An Electrophoretic Study of the Low-Molecular-Weight Components Of Myosin

By W. T. PERRIE and S. V. PERRY
Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham 15, U.K.

1. The low-molecular-weight components of myosin freshly prepared by the standard procedure from adult rabbit skeletal muscle migrated as four main bands Ml_1, Ml_2, Ml_3 and Ml_4 on polyacrylamide-gel electrophoresis in 8 M-urea. 2. The number of bands increased on storage. This change was accelerated by increasing the temperature and pH. 3. None of the bands had electrophoretic mobilities identical with those of the well-characterized proteins of the myofibril or with the sarcoplasmic proteins. 4. By varying the ionic conditions and concentration of muscle mince used for the initial extraction it was possible to change the relative proportions of the two electrophoretic bands of intermediate mobility, Ml_2 and Ml_3. 5. The four-band picture similar to that obtained with rabbit was observed with myosin isolated from skeletal muscle of the rat, mouse, hamster, pigeon and chicken. 6. Rabbit cardiac myosin gave only two bands on electrophoresis. Myosin from rabbit red muscle gave a pattern intermediate between cardiac and white-skeletal-muscle myosin, i.e. the two fastest bands were present in decreased relative amounts. 7. It is suggested that the differences in the low-molecular-weight components of myosin from different types of muscle are a consequence of differences in the isoenzyme composition of the myosins.

It has been known for some time that myosin does not migrate readily under the normal conditions of gel electrophoresis unless dissociating agents are present. In an early study Small, Harrington & Kielley (1961) reported that myosin migrated as a single band on acrylamide gel in 12 M-urea, whereas on starch gel in 8 M-urea Mueller & Perry (1962) observed a three-band pattern with a dense central band. It was not appreciated until the work of Stracher and collaborators (Gershmann, Dreizen & Stracher, 1966; Dreizen, Gershmann, Trotta & Stracher, 1967) that the material migrating under these conditions was the low-molecular-weight material responsible for the slowly sedimenting components observed when myosin preparations were studied in the ultracentrifuge in the presence of dissociating agents (Tsao, 1953; Wetlaufer & Edsall, 1960). Since the work described in the present paper was completed Florini & Brivio (1969) have reported conditions under which the large subunits of myosin will migrate on electrophoresis.

From electrophoretic and hydrodynamic studies Stracher and collaborators concluded that the myosin molecule contains three small units per molecule, although on hydrodynamic data alone Frederiksen & Holtzer (1968) suggested that there were two. From several studies (Gaetjens, Barany, Bailin, Oppenheimer & Barany, 1968; Locker & Hagyard, 1967, 1968; Florini & Brivio, 1969) the electrophoretic behaviour appears complex and the number present variable. Removal of these components from myosin results in a loss of adenosine triphosphatase activity, but Gaetjens *et al.* (1968) were unable to restore enzymic activity to the heavy component by readdition of the light components. On the other hand Frederiksen & Holtzer (1968) and Stracher (1969) have reported that enzymic activity can be restored in part on addition of the light components to the heavy fraction. This property clearly suggests an important role of the light components in the biological activity of myosin, as do the findings of Locker & Hagyard (1968) that the subunits obtained from acetylated myosin were characteristic for the type of myosin. The recent reports that a light component can be removed from myosin by treatment with 5,5'-dithiobis-2-(nitrobenzoic acid) without loss of enzymic activities (Weeds, 1969; Gazith, Himmelfarb & Harrington, 1970), however, indicates that the role and precise nature of the low-molecular-weight components of the myosin molecule are far from understood.

The present investigation was undertaken to

attempt to characterize more precisely the low-molecular-weight components with a view to studying their significance for the biological function of the myosin molecule. A consistent band pattern has been described that is characteristic of the muscle type but not of the species from which the myosin is prepared. It is suggested that the variation in band pattern obtained with myosin from different types of muscle arises from differences in isoenzyme composition of the protein. Some of the findings have already been briefly reported (Perrie, Perry & Stone, 1969).

METHODS

Preparation of myosin. Myosin was normally prepared from rabbit muscle by the method of Trayer & Perry (1966). This involves initial extraction with a medium containing $10\,mM\text{-}Na_4P_2O_7$ and $1\,mM\text{-}MgCl_2$ to decrease the amount of actomyosin extracted. Longissimus dorsi muscle was used for preparations from adult white muscle, the soleus and the semitendinosus muscles for adult red-muscle preparations and hearts were extracted for cardiac myosin. Unless otherwise stated all solutions used in the preparations contained $10\,mM$-mercaptoethanol.

