THE PRIMARY STRUCTURE OF L-1 LIGHT CHAIN OF CHICKEN FAST SKELETAL MUSCLE MYOSIN AND ITS GENETIC IMPLICATION

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Received 19 February 1981

1. Introduction

It is known that in general fast skeletal muscle myosin consists of 2 heavy chains and 4 light chains [1,2]. We have also recognized 4 components in the light chain fraction from chicken fast skeletal muscle myosin by Cellulogel electrophoresis at pH 8.3, and designated these light chains L-1, L-2, L-3 and L-4 light chains in the order of their increasing anionic mobilities [3]. 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 chains. L-3 is a phosphorylated L-2 light chain. The primary structures of L-2 and L-4 light chains of chicken fast skeletal muscle myosin have been reported in [4,5]. We describe here the primary structure of the L-1 light chain from chicken fast skeletal muscle myosin and its genetic implication.

2. Materials and methods

Myosin was extracted from fast skeletal muscle of Hubbard-type chickens as in [6]. The light chains were separated as in [3]. A carboxymethylation of L-1 light chain was done as in [7]. The digestion of the L-1 light chain with trypsin was done at 37°C, pH 8.0 for 4 h. The tryptic peptides in the digest were separated by chromatography on a column of Chromo Beads P (Technicon Corp.) and further purified by column chromatography on AG 1 × 2, paper chromatography and paper electrophoresis under the conditions in [8].

The amino acid sequences of the smaller tryptic peptides were determined directly and those of the larger tryptic peptides were determined after further partial hydrolysis. The procedures for digestion of larger tryptic peptides with chymotrypsin, thermolysin and subtilisin, and for further cleavage with 0.25 M acetic acid are described in [9]. Subtractive Edman degradation, Edmandansyl method and carboxypeptidase A and B digestion were done as in [9,10].

The digestion of the L-1 light chain with pepsin was done at 35°C (pH 2.0) for 2 h. The peptic peptides in the digest were separated by gel filtration on Sephadex G-50 and chromatography on Chromo Beads P under the conditions in [8].

3. Results and discussion

The L-1 light chain of myosin which was extracted from chicken fast skeletal muscle was digested with trypsin after *S*-carboxymethylation. The tryptic peptides in the digest were separated, purified and their amino acid sequences analyzed. The carboxymethylated L-1 light chain was also digested with pepsin. The peptic peptides in the digest were separated, purified and their partial amino acid sequences analyzed. As the N-terminal amino acid of the L-1 light chain was not identified by the dansylation, we assumed that the α-amino group of the N-terminal amino acid of the light chain was blocked. However, it was determined by amino acid analysis that tryptic peptide T-1 contains Pro (1) and Lys (1), but its N-terminal amino acid was not identified by subtractive Edman degradation or dansylation. Therefore T-1 was deduced to be X–Pro–Lys, supposing that the N-terminal is blocked. From these results, the primary structure of the L-1 light chain of chicken fast skeletal muscle myosin was determined as shown in fig.1. This protein is composed of 190 amino acids and its N-terminal proline might be blocked by some means.

The primary structure of A 1(L-1) and A 2 (L-4)

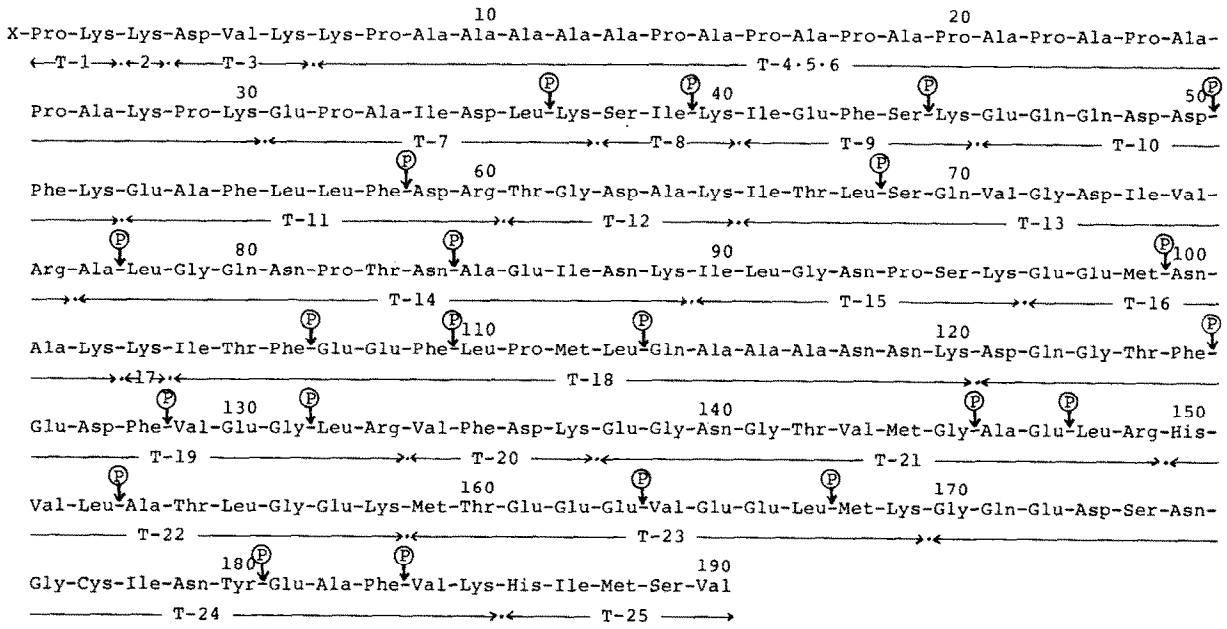

Fig.1. The primary structure of L-1 light chain of chicken fast skeletal muscle myosin: (T-1–T25) the final designation of tryptic peptides; (Ⓟ→) the peptide linkage cleaved with peptic digestion.

