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Isomyosins in human type 1 and type 2 skeletal muscle fibres

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Human myosin from different skeletal muscles was analysed in a non-denaturating gel system, and the isoenzyme composition correlated with the histochemical composition of the muscle. Two components (SM_1 and SM_2) were associated with type 1 (slow-twitch) fibres, and three (FM_1, FM_2 and FM_3) with type 2 (fast-twitch) fibres. Light-chain analysis was performed in sodium dodecyl sulphate/polyacrylamide gels. There are three light chains (LC^s_{1a}, LC^s_{1b} and LC^s_2) in type 1 fibres, and three (LC^f_1, LC^f_2 and LC^f_3) in type 2 fibres. LC^f_1 and LC^s_{1b} co-migrate in sodium dodecyl sulphate gels. The ratio LC^f_3/LC^f_2 is correlated with the distribution of the individual fast isoenzymes. These results explain apparent discrepancies in the literature concerning the light-chain distribution of human myosin.

The myosin molecule consists of two heavy chains (HC, mol.wt. approx. 200000) and four light chains. In lower animals it is firmly established that myosin from fast-contracting muscle contains three different kinds of light chain (LC^f_1, LC^f_2 and LC^f_3) which can be distinguished in SDS/polyacrylamide gels from the two kinds of light chain (LC^s_1 and LC^s_2) in slow-contracting muscle (Lowey & Risby, 1971; Sarkar *et al.*, 1971). Heterogeneity of LC^s_1 (composed of LC^s_{1a} and LC^s_{1b}) has been defined in rabbits and cats (Weeds, 1976), and rats (Whalen *et al.*, 1978). Fast and slow myosins may also be distinguished histochemically by using the myofibrillar ATPase reaction (pH 9.4), with which type 1 (slow) fibres are pale and type 2 (fast) fibres are dark (Guth & Samaha, 1969).

Pyrophosphate-gel electrophoresis has emerged as a powerful method for separating native myosin isoenzymes without denaturation. This technique has demonstrated the existence of three to four isomyosins in vertebrate fast-twitch muscle and one to two isomyosins in slow-twitch muscle (Hoh *et al.*, 1976; Hoh, 1978; Hoh & Yeoh, 1979; d'Albis *et al.*, 1979; Lowey *et al.*, 1979; Hoh *et al.*, 1980). This technique has not previously been used to analyse human myosin, work on which has been largely limited to histochemical studies, and analyses of component light chains (Sreter *et al.*, 1976; Bailin, 1976; Dalla Libera *et al.*, 1978; Samaha & Thies, 1979; Fardeau *et al.*, 1979), tryptic digests (Dalla Libera *et al.*, 1978), amino acid composition (Groschel-Stewart, 1971) and total ATPase activity (Samaha, 1973; Dalla Libera *et al.*, 1978). Penn *et al.* (1972) analysed human myosin in a pyrophosphate-gel electrophoretic system, but without buffer recirculation, and individual isomyosins were therefore not separated.

There are discrepancies between the different light-chain studies, and little attempt has been made to directly correlate their electrophoretic pattern with the histochemical properties of muscles. Unlike muscles from most laboratory animals, specific human muscles show a great deal of normal variation in histochemical profile between individuals (Johnson *et al.*, 1973). Samaha & Thies (1979) described only two light chains (designated LC_1 and LC_2) of estimated mol.wt. 26000 and 20000. Bailin (1976) described an additional light chain (designated L_3, estimated mol.wt. 15000). Sreter *et al.* (1976) had also described these three components, but noted that LC_2 was often a doublet. Since the faster-migrating component of this doublet was removed by prior 5,5′-dithiobis-(2-nitrobenzoic acid) treatment of the myosin, it was assumed, by analogy with rabbit myosin (Weeds & Lowey, 1971), to be a 'fast' light chain. A third component in the vicinity of LC_2 was also seen in the absence of thiol reducing agents, and was considered to be due to oxidized 'fast' light chain (Sreter *et al.*, 1974).

The study of Dalla Libera *et al.*, (1978) described only three definite light chains. Although LC_2 occasionally appeared as a doublet this was also attributed to partial thiol oxidation. There is no clear-cut evidence in the literature for the existence of two distinct classes of human myosin

Abbreviations used: ATPase, adenosine triphosphatase; SDS, sodium dodecyl sulphate.

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light chains specifically associated with the two classes of isomyosin of fibre types. No heterogeneity of LC_1 associated with slow fibres has been described.