Preparations were also made from muscle dissected from the back and legs of the rat and hamster, and breast of the pigeon and fowl. The whole eviscerated skinned carcass, from which the head and feet had been removed, was minced for the initial extraction of myosin from the mouse and the foetal and 1-day-old rabbit.

Preparation of actin. Actin was prepared by the method described by Johnson, Harris & Perry (1967).

Preparation of sarcoplasm. Sarcoplasm was prepared by homogenizing longissimus dorsi muscle of the adult rabbit in 3 vol. of $0.25\,M$-sucrose in a Waring Blendor, and centrifuging at $100000g$ for 2h to remove all particulate material.

Electrophoresis. Vertical polyacrylamide-gel slab electrophoresis was performed by a modification of the method of Akroyd (1967). The rubber-tubing spacers described were used only when large sample volumes were run. It was found to be more convenient to cast a gel with sample slots in the top with the aid of a toothed former.

Both running and gel buffers contained $20\,mM$-tris-glycine buffer, pH 8.6. The use of a 12-fold-concentrated stock solution of this buffer enabled gels containing 40% (v/v) of glycerol or $8\,M$-urea to be conveniently made up. Running buffers contained $6\,M$-urea for urea gels.

All gels described in this paper were 10% (w/v) Cyanogum 41. A 25ml volume of solution containing 20% (w/v) of Cyanogum and $0.25\,ml$ of β-dimethylaminopropionitrile was made up. For an $8\,M$-urea gel, $24\,g$ of urea was added, dissolved by stirring and the final volume made up to $50\,ml$. Immediately before pouring of the slab, $1\,ml$ of 5% (w/v) ammonium persulphate was added and the solution quickly stirred. Glycerol gels were prepared by adding $20\,ml$ of glycerol in place of the urea in the above procedure. Usually about $20\,\mu l$ samples in $8\,M$-urea containing $5\,mg$ of protein/ml were layered on to the gels. Myosin and tropomyosin samples had to be layered on to all gels in $8\,M$-urea because of their insolubility or high viscosity respectively at low ionic strength. Water-soluble components were layered on to glycerol gels with glycerol in the sample solutions to make them denser than the running buffers.

In the preparative procedure (see below) it was necessary to remove all the guanidine hydrochloride by dialysis before electrophoretic study of the fractions in $8\,M$-urea.

Chromatography. DEAE-cellulose chromatography of myosin was carried out in the presence of $10\,mM$-mercaptoethanol by the method of Trayer & Perry (1966). DEAE-Sephadex was used for separation of the low-molecular-weight components as described in the text. In all cases chromatography was carried out at 2–$4°C$.

Isolation of low-molecular-weight components of myosin. On some occasions, and particularly in the earlier studies, samples of mixtures of light components were prepared by treating myosin ($10\,mg/ml$) for 15h with the agent specified in the text (see the Results section) and then dialysing against three changes of a buffer prepared by diluting 15-fold the extraction buffe rused for the myosin preparation (Trayer & Perry, 1966). The precipitate obtained after dialysis was spun down, leaving low-molecular-weight components in the supernatant.

Better yields and some separation of individual components were obtained as follows. About $2.5\,g$ of myosin as the gel (in $0.05\,M$-KCl after the final precipitation stage) was suspended in water, and solid guanidine hydrochloride and mercaptoethanol were added to bring the final concentration to $5\,M$ and $10\,mM$ respectively in a total volume of $250\,ml$. The solution was allowed to stand in the cold for 3h.

The solution was then diluted, slowly and with stirring, first with $250\,ml$ of cold water and then with 1 litre of cold ethanol. The flocculent precipitate was removed by low-speed centrifugation. The supernatant contained the four components from myosin that migrated into an $8\,M$-urea gel on electrophoresis. The precipitate contained all the material that remained at the origin under these conditions and only a trace of the low-molecular-weight components.

Preparation of troponin and tropomyosin. 'Natural' actomyosin was extracted from myofibrils prepared by the method of Perry & Zydowo (1959) with the modification that the Weber's solution used for extraction contained $10\,mM$-mercaptoethanol, and precipitated as described by Schaub, Perry & Hartshorne (1967). The natural actomyosin was washed with sufficient cold water containing $10\,mM$-mercaptoethanol to decrease the ionic strength to 2×10^{-3}. After being kept overnight the suspension was sedimented at $33000g$ for 30min and the supernatant was freeze-dried. All procedures were carried out at 0–$2°C$.

