they contain 190 and 149 amino acids, respectively. The primary structure of the L-2 light chain of rabbit skeletal muscle myosin has been determined by Collins [2] and by Matsuda et al. [3]. The amino acid composition of the L-2 light chain of chicken skeletal muscle myosin has been found by Lowey and Holt [4] and its partial amino acid sequence reported by Jakes et al. [5]. We report here the primary structure of the L-2 light chain of chicken skeletal muscle myosin.

2. Materials and methods

Thirty adult, Hubbard-type chickens were used. Myosin was extracted from skeletal muscle according to [6]. The light chains were separated according to [7] with modification: The suspension of myosin was adjusted to the final concentration of 5 M and 2 mM by adding solid guanidine–HCl and 2-mercaptoethanol, respectively. It was left overnight at 4°C, then 2 vol. cold ethanol were added.

Precipitated heavy chains were removed by centrifugation; the supernatant containing the light chains was evaporated under reduced pressure and demineralized by Sephadex G-25 gel filtration. The four components in this light chain fraction were separated by DEAE-cellulose column chromatography. Elution was carried out through a 0.035 M KCl/4 M urea/25 mM Tris (pH 7.6) to 0.30 M KCl/4 M urea/25 mM Tris (pH 6.0) gradient. Lyophylized L-2 light chain was carboxymethylated according to [8]. The *S*-carboxymethylated L-2 light chain was digested with trypsin at 37°C, pH 8.0 for 3 h. The tryptic peptides were fractionated into 0.05 M pyridine-acetate buffer (pH 3.2) soluble and insoluble fractions. Peptides in the soluble fraction were separated by column chromatography using a Chromo Beads P ion exchanger, and pyridine-acetate buffers 0.05 M (pH 3.2), 0.2 M (pH 3.2) and 2.0 M (pH 5.0) as developers. Soluble fraction peptides were purified by Sephadex G-25 gel filtration, paper chromatography and paper electrophoresis. Peptides in the insoluble fraction were separated into four smaller fractions by Sephadex G-50 gel filtration. Peptides in each smaller fraction were separated and purified by DEAE-cellulose column chromatography.

The amino acid sequences of the tryptic peptides from *S*-carboxymethylated L-2 light chain of chicken skeletal muscle myosin were determined. The amino acid sequences of the smaller tryptic peptides were determined directly by subtractive Edman degradation [9], Edman-Dansyl method [10] and carboxypeptidase A and B digestion. The locations of amides in the peptides were deduced from the mobilities of the peptides during high voltage paper electrophoresis, at pH 6.4 [11]. Larger tryptic peptides were digested with chymotrypsin and thermolysin and cleaved at aspartic acid with 0.25 M acetic acid. The amino acid

sequences of the resulting peptide fragments from the larger tryptic peptides were determined as described above.

3. Results and discussion

Four components of the light chain fraction from chicken skeletal muscle myosin were eluted in the order from L-1, L-2, L-3 to L-4 by column chromatography as described above. Each component was collected separately and lyophilized. Comparison of the mean values of the weight ratio of L-1, L-2 + L-3, and L-4 shows it to be 1.3 : 2.0 : 0.7; i.e., the content of L-1 and L-4 is not equal. Thus it is hard to believe

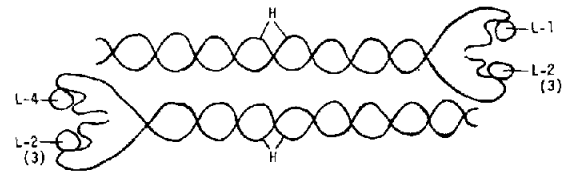

Fig.1. The subunit structure of the myosin in chicken skeletal muscle: H, heavy chain; L-1, L-1 light chain; L-2(3), L-2 or L-3 light chain; L-4, L-4 light chain.

L-1 and L-4 exist together within one myosin molecule. From these facts we assume, as fig.1 shows, that in chicken skeletal muscle there exist primarily two kinds of myosins, each constructed from two heavy

