LIGHT CHAIN DISTRIBUTION OF CHICKEN SKELETAL MUSCLE MYOSIN ISOENZYMES

J. F. Y. HOH

Department of Physiology, University of Sydney, Sydney, 2006, New South Wales, Australia

Received 7 April 1978

1. Introduction

Fast-twitch and slow-twitch muscle myosins contain 2 heavy chains and 2 pairs of light chains [1]. There are 3 types of light chains in fast-twitch muscle myosin: LC_1^f (alkali 1 light chain), LC_2^f (DTNB light chain) and LC_3^f (alkali 2 light chain). LC_2^f forms 2 mol/mol myosin while LC_1^f and LC_3^f together form 2 mol/mol myosin. Two types of light chains distinct from those in fast-twitch myosin are found in slow-twitch muscle myosin, LC_1^s and LC_2^s, and these occur in equal molar ratio. Analysis of intact myosin in pyrophosphate gels has revealed that myosin from both types of muscles are electrophoretically heterogeneous [2], there being 3 components in the fast-twitch posterior latissimus dorsi muscle myosin and 2 components in the slow-twitch anterior latissimus dorsi muscle myosin. It has been suggested that these multiple forms in each type of muscle are isoenzymes of myosin. In this work, the light chain distribution of these individual myosin forms are analysed by SDS–gel electrophoresis after separation in pyrophosphate gel. It will be shown that multiple forms in fast-twitch muscle myosins do differ in light chain distribution, thereby demonstrating their isoenzymic nature. Slow-twitch muscle myosins however, do not appear to differ in light chain distribution.

2. Experimental

Fast-twitch pectoralis muscle and the mixed plantaris muscle were taken from week 22 female white Leghorn chickens. The slow-twitch anterior latissimus dorsi muscle was from week 6–22 animals of the same sex and strain.

Myosin was prepared as a crude extract by methods similar to those in [2]. Washed myofibrils were minced in a solution containing 100 mM $Na_4P_2O_7$, 5 mM EGTA, 5 mM dithiothreitol, pH 8.8. The extract was centrifuged for 3 h at 108 000 × g. The supernatant was collected, mixed with an equal volume of glycerol and stored at −20°C.

The general procedure for the electrophoresis of intact myosin in pyrophosphate gels has been described [2]. Gels were 4% T and 3% C (notation from [3]) and were cast in 5 mm (i.d.) × 7.5 cm tubes. Electrophoresis buffer contained 2 mM cysteine in addition to 20 mM $Na_4P_2O_7$ and 10% (v/v) glycerol, pH 8.8 at 4°C. While electrophoretic patterns of chick myosins are apparently unaffected by the presence or absence of thiols [2], cysteine prevents blurring of electrophoretic bands with certain types of myosin. This reagent has therefore been routinely incorporated into the pyrophosphate electrophoresis system. Gels were loaded with 5–20 μg myosin. Electrophoresis was carried out at 80 V for 16 h at 4°C and with buffer recirculation.

Myosin bands were located by staining duplicate gels for 15 min in a 0.0025% (w/v) solution of Coomassie brilliant blue R in 7% (v/v) acetic acid. This enabled visualization of protein without destaining. Using the stained gel as a guide, bands containing myosin components in unstained gels were sliced. To each slice, measuring 1.5–2.0 mm thick, was added 50 μl solution containing 2% (w/v) sodium dodecyl sulphate (SDS) and 2% (v/v) 2-mercaptoethanol. The mixture was heated for 3 min in a small test-tube in a water bath at 100°C. The slice, together with residual solution thickened with an approximately equal volume of glycerol, was loaded onto SDS–gels for light chain analysis.

Light chains of myosin were analysed in polyacrylamide gels (12.5% T, 3% C) containing 0.1% (w/v) SDS, 2 mM cysteine, 50 mM sodium phosphate buffer, at pH 7.0. In order to accomodate gel slices adequately, 6 mm (i.d.) X 7.0 cm tubes were used. Electrophoresis was performed at 80 V for 3 h at 20°C. Gels were stained in 0.25% Coomassie brilliant blue R in 50% (v/v) methanol and 10% (v/v) acetic acid at 70°C for 2 h and destained in a diffusion destainer containing a solution of 15% (v/v) methanol and 7.5% (v/v) acetic acid. Gels were scanned at 550 nm using a Gilford spectrophotometer equipped with a linear transport.

