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TYPE-SPECIFIC PROTEINS OF SINGLE IIM FIBRES FROM CAT MUSCLE

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The fibre type IIM, present in the jaw-closer muscles of the cat, has a characteristic form of myosin. To analyse other proteins present in IIM fibres and the usual types I, IIA and IIB, ^{14}C-labelled typed single fibres were analysed by 2-dimensional gel electrophoresis. Whereas types I, IIA and IIB contained both α- and β- forms of tropomyosin, IIM fibres contained only a single form, shown by 1-dimensional peptide mapping to be different from the tropomyosins present in the other fibre types. This single form of tropomyosin, and two soluble proteins seen only in IIM fibres, were also found in IIM fibre-containing muscles from other species, demonstrating that IIM fibres are a distinct type.

The jaw-closer muscles of the cat have recently been shown to contain a distinct isoform of myosin, which is thought to be responsible for both their very fast contraction time and characteristic histochemical profile [1]. The common fibre types I, IIA and IIB are known to have characteristic forms of other myofibrillar proteins such as troponin and tropomyosin in addition to their respective isoforms of myosin [2, 3, 4, 5], and this study was undertaken to discover if a similar situation also existed in the IIM fibres of the cat jaw-closer muscles. To ensure that true fibre type specific differences were observed, a recently developed technique for the analysis of typed single muscle fibres by 2-D electrophoresis [6] was used to compare single IIM fibres of cat temporalis muscle with the type I, IIA and IIB fibres of the soleus and gastrocne-

Abbreviations used: 1-D: one dimensional; 2-D: two-dimensional; EDTA: ethylenedieminetetra-acetic acid; EGTA: ethylene-glycol-bis(2-amino-ethylether)-N,N'-tetra-acetic acid; TN-C: calcium-binding sub-unit of troponin; LC1s, LC2s: light chains 1 and 2 of slow myosins; LC1f, LC2f, LC3f: light chains 1, 2 and 3 of fast myosin; DTT: dithiothreitol; IEF: isoelectric focussing.

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mius muscles. In contrast to all the other three fibre types examined, IIM fibres consistently contained only one isoform of tropomyosin, and also two highly characteristic soluble proteins.

MATERIALS AND METHODS

Single fibres: Muscle pieces were obtained from the soleus, gastrocnemius, masseter and temporalis (posterior portion) muscles of three adult cats. The preparation and histochemical typing of the muscle fibres was done as described previously [6]. About 50 IIM fibres (most from temporalis and a few from the mixed muscle masseter) and 40 each of fibre types I, IIA and IIB (obtained from gastrocnemius and soleus) were taken for electrophoretic analysis.

Myofibrillar and soluble fraction extracts: These were prepared from bulk (1-2 g) pieces of soleus, flexor digitorum longus and posterior temporalis. Muscle pieces were homogenised briefly (3 x 10 sec) in about 10 ml ice-cold relaxing buffer (0.1 M KCl, 5 mM EDTA, 1 mM EGTA, 1 mM DTT, pH 7.0), using a Virtis blender. The homogenate was then spun for 3 min at 10,000 rpm to separate the soluble proteins from the myofibrils. Part of the soluble fraction was taken for ^{14}C-labelling (see below), and the rest immediately heated to 95°C for 5 min after addition of Laemmli sample buffer [7]. The myofibrillar fraction was washed twice with relaxing buffer + 0.1% (v/v) Tween 20. Part of the resulting pellet was taken for ^{14}C-labelling and the rest heated directly with Laemmli sample buffer.

Cryostat sections: 7 μm thick cryostat sections serial to those stained for myofibrillar ATPase activity [8] were dissolved in Laemmli sample buffer by heating to 95°C. Sections of muscle blocks of cat, dog and monkey masseter, and cat tensor tympani (all containing IIM fibres) were used.

^{14}C-labelling of single fibres, myofibrillar and soluble fraction proteins: This was done by reductive methylation following the method of Jentoft & Dearborn [9], with the adaptations for single fibres described in [6].