The freeze-dried powder was dissolved in $1\,M$-KCl containing $0.5\,mM$-dithiothreitol and the pH adjusted to 4.6. The precipitate was removed by centrifugation and the supernatant dialysed against $5\,mM$-tris–HCl buffer, pH 7.6, containing $0.5\,mM$-dithiothreitol. This supernatant contained about $4\,mg$ of protein/ml and consisted of principally troponin with some tropomyosin.

Preparative electrophoresis. An adaptation of the disc electrophoresis method of Davis (1964) was used as a preparative system. About $2.5\,cm$ of gel was set in a Quickfit B19 cone sealed at the bottom with Parafilm.

After removal of the Parafilm a short length of 24/32 Visking tubing was attached to the cone with an elastic band. This was immersed in the lower buffer tank and the top of the cone was fitted into an upper reservoir through a rubber bung. After running, the gel was sliced and stained in Amido Black to check that all the protein had run off the bottom. The dialysis tubing containing the material that ran in the gel was then carefully removed.

RESULTS

Electrophoresis of adult rabbit white-muscle myosin. On polyacrylamide-gel electrophoresis in 8 M-urea at pH 8.6, myosin prepared in the presence of 10 mM-mercaptoethanol and dissolved in 8 M-urea gave four main bands. Numbered in order of decreasing mobility these bands are referred to as Ml_1, Ml_2, Ml_3 and Ml_4 (see Fig. 1a).

In all electrophoretic runs of myosin, however, a considerable amount of material was left at the origin. Even in 8 M-urea gels, and when the myosin was treated with 8 M-urea for some time before application, most of the protein did not enter the gel. It was estimated that components Ml_1, Ml_2, Ml_3 and Ml_4 represented at the most about 10–15% of the total protein applied.

In myosin preparations that had been stored several faint bands moved a short distance into the gel in addition to the four bands described above. The very slow bands were absent or very faint in fresh myosin, but even in these preparations faint bands moving just in front of bands Ml_1 and Ml_4 were also often observed (see Fig. 1). The number of faint bands of fast mobility, however, markedly increased on storage and the intensity of bands Ml_1–Ml_4 simultaneously decreased until a very complex multiple-band picture was obtained.
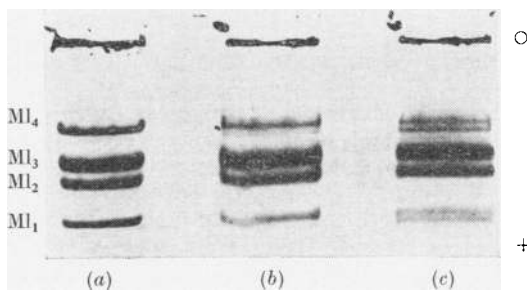

Fig. 1. Effect of storage at different pH values on the electrophoretic pattern of myosin from rabbit white skeletal muscle. Freshly prepared myosin gel was dissolved in 8 M-urea and 10 mM-mercaptoethanol. The pH was adjusted as indicated and samples were left overnight before electrophoresis. (a) No addition, pH 6.5; (b) adjusted to pH 9.0 by addition of solid tris; (c) adjusted to pH 11.0 by addition of solid Na_2CO_3.

These changes, particularly those occurring on storage in 8 M-urea, could be decreased by the addition of mercaptoethanol to the myosin samples. Likewise storage at low temperature and low pH values (Fig. 1) significantly decreased the rate at which the band pattern changed. It was concluded that, although skeletal-muscle myosin normally contained four electrophoretically distinct components, these were readily modified to material represented by the minor bands.

If myosin had been previously treated with 8 M-urea or simply applied in 8 M-urea solution to a 10% gel containing 20 mM-tris–glycine buffer, pH 8.6, the low-molecular-weight components moved into the gel. The pattern was diffuse, but could be sharpened by increasing the gel strength to 15–20%. Band separation was poor, however, but was improved by including 40% glycerol in the 10% urea-free gel. A four-band picture was obtained with myosin under these conditions, but the Ml_2 and Ml_3 bands were relatively fainter than in the pattern obtained with 8 M-urea present in the gel. The relative speeds of migration were different in the 40%-glycerol and 8 M-urea gels for in the former case if the gel was overloaded components Ml_3 and Ml_4 did not separate at all and stained as a single band (compare Fig. 2b with 2c and Fig. 2a with 2d). In general for a given preparation the band pattern obtained in 40% glycerol was less complex than that obtained in 8 M-urea, implying that resolution was better in the latter system. Myosin that had not previously been treated with a dissociating agent did not migrate into the glycerol gel at all.

