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Myofibrillar-protein isoforms and sarcoplasmic-reticulum Ca^{2+}-transport activity of single human muscle fibres

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In this study the polymorphism of myofibrillar proteins and the Ca^{2+}-uptake activity of sarcoplasmic reticulum were analysed in single fibres from human skeletal muscles. Two populations of histochemically identified type-I fibres were found differing in the number of light-chain isoforms of the constituent myosin, whereas the pattern of light chains of fast myosin of type-IIA and type-IIB fibres was indistinguishable. Regulatory proteins, troponin and tropomyosin, and other myofibrillar proteins, such as M- and C-proteins, showed specific isoforms in type-I and type-II fibres. Furthermore, tropomyosin presented different stoichiometries of the α- and β-subunits between the two types of fibres. Sarcoplasmic-reticulum volume, as indicated by the maximum capacity for calcium oxalate accumulation, was almost identical in type-I and type-II fibres, whereas the rate of Ca^{2+} transport was twice as high in type-II as compared with type-I fibres. It is concluded that, in normal human muscle fibres, there is a tight segregation of fast and slow isoforms of myofibrillar proteins that is very well co-ordinated with the relaxing activity of the sarcoplasmic reticulum. These findings may thus represent a molecular correlation with the differences of the twitch-contraction time between fast and slow human motor units. This tight segregation is partially lost in the muscle fibres of elderly individuals.

Myofibrillar proteins show an extensive polymorphism according to the tissue of origin [skeletal, cardiac or smooth muscle (Dhoot & Perry, 1979)]. In skeletal muscle, different isoforms of myosin are synthesized during development from embryonic to adult stage (Whalen *et al.*, 1979; Roy *et al.*, 1979; Gauthier *et al.*, 1982) and according to the specialization of adult muscle (i.e. fast-twitch or slow-twitch).

Although some of the differences may be related to post-translational events, such as methylation of histidine residues of fast-muscle myosin (Johnson & Perry, 1970), amino acid-sequence analysis demonstrated heterogeneities in the primary structure (Huszar & Elzinga, 1972), indicating that the different isoforms are indeed products of different genes. In recent years, recombinant-DNA techniques have provided definite evidence for the existence of different genes coding for myosin isozymes, and have suggested that muscle differentiation is linked to gene activation and repression rather than deletion and/or amplification (Nguyen *et al.*, 1983; Wydro *et al.*, 1983).

Adult human skeletal muscles are mixtures of fibres characterized by differences in twitch-contraction time, degree of resistance to fatigue [see references in MacComas (1977)], SR Ca^{2+}-uptake activity (Salviati *et al.*, 1982*b*) and histochemical myosin ATPase (Brooke & Kaiser, 1972). Biochemical analyses showed that myosin isolated from human muscle presents both fast and slow isoforms in electrophoretic gels either under non-denaturating conditions (Fitzsimmons & Hoh, 1981) or in SDS-containing gels (Sreter *et al.*, 1976; Dalla Libera *et al.*, 1978; Volpe *et al.*, 1981). Segregation of fast and slow myosin isoforms in individual fibres has been reported (Pette *et al.*, 1979; Billeter *et al.*, 1981; Takagi *et al.*, 1982). However, in these studies, an extensive heterogeneity in the expression of fast and slow myosin light chains is reported, which is difficult to reconcile with the stoichiometry of light chains of myosin isolated from the bulk muscle (Fitzsimmons & Hoh, 1981; Volpe *et al.*, 1981). Furthermore, only myosin iso-

Abbreviations used: SR, sarcoplasmic reticulum; TM, tropomyosin; TN, troponin; MHC, myosin heavy chain; MLC, myosin light chain.

enzymes were analysed, whereas immunohistochemical studies showed that, in addition to myosin, fast isoforms of TM and of TN I are segregated in fast fibres (Dhoot & Perry, 1979; Dhoot *et al.*, 1979) and, recently, the existence in human muscles of polymorphism of TM has also been suggested (Billeter *et al.*, 1982).

Mechanically and chemically skinned fibres have long been used to study the physiological properties of amphibian and mammalian skeletal and cardiac muscles. By using this preparation, two populations of fibres differing in the rate of Ca^{2+}-transport activity of the SR and its sensitivity to cyclic AMP were recently identified in human skeletal muscle (Salviati *et al.*, 1982*a*).

Here we present the results of a study on the isoform composition of myofibrillar proteins and on Ca^{2+}-uptake activity of SR of single fibres chemically skinned from several normal human muscles. Our data suggest that, in human muscle fibres, the segregation of fast and slow isoforms of myosin, TM and TN is correlated with the Ca^{2+}-transport activity of the SR. This co-ordinated control over the expression of the two key sites of regulation of the contraction–relaxation cycle is partially lost from muscles from elderly subjects.

Materials and methods

Materials

Acrylamide, *NN'*-methylenebisacrylamide and urea were purchased from Merck (Darmstadt, Germany); 2-mercaptoethanol, Nonidet P40 and glycerol were purchased from BDH Chemicals (Poole, Dorset, U.K.); *Staphylococcus aureus* V8 proteinase was purchased from Miles Laboratories (Stoke Poges, Slough, Berks., U.K.); Tris, glycine and ATP (vanadium-free) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); SDS was from Fisher Scientific Co. (Fair Lawn, NJ, U.S.A.) or BDH Chemicals (Poole, Dorset, U.K.). Ampholines were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals were of analytical grade.