2-D electrophoresis: 2-D electrophoresis was performed according to the method of O'Farrell [10], using a linear IEF pH gradient from 6.4 to 4.1 formed by ampholines pH 3.5 - 10 (80%) and pH 2.5 - 4 (20%). The second dimension acrylamide concentration was 15% and the ^{14}C-labelled proteins used for molecular weight calibration were from Amersham Ltd. ^{14}C-labelled proteins were visualised by fluorography [11, 12]. For a better separation of tropomyosin sub-units some second dimension gels were run in the presence of 3.5 M urea [13]. Unlabelled samples (ca. 50-100 μg per IEF gel) of the myofibrillar and soluble fraction extracts, and single cryostat sections were run under the same conditions as for the single fibres except that proteins were visualised by Coomassie Blue staining.

Peptide mapping: The one dimensional peptide analysis of tropomyosin from ^{14}C-labelled single muscle fibres (types I, II and IIM) was performed as described previously [5].

RESULTS

By comparing 2-D gels of myofibrillar and soluble fraction extracts of bulk muscle with those of single fibres it was possible to distinguish soluble proteins from those of myofibrillar origin. The migration of all the major

spots, and the microheterogeneity of some of them in the IEF dimension, was the same for unlabelled and ^{14}C-labelled samples, which is consistent with previous observations that reductive methylation affects neither the net charge [14, 15] nor molecular weight [9] of proteins. Since this labelling process is very efficient, and results in the methylation of all ε-amino groups [9], carbamylation of lysine residues [16] during IEF cannot be the cause of the microheterogeneity, which is probably due to post-translational modifications of proteins. LC2f was also consistently found to give multiple spots in the 2nd dimension, irrespective of the sample origin. Typical 2-D gels of single fibres of types I, IIB and IIM are shown in fig. 1a-c (IIA not shown because no difference could be detected between these fibres and IIB fibres). The protein patterns seen were always consistent for each type, even when the same type was obtained from different muscles (e.g. type I from gastrocnemius and soleus, IIM from temporalis and masseter). The myosin light chains, identified by comparison with the myofibrillar extracts (not shown), are like those described for the corresponding bulk myosins [1], with IIM fibres containing only 2 light chains both of which are different from the light chains of fibre type I, IIA and IIB. Even after long exposure times, no spot corresponding to LC3f was found in the IIM fibres. TN-C can be seen to exist in two forms with slightly different Mr and pI values, one found only in the type I fibres (fig. 1b), and the other in all the type II fibres, including IIM fibres (fig. 1a, 1c and 1d). The two forms can be compared directly in fig. 1e.

All fibres of the common fibre types I, IIA and IIB contained both α- and β-forms of tropomyosin (fig. 2a, 2c, 3d). The difference in migration between the two forms was small, though quite clear in the normal 2-D gels, and as is to be expected from the work of Montarras et al. [13], much more exaggerated in the SDS/urea gels (fig. 2f, 2g). In contrast, in IIM fibres only one form of tropomyosin was found (fig. 2h), and this migrated with the α-form on the normal 2-D gels (fig. 2e).