Non-identity of myosin band components with components of other muscle proteins. In view of the known strong tendency of the myofibrillar proteins to interact with each other and the consequent difficulties in obtaining pure preparations, an attempt was made to relate the electrophoretic band pattern obtained with myosin with those of other muscle proteins, which might be present as impurities in the myosin. This was carried out by studying the electrophoretic patterns obtained with preparations of the purified protein treated with urea, run alone and mixed with myosin on gels containing urea. Actin tended to give a complex picture, but the main component was slower than any of the main bands obtained with myosin. Likewise none of the four bands appeared to be identical with the components of the troponin complex (Schaub & Perry, 1969), tropomyosin or the major components of whole sarcoplasm.

If the bands represented contaminants it would be expected that myosin at different stages of purification would show different band patterns. This was not the case, for a very similar pattern was obtained at each of the three precipitation
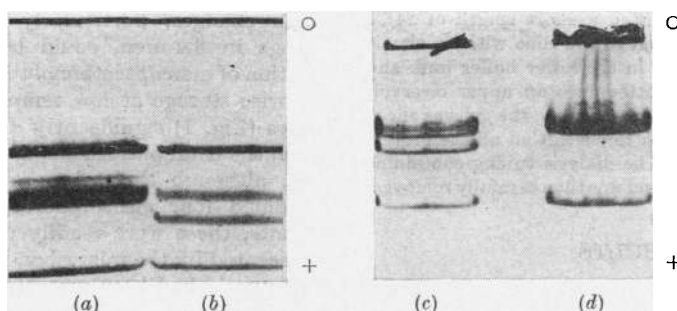
Fig. 2. Effect of ionic strength of buffer used for the initial extraction of muscle on the number of bands obtained on electrophoresis of the myosin obtained. Samples in 8 M-urea and 10 mM-mercaptoethanol were applied to gels indicated below. (a) Myosin extracted with 3 vol. of 0.15 M-KCl–75 mM-potassium phosphate, pH 6.5, and applied to an 8 M-urea gel; (b) myosin extracted with 3 vol. of standard buffer and applied to an 8 M-urea gel; (c) myosin prepared as in (b) applied to 40%-glycerol gel; (d) myosin prepared as in (a) applied to 40%-glycerol gel.

Table 1. *Effect of extraction conditions on the relative proportions of the low-molecular-weight components of myosin from rabbit skeletal muscle*

10 mM-Mercaptoethanol was present in all cases. Numbers of crosses indicate relative intensity of electrophoretic bands.

Extraction buffer	Muscle/buffer ratio (w/v)	Ml$_1$	Ml$_2$	Ml$_3$	Ml$_4$
0.3 M-KCl–0.15 M-potassium phosphate buffer (pH 6.5)–10 mM-Na$_4$P$_2$O$_7$–1 mM-MgCl$_2$	1:2	××	×	×××	××
0.3 M-KCl–0.15 M-potassium phosphate buffer (pH 6.5)–10 mM-Na$_4$P$_2$O$_7$–1 mM-MgCl$_2$	1:3	××	××	××	××
0.3 M-KCl–0.15 M-potassium phosphate buffer (pH 6.5)–10 mM-Na$_4$P$_2$O$_7$–1 mM-MgCl$_2$	1:10	××	×××	×	××
0.3 M-KCl–0.15 M-potassium phosphate buffer, pH 6.5	1:3	××	×	×××	××
0.3 M-KCl–0.15 M-potassium phosphate buffer, pH 6.5	1:10	××	××	××	××
0.15 M-KCl–75 mM-potassium phosphate buffer, pH 6.5	1:3	××		××××	××

stages of myosin preparation. On DEAE-cellulose chromatography of myosin the band pattern became complex. If these changes were diminished by the addition of mercaptoethanol it was apparent that the four-band pattern was little affected by chromatographic fractionation on DEAE-cellulose or DEAE-Sephadex A-25. To minimize changes in the band pattern most electrophoretic studies were carried out on freshly prepared myosin gel after the third precipitation step. Ammonium persulphate, used as the catalyst for polymerization of the polyacrylamide gel, has been reported (Brewer, 1967) as causing an increase in the number of bands on electrophoresis. After a 2 h pre-run of the gel the band patterns obtained with myosin were identical whether ammonium persulphate or riboflavin was used as a catalyst.