Methods

Two normal human pectoralis muscles were obtained from radical mastectomy. Vastus lateralis, vastus medialis and sartorius muscle biopsies were obtained from uninvolved tissue during surgery for a sarcoma. Normal biceps brachialis muscle was obtained from a biopsy of a volunteer healthy woman. The muscle biopsies, all obtained with informed consent, were immediately placed on ice, and when they reached the laboratory (15–20min) they were completely relaxed. Bundles containing several hundred fibres were excised from the bulk of the muscle after being tied to a wooden stick and stretched to 110–120% of slack length.

Each specimen was chemically skinned in 5mM-K_2EGTA/170mM-potassium propionate/2.5mM-Na_2K_2ATP/2.5mM-magnesium propionate/10mM-imidazole buffer, pH7.0, as reported previously (Salviati *et al.*, 1982*b*). After this treatment, fibres were almost completely devoid of soluble sarcoplasmic proteins. Fibres were stored at −20°C for several months in the same 'skinning' solution, but containing 50% (v/v) glycerol.

Single fibres were isolated under a dissecting microscope. After cutting small segments for histochemical assay of myofibrillar ATPase, fibres were solubilized by incubating overnight in 2.3% (w/v) SDS/5% (w/v) 2-mercaptoethanol/10% (v/v) glycerol/62mM-Tris/HCl, pH6.8. The electrophoretic protein pattern was unaffected by the solubilization at 90°C for 2min.

SDS/polyacrylamide gel electrophoresis was carried out essentially by the method of Laemmli (1970). The separating gel (0.5 or 0.75mm thick) was 5% acrylamide or a 10–20% (w/v)-polyacrylamide linear gradient. The electrophoresis buffer was 288mM-glycine/0.1% (w/v) SDS/32.5mM-Tris, pH8.3. Electrophoresis was carried out overnight at 50V. After electrophoresis the gel was stained with Coomassie Blue or silver as reported previously (Salviati *et al.*, 1982*a*). Cytochrome *c* (M_r 11700), carbonic anhydrase (29000), bovine serum albumin (68000) and phosphorylase (94000) were used as markers for M_r determinations.

The proteolytic digestion of myosin heavy chain (MHC) was carried out by the method of Cleveland *et al.* (1977) as previously described (Salviati *et al.*, 1982*a*).

Two-dimensional gel electrophoresis was done by the method of O'Farrell (1975), using, for the first dimension, 2% (v/v) Ampholines, pH3–10. In the second dimension, the acrylamide concentration of the separating gel (0.5 or 1mm thick) was 12.5%.

Histochemical myosin ATPase of small segments of single muscle fibres was assayed with the method of Padikula & Hermann (1965) after preincubation at pH10.4, 4.6 and 4.35 as described previously (Salviati *et al.*, 1982*a*).

Ca^{2+} uptake was measured at 25°C in 10% muscle homogenate in 0.3M-sucrose. The incubation medium (1.0ml) was 170mM-KCl/2.5mM-Na_2K_2ATP/2.5mM - $MgCl_2$/5mM - oxalate/5mM - NaN_3/10mM-imidazole buffer, pH7.0. Ca^{2+} concentration was kept at pCa6.4 with Ca–EGTA buffers (Salviati *et al.*, 1982*b*). Radioactivity was measured in the filtrates by liquid-scintillation spectrometry.

Ca^{2+} uptake by chemically skinned single muscle fibres was measured at 25°C by monitoring

the increase of light-scattering in the same incubation medium after the addition of 5mM-oxalate. Calibration of the light-scattering signal was performed with $^{45}Ca^{2+}$, as described previously (Salviati *et al.*, 1982*b*).

Myosin was purified from vastus lateralis muscle by the method of Szent-Gyorgyi (1951).

Protein was determined by the method of Lowry *et al.* (1951), in a final volume of 0.6ml, with bovine serum albumin as standard.

Results

In this study about 600 single fibres from several normal human skeletal muscles were analysed. A representative pattern of myofibrillar proteins after separation by one-dimensional SDS/polyacrylamide-gel electrophoresis is shown in Fig. 1. Since all human skeletal muscles are composed of a mixture of type-I and type-II fibres, each fibre was also typed by the histochemical reaction for myofibrillar ATPase.

Type II (fast) fibres (Fig. 1, lanes *a–c*, *f* and *g*) show the fast type of myosin light chains (MLC) (LC1F, LC2F and LC3F) with apparent M_r identical with those of rabbit fast MLC (i.e. M_r 25000, 18000 and 16000) as can be seen by comparison with MLC of a type-II fibre from rabbit psoas muscle (lane *e*). On the other hand, type-I (slow) fibres (lanes *h*, *j* and *k*) show the slow type of MLC, which is characterized by an M_r of 25500 and 19000 for LC1Sb and LC2S respectively. The stoichiometry of human slow MLC (Fig. 1) shows an abnormal prevalence of LC1Sb with respect to LC2S, because of the contamination of the light-chain band by the slow isoform of TN I, which has a very closely similar M_r (see, e.g., Fig. 1*d*, 1*i* and 1*h*, 1*j*, and 1*k*). When the two protein bands are resolved by two-dimensional electrophoresis (Fig. 3*a*), the ratio of the two slow MLCs is about 1:1.

Heterogeneity of myosin light chains

As shown in Fig. 2, both type-II and -I human fibres are heterogeneous with respect to MLC pattern.

