CHARACTERIZATION OF MYOSIN LIGHT CHAINS FROM HISTOCHEMICALLY IDENTIFIED FIBRES OF RABBIT PSOAS MUSCLE

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1. Introduction

It is now clearly established that rabbit myosin, isolated from skeletal muscles defined physiologically as of the fast-twitch class, contains two chemically related but phenotypically distinct light chains (termed LC1 and LC3). These 'Alkali light chains'—so called because of their release at high pH—differ in mol. wt. by 4000, but have an identical amino acid sequence over their C-terminal 141 residues [1]. Together with the unrelated 'DTNB light chain' (LC2), the alkali light chains give a characteristic band pattern on polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS), which is different from the light chain pattern of myosin from slow-twitch muscles [2,3]. Chemically related alkali light chains are present in fast-twitch muscle myosin from chicken [3], sheep and cat [4]. Their function remains unclear, though they appear to be essential for the enzymatic activity of the myosin [5]. In support of this, it is notable that cross-reinnervation of fast-twich and slow-twitch muscles results in a reciprocal transformation of the myosin light chains [6,7] which closely parallel the changes occurring in the twitch characteristics: for review of these latter, see [8]. The relative amounts of the two light chains have been determined by densitometry of the bands on SDS polyacrylamide gels, and results indicated that rabbit myosin contains 1.35 moles of the larger LC1 light chain and 0.65 moles of LC3 per mole of myosin [9]. These unequal and non-integral yields of the two homologous light chains suggest that there must be at least two populations of myosin in fast-twitch muscles.

Histochemical studies of a wide range of mammals (reviewed [8]) have shown the presence of several different fibre types—as characterized by the activities of a variety of enzymes and the cytological patterns of their distribution—even within muscles of uniformly fast-twitch function. This is as true for the rabbit [10,11] as for other animals. Accordingly, we decided: a) To examine the myosin from single rabbit muscle fibres, to see whether both light chains are present within a given muscle cell - these fibres to be taken from an area of exceptional histochemical uniformity. b) To check the stoichiometry of the light chains in myosin prepared from muscles isolated from a single animal, and showing the same histochemical uniformity as above.

2. Materials and methods

The tests of 'histochemical uniformity' which are directly relevant are those which throw some light upon the nature of the myosin in the fibre. We chose the Ca^{2+}-activated ATPase reaction, carried out at pH 9.4 after either pH 10.4 or pH 4.35 preincubations [12]. In addition we tested for succinic dehydrogenase (SDH) [13]. A strong correlation has been established, for adult mammalian muscle, between differing twitch characteristics and contrasting pH-stabilities of the de-