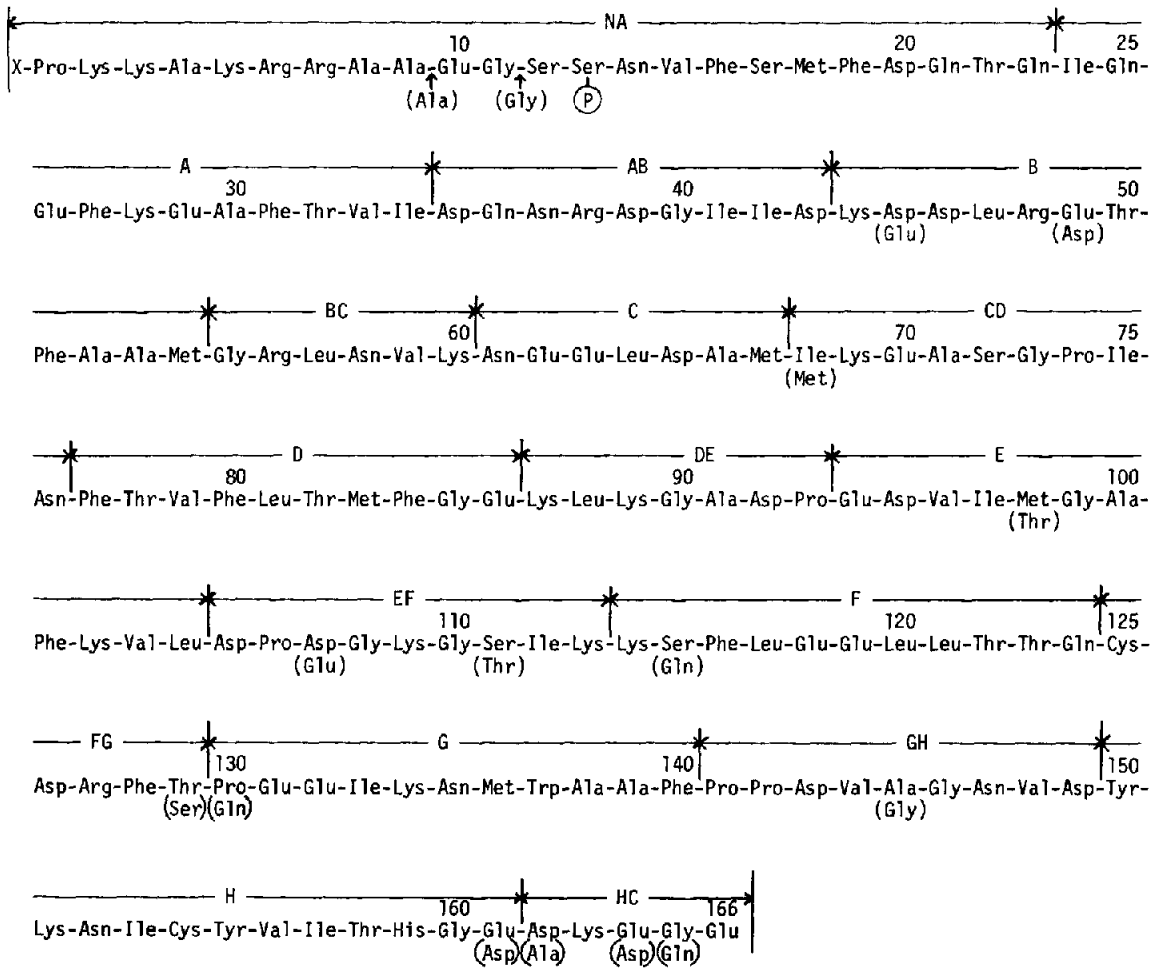

Fig.2. The amino acid sequence of L-2 light chain of chicken skeletal muscle myosin. Shown below are the amino acids inserted (arrows) and the amino acids substituted in the case of the L-2 light chain of the rabbit skeletal muscle myosin.

chains and two light chains. It has not been demonstrated so far whether or not the L-2 [3] light chains contained in the two kinds of myosins, as shown in this figure, are quite the same. Further it has not been shown whether or not the heavy chains contained in these two kinds of myosins are quite the same.