In type-II fibres the heterogeneity is dependent mainly on variations in the amount of LC3F. The content of LC3F in human fast myosin is generally

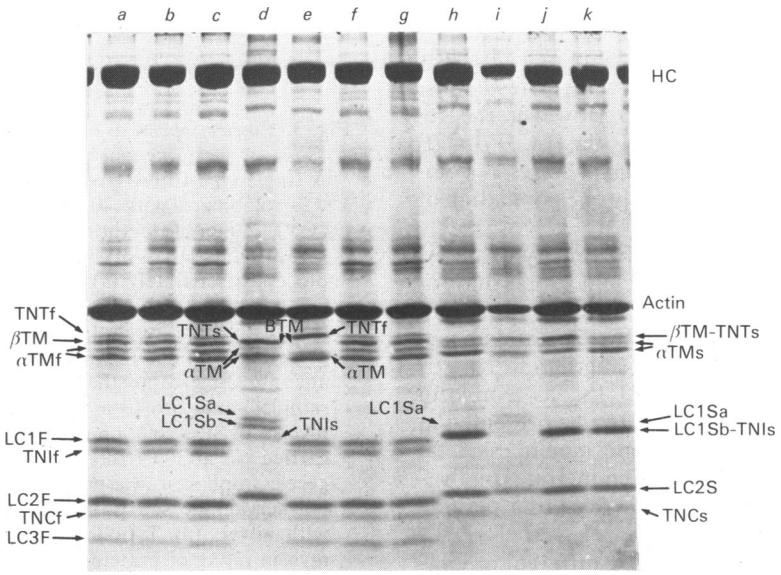

Fig. 1. *Myofibrillar protein patterns of single human muscle fibres*

A bundle of fibres from human biceps brachialis muscle was chemically skinned as described in the Materials and methods section. Isolated single fibres were cut into four segments. Three segments were assayed for histochemical myofibrillar ATPase. The longer segment (about 5mm) was solubilized by overnight incubation with 20μl of 2.3% SDS/5% 2-mercaptoethanol/10% glycerol/62.5mM-Tris/HCl, pH6.8. Electrophoresis was carried out on 10–20% (w/v)-polyacrylamide linear gradient gels. Gels were stained with Coomassie Blue. Human proteins were identified by co-migration with myofibrillar proteins from rabbit muscles. *e*, type-II fibre from rabbit psoas muscle; *a–c*, *f*, *g*, type II human fibres; *d* and *i*, type-I fibres from rabbit semitendinosus muscle; *h*, *j* and *k*, type-I human fibres. Abbreviations used: f, fast isoforms; s, slow isoforms; HC, myosin heavy chains; LC, myosin light chains; TM, tropomyosin; TN T, I and C: troponin T, I and C.

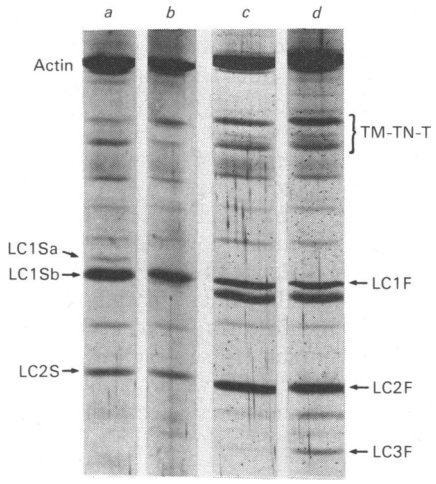

Fig. 2. *Heterogeneity of MLC patterns of single human muscle fibres*

SDS/polyacrylamide-gel electrophoresis was carried out as in Fig. 1. Gels were stained with silver. *a* and *b*, type-I fibres; *c* and *d*, type-II fibres. Other abbreviations are as for Fig. 1. Only the region of MLCs is shown. In this Figure, representative patterns of MLC of single fibres obtained from different electrophoretic separations were combined.

low (6% of total LC material as compared with 20% of rabbit fast myosin), but there are fibres almost lacking this light chain (Fig. 2*c*). However, the frequency-distribution pattern of the LC3F/LC2F molar ratio among type II human fibres is compatible with the existence of only one population of fast fibres (result not shown).

On the other hand, the heterogeneity of type-I fibres is due to the presence, in some fibres only, of an additional light chain (LC1Sa) with an apparent M_r of 26000 (Figs. 1*h* and 1*k*; Fig. 2*a*), and an isoelectric point higher than 6.0 (Fig. 3*a*).

The identification of the LC1Sa as a myosin light chain is supported by the results presented in Fig. 3. Myosin purified from human skeletal muscle contains a peptide that co-migrates on two-dimensional gel electrophoresis with the peptide identified in type-I fibres (Fig. 3*c*).

Different human muscles have different proportions of fibres showing the LC1Sa light chain. As shown in Table 1, in biceps branchialis and in pectoralis minor muscles about 50% of type-I fibres contain this chain; in pectoralis major the percentage is decreased to 25%, whereas in leg muscles (vastus lateralis and vastus medialis) it is 75%.

