AMINO ACID SEQUENCES OF THE CARDIAC L-2A, L-2B AND GIZZARD 17 000-M_r LIGHT CHAINS OF CHICKEN MUSCLE MYOSIN

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1. Introduction

Myosins separated from various vertebrate muscles contain 2 heavy chains of ~200 000-M_r and 4 light chains of ~20 000-M_r [1–4]. In connection with a relationship between the light chain structure and mechanism of the muscle contraction, we have studied the primary structures of the light chains of the fast skeletal, cardiac and gizzard muscle myosins from chicken [5–11].

Chicken fast skeletal muscle myosin has 4 kinds of light chains designated L-1–L-4 in order of their increasing anionic mobilities at pH 8.3 [5,6,8–10]. The L-1 and L-4 light chains are also called alkali light chain 1 (A1) and alkali light chain 2 (A2), respectively. The L-2 and L-3 light chains are also known as DTNB light chains. L-3 is a phosphorylated L-2 light chain [1–3]. The primary structures of these light chains have been reported [5,6,8–10].

Chicken cardiac muscle myosin has 2 kinds of light chains designated L-1 and L-2, and the primary structure of the L-1 light chain has been already reported [7,8]. We have also recognized 2 components in the L-2 light chain fraction by cellulogel electrophoresis at pH 8.3 after performic acid oxidation, and designated these components L-2A and L-2B.

Chicken gizzard muscle myosin contains 2 kinds of light chains. One of them is called 20 000-M_r light chain or GI light chain. It is also called regulatory light chain because it has calcium binding ability [4]. The other is called 17 000-M_r light chain or GII light chain [4]. The primary structure of the 20 000-M_r light chain has been reported [11].

Here, we present the primary structures of the L-2A and L-2B light chains of chicken cardiac muscle myosin and the 17 000-M_r light chain of chicken gizzard muscle myosin, and compare the primary structures of the light chains of chicken fast skeletal, cardiac and gizzard muscle myosins.

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2. Materials and methods

About 600 adult Hubbard-type chickens were used for this experiment. Cardiac myosin was extracted from ventricular muscle according to [12]. The light chain fraction was separated from the heavy chain as in [1]. The L-1 and L-2 light chains in the light chain fraction were separated as in [7]. After performic acid oxidation as in [13], we recognized 2 components which were designated L-2A and L-2B in the L-2 light chain fraction by cellulogel electrophoresis at pH 8.3. These L-2A and L-2B light chains were separated by DEAE-cellulose column chromatography. The elution was carried out through a 50 mM KCl/4 M urea/50 mM Tris–HCl buffer (pH 8.5) to a 100 mM KCl/4 M urea/50 mM Tris–HCl buffer (pH 8.5) gradient.

Gizzard myosin was extracted from chicken gizzard muscle according to [14]. The light chain fraction was separated from the heavy chain as in [1]. The two light chain components, 20 000 and 17 000-M_r light chains, in the light chain fraction were separated by DEAE-cellulose column chromatography after performic acid oxidation. The elution was done as in [11]. The sequence studies were done as in [15,16].

3. Results and discussion

The weight ratio of the L-2A and L-2B light chains of chicken cardiac muscle myosin which were separated from chicken ventricular muscle was almost the

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same. The oxidized L-2A light chain was digested with trypsin at 37°C (pH 8.0 ~ 8.5) for 4 h. The tryptic peptides in the digest were separated, purified and their amino acid sequences analyzed. The oxidized L-2A light chain was also digested with pepsin at 37°C (pH 2.0) for 2 h. The peptic peptides in the digest were separated, purified and their partial amino acid sequences analyzed. From these results, the primary structure of L-2A light chain was determined (fig.1). The protein is composed of 163 amino acids and its N-terminal proline might be blocked by some means. The primary structure of L-2B light chain was analyzed in almost the same way as L-2A light chain. The result is shown also in fig.1.

The oxidized 17 000-M_r light chain was digested with trypsin at 37°C (pH 8.0 ~ 8.5) for 6 h. The tryptic peptides in the digest were separated, purified and their amino acid sequences analyzed. The alignment of these tryptic peptides in the light chain was deduced from the amino acid compositions and from the partial sequences of peptic peptides of the oxidized light chain. The established primary structure of the 17 000-M_r light chain is shown in fig.2. The protein is composed of 150 amino acids and its N-terminal cysteine might be blocked by some means.

