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# Light Chains of Myosins from White, Red, and Cardiac Muscles

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**ABSTRACT** **Purified preparations of rabbit skeletal white, red, and cardiac muscle myosin (WM, RM, and CM) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Significant differences in both the molecular weights and number of light chains in these myosins were found. WM has three distinct light-chain components ($LC_{1W}$, $LC_{2W}$, $LC_{3W}$) having molecular weights of 25,500, 17,400, and 15,100, respectively. No component with a molecular weight around 15,000 is present in RM or CM. RM and CM contain components of identical molecular weights close to 25,000 and 17,000 ($LC_{1CR}$ and $LC_{2CR}$) which, however, clearly differ in molecular weight from the corresponding subunits in WM. RM has an additional component ($LC_{1R}$) having a slightly higher molecular weight than $LC_{1W}$ and $LC_{1CR}$. Thus differences and similarities in many biochemical properties between WM, RM, and CM, which have been described earlier, are also reflected in the light-chain components. The present results support the hypothesis that different sets of genes are active in producing components of myosin that make up different isozymic forms characteristic of each muscle type.**

It is generally accepted that the myosin molecule contains two identical polypeptide chains, each having a molecular weight of about 200,000, referred to as heavy chains, and the so-called light-chain component(s), constituting about 10% of the mass of the myosin molecule (1–3). Dissociation of the molecule can be brought about by a number of procedures and is accompanied by the loss of ATPase and actin-binding activity (for a review see ref. 4). Reconstitution of myosin from dissociated light and heavy chains, with partial restoration of actin-binding and ATPase activities, has been reported (5–7).

Myosins isolated from red (or slow) and white (or fast) skeletal muscle exhibit differences with respect to ATPase activity (8–11), their stability at acid and alkaline pH (8, 10, 12), susceptibility to tryptic digestion (11, 13), and the structure of the light meromyosin portion as revealed by electron microscopy of negatively stained aggregates (14). On the other hand, CM and RM are similar with respect to these parameters (12–14). Several reports indicate that these similarities and differences are also reflected in the light-chain patterns (15–17). There is, however, considerable disagreement among these reports. The number of electrophoretically distinguishable light-chain components present in myosin preparations from different types of muscle in various species ranges from one to six (2, 15–28). Estimated molecular weights fall in the range of 15,000–30,000 (3, 5, 15, 16, 19, 20, 23–28).

In view of the availability of the technique of SDS–polyacrylamide gel electrophoresis (29), extensively used for the separation of various proteins and the determination of their molecular weights, we decided to apply this method to a reinvestigation of the light chains of rabbit WM, RM, and CM. Our results clearly show that both quantitative and qualitative differences exist between light chains in the different types of muscle; these will be discussed in terms of the isozymic nature of myosin.

## MATERIALS AND METHODS

### Preparation of myosin

Rabbit soleus, semitendinosus, crureus, and intertransversarius (red muscles); the outer layers of vastus lateralis and adductor magnus (white muscles); and heart ventricle (cardiac muscle) were used for the preparation of myosin (8). Myosin was further purified by column chromatography on DEAE-Sephadex A-50, essentially by the methods of Richards *et al.* (30). 150–200 mg of myosin, dissolved in a solution containing 0.04 M $Na_4P_2O_7$ (pH 7.5), 0.1 mM EDTA, and 0.1 mM dithiothreitol (buffer I), were applied to a column (2.5 × 35 cm) of DEAE-Sephadex A-50 equilibrated with the same buffer. The column was then washed with 2–3 column volumes of buffer I. Under these conditions myosin remained on the column, but other proteins (5–10% of the input) were eluted. A linear gradient of 0–0.5 M NaCl in buffer I was then applied. Myosin was eluted as a sharp single peak at 0.13–0.15 M NaCl and concentrated by precipitation either at low ionic strength after dilution or with $(NH_4)_2SO_4$ at 50% saturation. Myosin was finally dissolved in a solution containing 0.01 M sodium phosphate (pH 7.0), 0.5 M NaCl, 0.1 mM EDTA, and 0.1 mM dithiothreitol (buffer II). For the determination of ATPase activities of column-purified myosin, $K_4P_2O_7$ and KCl were used in the elution buffers for chromatography and myosin was finally dissolved in a solution containing 0.5 M KCl, 1 mM EDTA, and 10 mM *N*-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid (TES), pH 7.0.

