ISOENZYMES OF RABBIT SLOW MYOSIN

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1. Introduction

Pyrophosphate gel electrophoresis has been established as a sensitive and reliable method of resolving myosin isozymes. The existence of 3 isozymes of vertebrate fast myosin differing in their alkali light chain distribution is well documented. In order of increasing mobility they represent the $(LC_1)_2(LC_2)_2$, $(LC_1)(LC_2)_2(LC_3)$ and $(LC_3)_2(LC_2)_2$ species [1,2,3]. However, in rat ventricular myosin, where only one light chain is analogous to the alkali light chains of fast skeletal myosin, the isozymes differ with respect to the iso-forms of 2 distinct heavy chains [4,5]. Fast skeletal myosin isozymes differ in the heavy chain [6–9].

In case of rabbit slow myosin no isozymes were distinguishable on pyrophosphate gel [10]. The existence of 2 different light chains (LC_{1a},LC_{1b}) analogous to the alkali light chains of fast myosin [11], and as reported here, the existence of 2 forms of slow subfragment-1 (S-1) separated in pyrophosphate gel suggests the presence of the isozymes analogous to the fast myosin isozymes. On the basis of analysis in pyrophosphate gels of single fibers, some of which contain predominantly either LC_{1a} or LC_{1b}, there are at least 2 isozymes of rabbit slow myosin namely, $(LC_{1a})_2(LC_2)_2$ and $(LC_{1b})_2(LC_2)_2$ homodimers.

2. Methods and materials

Slow myosin was prepared from combined rabbit soleus and semitendinosus muscles, rabbit cardiac myosin was prepared from the left ventricle and fast myosin was prepared from rabbit adductor magnus. The preparation of myosins was done as in [12].

Myosin S-1 fragment was obtained after chymotryptic digestion according to [13].

Pyrophosphate gel electrophoresis was done as in [14]. A larger amount of protein (50–100 μg) was loaded when re-electrophoresis of the separated S-1 bands in SDS gel was to be carried out. In that case the bands were cut out from the gel after quick staining (<5 min) in 0.04% Coomassie brilliant blue G-250 in 3.5% perchloric acid [15]. The 0.8–1.2 mm thick slices were then soaked in a large volume of a solution containing 40 mM Na-pyrophosphate (pH 8.8) and 10% glycerol for 30–60 min, following which each slice was allowed to stand overnight at 4°C with 6 μl of a solution containing 5% SDS, 0.5 M Tris–HCl (pH 8.8) and 5% mercaptoethanol.

Electrophoresis on slab gels containing SDS was done following [16] using a 14% separating gel and 4.3% stacking gel. For re-electrophoresis of bands separated on pyrophosphate gel, slices cut out of latter were pushed into the wells of the stacking gel. Gels were stained with 0.1% Coomassie brilliant blue R-250 in 50% methanol and 10% acetic acid.

Rabbit single fibers were isolated and treated for both SDS and pyrophosphate gel electrophoresis as in [9]. Occasionally the fibers were divided into 2 to extract the myosin from the same fiber for both pyrophosphate and SDS gel analysis.

3. Results and discussion

We attempted to separate the putative slow myosin isozymes in pyrophosphate gels. In contrast to the well-known, clear separation of the fast myosin isozymes [$(LC_1)_2$, $(LC_1)(LC_3)$ and $(LC_3)_2$] the slow myosin migrated consistently as one band regardless of the amount of protein loaded or the time of electrophoresis (fig.1A). It should be noted, however, that the width is greater than that of any subbands of the fast myosin or of the single cardiac myosin band

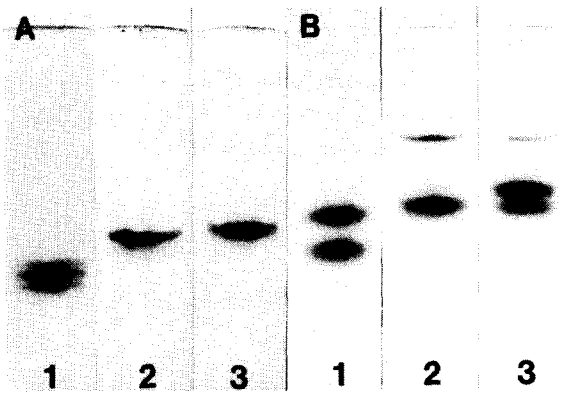

Fig.1. Gel electrophoresis in non-dissociating conditions of myosin and S-1: (A) 1, fast myosin; 2, cardiac myosin; 3, slow myosin; (B) 1, fast S-1; 2, cardiac S-1; 3, slow S-1. Gels in fig.1A (60 × 5 mm) were run in 3.6% acrylamide at constant voltage of 40 V for 24 h, and gels in fig.1B were run in 5% acrylamide for 8 h. Protein loaded was 1–2 μg.

we always find in adult rabbit heart (fig.1A). The wider band of the slow myosin suggests a slight difference in mobility between isozymes.

S-1 prepared from slow myosin readily separated into two bands in pyrophosphate gel (fig.2B). However, the separation is still less than in case of fast myosin S-1 (fig.1B). The fact that S-1 of cardiac myosin is homogeneous on pyrophosphate gel (fig.1B), shows that the digestion itself does not cause heterogeneity among the S-1.

The two bands of the skeletal S-1s differ in their light chains, as shown by SDS gel electrophoresis of the bands cut out from the pyrophosphate gels. The 2 slow S-1s contain LC_{1a} and LC_{1b}, respectively (fig.2A), while the two bands of fast S-1 differ with respect to LC_1 and LC_3 (fig.2B). The cardiac S-1 contains the intact cardiac type LC_1 (fig.2C). None of the S-1 contains LC_2, because it is degraded during the digestion.