Myosin heavy chains

It has been previously shown that the heavy chains of fast and slow human myosin differ in

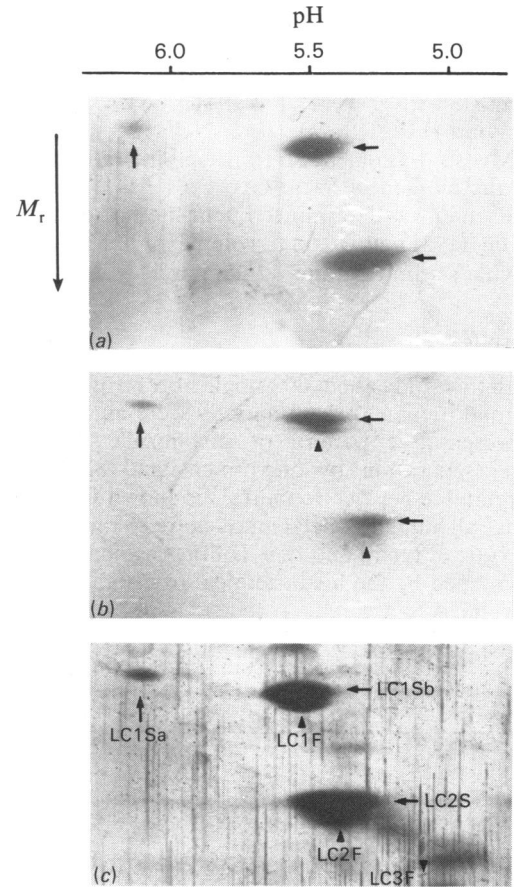

Fig. 3. *Co-electrophoresis of type-I-fibre protein and purified human myosin*

A single type-I fibre from pectoralis minor muscle was solubilized with 20 μl of 2.3% (w/v) SDS/5% (v/v) 2-mercaptoethanol/10% (v/v) glycerol/62.5 mM-Tris/HCl, pH 6.8. a portion (10 μl) was processed separately (*a*). The second 10 μl portion was combined with 10 μg of purified human myosin (*b*). Just before isoelectrofocusing, 20 μl of 9.5 M-urea, 2% Nonidet P40, 2% Ampholine (pH 3–10) and 5% 2-mercaptoethanol were added to these samples, and also to a sample (10 μg) of purified myosin in SDS solution (*c*). Isoelectrofocusing was carried out at 300 V for 18 h. SDS/polyacrylamide-gel electrophoresis was performed on a 12.5% (w/v)-polyacrylamide separating gel. Gels were stained with silver. (*a*) Type-I single fibre; (*b*) purified myosin (10 μg of protein); (*c*) co-electrophoresis of type-I fibre protein and purified myosin (10 μg of protein). Only the region of MLCs is shown. Abbreviations used: LCS, slow myosin light chains; LCF, fast myosin light chains.

both immunological reactivity (Billeter *et al.*, 1980) and peptide mapping (Billeter *et al.*, 1981). Figs. 4(*a*) and 4(*b*) demonstrate that when SDS/poly-

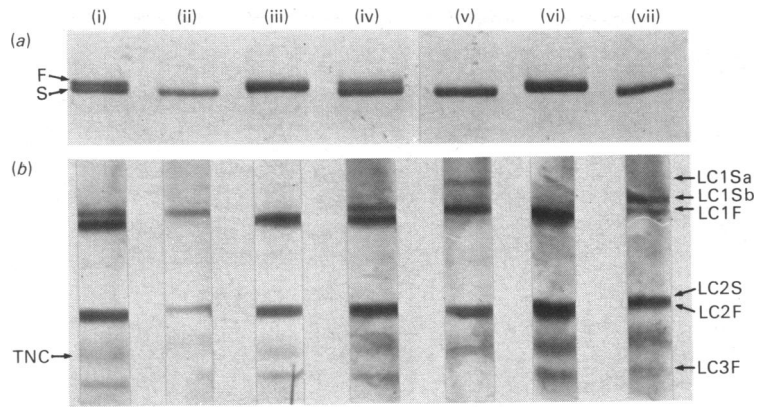

Fig. 4. *Myosin hybrids and coexistence of fast (F) and slow (S) MHCs in single human muscle fibres*
Single fibres were isolated from sartorius muscle and were solubilized with 20 μl of the solubilizing solution (see the Materials and methods section). Portions (5 μl) were used for the analysis of heavy chains, and the remaining portions for that of light chains. (*a*) SDS/polyacrylamide-gel electrophoresis of 5% polyacrylamide gels; only the region of MHC is shown. (*b*) SDS/polyacrylamide-gel electrophoresis on a 10–20% polyacrylamide linear gradient. Gels were stained with silver. Only the region of MLC is shown: (i) and (vi), intermediate single fibres; (ii), (iii) and (vii) type-I single fibres; (iii) and (vi), type-II single fibres.

acrylamide-gel electrophoresis is performed on 5% polyacrylamide gels, i.e. under conditions suitable for the analysis of high-M_r peptides such as MHC (M_r 200000) (Rushbrook & Stracher, 1979; Carraro & Catani, 1983), human fast and slow MHC show different electrophoretic mobilities. This method, however, appears to be inadequate to show differences between the heavy chains of myosin from type II A and type II B human fibres, although they have been demonstrated by peptide mapping (Billeter *et al.*, 1981).

Regulatory proteins

The electrophoretic analysis of human skeletal-muscle fibres shows that different isoforms of TN T, I and C and of TM, are expressed in type-II and -I fibres (Figs. 1, 5 and 6). Fast TN T and TN C have an higher, and fast TN I a lower, M_r than the corresponding slow isoforms.

α-TM is composed of two subunits (α and α') both in type-I and type-II fibres (Fig. 6). However, the two α-subunits of fast TM have apparent M_r values different from those of the corresponding slow subunits, since they do not co-migrate when they co-exist in the same fibre (Fig. 5). Small differences in the apparent M_r between the fast and the slow β-TM subunits can also be recognized (Figs. 6*a*–6*h*).