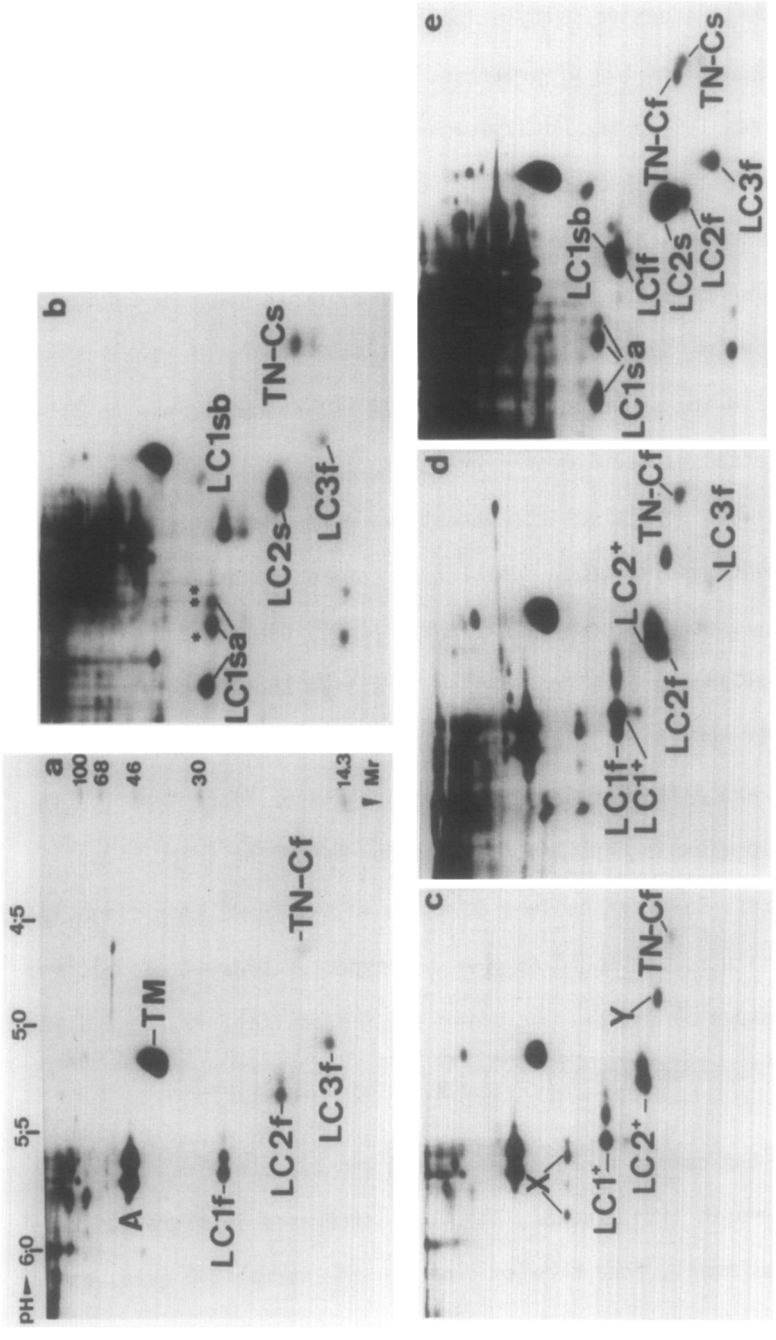

Figure 1. 2-D gels of ^{14}C-labelled single fibres of types IIB (a), I (b), IIM (c), IIB + IIM (d) and I + IIB (e). A: actin, TM: tropomyosin, LC1f, LC2f, LC3f: fast LC, LCs1a, LCs1b, LC2s: slow LC, LC1$^+$, LC2$^+$: LC of IIM fibres, TN-Cf, TN-Cs: fast and slow forms of TN-C, X, Y: unidentified soluble proteins characteristic of IIM fibres. The small spot identical to LC3f was found in all type I fibres from both soleus and gastrocnemius. Small amounts of fast LC have also been found in human type I fibres [6]. The spots labelled * and ** are minor forms of LC1sa, the spot marked * could be detected on heavily loaded Coomassie stained gels of myofibrillar samples. The low molecular weight protein (Mr ca. 14000 daltons) with pI values of 6.8 for the major spot and 6.6 for the minor spot which can be seen in (b) is a soluble protein found only in type I fibres. pH scale of first dimension and Mr scale of second dimension are indicated on (a). Mr standards are given in kilodaltons. Proteins were visualised by fluorography.