The purpose of the present study was therefore (1) to define the isomyosins in human skeletal muscle, and the variation in their proportions according to the muscle's histochemical profile, and (2) to relate the light-chain composition to both the isomyosin composition and the histochemical profile.

Experimental

Muscles for myosin analysis were obtained (1) by needle biopsy (Edwards, 1971) of the vastus lateralis of normal human volunteers, (2) by biopsy incidental to surgery from patients not known to be suffering from any neuromuscular disorder and (3) as portions of muscle biopsies performed for diagnostic purposes. Myosin was prepared, either after the removal of soluble proteins (Hoh *et al.*, 1976) or directly as a crude extract (Hoh, 1978). Since it was found that omitting the partial myosin purification before electrophoresis did not affect the myosin pattern, this step was not included in routine analyses. Pyrophosphate electrophoresis was performed as previously described (Hoh *et al.*, 1976), except that polyacrylamide gels were 4.5%T (notation according to Hjerten, 1962), electrophoresis was performed for 24–27h, and 2mM-cysteine was included in the electrophoresis buffer. It was found that these alterations to the original method resulted in improved resolution of human myosins. The relative Ca^{2+}-activated ATPase activities of the different isomyosins were measured as described (Hoh *et al.*, 1976), except that the concentration of $CaCl_2$ in the assay medium was 12mM, and 2mM-cysteine was included in all electrophoresis and incubation buffers.

The light chains of electrophoretically purified total myosin were analysed by slicing the myosin-containing band from pyrophosphate gels after a short (4–5h) electrophoresis, and subjecting these gel slices to SDS/polyacrylamide-gel electrophoresis [as described by Hoh (1978), except that the concentration of the sodium phosphate buffer was 0.1M].

Histochemical analysis was performed in most cases on specimens of muscle immediately adjacent to the muscle used for electrophoretic analysis. The Ca^{2+}-activated myosin ATPase activity was visualised at pH9.4 by using standard histochemical techniques (Padyluka & Herman, 1955).

Specimens of muscle from both extremes of the histochemical profile (i.e. all type 1 fibres or nearly all type 2 fibres), as well as specimens with approximately equal fibre proportions, were selected for analysis. Since normal human skeletal muscle is usually not histochemically homogeneous it was necessary to use diseased muscle to study the extremes. Eight muscle samples with type 1 homogeneity were analysed. These were obtained from patients with six different diseases. Four muscle samples, each associated with a different disease, and which consisted almost entirely of type 2 fibres, where also analysed. Since all the myosins in each of these two histochemical categories consistently exhibited electrophotetic patterns characteristic of the group it was concluded that the isomyosin distribution was a function of the histochemical profile, and not of any particular disease process *per se*.

In addition the relative area occupied by the type 1 fibres was estimated from photomicrographs of 13 normal skeletal muscle histochemical preparations. All the fibres within a given photographic field were counted, and the calculated result was correlated with the percentage of slow isomyosins ($SM_1 + SM_2$) present, as estimated from densitometer tracings. This percentage of slow myosin ranged from 15 to 82%. For the purpose of the area calculations, fibre diameters were measured by the method of Dubowitz & Brooke (1973). The number of fibres counted ranged from 176 to 397. The percentage, $[\Sigma(d_1)^2/\Sigma(d_1)^2 + \Sigma(d_2)^2] \times 100\%$, where d_1 represents the diameter of the type 1 fibres, and d_2 the diameter of the type 2 fibres, was calculated in each case as a measure of the percentage area occupied by the type 1 fibres.

Results and discussion

Sodium pyrophosphate electrophoresis

Myosin from histochemically mixed muscle separated into five components (Figs. 1 and 2). Only the two slower-migrating components (SM_1 and SM_2), which were closely apposed in the gels, were present in muscle consisting entirely of type 1 fibres. In general the amount of SM_2 present was greatly in excess of SM_1.

SM_1 and SM_2 were widely separated from the three faster-migrating isomyosins (FM_1, FM_2 and FM_3). In pathological muscle, in which the area of type 2 fibres exceeded 90% of the total, these fast isomyosins were present, together with only small amounts of slow myosin. The ratios of FM_1, FM_2 and FM_3 varied, but almost always FM_3 was present in greatest, and FM_1 in least amount.

In normal muscle the percentage of slow myosin closely paralleled that of the area occupied by the type 1 fibres in histochemical preparations of adjacent muscle (correlation coefficient, $r = 0.93$). A plot of the percentage of slow myosin present against the percentage of the area occupied by the type 1 fibres (ordinate) could be fitted by an equation $y = 6.44 + 0.92x$.