Further heterogeneity is found in relation to the stoichiometry of α- and β-subunits in human fibres. In type-II fibres α-TM is always the predominant form, whereas two populations of type-I fibres can be identified, one showing the predominance of the β-TM subunit and the other that of the α-subunit. This latter population is represented by those type-I fibres that contain the LC1Sa myosin light chain (Figs. 6*b*, 6*c* and 6*h*).

Other myofibrillar proteins

Fig. 7 shows the pattern of M- and C-proteins in SDS/polyacrylamide/urea gels. The two subunits of M-protein are found to differ by apparent M_r in type-II and type-I fibres, whereas of the two peptides of C-protein, one is shared by the two types of fibres and the second is different.

Ca^{2+}-transport activity

Since preliminary results showed a very good correlation between the histochemical reaction for myofibrillar ATPase and the pattern of myosin light chains of human single fibres, we used this criterion to classify the fibres where Ca^{2+}-uptake activity was measured. Maximum Ca^{2+} accumulation in the presence of 5mM-oxalate by single human muscle fibres is similar, on a protein basis, to that measured in total muscle homogenate from normal pectoralis minor muscle (60 μmol of Ca^{2+}/15min per g of muscle protein). Fast fibres, i.e. fibres showing the fast type of MLC, have a capacity for Ca^{2+} oxalate that is 30% higher than that of slow fibres [84 $\pm$ 11 (S.E.M.) μmol/g of fibre protein ($n = 17$) and 62 + 5 (S.E.M.) μmol/g of fibre protein ($n = 9$) respectively]. On the other hand, the mean rate of Ca^{2+}-transport at pCa 6.4 of fast fibres is 6.4 μmol/min per g of fibre protein as compared with 2.4 μmol/min per g of fibre protein of

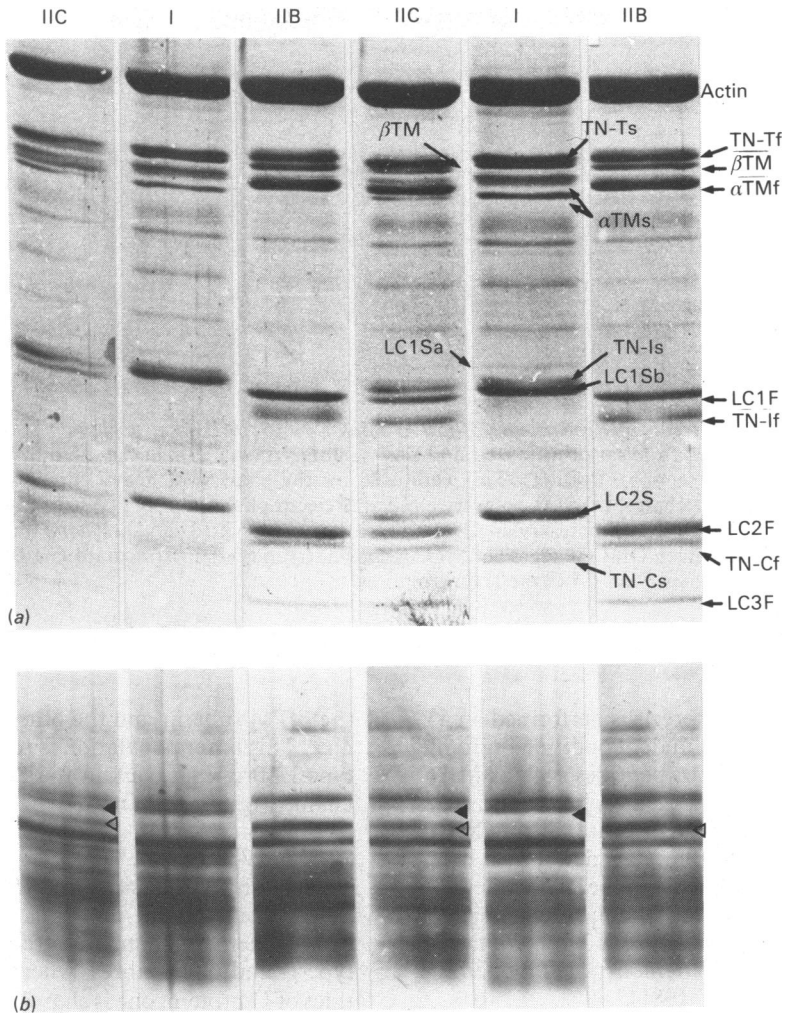

Fig. 5. *Coexistence in type IIC of fast and slow isoforms of both heavy and light chains of myosin*
Single fibres from human pectoralis major were typed by histochemical assay of myofibrillar ATPase. Three to four fibres of the same histochemical type were combined and analysed by SDS/polyacrylamide-gel electrophoresis as reported in Fig. 1. (*a*) Patterns of MLC after staining with Coomassie Blue. Abbreviations are as in Fig. 1. (*b*) Peptide mapping of MHC. Protein bands corresponding to MHC were cut out from the electrophoretic gel shown in (*a*) and were loaded on a second gel after equilibration for 30min in 20ml of 1mM-EDTA/1mM-2-mercaptoethanol/1% SDS/125mM-Tris/HCl, pH6.8 (Cleveland *et al.*, 1977). Peptide mapping of MHC was obtained by digestion with 12 μg (total) of *Staphylococcus aureus* V8 proteinase during the migration in the stacking gel (90min). Under these conditions, extensive proteolysis of MHC was achieved, since almost all peptides exhibit M_r values lower than 20000. Peptides were separated on 20cm of a 15–22.5% (w/v)-acrylamide linear gradient gel. The gel was stained with silver. ▶, Peptide specific to type-I-fibre MHC; ▷, peptide specific to type-II-fibre MHC.