There are two kinds of L-2 light chains of chicken cardiac muscle myosin. The same fact has been also recognized on cardiac muscle myosins from rabbit, human, baboon and dog [17,18]. It might have something to do with the periodical cardiac contraction. Comparison of the primary structures of the L-2A and L-2B light chains of chicken cardiac muscle myosin shows 1 amino acid deletion and 1 insertion each (fig.1). Furthermore, 16 amino acid substitutions are recognized between the L-2A and L-2B light chains

It is supposed that the 17 000-M_r light chain of

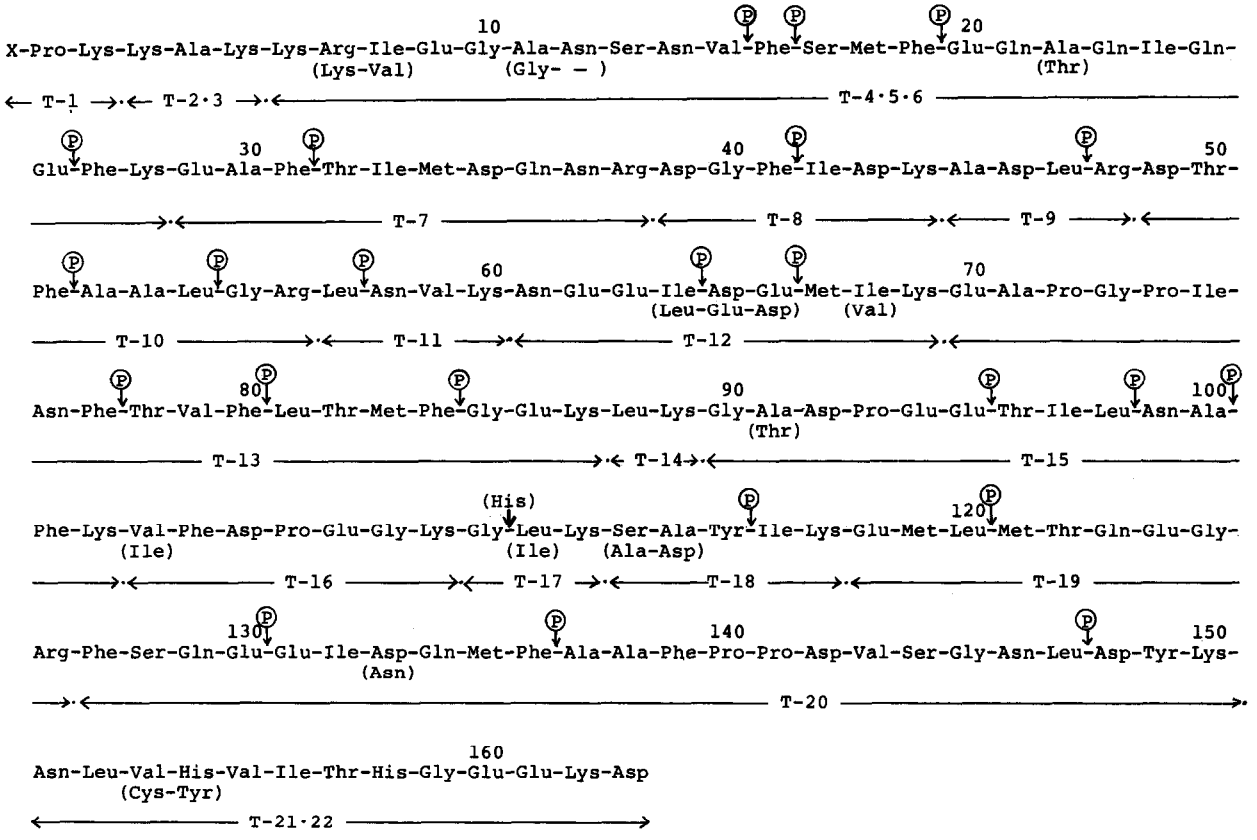

Fig.1. The primary structures of the L-2A and L-2B light chains of chicken cardiac muscle myosin. Amino acid sequence of the L-2A is originally shown. Amino acid residues parenthesized are the amino acids substituted, inserted (→) and deleted (–) in the case of the L-2B. The marks (T-1, · · · , T-21 · 22) show the final designations of tryptic peptides. The mark (Ⓟ →) peptide linkages cleaved with peptic digestion.