The L-2 light chain, after *S*-carboxymethylation, was digested with trypsin. This tryptic digest was fractionated into soluble and insoluble fractions. Twenty one peptides from the soluble fraction and ten from the insoluble fraction were separated and purified by the methods described above. The amino acid compositions of all the tryptic peptides thus obtained from the *S*-carboxymethylated L-2 light chain were analysed, and the amino acid sequences determined. The primary structure of the L-2 light chain of chicken skeletal muscle was determined to be as shown in fig.2 by comparing the amino acid sequences of these tryptic peptides with the sequence of the L-2 light chain from rabbit skeletal muscle myosin. This chicken protein is composed of 166 amino acids and comparison of the primary structures of the chicken and rabbit L-2 light chains shows two amino acid insertions in the latter. In the rabbit protein there are inserted one Ala between Ala(9) and Glu(10) and one Gly between Gly(11) and Ser(12) of the chicken L-2 light chain. Further, fourteen amino acid substitutions are recognized between the two as follows (the first residue and the number refer to chicken and the second residue to rabbit): Asp(45) ↔ Glu, Glu(49) ↔ Asp, Ile(68) ↔ Met, Met(98) ↔ Thr, Asp(107) ↔ Glu, Ser(111) ↔ Thr, Ser (115) ↔ Gln, Thr(129) ↔ Ser, Pro(130) ↔ Gln, Ala(145) ↔ Gly, Glu(161) ↔ Asp, Asp(162) ↔ Ala, Glu(164) ↔ Asp and Gly(165) ↔ Gln. All these amino acid substitutions can be explained by single base changes, except Ser(115) ↔ Gln and Gly(165) ↔ Gln. No amino acid substitution occurred at Ser(13) which was assumed [12] to be the position phosphorylated.

The sections marked A, B, C, D, . . ., H in fig.2 have an α-helix structure and AB, BC, CD, DE, . . ., GH are loops between two helices. These were arrived at by comparison of carp muscle calcium-binding protein [13,14] and rabbit L-2 light chain [2,3] structures. From its primary structure Collins [2] suggested that the L-2 light chain of rabbit skeletal muscle myosin might be able to be linked to at least one Ca^{2+}. The same conclusion can be reached from the primary structure of the L-2 light chain of chicken skeletal muscle myosin. The linkages are Asp(35), Asn(37), Asp(39), Ile(41), Asp(43) and Asp(46) at the

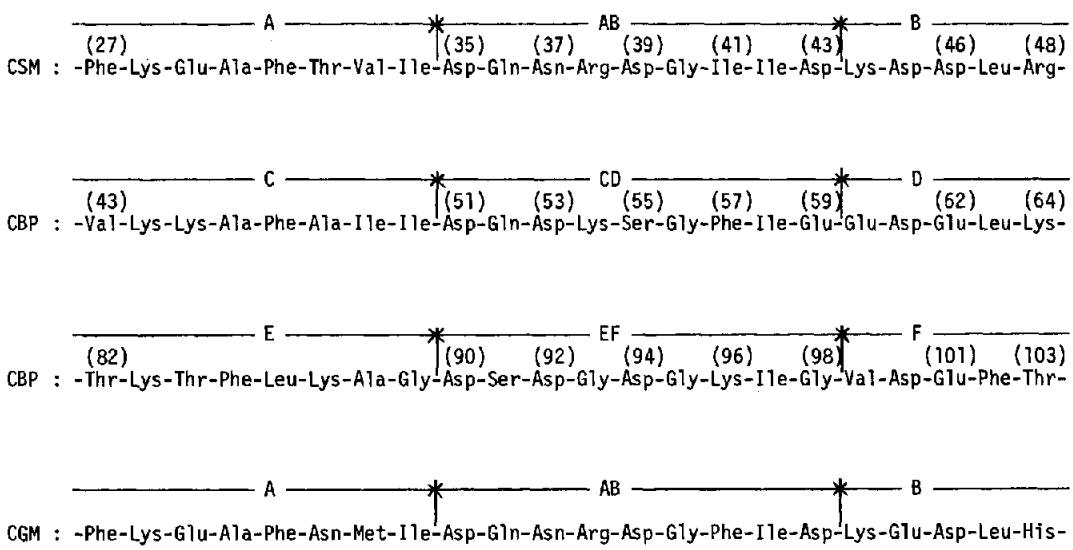

Fig.3. Comparison of the amino acid sequences of the L-2 light chains and carp muscle calcium-binding protein: CSM, L-2 light chain of chicken skeletal muscle myosin; CBP, carp muscle calcium-binding protein; CGM, L-2 light chain of chicken gizzard myosin.

center of the AB loop. Then, very great similarities were recognized if the primary structure of the center part of the AB loop of chicken L-2 light chain, and the center parts of the CD loop and the EF loop, where Ca^{2+} is assumed to be linked, of carp muscle calcium-binding protein were compared (fig.3). Moreover it is suggested that the light chain of chicken gizzard myosin might participate in calcium regulation in muscle contraction. As the partial primary structure of the L-2 light chain of this myosin had been reported [5], we show the comparison between the corresponding parts of the primary structures of the L-2 light chain of chicken skeletal muscle myosin and chicken gizzard myosin in fig.3. Very great similarities are recognized between the L-2 light chains of chicken skeletal and chicken gizzard myosins.

Acknowledgements

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