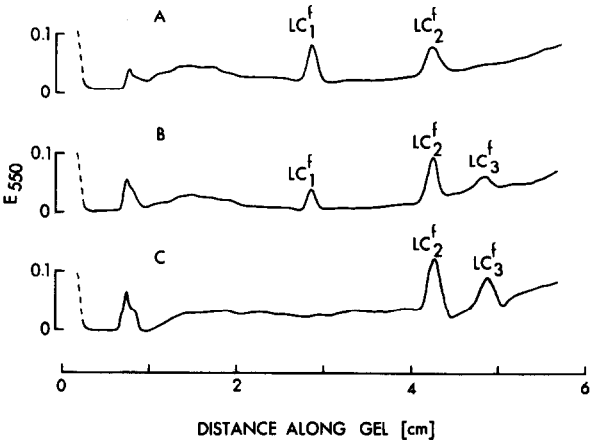

Fig.1. SDS–gel electrophoresis of individual fast-twitch myosin isoenzymes fractionated by pyrophosphate gel electrophoresis. Densitometer tracings of fast-twitch myosin light chains in FM_3 (A), FM_2 (B) and FM_1 (C). The light chain compositions of the different fractions, averaged from n experiments and normalized to 2 mol LC_2^f were: (A) 1.9 ± 0.3 (SEM, n = 5) mol LC_1^f in FM_3, (B) 1.4 ± 0.2 (SEM, n = 4) mol LC_1^f and 1.0 ± 0.2 (SEM, n = 4) mol LC_3^f for FM_2 and (C) 1.6 ± 0.2 (SEM, n = 3) mol LC_3^f in FM_1. Molecular weights used were based on amino acid sequences of fast-twitch light chains: LC_1^f, 20 700 [12]; LC_2^f, 18 600 [13]; LC_3^f, 16 500 [12].

3. Results and discussion

Pectoralis myosin upon electrophoresis and pyrophosphate gel showed 3 components which comigrated with the 3 components of the fast-twitch muscle myosin in posterior latissimus dorsi as described in [2]. Three components of fast-twitch myosin are referred to as FM_1, FM_2 and FM_3, in the order of decreasing mobility. These components form 37%, 34% and 29%, respectively, of the total myosin in pectoralis. In the plantaris, a mixed muscle containing all 5 myosin components, the distribution of fast-twitch myosin markedly favour FM_3, the ratio FM_1 : FM_2 : FM_3 being 4 : 26 : 70.

The results of SDS–gel electrophoresis of isolated fast-twitch myosin components are presented in fig.1. FM_1 contained LC_2^f and LC_3^f only. FM_2 contained all 3 light chains while FM_3 contained LC_1^f and LC_2^f. These results demonstrate the isoenzymic nature of these myosin fractions. The stoichiometric ratios of the light chains of these isoenzymes are consistent with the following structures : $(LC_2^f)_2$ $(LC_3^f)_2$ for FM_1, LC_1^f $(LC_2^f)_2LC_3^f$ for FM_2 and $(LC_1^f)_2$ $(LC_2^f)_2$ for FM_3.

Subfragment 1 (S-1) prepared from fast-twitch myosin can be fractionated into LC_1^f containing and LC_3^f containing isoenzymes [4,5]. It follows that FM_1 and FM_2, which contain only one type of alkali light chain, have an identical pair of light chains in each myosin head; they are homodimers of myosin. The existence of myosin homodimers has been demonstrated by other techniques [5,6]. Using antibodies specific for LC_1^f coupled to Sepharose as an immunoabsorbant, chicken fast-twitch heavy meromysin (HMM) and myosin can be separated into a fraction containing LC_1^f and LC_2^f and another contaning LC_2^f and LC_3^f with smaller amounts of LC_1^f [5]. Using an ADP affinity column, 3 fractions of chicken fast-twitch HMM have been separated [6]. These fractions have similar, though less clear-cut light chain distribution than the electrophoretically separated myosin isoenzymes described here. The Ca^{2+}-activated ATPase activity of these HMM fractions is the same, in agreement with that of myosin isoenzymes [2]. Since actin-activated myosin ATPase activity of LC_3^f containing S-1 [4] and HMM [6] is about twice that of the LC_1^f containing counterpart, it is expected that FM_1 would have an actin-activated myosin ATPase activity about twice that for FM_3. The actin-activated ATPase activity of myosin is correlated with the speed of muscle contraction [7]. The functional significance of fast-twitch myosin isoenzymes would be that variation in distribution of isoenzymes could result