Typical values for $Ca^{++}$-activated ATPase activities for WM, RM, and CM were 1.0, 0.25, and 0.35 μmol/mg per min, respectively. The corresponding values for $K^+$–EDTA-activated ATPase activities were 3.2, 1.6, and 1.1 μmol/mg per min, respectively. These values for ATPase activities are

Abbreviations: SDS, sodium dodecyl sulfate; WM, white muscle myosin; RM, red muscle myosin; CM, cardiac muscle myosin; $LC_{1W}$, $LC_{2W}$, $LC_{3W}$, light chains of WM in order of increasing electrophoretic mobility; $LC_{1R}$, light chains found in RM only; $LC_{1RC}$, $LC_{2RC}$, light chains common to RM and CM; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

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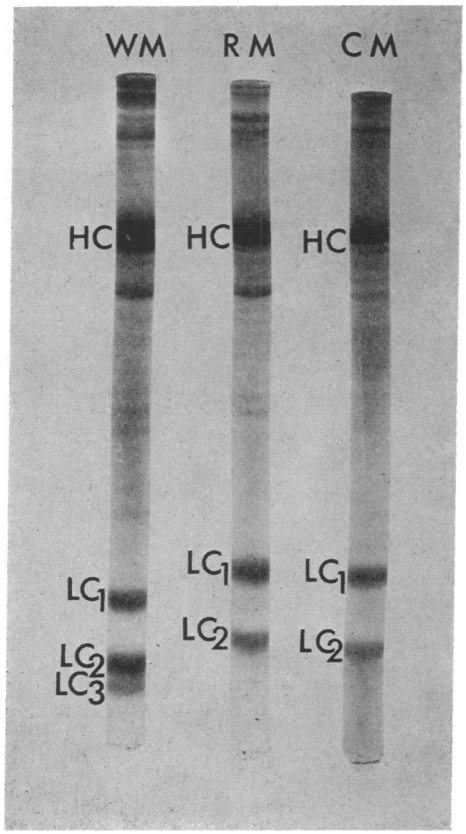


FIG. 1. Electrophoresis of white and red skeletal and cardiac myosins in 4% polyacrylamide gels. Electrophoresis was carried out in 0.10 M sodium phosphate (pH 7.0) containing 0.1% SDS, at a constant current of 8 mA per gel for 80 min. The length of the gels was 10 cm; they were stained with Coomassie brilliant blue (29). Amount of protein applied: 15 $\mu$g for WM, 15 $\mu$g for RM, and 11 $\mu$g for CM. HC, heavy chain; LC, light chain.

in good agreement with reported values published earlier (8, 12). Protein concentration was determined by the biuret reaction and ATPase activities as previously described (8).

### Isolation of light chains by alkali treatment

Light chains were isolated essentially according to Gershman and Dreizen (18). To WM in buffer II, 15–20 mg/ml, an equal volume of 0.5 M NaCl–0.1 M $Na_2CO_3$ was added, and the pH was adjusted to 11.0 with 0.1 N NaOH. After 40 min at 0°C, 10 volumes of water were added and the pH was readjusted to 7.0 by addition of 0.1 M HCl. The precipitated material containing the heavy chains was sedimented at 20,000 $\times$ $g$ for 20 min and the supernatant was dialyzed against a solution containing 0.10 M sodium phosphate. The light-chain material in the supernate was concentrated to about 2 mg/ml by lyophilization and final dialysis against a solution of 0.1 M sodium phosphate, pH 7.0. For electrophoresis, SDS and $\beta$-mercaptoethanol were added.

