TWO KINDS OF SLOW SKELETAL MUSCLE FIBERS WHICH DIFFER IN THEIR MYOSIN LIGHT CHAIN COMPLEMENTS

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1. Introduction

Several protocols have been devised for the histochemical typing of mammalian muscle fibers (reviewed [1,2,3]). Each distinguishes 3 types of fibers, 2 which are physiologically fast-twitch and 1 which corresponds to a physiologically slow-twitch fiber containing slow muscle protein isozymes and relying primarily on oxidative phosphorylation for the generation of ATP. That latter fiber type, which we will refer to as slow-oxidative or SO after [2], is believed to be the same regardless of the muscle in which it is identified [1,4]. In contrast to this histological view of a single type of SO fiber, physiological studies suggest that SO fibers in slow muscles which are composed predominantly of SO fibers differ from those in muscles composed of a mixture of fiber types [5,6]. In the course of studies on myosins from several muscles of the rabbit we observed that the myosin light chain (MLC) complement of the mixed muscles diaphragm and masseter was not the sum of the fast and slow MLCs. One of the slow MLCs was lacking. That observation suggested that 2 types of SO fibers, those from slow muscles as opposed to those from mixed muscles, could be distinguished on the basis of their MLC complement. Studies on single fibers have confirmed the presence of 2 kinds of SO fibers. The differences in myosins between these 2 types of SO fibers may explain the differences in their physiological properties.

2. Materials and methods

Muscle samples were taken from adult female New Zealand White rabbits (2–4 kg). Myofibrils were prepared as in [7] and a myosin-enriched 40% ammonium sulfate precipitate prepared. Myosin extracted from untreated fibrils was purified as in [8]. Sodium dodecyl sulphate (SDS)–polyacrylamide gels were run as in [9] and alkaline urea-polyacrylamide gels as in [10]. Silver staining was as in [11]. Densitometry was as in [12]. Single fibers were dissected under a dissecting microscope and washed in mammalian Ringers supplemented with 0.5% Triton X-100 before being placed in SDS sample buffer.

3. Results

3.1. *Variation in the myosin light chain complement in different rabbit skeletal muscles*

We analyzed the patterns of MLC expression in several rabbit skeletal muscles in preparations from myofibrils (the 40% ammonium sulfate precipitate was used to minimize interference from low M_r proteins). Fig.1 shows the results of SDS electrophoresis of those preparations. The 3 fast MLCs designated LC_{1f}, LC_{2f} and LC_{3f} and the slow MLC LC_{1s}, LC_{2s} and LC_{3s} are identified from preparations of purified myosin from a fast muscle, longissimus dorsi (fig.1A), and from a slow muscle, soleus (fig.1B). Inspection of the MLCs from myofibrils preparations of several muscles shows 3 patterns of variation: the fast muscle longissimus dorsi (like the myosin purified from it) shows only the 3 fast MLCs (fig.1C); the 4 slow hind limb muscles, soleus, adductor longus, vastus intermedius, and semitendonosis contain all 3 slow MLCs (fig.1F–I, respectively) and, the mixed muscles diaphragm and masseter possess 5 of the 6 myosin light chains, lacking any significant quantity of a MLC corresponding in mobility to LC_{1s}. This result was observed in 7 of 7 adult rabbits.

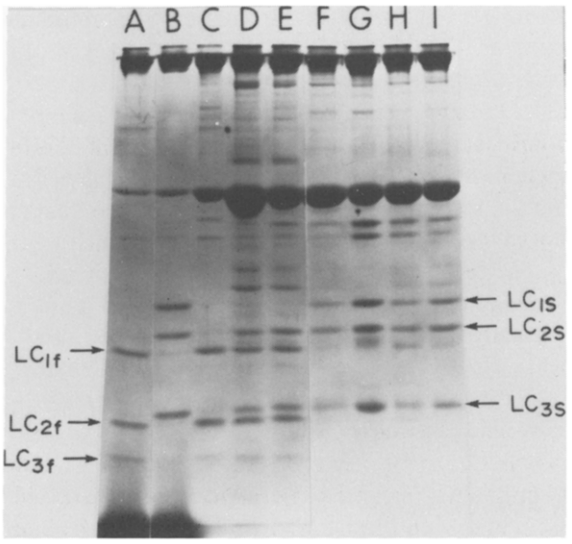

Fig.1. SDS–polyacrylamide gel electrophoresis: (A) purified myosin from longissimus dorsi; (B) purified myosin from soleus, 40% ammonium sulfate precipitates of myofibrils from (C) longissimus dorsi, (D) diaphragm (E), masseter (F) soleus, (G) semitendonosis, (H) adductor longus and (I) vastus intermedius. Fast and slow MLCs are designated as in section 3. Proteins were stained with Coomassie brilliant blue.