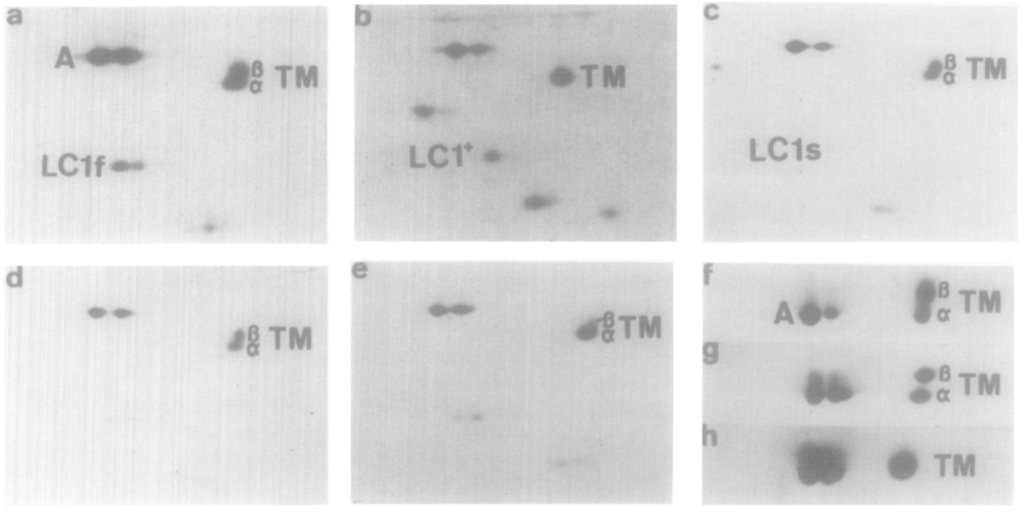

Figure 2. Portions of normal 2-D gels (a-e), and 2-D gels with the second dimension run in the presence of 3.5 M urea (f-h) showing tropomyosins in ^{14}C-labelled single fibres of types IIB (a), IIM (b), I (c), I + IIB (d), IIM + IIB (e), I (f), IIB (g) and IIM (h). A: actin. TM: tropomyosin. Proteins were visualised by fluorography.

On comparison of 1-D peptide maps of the β- and α-tropomyosin spots of fibre types I (fig. 3a, 3b) and II (fig. 3c, 3d) with the map of the single tropomyosin spot of IIM fibres, differences between the IIM form of tropomyosin and the others could be seen, indicating that this is a distinct isoform.

The most striking type-specific difference in the soluble fraction proteins was in the IIM fibres which all contained quite large amounts of two highly characteristic soluble proteins, which are shown labelled as X and Y on fig. 1c, as their identity is not known. The type specificity of these two proteins, and the single form of tropomyosin, was further confirmed by their presence also in the cryostat sections of other IIM fibre containing muscles of the cat (masseter and tensor tympani), dog and monkey (masseter) (not shown).

DISCUSSION

Although the two IIM type-specific soluble proteins could not be identified, it is possible to say that they are unlikely to be involved in oxidative metabolism, because they were present in similar amounts in the cryostat sec-

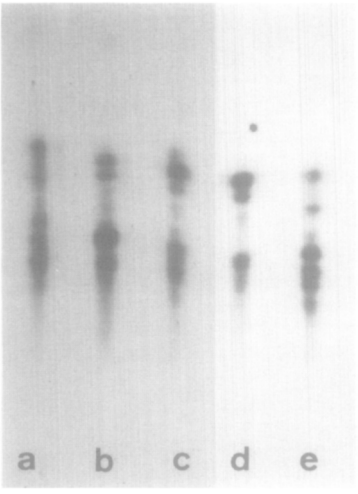

Figure 3. Comparison of the peptide fragments of β- (a) and α- (b) tropomyosins from ^{14}C-labelled single type I muscle fibres, β- (c) and α- (d) tropomyosins of type II fibres and (e) the single tropomyosin spot of IIM fibres. Digestion was done with Staphylococcus aureus V8 protease (0.03 μg). Proteins were visualised by fluorography.

tions of all IIM-containing muscles despite big differences in these muscles' oxidative capacity.

It has been known for some time that the fast and slow forms of tropomyosin are antigenically different [4]. This was originally thought to be associated with the different ratios of α- and β-forms present, but more recent work with human muscle has shown that the α- and β-forms, both of which are present in type I and II fibres, are structurally different [5], and this probably accounts for the antigenic differences. However, it is characteristic of IIM fibres that only one form of tropomyosin is present, and in this respect they resemble cardiac muscle which in small mammals like the cat contains only one form of tropomyosin [17], rather than the other skeletal muscle fibres which contain both the α- and β-forms. The tropomyosin of IIM fibres was found by peptide mapping to be different from the α- and β-tropomyosins of type I and II fibres, which suggests a difference in primary structure, though techniques other than the electrophoretic ones used here would be required to confirm this. Together, the type-specific characteristics of IIM fibres establish that they

are indeed a true fibre type, having a set of type-specific soluble and myofibrillar proteins comparable to those found in the usual type I, IIA and IIB fibres.

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