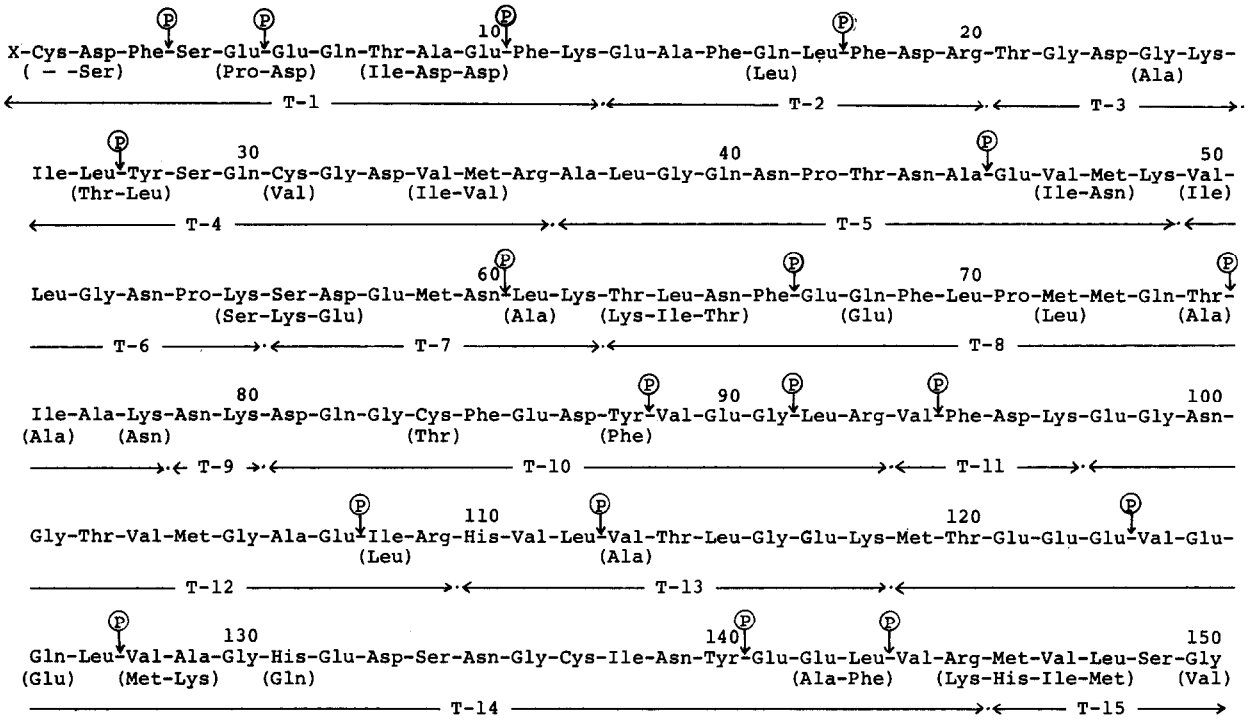

Fig.2. The primary structure of the 17 000-M_r light chain of chicken gizzard muscle myosin. Amino acid residues in parentheses are the amino acids substituted and deleted (–) in the case of the L-4 light chain of chicken fast skeletal muscle myosin. The other marks are the same as in fig.1.