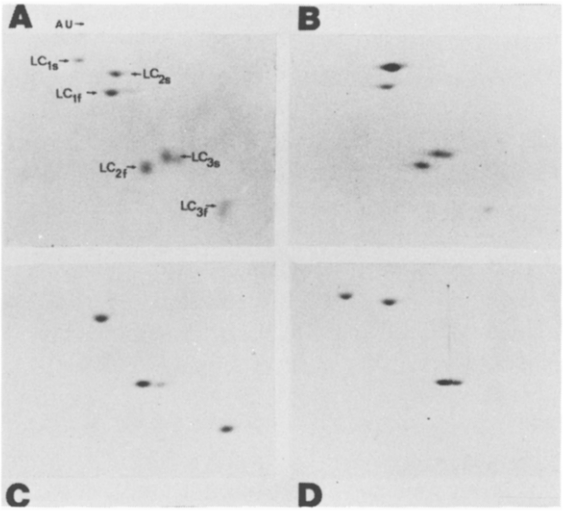

Fig.2. Two-dimensional alkaline urea–SDS–polyacrylamide gel electrophoresis of myosin light chains of myosin from: (A) longissimus dorsi and soleus; (B) masseter; (C) longissimus dorsi; (D) soleus. Splitting of LC_{3s} and LC_{2f} is due to partial phosphorylation of those light chains. The directions of alkaline–urea (AU) and sodium dodecyl sulfate (SDS) electrophoresis are indicated by arrows. Proteins were stained with Coomassie brilliant blue.

3.2. *Electrophoresis of the mixed muscle light chains in two dimensions*

To determine whether there were other differences in the MLCs from mixed as opposed to fast or slow muscle that might not be detected by SDS electrophoresis, we compared the patterns of the light chains of purified masseter myosin with those of soleus and longissimus dorsi by two-dimensional alkaline urea–SDS–polyacrylamide gel electrophoresis. The results in fig.2 show that the pattern of light chains identified as LC_{2s}, LC_{3s}, LC_{lf}, LC_{2f} and LC_{3f} of masseter (fig.2B) are superimposable on corresponding fast (fig.2C) and slow MLCs (fig.2D) as revealed in the mixture of fast and slow MLCs (fig.2A). This confirmed the identification of the mixed muscle MLCs and shows that using a physical basis for electrophoretic separation different from SDS electrophoresis, no further differences in the fast and slow MLCs from masseter other than the absence of LC_{1s} is detectable (the result was also confirmed by co-electrophoresis in one dimension, not shown). The diaphragm MLCs behaved identically (not shown).

3.3. *Myosin light chain patterns in individual muscle fibers*

The observations on myofibril preparations allowed

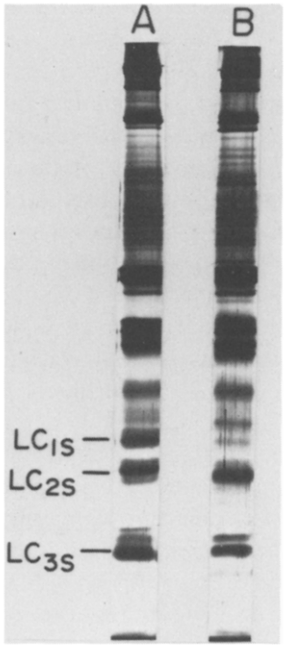

Fig.3. Electrophoresis in the presence of SDS of representative slow muscle fibers from (A) soleus and (B) diaphragm. Proteins were stained by the silver technique.

several possibilities for the composition of single SO fibers:

(i) Soleus might contain 2 types of fibers, one with LC_{1s} and LC_{3s} the other with LC_{2s} and LC_{3s};
(ii) Its SO fibers might contain all 3 slow MLCs;
(iii) The mixed muscle SO fibers must contain only LC_{2s} and LC_{3s}, however, they might also be 'promiscuous', containing some fast MLCs.