slow fibres. Thus the fractional rate of filling the SR (rate of loading normalized to the maximum capacity) of fast fibres is twice as much that of slow fibres ($0.07\,min^{-1}$ and $0.03\,min^{-1}$ respectively). Both fast and slow fibres show a certain degree of variability of maximum capacity and rate of loading. However, in agreement with previous results (Salviati *et al.*, 1982*b*), the frequency distribution of the fractional rate of filling of the SR (min^{-1}) (Fig. 8), shows that fast and slow fibres represent two distinct populations of fibres.

Myosin hydrids and coexistence of fast and slow myosin in single human fibres

The segregation of fast and slow isoforms of MLC in human muscle fibres is very strict. How-

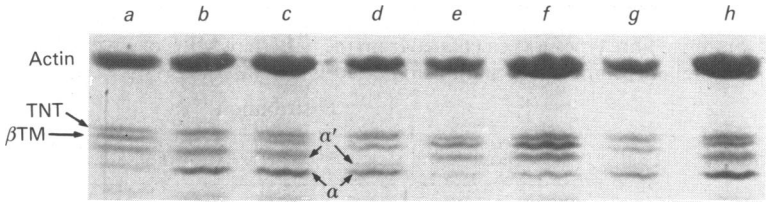

Fig. 6. *Polymorphism of TM subunits in fast and slow human fibres*
SDS/polyacrylamide-gel electrophoresis of single fibres was performed as described in Fig. 1. The gel was stained with Coomassie Blue. Only the region corresponding to TM subunits is shown. Abbreviations used: TN T, troponin T; *a* and *e*, type-I fibres showing LC1Sb and LC2S myosin light chains; *b*, *c*, *f*, and *h*, type I fibres showing LC1Sa, LC1Sb and LC2S myosin light chains; *d* and *g*, type-II fibres.

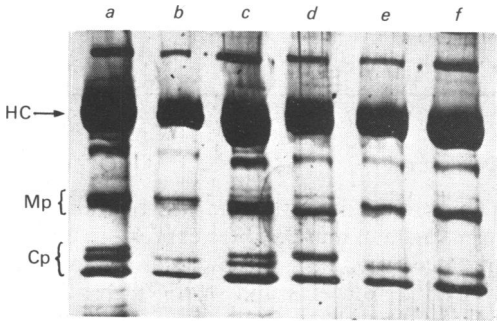

Fig. 7. *Segregation of specific isoforms of M(Mp)- and C(Cp)-proteins in fast and slow human fibres*
SDS/polyacrylamide-gel electrophoresis of single human fibres from vastus lateralis muscle was carried out on a 7–15% (w/v)-polyacrylamide linear gradient gel. Urea (6M) (Sender, 1971) was present in the solubilizing solution and in the stacking and separating gel. The gel was stained with silver. Only the region of high M_r is shown. M- and C-proteins were identified on the basis of M_r and of the co-migration with the corresponding proteins from rabbit muscle fibres (Salviati *et al.*, 1982*a*). *a* and *c*, Intermediate fibres; *b*, *e* and *f*, type-I fibres; *d*, type-II fibres. Abbreviation used: HC, heavy chain.

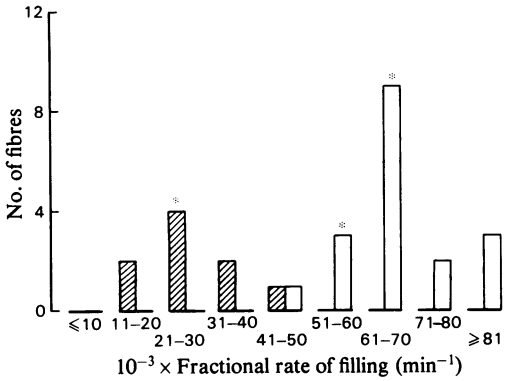

Fig. 8. *Differences of* Ca^{2+}*-uptake activity of fast and slow human fibres*
SR Ca^{2+}-uptake activity of single human muscle fibres was measured in the presence of 5mM-oxalate by a light-scattering technique, as reported previously by Salviati *et al.* (1982*b*). After Ca^{2+}-uptake measurements, fibres were solubilized and electrophoresed as described in Fig. 1. Fibres were typed according to the pattern of MLC. Fractional rate of filling the SR is the ratio between the rate of transport (μmol of Ca^{2+}/min per g of protein) and the maximum capacity for calcium oxalate (μmol/g of protein) ▨, slow fibres; □, fast fibres; *, intermediate fibres, showing coexistence of fast and slow MLC. Each asterisk indicates results for a single intermediate fibre.

ever, a small percentage of fibres (about 2%) (Table 1) present a pattern of MLC characterized either by the presence of three fast and two slow MLCs (the LC1Sa light chain is never found in these fibres) or by the presence of MLC of one type and an extra chain of the other type (Table 1). Usually, fibres with coexistence of all fast and slow MLCs give an histochemical reaction characteristic of type-IIC fibres, i.e. the ATPase activity is not inhibited either by alkaline (pH 10.4) or by acid (pH 4.35) preincubation, whereas fibres with the extra light chain are histochemically either type-II (A or B) or type-I fibres. This different histochemical reactivity is explained by the results reported in Fig. 4 and Fig. 5. Histochemical type-IIC fibres contain both fast and slow MHC, although in different proportions, as shown by one-dimensional SDS/polyacrylamide-gel electrophoresis (Figs. 4*a* and 4*d*). Furthermore, peptide mapping after digestion of MHC (Fig. 5) with the V8 proteinase from *Staphylococcus aureus* shows coexistence in type-IIC fibres of peptides specific for both type-I and type-II MHC.