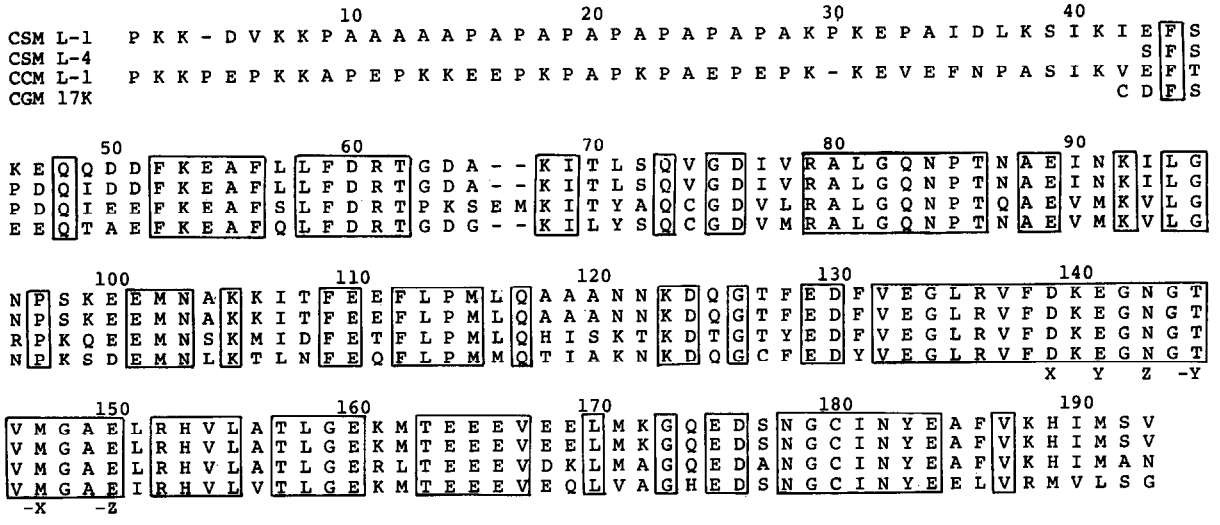

Fig.3. The comparison of the primary structures of the catalytic light chains of various chicken muscle myosins. *Abbreviations:* CSM, chicken fast skeletal muscle myosin; CCM, chicken cardiac muscle myosin; CGM 17 K, the 17 000-M_r light chain of chicken gizzard muscle myosin. The identical residues in the 4 light chains are squared; X, Y, Z, –Y, –X, –Z, residues which might be involved in the binding to divalent metal ions in III homologous region.

chicken gizzard muscle myosin corresponds to the L-4 light chain of chicken fast skeletal muscle myosin from the homology of the primary structures of these light chains. Comparing these primary structures, there is one amino acid deletion at the N-terminal in the latter, and a further 43 amino acid substitutions between them.

The detailed physiological functions of the light chains of various muscle myosins are still not clearly understood. However it is generally believed that the L-1 and L-4 light chains of the fast skeletal muscle myosin may be somehow involved in the ATPase activity [19]. The L-1 and L-4, therefore, might be classified as catalytic light chains of myosin. The L-1 light chain of cardiac muscle myosin and the 17 000-M_r light chain of gizzard muscle myosin might also be classified in the same class as the L-1 and L-4. The primary structures of these catalytic light chains are compared in fig.3. The 91 invariable amino acid residues among these catalytic light chains are recognized (fig.3). These light chains contain four homologous regions (I–IV) of the calcium-binding structure in parvalbumin [20–23]. Among these regions, it is suggested from the primary structures that III region is most invariable and has the binding-ability to divalent metal ions.

The L-2 light chains of the fast skeletal and the cardiac muscle myosins and 20 000-M_r light chain of gizzard muscle myosin are considered to be a homolog of the EDTA light chain of molluscan myosin because of having the calcium-binding ability and of the existence of a phosphorylated form [24–27]. They, therefore, might be classified as regulatory light chains of myosins. The primary structures of these regulatory light chains is compared in fig.4. The 75 invariable amino acid residues among these regulatory light chains are recognized as shown in this figure. These light chains also contain 4 homologous regions (I–IV) of the calcium-binding structure in parvalbumin [20–23]. Among these regions, I and II regions are more conservative than III and IV, and from the primary structures I region is suggested to have the binding-ability to Ca^{2+}. The phosphorylated residue is supposed to be Ser at 19th from N-terminal in this figure.

It might be expected that a systematic nomenclature of the light chains of various muscle myosins would be arranged. We also hope to further elucidate the relationship between these light chain structures and the biochemical functions of various muscle myosins.