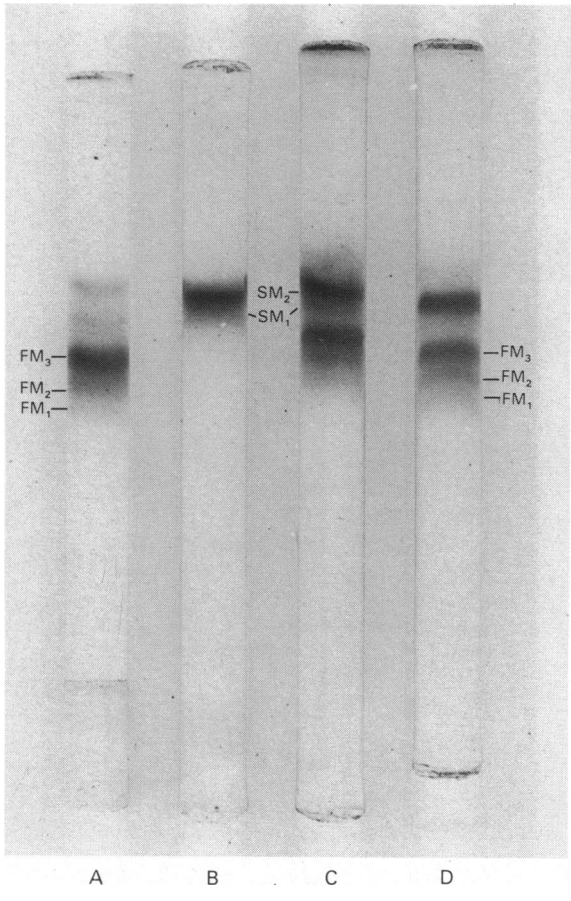

Fig. 1. *Pyrophosphate gel electrophoresis*
(A) Myosin from muscle containing mostly type 2 fibres. Most of the myosin is fast-migrating. This fast-migrating myosin consists of three bands (FM_1, FM_2 and FM_3). There is a small amount of slow-migrating myosin. (B) Myosin from muscle consisting entirely of type 1 fibres. There are two components, a dominant slow-migrating component (SM_2), and a clearly separated lesser component (SM_1). (C) Mixture of the myosins demonstrated in (A) and (B). SM_1 and SM_2 are widely separated from FM_1, FM_2 and FM_3. (D) Myosin from histochemically mixed muscle with approx. equal proportions of type 1 and 2 fibres. There are five components (FM_1, FM_2, FM_3, SM_1 and SM_2).

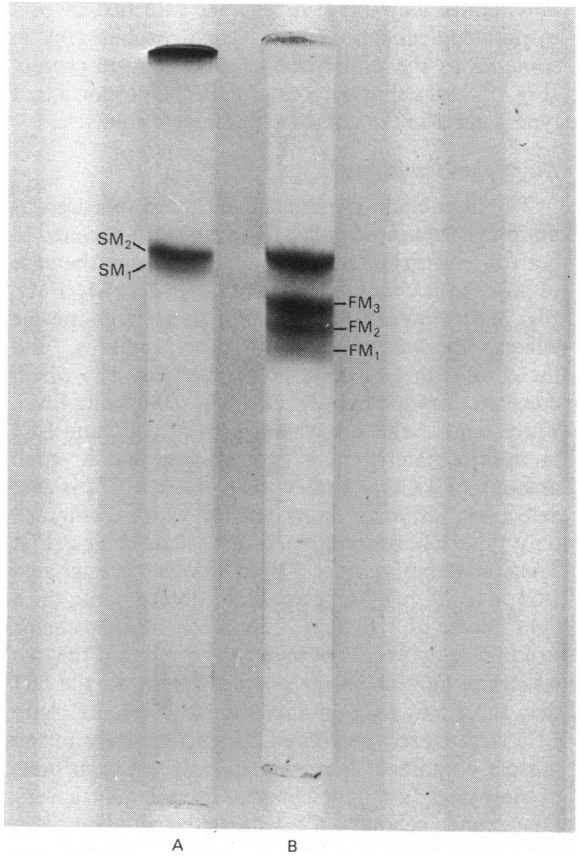

Fig. 2. *Pyrophosphate gel electrophoresis*
(A) Myosin from muscle containing only type 1 fibres consists of two components (SM_1 and SM_2). (B) Myosin from histochemically mixed muscle, showing an additional three components (FM_1, FM_2 and FM_3).