Effect of initial extraction conditions on the electrophoresis pattern of myosin. Myosin extracted under the standard conditions (0.3 M-potassium chloride, 0.15 M-potassium phosphate buffer, pH 6.5, 10 mM-sodium pyrophosphate and 1 mM-magnesium chloride; I approx. 0.6) always gave a four-band pattern on electrophoresis in 8 M-urea. If the initial extraction was carried out in 0.15 M-potassium chloride–75 mM-potassium phosphate buffer, pH 6.5 (I approx. 0.3), a three-band pattern was obtained on electrophoresis. The missing band was Ml$_2$, and band Ml$_3$ was denser relative to bands Ml$_1$ and Ml$_4$ than in the normal four-band pattern (Fig. 2a). A gradual change in the pattern from the three-band to four-band occurred if the ionic strength of the extraction buffer varied between these limits so long as the concentration of the muscle was kept
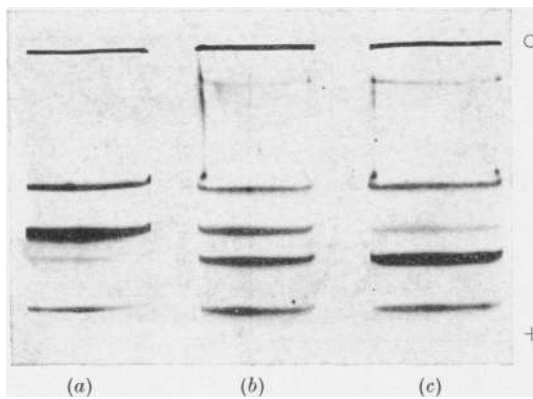
Fig. 3. Effect of concentration of muscle during initial extraction on the relative proportions of components Ml_2 and Ml_3 in myosin. Minced muscle was extracted with the number of volumes of standard extraction buffer indicated, and preparation then carried out in the normal manner (see the Methods section). (a) 2 vol.; I approx. 0.45; (b) 3 vol.; I approx. 0.50; (c) 10 vol.; I approx. 0.69. Samples were applied to 8 M-urea gel.

constant (Table 1). Ionic strength was not the only factor determining the proportions of components Ml_2 and Ml_3 present, for it was noted that, even when extraction was carried out at the higher ionic strength, preparations containing more component Ml_3 than component Ml_2 could be obtained if the muscle concentration was high, i.e. 1:2 (w/v). On the other hand if minced muscle was extracted with excess of buffer (1:10 w/v), at high ionic strength very little component Ml_3 was present whereas the Ml_2 component was much increased (Fig. 3 and Table 1). No significant differences in the adenosine triphosphatase activity measured in 25mM-tris-hydrochloric acid buffer (pH 7.6)–2.5mM-tris ATP–2.5mM-calcium chloride (Schaub & Perry, 1969) were observed in myosin preparations containing various proportions of components Ml_2 and Ml_3.

Thus the relative amounts of components Ml_2 and Ml_3 changed as though they were interconvertible, the amounts of each form depending on the initial extraction conditions of the myosin from the minced muscle. In general extraction at lower ionic strengths favoured greater relative proportions of component Ml_3 whereas the relative amounts of component Ml_2 were greater when the concentration of muscle was low. No obvious changes in the relative amounts of components Ml_1 and Ml_4 were apparent in the extraction conditions modifying the relative amounts of components Ml_2 and Ml_3.

Separation of the low-molecular-weight components of myosin. A number of methods were investigated for separation of the low-molecular-weight components from the heavy chains. Some separation

was obtained of all four light components by preparative electrophoresis in 8 M-urea. If electrophoresis was carried out in 20mM-tris–glycine buffer, pH 8.6, mainly bands Ml_1 and Ml_4 moved into the gel. Although the method offered promise of effective separation, deterioration of the band pattern occurred during preparative electrophoresis.

Precipitation of the heavy fraction from myosin dissolved in 5 M-guanidine hydrochloride, leaving the light components in solution, could be achieved by ethanol (see the Methods section) or saturated ammonium sulphate. However, band modification occurred in the latter case. On dialysis of the solution of the light components in 5 M-guanidine against a solution containing 20mM-potassium chloride, 10mM-potassium phosphate buffer, pH 6.5, and 10mM-mercaptoethanol components Ml_2 and Ml_3 were preferentially precipitated, leaving the bulk of components Ml_1 and Ml_4 in solution. Further fractionation of the supernatant could be obtained by chromatography on DEAE-Sephadex A-25. Although this procedure gave good separation of components Ml_1 and Ml_4, more convenient fractionation into all four low-molecular-weight components was obtained by applying the supernatant containing all four low-molecular-weight components to DEAE-Sephadex A-25 (Fig. 4a).