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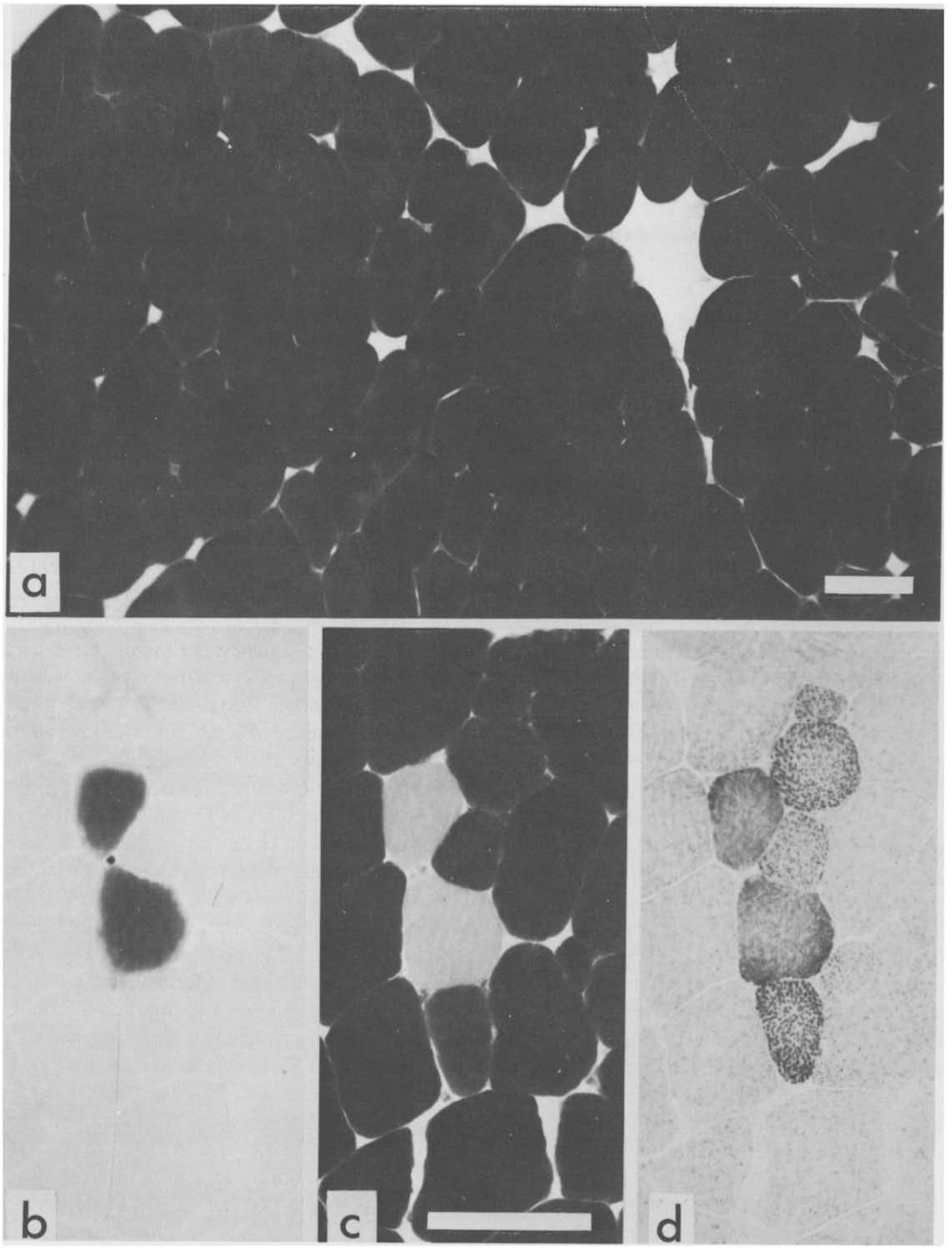

Fig.1. a) Psoas muscle, central region, transverse section; alkaline pre-incubated ATPase reaction showing uniform intense activity. b)–d) Medial edge of psoas muscle, serial sections used as controls for the uniformity of a) two fibres with alkali-labile ATPase c) are acid-stable b). Reacted for SDH d) these fibres stain with moderate intensity, as do four others, but the deposits in these latter have a different texture. The white bars on a) and c) = 100μm.

monstrated ATPases: the latter in turn match the respective pH-stabilities of the isolated myosins, [10], and refs. therein. Recent indications [14,15] that the reaction is positive not only to myofibrillar but to certain mitochondrial ATPases, do not seriously weaken the test for our purpose; in searching for uniformity we might well, because of this effect, make unnecessary rejections, but false acceptances are improbable. The SDH reaction also helps here. Obviously the mean density of reaction product gives information about the oxidative capability of each fibre; in addition the pattern of the deposits (ref. in [8]; also [16])–effectively the pattern of mitochondrial distribution–has independently been found to correlate with twitch-characteristics, and so provides a check upon the conclusions drawn from the ATPase reactions.