### Gel electrophoresis

SDS–polyacrylamide gel electrophoresis of myosin was carried out according to the procedure of Weber and Osborn (29), in both 4% and 12.5% polyacrylamide gels (28). $\beta$-Mercaptoethanol and SDS were added to myosin (4–5 mg/ml in buffer II) to a final concentration of 1%. The solution was allowed to stand at 37°C for 60 min, and then at 25°C at

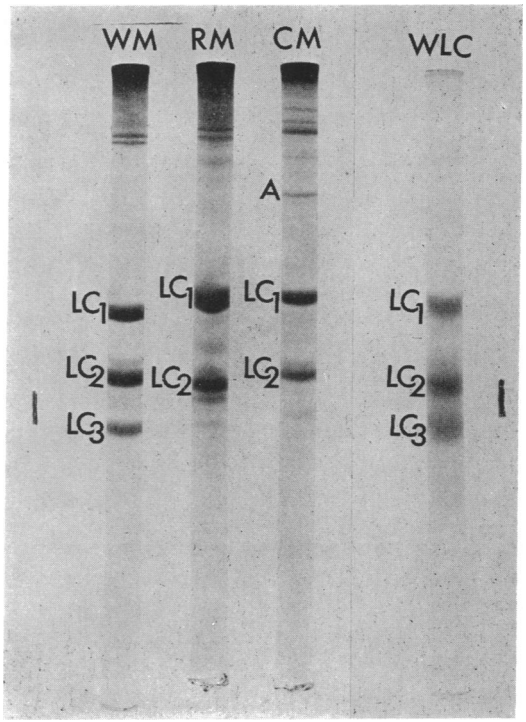


FIG. 2. Electrophoresis of myosin samples in 12.5% polyacrylamide gels. Conditions as for Fig. 1 except that the time of electrophoresis was 170 min. WLC refers to the electrophoretogram of a preparation of light chains obtained from WM by treatment with alkali (see *Methods*). Amount of protein applied: 30 $\mu$g for WM, 29 $\mu$g for RM, 25 $\mu$g for CM, and 7 $\mu$g for light chains of WM. LC, light chain; A, actin.

least overnight, before gel electrophoresis. The amount of protein used in an electrophoretic run was 10–15 $\mu$g for the study of heavy chains (using 4% gels) and 20–30 $\mu$g for the light chains (using 12.5% gels).

Purified samples of rabbit skeletal actin, tropomyosin, and troponin were the kind gifts of Dr. M. Greaser.

### Determination of molecular weights

Molecular weights were determined with the use of a calibration curve obtained by plotting the electrophoretic mobilities of various highly purified proteins against the logarithm of their known polypeptide chain molecular weights (28, 29). Standards were run simultaneously with each set of unknowns.

## RESULTS

### Gel electrophoresis

Fig. 1 shows the electrophoretograms of WM, RM, and CM in 4% acrylamide gels. The strong bands that migrated to about one-fourth of the length of the gel presumably correspond to the heavy chains of these myosins. The light chains appeared as two bands near the bottom of the gel. In the case of WM, the faster light-chain component showed indications of an incompletely resolved leading band. This suggested that WM might have an additional light-chain component. Additional bands with migration velocities both slower and faster than that of the heavy chain are also seen in the electrophoretograms. Their amount is small and variable, and they probably represent aggregates of the heavy and light chains, as well as contaminating proteins not integrally related to myosin.