Band pattern in different species and muscle types. The four-band pattern present in rabbit skeletal-muscle myosin was consistently observed with myosins from the skeletal muscle of several species including mouse, rat, hamster, pigeon and chicken. The four bands of the three mammalian species were very similar in mobility, whereas in the chicken bands Ml_1, Ml_2 and Ml_3 and in the pigeon all four of the bands ran faster than their rabbit equivalent (Fig. 5).

On the other hand the electrophoretic patterns of myosin isolated from red and cardiac muscles of the adult rabbit by initial extraction in the standard buffer showed clear differences from myosin of white muscle both in 8 M-urea and glycerol gels. In myosin from red muscle the Ml_1 and Ml_2 components were diminished in intensity and they were absent from cardiac myosin (Fig. 6). The latter had two main bands, one corresponding to band Ml_4 and the other probably to band Ml_3. In cardiac myosin several minor bands were also observed, although these may have been derived from the material that was responsible for the major bands.

Myosin isolated from skeletal muscle of the 1-day-old rabbit gave a similar electrophoretic pattern to that from adult red muscle. Neonatal or foetal muscle myosin gave a more complex band pattern than that obtained with adult white-muscle myosin, which usually increased in complexity on attempted purification. If the elution
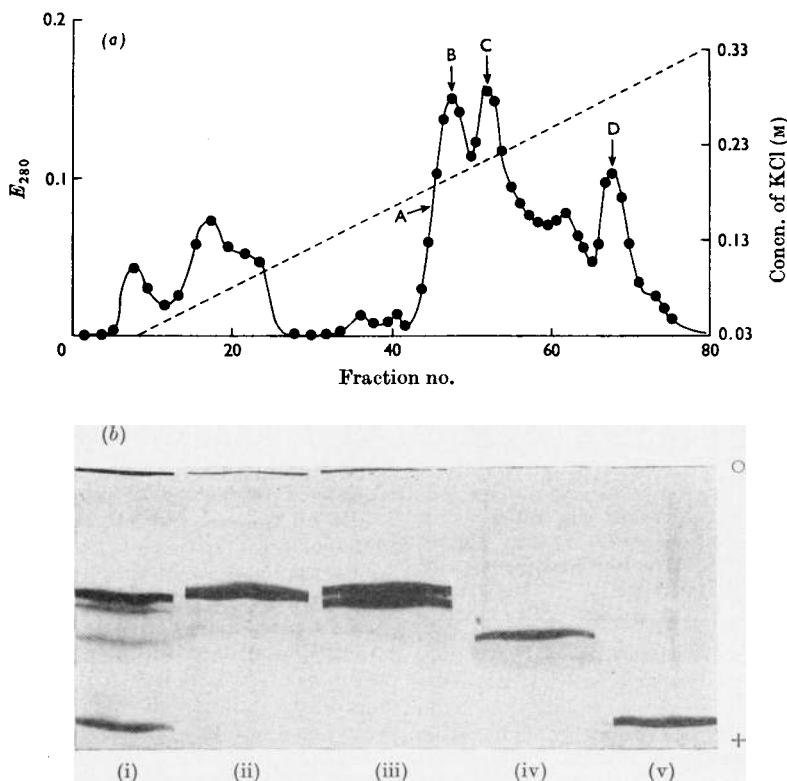
Fig. 4. Fractionation of low-molecular-weight components of rabbit skeletal-muscle myosin on DEAE-Sephadex A-25. A 200 ml portion of whole fraction (1.1 mg/ml) in 30 mM-KCl–25 mM-tris–HCl buffer (pH 7.6)–10 mM-mercaptoethanol was applied to a 2.5 cm × 30 cm column equilibrated against the same buffer. A linear gradient was applied to 0.33 M-KCl–25 mM-tris–HCl buffer (pH 7.0)–10 mM-mercapto-ethanol as indicated by the dotted line. (a) Elution profile; (b) electrophoretic patterns of fractions at points marked by arrows A, B, C and D. (i) Total low-molecular-weight fraction applied to column; (ii) fraction A, component Ml_4; (iii) fraction B, components $Ml_3 + Ml_4$; (iv) fraction C, component Ml_2; (v) fraction D, component Ml_1. Samples were applied to 40%-glycerol gel.

was sufficiently rapid, however, the band picture was simplified after DEAE-cellulose column chromatography. Myosin from the 1-day-old rabbit purified in this way gave an electrophoretic picture similar to that of adult red muscle, although it was somewhat obscured by additional fine bands.