In trying to determine the number of isozymes present in slow muscle, we made use of single fibers, some of which have either very low or very high LC_{1a}/LC_{1b} ratios. In fig.3 myosin extracted from single fibers, in which either LC_{1a} or LC_{1b} is predominant (1,2), appears on pyrophosphate gel as a single band, much sharper than the myosin band of a fiber (3) that contains the two LC_1 in roughly equal amounts. Myosin containing chiefly LC_{1a} migrates somewhat more slowly than its LC_{1b} dominated counterpart (fig.3B(1,2)). These myosins would correspond to $(LC_{1a})_2$ and $(LC_{1b})_2$ homodimers. The lack of a zone of low staining intensity in the center of the myosin band from a fiber in which the amount of LC_{1a} and LC_{1b} is rather equal (fig.3B(3)) suggests

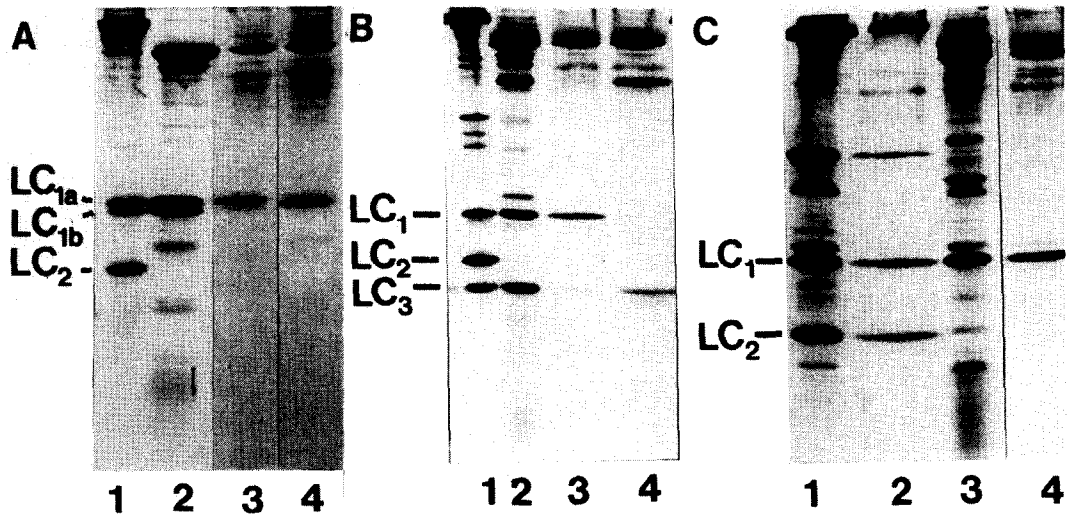

Fig.2. SDS gel electrophoresis of myosin, S-1 and the re-electrophoresis of S-1 bands cut out from pyrophosphate gel: (A) 1, slow myosin; 2, slow S-1; 3, S-1 of the slower moving band; 4, S-1 of the faster moving band cut out of pyrophosphate gel; (B) 1, fast myosin; 2, fast S-1; 3, S-1 of the slower moving band; 4, S-1 of the faster moving band cut out of pyrophosphate gel. (C) 1, cardiac myosin (crude extract); 2, the re-electrophoresis of the same cardiac myosin purified in pyrophosphate gel; 3, cardiac S-1; 4, the re-electrophoresis of the S-1 band cut out of pyrophosphate gel.

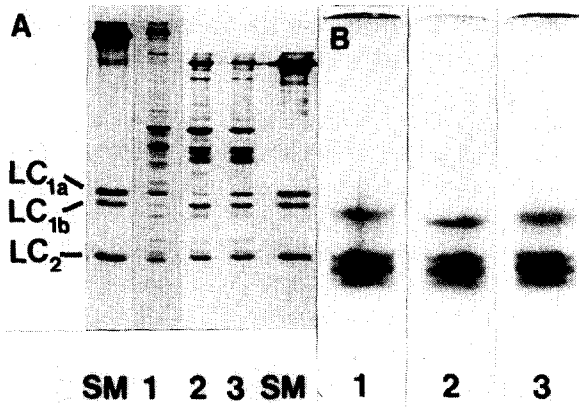

Fig.3. SDS and pyrophosphate gel electrophoresis of single fibers. One portion of a fiber was used for SDS gel and the other for pyrophosphate gel. (A) SDS gel electrophoresis of: 1, LC_{1a} dominant fiber; 2, LC_{1b} dominant fiber; 3, fiber which contains LC_{1a} and LC_{1b} equally. At both edges standard slow myosins are shown. (The pictures were taken from two different slabs.) (B) 1, LC_{1a} dominant fiber; 2, LC_{1b} dominant fiber; 3, LC_{1a} and LC_{1b} are equal in the fiber. Fast myosin was used as marker in each case.

the existence of a myosin species with mobility intermediate between the 2 homodimers – presumably the heterodimer. It appears that the difference in mobility imposed by the different light chains on slow myosin isozymes is less than in the case of fast myosin. This smaller influence of the light chains is also reflected in the less separation of the slow iso-S-1 in comparison with their fast counterparts.

While we report the presence of slow myosin isozymes related to different LC_1, the existence of 2 distinct kinds of heavy chains, each having strong affinity to the particular light chain (LC_{1a} or LC_{1b}) cannot be ruled out. The slight contamination by the heterologous light chain observed when we re-electrophoresed the separated S-1 fractions of either fast or slow myosin in SDS gel, and a similar observation [2,3] on fast myosins, points in this direction.

Acknowledgements

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