We performed these histochemical tests with customary controls, on serial, transverse, cryostat sections from a number of fast-twitch muscles of adult rabbits. In each of the six animals studied, an extensive region of the psoas major was almost totally homogeneous, more so than a comparable volume of any other muscle examined. Though both medial and lateral edges are appreciably heterogeneous, a central band, representing about $\frac{2}{3}$ of the muscle cross-section at midlength, consisting 99.6–100% (according to animal) of fibres showing high and uniform alkali-stable/acid-labile ATPase activity (fig1a; cf b & c); under SDH, 92–96% would be as pale as the background fibres of fig.1d, and only 0–2% as dark as the central group. We concluded that, in the central band of psoas, at least 98% of fibres could be classied as of the 'fast-twitch-glycolytic' type [11].

As single psoas fibres are relatively easily isolated by dissection, this region of the psoas was chosen for part 'a' of the biochemical work, though, because of the geographical separation of our laboratories, not from the same individual animals as had been used for histochemistry. Fibres up to 6 cm in length were isolated and transferred to small tubes. 5 μl of 10% SDS were added and the samples ultrasonicated for 1–2 min, then placed in a boiling water bath for about 3 min to denature the proteins. At this point samples were usually frozen overnight, which appeared to improve the extractability of the proteins, then after addition of 2 μl of a 'sample buffer' (containing 1% SDS, 10% β-mercaptoethanol, 25% glycerol and 10 mM Tris–bicene pH 8.3, plus Bromophenol Blue indicator) they were again placed in boiling water for 2 min before being applied to 10% polyacrylamide gels. These gels were prepared as described previously [3], except that the gel buffer used throughout was 0.1 M Tris–bicene in place of sodium phosphate. The gels were polymerised in 200 μl 'Yankee' micropipettes of diameter less than 2 mm, which had been washed in chromic acid and treated with DC34 surface active agent (Welton Laboratories) to facilitate their removal from the tubes. Electrophoresis was carried out at a constant voltage of 70 V and gels were stained with 0.2% Coomassie Brilliant Blue in 50% methanol, 10% acetic acid for 30 min at 45°C. Destaining was carried out initially in the same solvent for 30 min and then in 5% methanol and 10% acetic acid. Gels were also run with 2 μg of rabbit myosin light chains as a marker.

3. Results and discussion

Fig.2 shows gels of single fibres together with light chain markers. The presence of both LC1 and LC3 could be seen in over 30 individual fibre preparations and mixtures with added light chain marker show reinforcement of these bands. No attempt has been made to mark the proteins of higher molecular weight, but the intense bands in the middle of the gels clearly indicate the presence of actin and tropomyosin, while the myosin heavy chains can be seen at the tops of some gels. There was no evidence that the presence of glycolytic enzymes and other soluble proteins had obscured the light chain regions, since gels run on glycerinated single fibres were identical to those shown. However, these fibres were much more fragile to handle, which made isolation of long fibres difficult. Other myofibrillar proteins which are expected to occur in the light chain region include two troponin components, troponin-I and troponin-C. Thin filaments have been isolated from rabbit psoas muscle (methods as [17]) to check the mobility to the additional low molecular weight band seen in fig.2 just below LC1, while troponin-C had a mobility coincidental with LC2.

The presence of proteins with molecular weights corresponding to LC1 and LC3 in single fibres isolated from both fresh and glycerinated psoas muscles leads firstly to the conclusion that the existence of both light chains in a multifibre preparation need not be ascribed to the presence of different fibre types.