In order to achieve a satisfactory resolution of the light chains, we analyzed samples of different myosins by electrophoresis using 12.5% polyacrylamide gels (28). The electrophoretograms in Fig. 2 show that the heavy chain did not enter the gel, while the light chains migrated as distinct bands. Electrophoresis of WM gave three well-resolved light-chain bands (Fig. 2). These bands are referred to as $LC_1$, $LC_2$, and $LC_3$ in order of increasing mobility. The two slower-moving bands, $LC_1$ and $LC_2$, seemed to be the major ones, while the faster band, $LC_3$, although always present in WM, appeared in relatively smaller amounts than the other two bands. The identification of these main bands as light chains of myosin is supported by comparison of the electrophoretogram of WM with that of a preparation of light chains obtained from WM by exposure to pH 11 (see *Methods*). As shown in Fig. 2 (see the electrophoretogram marked WLC), the light-chain preparation contains three bands corresponding to $LC_1$, $LC_2$, and $LC_3$. This result would exclude the possibility that what we referred to as the light chains of myosin were due to decomposition of myosin in SDS, perhaps by proteolytic enzymes that retained their activity. RM gave two major bands of light chains whose mobilities were similar to those of $LC_1$ and $LC_2$ of WM (Fig. 2). The $LC_1$ band of RM could be further resolved, resulting in a double band, when electrophoresis was continued for a longer time or when smaller amounts of myosin were used. (These bands are marked with double arrows in Fig. 4 and are referred to as $LC_{1a}$ and $LC_{1b}$ in Table 1.) Judged by these criteria, the $LC_1$ band of RM consists of two polypeptide chains of very similar mobility and molecular weight. Cardiac myosin gave two well-resolved bands, $LC_1$ and $LC_2$.

In addition to the light-chain bands described above, the electrophoretograms showed the presence of several minor bands (Fig. 2). The number of these bands was highly variable in different preparations and increased on storage. One of them, present in CM, had a mobility corresponding to that of actin.

### Molecular weights of light chains

The molecular weights of the light chains of WM, RM, and CM, estimated with the use of standard plots (see *Methods* and ref. 28) are listed in Table 1. The molecular weights obtained for WM are: 25,500 for $LC_1$; 17,400 for $LC_2$; and 15,100 for $LC_3$. These values are essentially identical with the molecular weights of the three light chains of chicken skeletal myosin, namely, 25,500; 17,600; and 15,200 (28). It is difficult to reconcile the present results with the recent report by Paterson and Strohman (25) showing the presence of only two kinds of subunits in WM—with molecular weights of 19,000 and 32,000 in SDS-gel electrophoretograms. Presumably the two components of lower molecular weight were not resolved and some aggregation may account for the higher molecular weight of the slower component.

According to the data in Table 1, the molecular weights of $LC_1$ and $LC_2$ in WM differ from those of the corresponding components of both RM and CM; the differences are larger for the $LC_1$ bands of the three myosins. It appears that apart from the slower component of the doublet $LC_1$ band of RM, namely, $LC_{1a}$ in Table 1, the molecular weights of the other light-chain components of RM and CM are very similar.

For comparison we have listed in Table 1 molecular weights of a number of other myofibrillar proteins, determined by us with the use of the SDS–gel method. These are in good agreement with published values in the literature (31–33). The minor bands due to proteins or aggregates of high molecular weight (see Figs. 1 and 2) have mobilities corresponding to molecular weights different from those of other known myofibrillar proteins (compare Table 1).

### Electrophoresis of mixed samples of RM, WM, and CM

The above results suggested that $LC_1$ and $LC_2$ of WM differ in molecular weight from the corresponding components of RM and CM. In order to bring out the differences and similarities among the light chain components more clearly, we subjected various mixtures of myosins to electrophoresis.

Fig. 3*A* shows the electrophoretogram of a mixed sample of WM and RM. The light chains of $LC_1$ of WM and RM migrated as three distinct, resolved bands, and the $LC_2$ components of these two myosins were also resolved. Similarly, when a mixture of WM and CM was analyzed, differences between the corresponding light chains became evident (Fig. 3*B*). In both cases the gel pattern of the mixtures contained $LC_3$ of WM, which was not present in the light-chain fractions of either CM or RM.

When samples of RM and CM were run together (Fig. 3*C*), the electrophoretograms revealed that: (*a*) the cardiac light-chain $LC_1$ comigrated with the faster component of the "doublet" band of $LC_1$ ($LC_{1b}$ in Table 1) of RM; (*b*) the $LC_2$ components of RM and CM migrated together in a single unresolved band. These features remained unaltered even when the time of electrophoresis was increased to 7 hr. These results support the conclusion that the $LC_2$ light-chain components of RM and CM have the same molecular weight, while the single $LC_1$ component of CM is identical with the smaller of the two $LC_1$ components of RM.