DISCUSSION

The variable reports in the literature on the number of components of low molecular weight present in myosin isolated from skeletal muscle have probably arisen because of the failure of investigators to appreciate the inherent instability of the components and the effect of the conditions for the initial extraction of the muscle mince on their relative proportions. When myosin was extracted from skeletal muscle under the standard

conditions (see the Methods section) a similar pattern consisting of four distinct electrophoretic components was obtained in all species studied, although some general differences in mobilities were apparent between avian and mammalian species. Minor bands, present in greater numbers in aged preparations and those in which special precautions (see the Results section) had not been taken, are presumed to be due to modification of the main components Ml_1–Ml_4. The variation in pattern in myosin extracted from slower and less specialized muscles such as the red and foetal skeletal and cardiac types can be explained as being due to differences in the proportions of isoenzyme forms in the myosin present in these muscles (Perrie *et al.* 1969; Perry, 1969, 1970).

The effect of ionic strength of the extraction medium and the concentration of muscle on the
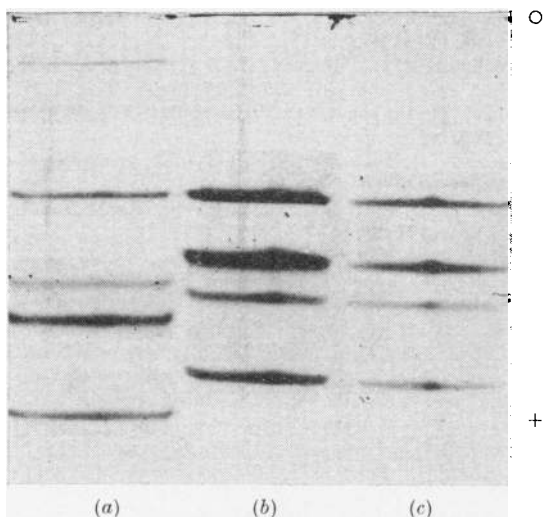
Fig. 5. Electrophoretic patterns of myosin isolated by the standard extraction procedure from the skeletal muscle of different species. Samples of myosin gel in 8 M-urea were applied to an 8 M-urea gel. (a) Chicken; (b) rat; (c) rabbit.
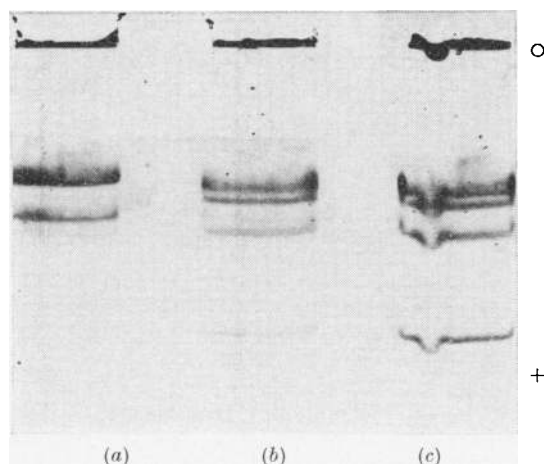

Fig. 6. Electrophoretic patterns of myosin isolated by the standard extraction procedure from different muscles of the rabbit. Samples of myosin gel in 8 M-urea were applied to 40%-glycerol gel. (a) Cardiac; (b) red skeletal (mixed soleus and semitendinosus); (c) white skeletal (longissimus dorsi).

number and relative proportions of the low-molecular-weight components in myosin raises the question whether all the four electrophoretically distinct bands represent essential components of the molecule. The fact that myosin that is enzymic-ally active can be prepared from which either component Ml_2 or Ml_3 is absent or barely detectable suggests that neither component is essential for biological activity. The reports (Weeds, 1969; Gazith et al. 1970) that a low-molecular-weight component can be removed from myosin by treatment with 5,5'-dithiobis-(2-nitrobenzoic acid) without loss of enzymic activity are compatible with this observation. In the preliminary report by Weeds (1969) details of the electrophoretic conditions are not given, but the carboxymethylated derivative of the low-molecular-weight component removed by 5,5'-dithiobis-(2-nitrobenzoic acid) appears to have a mobility intermediate between that of the other two low-molecular-weight components. This component is probably an unresolved mixture of components Ml_2 and Ml_3.