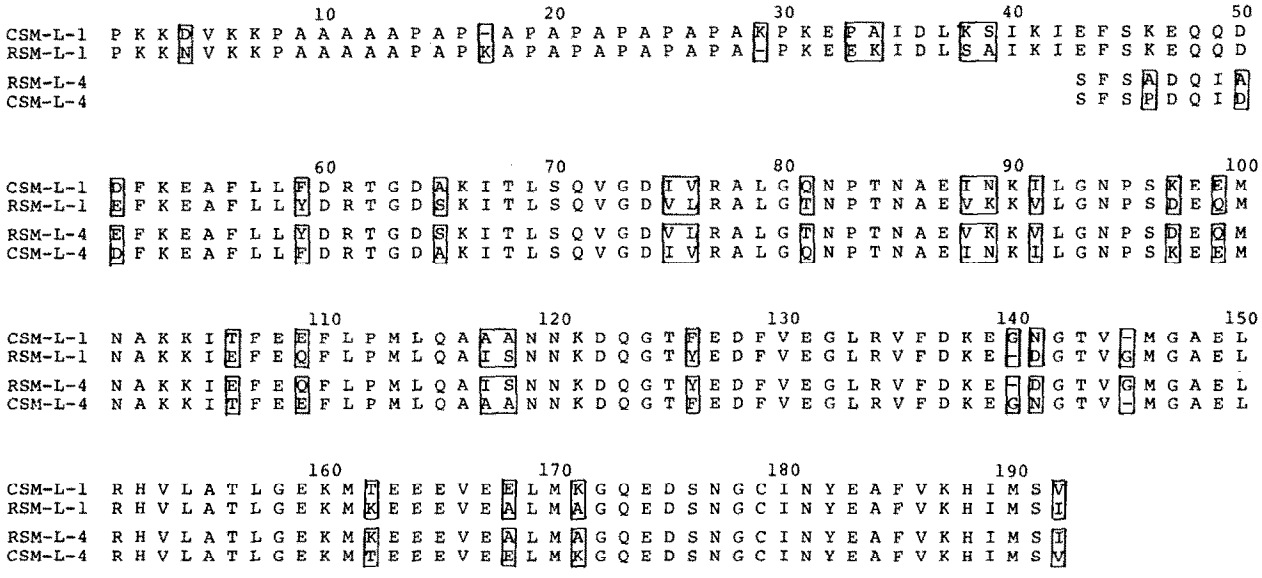

Fig.2. The comparison of the primary structures of L-1 (A1) and L-4 (A2) light chains between chicken fast skeletal muscle myosin (CSM) and rabbit fast skeletal muscle myosin (RSM). Both N-terminal Pro of L-1 (A1) light chains of CSM and RSM are blocked and both N-terminal Ser of L-4 (A2) are acetylated.

light chains of rabbit fast skeletal muscle myosin have been already determined [11]. Fig. 2 shows the comparison of the primary structures of L-1 (A1) and L-4 (A2) light chains between chicken and rabbit fast skeletal muscle myosins. In fig.3 amino acid residues of these proteins are aligned so as to maximize homology by introducing the necessary gaps. As fig.3 shows, between chicken L-1 (A1) and rabbit L-1 (A1), and between chicken L-4 (A2) and rabbit L-4 (A2) there are 30 and 25 amino acid substitutions, respectively. The most important point is following. In the C-terminal 142 amino acid residues including a gap of L-1 (A1) and L-4 (A2) chains, the amino acid sequences of chicken L-1 (A1) and chicken L-4 (A2) and those of rabbit L-1 (A1) and rabbit L-4 (A2) are, respectively, identical; furthermore the position of amino acid substitution and the kind of the substituted amino acids between chicken L-1 (A1) and rabbit L-1 (A1) and between chicken L-4 (A2) and rabbit L-4 (A2) are identical.

On the contrary, however, in the N-terminal 50 amino acid residues including a gap of L-1 (A1) chains and N-terminal 8 amino acid residues of L-4 (A2) chains, the amino acid sequences of chicken L-1 (A1) and chicken L-4 (A2) and those of rabbit L-1 (A1) and rabbit L-4 (A2) are, respectively, different; furthermore the position of amino acid substitutions and the kind of the substituted amino acids between chicken L-1 (A1) and rabbit L-1 (A1) and between chicken L-4 (A2) and rabbit L-4 (A2) are quite different.

From these facts we can assume that both C-terminal 142 amino acid residues of L-1 (A1) and L-4 (A2) light chains of fast skeletal muscle myosin are encoded in a common DNA segment on one gene, and that furthermore the N-terminal 50 amino acid residues of L-1 (A1) and the N-terminal 8 amino acid residues of L-4 (A2) are encoded in 2 separate DNA segments on the same gene. It can be also presumed that L-1 (A1) mRNA and L-4 (A2) mRNA were separately synthesized by RNA–RNA splicing from hnRNA which transcripted from one common structural gene, and furthermore L-1 (A1) and L-4 (A2) light chains were translated from these separated mRNA. Besides we assume that these alkali light chains consist of at least two domains corresponding to these N-terminal and C-terminal regions, and these domains might be functionally different from each other. On the other hand these facts may also suggest the mechanism of the appearance of new proteins on molecular evolution.

Acknowledgement

We wish to thank Miss Michiko Ishibashi for her help in preparing this paper.

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