The relative Ca^{2+}-activated ATPase activity of the different myosins was $FM_1/FM_2/FM_3/slow = 1:2:2.7:1.5$. Since FM_3 has the greatest activity under the alkaline conditions of the assay, and is also present in greatest amount of the fast isomyosins, it is presumably responsible for the relatively dark staining of type 2 fibres compared with type 1 fibres, when the histochemical myosin ATPase reaction is performed at pH9.4. (This histochemical profile is reversed if the specimen is preincubated at acid pH.) It is emphasized that these results *in vitro* may not accurately reflect the actin-activated ATPase activity *in vivo*.

These results do not exclude the possibility that fast and slow myosins may coexist in the one fibre. Such a coexistence could be predicted on the basis that myosin properties can be altered by changing the muscle's activity or its innervation or electrical stimulation pattern (Sreter *et al.*, 1973; Hoh *et al.*, 1980).

This implies a state of transition in which the fibres will contain both classes of myosin. Both fast and slow light chains were found to coexist in fibres undergoing such changes (Pette & Schnez, 1977). Pathological muscle may be especially susceptible to alteration in fibre typing. Coexistence of small

amounts of slow myosin together with fast myosin in pathological type 2 fibres could explain why in some cases the proportion of slow myosin present (Fig. 1C) was slightly greater than the proportion of type 1 fibres in the adjacent speciment of muscle.

Light-chain analysis

The light-chain pattern in SDS gels appeared as up to five separate bands. The pattern was related to both the histochemical fibre typing, and in the case of 'fast' light chains to the ratio of $FM_1/FM_2/FM_3$. Fig. 3(B) shows the light-chain pattern from muscle which consisted mostly of type 2 fibres, and therefore with an isomyosin profile (Fig. 1C) which consisted predominantly of FM_1, FM_2 and FM_3. Three light chains are clearly present: LC_1^f and LC_2^f in approximately equal proportions, and a small amount of LC_3^f. Another light chain, LC_2^s, is just detectable. Myosin from muscle which contained only type 1 fibres contained three light chains (Fig. 3A), including a closely split doublet (designated LC_{1a}^s and LC_{1b}^s by analogy with rabbit), and a third light chain, LC_2^s. Co-electrophoresis of light chains from these two kinds of myosin showed that the two classes of light chain are distinct except for the fact that LC_{1b}^s and LC_1^f co-migrate. However, LC_2^s and LC_2^f were clearly separated. Histochemically mixed muscle contained appropriately mixed proportions of slow and fast light chains. Occasionally a very faint band behind LC_2^s was detected. This also appears in some gels illustrated by Dalla Libera *et al.* (1978) and is analogous to a rabbit myosin band, which is considered by Weeds (1976) to be a breakdown product consequent upon myosin storage.

The distribution of the light chains of fast myosin can be correlated with the distribution of the individual fast isomyosins. The proportion of LC_3^f present was generally low. This correlates with the observation that the isomyosin profile usually shows a prominent FM_3 relative to a low but variable FM_1. The ratio LC_3^f/LC_2^f was proportional to the relative amount of FM_1 present in the fast isomyosins. LC_3^f was not detected if the proportion of FM_1 in the total myosin was very low, e.g. if there was both a preponderance of slow myosin and a particularly low FM_1/FM_3 ratio. The variability of these isomyosin proportions explains the apparently contradictory results in the literature concerning the presence of LC_3^f in human myosin.

It has been shown for a number of species that the individual fast isomyosins differ in light-chain composition, the light-chain complement of FM_1, FM_2 and FM_3 being $(LC_2^f)_2$ $(LC_3^f)_2$, LC_1^f $(LC_2^f)_2$ LC_3^f and $(LC_1^f)_2$ (LC_2^f) respectively (Hoh, 1978; Hoh & Yeoh, 1979; d'Albis *et al.*, 1979; Hoh *et al.*, 1980). The correlation between light-chain distribution and fast isomyosins in human muscle is consistent with the hypothesis that the light-chain distribution of these isomyosins is the same as that in other species.