The relations among components in the $LC_1$ and $LC_2$ bands of different myosins is best shown in an electrophoretogram of the mixture of three myosins. The electrophoresis experiment in Fig. 3*D* was run for a sufficiently long time to achieve optimal resolution of all components; this, however, caused the $LC_3$ component of WM to move out of the gel. The $LC_1$

TABLE 1. *Molecular weights of myosin light chains and of myofibrillar proteins*

| Protein | Molecular weight |
|---|---|
| White muscle myosin $LC_1$ (5) | 25,500 ± 140 |
| White muscle myosin $LC_2$ (5) | 17,400 ± 150 |
| White muscle myosin $LC_3$ (5) | 15,100 ± 100 |
| Red muscle myosin $LC_{1a}$ Doublet (4) | 27,500 ± 200 |
| Red muscle myosin $LC_{1b}$ Doublet (4) | 26,600 ± 105 |
| Red muscle myosin $LC_2$ (4) | 18,000 ± 295 |
| Cardiac muscle myosin $LC_1$ (4) | 26,800 ± 100 |
| Cardiac muscle myosin $LC_2$(4) | 17,800 ± 240 |
| Actin (3) | 46,300 ± 210 |
| Tropomyosin (3) | 34,300 ± 410 |
| Troponin fraction 3 (3) | 35,300 ± 460 |
| Troponin fraction 2 (3) | 23,700 ± 780 |
| Troponin fraction 4 (3) | 20,500 ± 640 |
| Troponin fraction 1 (3) | 14,700 ± 420 |

The molecular weights were determined from SDS-polyacrylamide gel electrophoresis (12.5% gels) under conditions described in the legends to Figs. 2 and 3 (see *Methods*). The values in parentheses represent the number of determinations. The average molecular weights are listed with their standard errors.