in variation in the speed and power of fast-twitch muscle fibres.

The presence of a myosin heterodimer (i.e., molecules with non-identical light chain pair in each head) has not been demonstrated, though results consistent with its existence have been obtained [5,6]. The present work provides strong evidence that FM_2 is a heterodimer. Electrophoretic homogeneity in gels of a wide range of concentrations constitutes a very rigorous criterion for protein homogeneity [8]. FM_2 is electrophoretically homogeneous in 3–5.5% gels [2]. Further, the ratio of alkali light chains in FM_2 is not significantly different from 1:1. An alternative hypothesis for the structure of FM_2 is that it is composed of an approximately equal mixture of LC_1^f containing and LC_3^f containing homodimers having the same electrophoretic mobility. It would follow from this hypothesis that light chains do not contribute towards the electrophoretic heterogeneity, which would then be due to 3 types of heavy chain cores. Although limited peptide sequence studies have revealed the presence of at least 2 types of heavy chains [9,10], this hypothesis of the structure of FM_2 is unlikely in view of the finding that the heavy chain type is not specifically associated with a particular alkali light chain [11].

Myosin from the slow-twitch anterior latissimus dorsi muscles of week 6–22 animals all show only 2 components in pyrophosphate gels. These components migrate slower than FM_3 and are also present in chick plantaris. In keeping with the nomenclature suggested for fast-muscle myosin components, these slow muscle myosin components may be called SM_1 and SM_2, in the order of decreasing mobility. In week 22 chicks, SM_1 constitutes 12% of the total myosin in the anterior latissimus dorsi. In week 12 and week 6 animals, this component formed, respectively, 23% and 28% of the total myosin. Because of the more favourable distribution of components, myosins from these younger animals were used for light chain analysis.

Figure 2 shows densitometer scans of light chains after SDS–gel electrophoresis of the 2 fractions in slow-twitch muscle myosin. Both SM_1 and SM_2 showed the same two light chains : LC_1^s (mol. wt 27 000) and LC_2^s (mol. wt 18 000). Further, these light chains occurred in approximately equal stoichiometric ratios for SM_1 and SM_2. These results

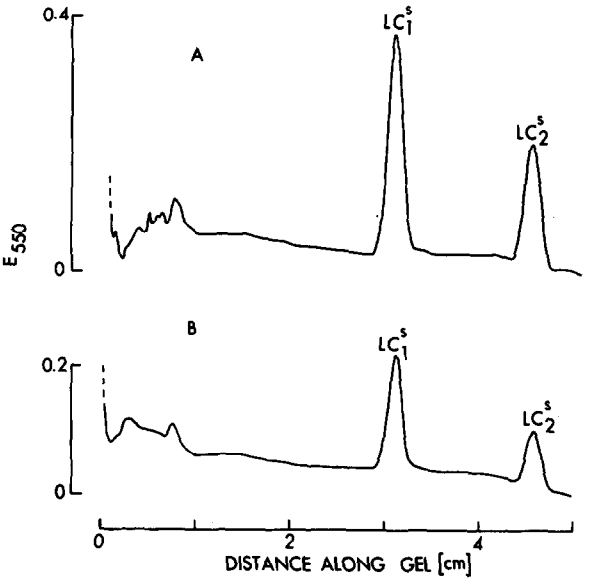

Fig.2. SDS–gel electrophoresis of individual slow-twitch muscle myosin components from the anterior latissimus dorsi of a week 12 chick fractionated by pyrophosphate gel electrophoresis. Densitometer tracings of slow-twitch myosin light chains in SM_2 (A) and SM_1 (B). The contents of LC_2^s of these fractions, normalized to 2 mol LC_1^s and averaged from n experiments were 1.7 ± 0.1 (SEM, n = 6) mol for SM_2 and 1.9 ± 0.8 (SEM, n = 5) mol for SM_1. Molecular weights used were : LC_1^s, 27 000; LC_2^s, 18 000. These values were obtained from their mobilities in these SDS–gels and molecular weight : mobility relation of marker proteins.