Fig.3A shows a typical single fiber from soleus. It contains all 3 slow MLCs. A typical SO fiber from diaphragm is shown in fig.3B. Although with the enhanced sensitivity of silver staining it was not possible to exclude the presence of LC_{1s}, densitometeric analysis revealed that $\leqslant 5\%$ of the mass of LC_{2s} could be detected in the region of LC_{1s}. No fast MLCs were detected. Given the apparent absence of LC_{1s} in the purified myosins and myofibril preparations (sections 3.1 and 3.2), it is probable that the material in the region of LC_{1s} represents another protein which is now detectable in the absence of LC_{1s}.

4. Discussion

In 1965 Wuerker et al. [5] pointed out differences in the contraction time of SO fibers from slow and mixed muscles of the cat. The observed correlation between the V_{max} of the myosin ATPase and shortening velocity [13] suggests that the physiological differences in SO fibers could be due to variations in myosin. In these studies on muscles from the rabbit, we show that 2 kinds of slow myosins and 2 kinds of SO fibers can be distinguished on the basis of their myosin light chain complements. One kind of SO fibers contains all 3 of the slow myosin light chains in [14] and is typical of SO fibers in the slow hindlimb muscles of the rabbit. The other, contains only 2 of the slow myosin light chains, LC_{2s} which is an alkali-like light chain and LC_{3s} which can be phosphorylated [14]. That kind of fiber is found in the mixed muscles diaphragm and masseter of adult rabbits. Without being able to separate the slow myosin species of mixed muscle to compare its enzymatic activity to that of slow muscle myosin, we can only suggest that the differences in myosins are the basis of physiological differences seen in SO fibers [5,6]. But, there can be no doubt that the concept of a unitary SO fiber type is no longer valid on the basis of molecular considerations.

The regulatory phenomenon that determine the expression of these different sets of slow myosin light chains are of great interest to us. Both the reproducibility of this observation and the ability to exclude heterozygosity at a myosin light chain locus, since all of these results come from muscles of the same animal, suggest that it is the result of basic developmental or physiological processes. We have observed 2 other sets of differences in SO fibers: those that are not in regulatory components and those which we ascribe to developmental affects because they are found in very young animals. Those variations are currently under investigation in the hope that they will yield insights into the processes responsible for regulating slow myosin light chain expression in slow-oxidative fibers.

Acknowledgements

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References

[1] Close, R. I. (1972) Physiol. Rev. 52, 129–197.
[2] Peter, J. B., Barnard, R. J., Edgerton, V. R., Gillespie, C. A. and Stempel, K. E. (1972) Biochemistry 11, 2627–2633.
[3] Dubowitz, V. and Brooke, M. H. (1973) Muscle Biopsy: A Modern Approach, W. B. Saunders, London.
[4] Duhoot, G. K. and Perry, S. V. (1979) Nature 278, 714–718.
[5] Wuerker, R. B., McPhedran, A. M. and Henneman, E. (1965) J. Neurophysiol. 28, 85–99.
[6] Zeeman, R. J. and Woods, D. S. (1980) J. Histochem. Cytochem. 28, 714–715.
[7] Etlinger, J. D., Zak, R., Fischman, D. A. and Rabinowitz, M. (1975) Nature 255, 259–261.
[8] Harris, H. E. and Epstein, H. F. (1977) Cell 10, 709–719.
[9] Laemmli, U. K. (1970) Nature 227, 680–685.
[10] Grand, R. J. A., Perry, S. V. and Weeks, R. A. (1979) Biochem. J. 177, 521–529.
[11] Oakley, B. R., Kirsch, D. R. and Morris, N. R. (1980) Anal. Biochem. 105, 361–363.
[12] Schachat, F. H., Harris, H. E. and Epstein, H. F. (1977) Cell 10, 721–728.
[13] Barany, M. (1967) J. Gen. Physiol. 50, 197–216.
[14] Frearson, N. and Perry, S. V. (1975) Biochem. J. 151, 99–107.