The results strongly suggest the interconversion of components Ml_2 and Ml_3, and that the conditions of initial extraction of the muscle determine the relative proportion of these two components. Nevertheless once the myosin has been extracted it has not yet been possible to bring about further interconversion by treatment with dissociating agents or high ionic strength. It would appear that once the myosin has been extracted and separated from the muscle the interconversion of components Ml_2 and Ml_3 is in some way prevented. The evidence available does not allow a definite conclusion to be made about the relationship between components Ml_2 and Ml_3, but suggests that one is a modified form of the other. The slower component may be an aggregate of the faster or possibly the result of enzymic modification leading to a lower net negative charge. The evidence that high muscle concentrations lead to the extraction of myosin with component Ml_3 and low concentrations favour preparations of myosin with high content of component Ml_2 supports this view.

When present, all four low-molecular-weight components of myosin are very tightly bound to the heavy part of the molecule and can only be removed under strong dissociating conditions. After dissociation, low ionic strength does not lead to selective precipitation of the heavy fraction as recombination or co-precipitation occurs when the concentration of dissociating agent falls. The ethanol precipitation procedure in 5 M-guanidine carried out in mercaptoethanol is an effective means of separation with minimum modification of the low-molecular-weight components. Some differences can be observed between the components, for the Ml_1 and Ml_4 components tend to be more readily removed from the heavy fraction and rather more soluble than components Ml_2 and Ml_3.

Whatever may be the significance of components Ml_2 and Ml_3 for the structure and function of myosin, it is suggested that components Ml_1 and

Ml_4 are characteristic of the adult and foetal cardiac forms of isoenzyme respectively. White skeletal muscle contains both in roughly similar amounts; in the rabbit the amount of component Ml_1 is much less in slower muscles such as red skeletal muscles and is absent from heart. This view is supported by recent findings (G. E. Lobley & S. V. Perry, unpublished work; Perry, 1970) that in conditions of vitamin E dystrophy where the skeletal muscle reverts to a more foetal type, the Ml_1 component selectively falls in amount.

REFERENCES

Akroyd, P. (1967). *Analyt. Biochem.* **19**, 399.

Brewer, J. M. (1967). *Science, N.Y.*, **156**, 256.

Davis, B. J. (1964). *Ann. N.Y. Acad. Sci.* **121**, 404.

Dreizen, P., Gershmann, L. C., Trotta, P. P. & Stracher, A. (1967). *J. gen. Physiol.* **50** (Suppl.), 85.

Florini, J. R. & Brivio, R. P. (1969). *Analyt. Biochem.* **30**, 358.

Frederiksen, D. W. & Holtzer, A. (1968). *Biochemistry, Easton,* **7**, 3935.

Gaetjens, E., Barany, K., Bailin, G., Oppenheimer, H. & Barany, M. (1968). *Archs Biochem. Biophys.* **132**, 82.

Gazith, J., Himmelfarb, S. & Harrington, W. F. (1970). *J. biol. Chem.* **245**, 15.

Gershmann, L. C., Dreizen, P. & Stracher, A. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 966.

Johnson, P., Harris, C. I. & Perry, S. V. (1967). *Biochem. J.* **105**, 361.

Locker, R. H. & Hagyard, C. J. (1967). *Archs Biochem. Biophys.* **120**, 454.

Locker, R. H. & Hagyard, C. J. (1968). *Archs Biochem. Biophys.* **127**, 370.

Mueller, H. & Perry, S. V. (1962). *Biochem. J.* **85**, 431.

Perrie, W. T., Perry, S. V. & Stone, D. (1969). *Biochem. J.* **113**, 28 P.

Perry, S. V. (1969). *Symp. Physiology and Biochemistry of Muscle as a Food.* Madison: University of Wisconsin Press.

Perry, S. V. (1970). *J. neurol Sci.* (in the Press).

Perry, S. V. & Zydowo, M. (1959). *Biochem. J.* **71**, 220.

Schaub, M. C. & Perry, S. V. (1969). *Biochem. J.* **115**, 993.

Schaub, M. C., Perry, S. V. & Hartshorne, D. J. (1967). *Biochem. J.* **104**, 263.

Small, P. A., Harrington, W. F. & Kielley, W. W. (1961). *Biochim. biophys. Acta,* **49**, 462.

Stracher, A. (1969). *Biochem. biophys. Res. Commun.* **35**, 519.

Trayer, I. P. & Perry, S. V. (1966). *Biochem. Z.* **345**, 87.

Tsao, T.-C. (1953). *Biochim. biophys. Acta,* **11**, 368.

Weeds, A. G. (1969). *Nature, Lond.,* **223**, 1362.

Wetlaufer, D. B. & Edsall, J. T. (1960). *Biochim. biophys. Acta,* **43**, 132.