therefore failed to reveal differences in size or distribution of light chains in SM_1 and SM_2 though possible differences in charge cannot be excluded. The moderate difference in mobility between SM_1 and SM_2 raised the possibility that SM_2 may be a dimer of SM_1. This possibility was investigated by analysis of electrophoretic mobility in gels of different concentrations.

The retardation coefficient (K_r) obtained from a plot of the logarithm of mobility against gel concentration (Ferguson plot) is a measure of the effective molecular surface area [8]. It has been estimated that a myosin dimer would have a surface area, and therefore of K_r, in excess of 40% of that for a monomer [2]. Figure 3 shows Ferguson plots for SM_1 and SM_2, the K_r obtained for these myosin components being 0.33 and 0.36, respectively. These values are comparable to those previously found for

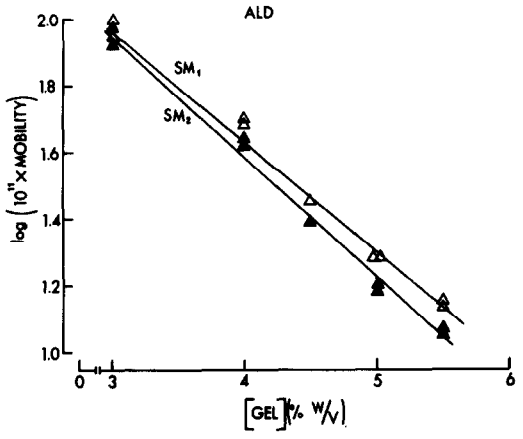

Fig.3. Ferguson plots for the 2 slow-twitch muscle myosin components from anterior latissimus dorsi. The logarithm of 10^{11}-times the mobility ($m^2 \cdot V^{-1} \cdot s^{-1}$) of SM_1 (△) and SM_2 (▲) is plotted against gel concentration. The lines are fitted by the method of least squares, $r = -0.994$ for SM_1 and $r = -0.996$ for SM_2.

fast-twitch myosin isoenzymes [2]. The difference between these values for SM_1 and SM_2 is too small to implicate SM_2 as a dimer of SM_1. These results suggest that SM_1 and SM_2 may be isoenzymes which differ in heavy chains.

Acknowledgements

This work was supported by a grant from the National Health and Medical Research Council of Australia. I wish to thank Miss B. H. Kim for technical assistance.

References

[1] Lowey, S. and Risby, D. (1971) Nature 234, 81–85.
[2] Hoh, J. F. Y., McGrath, P. A. and White, R. I. (1976) Biochem. J. 157, 87–95.
[3] Hjerten, S. (1962) Arch. Biochem. Biophys. Suppl. 1, 147–151.
[4] Weeds, A. G. and Taylor, R. S. (1975) Nature 257, 54–56.
[5] Holt, J. C. and Lowey, S. (1977) Biochemistry 16, 4398–4402.
[6] Wagner, P. D. (1977) FEBS Lett. 81, 81–85.
[7] Close, R. I. (1972) Physiol. Rev. 52, 129–197.
[8] Rodbard, D. and Chrambach, A. (1971) Anal. Biochem. 40, 95–134.
[9] Weeds, A. G. (1967) Biochem. J. 104, 44P.
[10] Starr, R. and Offer, G. W. (1973) J. Mol. Biol. 81, 17–31.
[11] Pope, B., Wagner, P. D. and Weeds, A. G. (1977) J. Mol. Biol. 109, 470–473.
[12] Frank, G. and Weeds, A. G. (1974) Eur. J. Biochem. 44, 317–334.
[13] Matsuda, G., Suzuyama, Y., Maita, T. and Umegane, T. (1977) FEBS Lett. 84, 53–56.