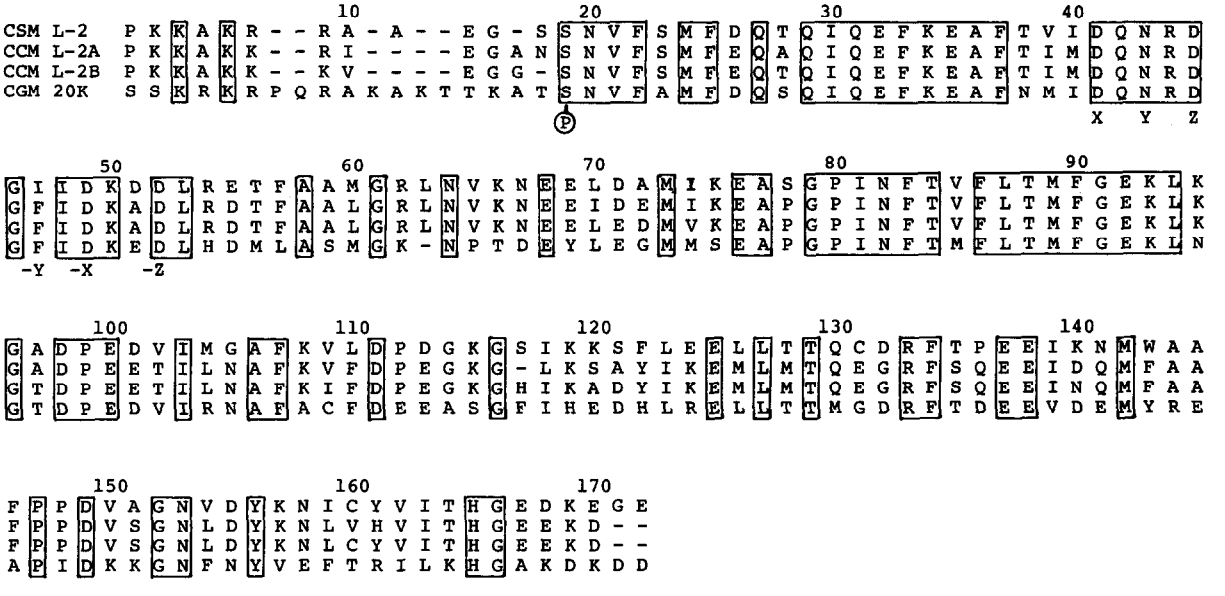

Fig.4. The comparison of the primary structures of the regulatory light chains of various chicken muscle myosins. *Abbreviations:* CGM 20 K, the 20 000-M_r light chain of chicken gizzard muscle myosin; X, Y, Z, –Y, –X, –Z, residues which might be involved in the binding to Ca^{2+} in I homologous region. The other marks are as in fig.3.

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References

[1] Perrie, W. T. and Perry, S. V. (1970) Biochem. J. 119, 31–38.
[2] Weeds, A. G. and Lowey, S. (1971) J. Mol. Biol. 61, 701–725.
[3] Lowey, S. and Risby, D. (1971) Nature 234, 81–86.
[4] Kendrick-Jones, J. (1973) Phil. Trans. Roy. Soc. Lond. B 265, 183–189.
[5] Matsuda, G., Suzuyama, Y., Maita, T. and Umegane, T. (1977) FEBS Lett. 84, 53–56.
[6] Suzuyama, Y., Umegane, T., Maita, T. and Matsuda, G. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 119–127.
[7] Maita, T., Umegane, T., Kato, Y. and Matsuda, G. (1980) Eur. J. Biochem. 107, 565–575.
[8] Matsuda, G., Maita, T., Umegane, T. and Kato, Y. (1980) in: Frontiers in Protein Chemistry (Liu, T. Y. et al. eds) pp. 323–334, Elsevier/North-Holland, Amsterdam, New York.
[9] Maita, T., Umegane, T. and Matsuda, G. (1981) Eur. J. Biochem. 114, 45–49.
[10] Matsuda, G., Maita, T. and Umegane, T. (1981) FEBS Lett. 126, 111–113.
[11] Maita, T., Chen, J. and Matsuda, G. (1981) Eur. J. Biochem. 117, 417–424.
[12] Weeds, A. G. and Hartley, B. S. (1968) Biochem. J. 107, 531–548.
[13] Hirs, C. H. W. (1956) J. Biol. Chem. 219, 611–621.
[14] Ebashi, S. (1976) J. Biochem. (Tokyo) 79, 229–231.
[15] Matsuda, G., Maita, T., Suzuyama, Y., Setoguchi, M. and Umegane, T. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 357–368.
[16] Matsuda, G., Maita, T., Suzuyama, Y., Setoguchi, M. and Umegane, T. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 629–640.
[17] Frearson, N. and Perry, S. V. (1975) Biochem. J. 151, 99–107.
[18] Price, K. M., Littler, W. A. and Cummins, P. (1980) Cardiovasc. Res. 14, 555–560.
[19] Dreizen, P. and Richard, D. H. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 29–45.
[20] Kretsinger, R. H. and Nockolds, C. E. (1973) J. Biol. Chem. 248, 3313–3326.
[21] Collins, J. H. (1974) Biochem. Biophys. Res. Commun. 54, 301–308.
[22] Weeds, A. G. and McLachlan, A. D. (1974) Nature 255, 646–649.
[23] Goodman, M., Pechere, J. F., Haiech, J. and Demaille, J. G. (1979) J. Mol. Evol. 13, 331–352.
[24] Kendrick-Jones, J., Szentkiralyi, E. M. and Szent-Györgyi, A. G. (1972) Cold. Spring Harbor Symp. Quant. Biol. 37, 47–53.
[25] Perrie, W. T., Smillie, L. B. and Perry, S. V. (1973) Biochem. J. 135, 151–164.
[26] Morimoto, K. and Harrington, W. F. (1974) J. Mol. Biol. 88, 693–709.
[27] Jakes, R., Northrop, F. and Kendrick-Jones, J. (1976) FEBS Lett. 70, 229–234.