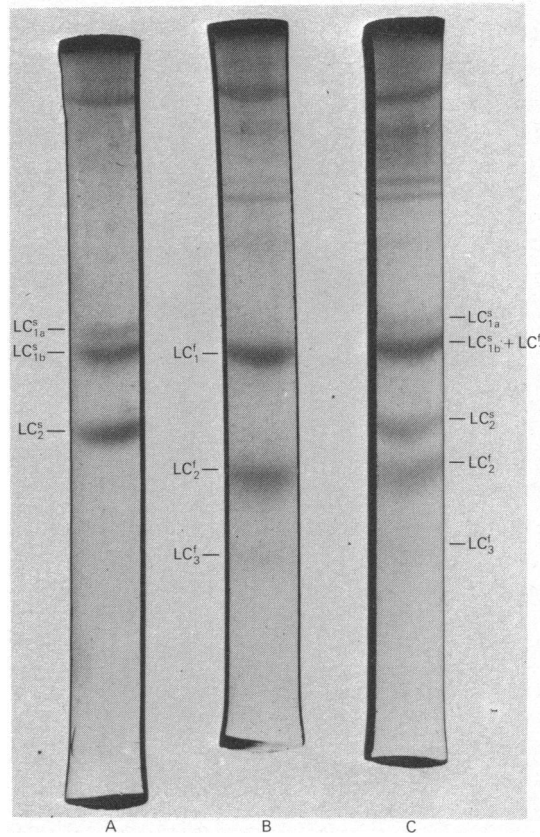

Fig. 3. *Myosin light chains: SDS electrophoresis* (A) Light-chain pattern of myosin from type 1 fibres. LC_1 is heterogeneous. The dominant component of LC_1 is the faster-migrating band (LC_{1b}^s), which was clearly separated from LC_{1a}^s. The pyrophosphate electrophoretic pattern was similar to that illustrated in Fig. 1(B). (B) Light-chain pattern from muscle containing mostly type 2 fibres. LC_1^f appears homogeneous. LC_2^f migrates faster than LC_2^s, which is present in trace amount. There is an additional component (LC_3^f) which is not seen in slow-twitch muscle. The pyrophosphate electrophoretic pattern is illustrated in Fig. 1(C). FM_1 constituted a small proportion of the total fast myosin. (C) Light chains of a mixture of the myosins shown in (A) and (B). LC_1^f co-migrates with LC_{1b}^s. LC_2^f is clearly separated from LC_2^s.

Heterogeneity of LC_1^s was clearly demonstrated in the present study. This has not been previously described in human muscle, although less clearly resolved bands are seen in some photographs of presumably slow myosin by Sreter *et al.* (1976; Figs. 7D and E). Gel concentration and duration of

electrophoresis may be critical in resolving individual bands.

Fardeau *et al.* (1979) included a brief description of two-dimensional analysis of myosin light chains in their paper on congenital myopathies. In the myosin sample analysed from normal histochemically mixed muscle there were three spots in the vicinity of LC_1. One of these spots is unlabelled, and can also be seen in pure 'slow' myosin (their Fig. 6*b*). It is likely to correspond to our LC^s_{1a} since it migrates marginally slower than their 'LC^s_1' in SDS, although their electrophoretic technique gave poor resolution in this dimension. Analysis of light chains from predominantly 'fast' muscle was not reported.

They also described two components of LC_1 in pathological muscle consisting of type 1 fibres (their Fig. 6*c*). One spot was considered to be LC^f_1, and since LC^f_2 was not present, the myosin was considered to be structurally abnormal or hybrid. However, on the basis of the illustrations presented, the spot designated as LC^f_1 could equally well be LC^s_{1a}. If so, their myosin data would be entirely consistent with the fibre typing, and it would not be necessary to postulate an abnormal or hybrid myosin [such as that described in cross-reinnervated soleus (Hoh *et al.*, 1980)] to explain their results.

No heterogeneity of LC^f_1 was detected. Such heterogeneity was suggested by Pette *et al.* (1979) as an explanation for the two proteins in the vicinity of LC_1 in electrophoresed myofibrillar preparations of individual type 2 fibres. In other species LC^f_1 appears to be homogeneous, and it seems probable that the extra band observed is a non-myosin protein.

The LC^s_1 heterogeneity may be correlated with the fact that slow myosin can be resolved into two components, SM_1 and SM_2. Since LC^s_{1b} is more prominent than LC^s_{1a}, and SM_2 is more prominent than SM_1 in all samples showing a predominance of slow myosin, it appears likely that LC^s_{1b} is associated with SM_2, and LC^s_{1a} with SM_1. However, the possibility that light-chain hybrids exist, which are unresolved by these techniques, cannot be excluded. Rabbit LC^s_1 is heterogeneous (Weeds, 1976), but only one slow myosin has been detected with pyrophosphate electrophoresis (Hoh & Yeoh, 1979). Myosin heterogeneity may also result from differences in heavy chains.

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