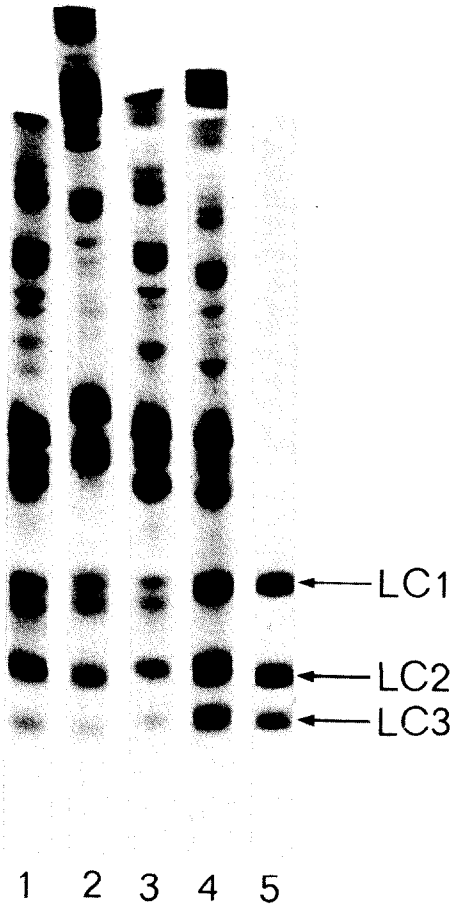

Fig.2. Polyacrylamide gel electrophoresis of single fibres from the central region of psoas muscle. 1–3 are gels of single fibres and 5 contains 2 μg of rabbit light chain marker. 4 contains a single fibre preparation with added light chain marker to show reinforcement of the appropriate bands. The tops of gels 1 and 3 were broken and the heavy chain band is missing.

Secondly, taken as a non-quantitative finding, it suggests either that the two alkali light chains which occur in each myosin molecule [18] are one of each kind, or that there must be at least two populations of myosin within a single fast-twitch, glycolytic fibre. The second of these conclusions is indicated by the stoichiometric determinations quoted earlier [9]; however, the methodological reservation must be made that the relative staining intensity of proteins on gels may depend on factors other than their concentration for example, the respective numbers of basic residues. For this reason we checked the stoichiometry by an independent method, based on the knowledge that both LC1 and LC3 contain the same single thiol sequence [1]. A quantitative reaction of this thiol group thus permits determination of the relative concentrations of the two proteins.

Rabbits psoas myosin, prepared from the central band of the muscle of a single animal, was labelled with [C^{14}]iodoacetic acid, as described previously, and dialysed against 1% acetic acid to remove excess reagents [1]. The incorporation of label was determined by scintillation couting and corresponded to 91% of the total thiol groups labelled. 4 mg SDS/mg myosin was added and the protein freeze dried to remove the acetic acid. The protein was dissolved in the 'sample buffer' already described and placed in boiling water for 5 min. Aliquots of 50 μg to 400 μg were applied to polyacrylamide gels which were run and stained as described previously [3], except that Tris–bicene was used as buffer throughout. The light chain bands were cut out with a razor blade, and chopped carefully into 5 ml scintillation vials. 0.4 ml of 30% volume hydrogen peroxide was added, the vials sealed and incubated at 45°C for 6 hr to disintegrate the gel and extract labelled protein. Bray's scintillation fluid was added and samples counted to give a minimum of 7000 counts per sample. 15 gels were analysed and the ratio of counts indicated a stoichiometry of 1.31 moles of LC1 and 0.69 moles of LC3 per mole of myosin assuming a total of two moles of alkali light chain [18]. These results indicate that myosin from the fast-twitch glycolytic fibres of psoas muscle contains LC1 and LC3 in a molar ratio of 2:1. Similar experiments, using 11 gels, carried out with conventionally prepared rabbit skeletal muscle myosin as a control, gave values for LC1 of 1.38 and for LC3 of 0.68, while LC2, which contains two thiol groups gave 1.95 moles/mole of myosin. The mixed fast muscles used in this preparation contain also glycolytic-oxidative fibres, and the results suggest that there is no significant difference between the myosin light chains of these two fibre types. Furthermore these results are in good agreement with the stoichiometry determined by densitometric methods [9]. Although no quantitative densitometric measurements were carried out on the single fibre preparations, it is encouraging to note that every gel stained lighter in the LC3 band than in the LC1.

Our interpretation of these findings is that rabbit

fast-twitch glycolytic fibres, and probably fast-twitch glycolytic-oxidative fibres too, each contain two or three isoenzymes of myosin, differing in respect of their alkali light chains. If individual myosin molecules are homogeneous in their light chain content, so that the number of isoenzymes is two, the population ratio is about two: one in favour of LC1. The significance of these two isoenzyme populations within a single cell remains to be established.

Acknowledgements

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