Table 1. *Fibre composition of normal human skeletal muscles*
The percentage distribution of fibre types was calculated from the pattern of MLC of single human fibres after SDS/polyacrylamide-gel electrophoresis and silver stain as reported under 'Methods'.

Muscle	(No. of fibres)	Distribution (%)								
		Fast	Slow		Intermediate					
		LC1F LC2F LC3F	LC1Sb LC2S	LC1Sa LC1Sb LC2S	LC1S LC2S LC1F	LC1S LC2S LC2F	LC1S LC2S LC3F	LC2S LC1F LC2F LC3F	LC1S LC2S LC1F LC2F LC3F	MLC pattern
Biceps	(122)	57.4	19.7	19.7	—	—	—	1.6	1.6	
Pectoralis minor	(262)	48.5	24.0	25.6	—	—	—	0.4	1.5	
Pectoralis major	(56)	32.1	10.7	3.6	—	1.8	—	12.5	39.3	
Sartorius	(62)	14.5	37.1	17.8	16.1	1.6	—	4.8	8.1	
Vastus lateralis	(42)	14.3	11.9	40.5	33.4	—	—	—	—	
Vastus medialis	(41)	17.1	7.3	36.6	7.3	—	—	2.4	7.3	

In contrast, those fibres having only one extra light chain show only one type of heavy chain (Fig. 4, vii), indicating that the constituent myosin is a molecular hybrid, since light chains of one type are assembled with heavy chains of the other type. In these fibres the myofibrillar ATPase is inhibited either by alkaline or acid preincubation, depending on the type of MHC.

In these intermediate fibres, coexistence of the regulatory proteins TM and TN (Fig. 5) and of other proteins (M- and C-proteins; Figs. 7*a* and 7*c*) can also be found. On the other hand, the rate of SR Ca^{2+}-uptake activity in intermediate fibres is variable. As shown in Fig. 8, in one fibre the fractional rate of filling the SR is $0.07\,min^{-1}$, i.e. it is in the range of that shown by fast fibres, whereas in two other fibres the rate is intermediate or in the range of that shown by slow fibres (0.056 and $0.03\,min^{-1}$ respectively).

Myosin composition of single fibres in muscle of elderly individuals

Table 1 shows the MLC composition of single fibres from pectoralis major, sartorius, vastus medialis, and vastus lateralis muscle biopsies that were obtained from two women who were over 70 years old. All muscles have a low content of fast fibres and a concomitant high content of intermediate fibres (true mixed or fibres with myosin hybrids). These results are in contrast with the low amount of type-IIC fibres in normal human muscles (Brooke & Kaiser, 1972) and with the low content of intermediate fibres shown by biceps brachialis and pectoralis minor muscle biopsies (Table 1) that were obtained from younger individuals.

Discussion

The present investigation has established that normal human muscle fibres express, in a very co-ordinated way, fast or slow isoforms of several myofibrillar proteins and of SR membrane proteins, and that the co-ordination is partially lost in muscles of elderly individuals.

Myosin light and heavy chain composition

On the basis of the histochemical staining for myofibrillar ATPase, human muscles are mixture of type-I, type-IIA and type-IIB fibres. The differential staining for myosin ATPase at different pH values implies that different types of myosin are located in each fibre (Weeds & Taylor, 1975). Immunohistochemical studies (Billeter *et al.*, 1980), as well as biochemical characterization of myosin of single fibres, have confirmed that different isoforms of myosin are present in different fibre types (Pette *et al.*, 1979; Billeter *et al.*, 1981; Takagi *et al.*, 1982).

The present investigation has demonstrated that the differences between fast and slow heavy chains of human myosin are sufficient to permit their separation by one-dimensional SDS/polyacrylamide-gel electrophoresis, providing a suitable acrylamide concentration is used for the analysis of protein of high M_r (Rushbrook & Stracher, 1979).

We have recently shown that rabbit muscle type-IIA fibres are characterized by a lower content of LC3F myosin light chain (Salviati *et al.*, 1982*a*). Pons *et al.* (1983) reported that myosin isolated from human muscles with predominance of type-IIA fibres shows a lower content of this chain. Our results, however, indicate that myosin of type-IIB

and type-IIA human fibres has a similar content of LC3F. Since the biopsy used by Pons *et al.* (1983) was from a pathological muscle, it is possible that the low content of LC3F of the isolated myosin is due mainly to the disease process and not to the predominance of type-IIA fibres. Preliminary results on hypothyroid muscles seem to support such an interpretation.