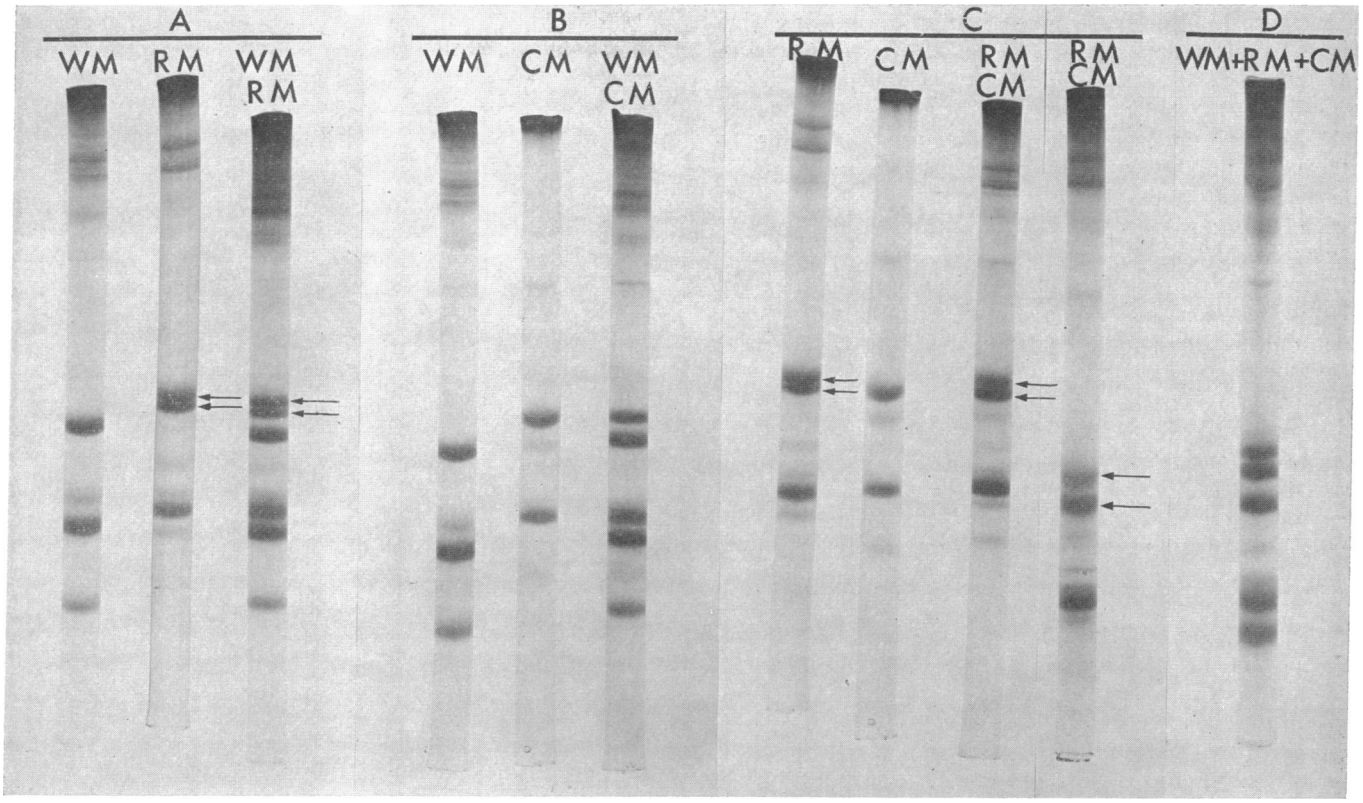


FIG. 3. Electrophoresis of various mixtures of different myosins on 12.5% polyacrylamide gels. The following amounts of protein were used: Panel *A:* WM (19 $\mu$g), RM (21 $\mu$g), and a mixture of WM (19 $\mu$g) and RM (21 $\mu$g). Panel *B*: WM (19 $\mu$g), CM (20 $\mu$g), and a mixture of WM (19 $\mu$g) and CM (20 $\mu$g). Panel *C*: RM (21 $\mu$g), CM (20 $\mu$g), and two mixed samples of RM (21 $\mu$g) and CM (20 $\mu$g). Panel *D*: A mixture of WM (19 $\mu$g), CM (20 $\mu$g), and RM (21 $\mu$g). Conditions as for Fig. 1 except that the time of electrophoresis was 4.5 hr. The time was further increased to 7 hr for the second mixed sample of RM and CM in panel *C* and for the gel shown in Panel *D*.

complex appeared as a triplet. The slowest band was contributed by RM, the middle one by RM and CM, and the fastest by WM alone. The $LC_2$ complex migrated as a well-resolved doublet whose first band corresponded to $LC_2$ of RM and CM, and the second to $LC_2$ of WM. Thus, the results of these experiments on various mixtures of myosins are consistent with the calculated molecular weights of the different light chains of WM, RM, and CM listed in Table 1.

In view of these results, it may be convenient to modify our nomenclature as follows. In the *Discussion* we shall refer to the three unique WM light-chain bands as $LC_{1W}$, $LC_{2W}$, and $LC_{3W}$. The bands common to CM and RM will be referred to as $LC_{1CR}$ and $LC_{2CR}$. Finally, the slowest of the RM bands, hitherto referred to as $LC_{1a}$, will be called $LC_{1R}$. The notation is, we hope, self-explanatory.

## DISCUSSION

The present results, based on electrophoresis of myosins in SDS–gels, clearly show that none of the three light-chain components in WM is identical with any light chain in RM or CM. Secondly, apart from an additional slower component present in the doublet band of RM, namely, $LC_{1R}$, the light chains of RM and CM appear to be identical. Differences in electrophoretic mobilities between the light chains of WM and those of RM and CM of rabbit have been reported by several authors (15–17). Although the mobilities of some components in the WM pattern were found to be similar to those of components in RM and CM, no definitive conclusion could be reached as to whether they were identical. Mobilities determined in the absence of SDS, as was the case in these previous studies, depend both on charge and molecular weight of proteins; further complications may arise from aggregation or from incomplete dissociation of protein complexes.

Recently Gazith *et al.* (2) and Weeds (22, 27) have shown that one light-chain component can be removed from rabbit skeletal myosin by treatment with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) without loss of ATPase activity. Subsequent treatment of the DTNB-treated myosin with alkali (pH 11) results in the release of two additional light-chain components, with loss of ATPase activity (22, 27). Our preliminary experiments indicate that the 17,000-dalton subunit ($LC_{2W}$) is removable by DTNB, and the 25,000- and 15,000-dalton subunits ($LC_{1W}$ and $LC_{3W}$) are preferentially removed by alkali. Lowey (26) has reported two light chains in skeletal myosin, which is predominantly WM, having molecular weights of 25,000 and 17,000; of these two, the 17,000-dalton component was identified as the subunit removed by DTNB, in agreement with our data. Weeds (27) has reported molecular weights of 17,000 and 21,000, based on amino acid analysis, for the two light chains removed by alkali. Preliminary results indicate that the $LC_2$ subunits of CM, although similar in molecular weight to the DTNB-removed subunit of WM, are not removed by DTNB treatment. Lack of a DTNB-removable subunit in CM has also been reported by Weeds (27). This suggests that subunits of similar size may have different properties in different myosins.

It is interesting that when major differences exist in the light-chain patterns, the heavy chains are also different; and

when the light-chain patterns are similar, the heavy chains are similar. Differences exist between WM on the one hand and RM and CM on the other (14), as regards sensitivity to trypsin (11, 13) and the electron-microscopic appearance of light meromyosin aggregates (14). Conversely, the heavy chains of CM and RM, whose light-chain patterns are similar, are similar with respect to these properties.

It has been suggested that skeletal myosin has two isozyme forms, one corresponding to red and the other to white muscle (22), and that the complex band pattern of the light chains of WM is due to some component originating in white-type fibers and others originating in red-type fibers. Our results showing that all the light-chain bands present in WM are distinguishable from those in RM do not support this view, but indicate that the multiple light-chain band patterns are due to a set of polypeptide chains specific for each fiber type. The molecular basis of myosin isozymes would thus differ from that of many well-characterized isozymic systems, including, for example, lactic dehydrogenase. In the latter case, differences in the isozymic patterns found in different types of muscle cells are due to differences in the distribution of the same two subunits (for a review see ref. 34). Differences in the light-chain pattern of CM and RM are rather small, and in view of the similarities with respect to many other properties of myosin, the existence of distinct isozymes in these two muscle types must await further work. Since there are also differences between heavy chains of WM and RM (11–14), the different isozymic forms found in the two fiber types represent heteropolymers of subunits characteristic for each fiber type.

Weeds reported that there are nearly two DTNB-removable light chains per myosin molecule and two alkali-removable light chains, one of which is present in a larger amount than the others (27). He suggested that myosin molecules contain pairs of identical alkali-removable chains. More work is required to determine the stoichiometry of the various light chains in different types of myosins and to settle the question of the number and type of subunit within a myosin molecule.

As mentioned before, the ATPases of WM, on the one hand, and RM and CM, on the other, differ in their specific activity (8–11) as well as their stability on exposure to mild alkaline conditions (8, 10, 12). Whether these differences reside in the light chains alone or whether differences in the heavy-chain region that may participate in the active site (3, 35) are also involved remain to be determined.

Recent studies by one of us (28) show that in an *in vitro* cell-free polysome system, the heavy chain and three light chains of myosin are synthesized by polysomes that differ in size (50–60 monosomes for the heavy chain and 4–7 monosomes for light chains). This suggests that heavy and light chains are products of separate genes. In terms of the results discussed above, it appears that different sets of genes are active in producing components of myosin that make up the isozymic forms characteristic of each muscle type.

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