Previous works on isolated myosin (Volpe *et al.*, 1981) and on single fibres (Pette *et al.*, 1979; Billeter *et al.*, 1981; Takagi *et al.*, 1982) provided evidence for the existence, in slow human myosin, of two light chains, LC1S and LC2S. The results reported here show that human slow myosin contains a third light chain with molecular properties similar to those of the LC1Sa light chain of rabbit, rat and cat slow myosin (Biral *et al.*, 1982; Carraro *et al.*, 1981; Weeds, 1976). Further support for the interpretation that LC1Sa is indeed a slow myosin light chain comes from the results of Fitzsimmons & Hoh (1981), who demonstrated that the LC1Sa light chain co-purifies with human slow myosin in a non-denaturing gel-electrophoresis system.

Human type-I fibres, at variance with rabbit slow fibres, where a continuous variation in the amount of LC1Sa has been described among type-I fibres (Schachat *et al.*, 1980; Pinter *et al.*, 1981; Salviati *et al.*, 1982*a*), either do or do not contain this light chain. Factor(s) that regulate the synthesis of this light chain apparently seem to regulate also the synthesis of slow α-TM, since this subunit predominates in those fibres showing the LC1Sa light chain.

Regulatory proteins

In species such as the rabbit, slow TM is characterized by two distinct α-subunits differing in M_r as well as in isoelectric point (Salviati *et al.*, 1982*a*; Heeley *et al.*, 1983). Our results show that, in human muscle, splitting of α-TM is shown by both type-I and type-II (A and B) fibres. On the other hand, on the basis of the apparent M_r and in agreement with previous peptide-mapping and sequence studies (Billeter *et al.*, 1982; Romero-Herrera *et al.*, 1982), one can conclude that fast and slow human α-TM subunits are different peptides.

It has been reported (Billeter *et al.*, 1982) that in type-I and type-II human fibres the amounts of α- and β-TM are equivalent. However, our results show that the stoichiometry of the two subunits in human type-I and type-II fibres resembles that of the corresponding fibres of other species by showing predominance of β-TM and α-TM respectively. An exception to this trend is represented by those type-I fibres that contain myosin LC1Sa light chain, where a clear predominance of α-TM is seen. These differences in the pattern of TM subunits, together with the differences of the pattern of TN isoforms, may explain the differences in Sr^{2+} affinity found in type-I and type-II human fibres (Takagi *et al.*, 1978). Preliminary results obtained by analysing the pCa–tension relationship demonstrate that the tension developed by type-I and type-II human fibres at pCa 6.4 is 34 and 6% of the maximal tension (P_0) respectively.

The results reported here have demonstrated the existence, in type-I and type-II human fibres, of distinct isoforms of other myofibrillar proteins such as M- and C-protein. The presence of a polymorphism of the M-protein may correlate with the reported differences of the ultrastructural appearance of the M-line in fast and slow human fibres (Sjostrom *et al.*, 1982). On the other hand, polymorphism of the C-protein was recently demonstrated in fast and slow chicken muscles (Reinach *et al.*, 1982).

Ca^{2+} uptake by SR

The study of Ca^{2+}-uptake activity demonstrates that the main differences between the type-I and type-II human fibres are not related to the SR volume, as indicated by the small difference in maximum capacity for calcium oxalate (Briggs *et al.*, 1977), but to the rate of Ca^{2+} transport, which is about one-half in type-I fibres compared with type-II fibres (Salviati *et al.*, 1982*b*). The analysis of the frequency distribution of the fractional rate of filling of the SR clearly shows that type-II fibres are a homogeneous population, indicating that differences in Ca^{2+}-uptake activity between type-IIA and type-IIB fibres should be relatively small or zero. This conclusion is supported by the results obtained by MacComas (1977), which show that, in human muscles, the twitch-contraction times of fast fatigue-resistant motor units (type-IIA fibres) and fast fatigue-sensitive motor units (type-IIB fibres) are superimposable.

We have already demonstrated that the differences in the rate of Ca^{2+} uptake between fast and slow fibres cannot be accounted for by differences in affinity of the transport ATPase for Ca^{2+}, but are dependent on differences in the turnover rate of the Ca^{2+} pump (Salviati *et al.*, 1982*b*). Furthermore, Ca^{2+}-transport activity of type-I fibres is modulated by the activity of a cyclic AMP-dependent protein kinase (Salviati *et al.*, 1982*b*). The sensitivity to this modulatory system exhibited by only type-I-fibre SR suggests intrinsic differences in the Ca^{2+}-transport systems of the two types of fibre. By analogy with the different immunological reactivities of the Ca^{2+}-pump protein from fast and slow rabbit SR (Damiani *et al.*, 1981), one may suggest the existence of specific isoforms of the Ca^{2+}-pump protein in human type-I and type-II fibres. Results of an immunofluorescence study on the reactivity of type-II and type-I human fibres

with anti-(rabbit fast SR Ca^{2+}-ATPase) antibody support this hypothesis (Salviati *et al.*, 1984).

All together these results indicate that the expression of fast and slow isoforms of myofibrillar and of SR proteins, which represents the molecular correlates of the differential physiological characteristics of human fast and slow motor units, is under the same co-ordinated control. The co-ordination is apparently lost in muscles of elderly individuals. In these muscles there is an increased number of fibres showing coexistence of fast and slow isoforms of both myosin and other myofibrillar proteins, and of fibres with myosin hybrids. The fact that these hybrids are found not only in type-I fibres (Billeter *et al.*, 1981; Takagi *et al.*, 1982) but also in type-II (A and B) fibres and that almost all the possible patterns of light chains are found in these conditions, suggests that various mechanisms may affect the synthesis and/or the catabolism of MHC and MLC.

The intermediate fibres are interpreted as transitional fibres, i.e. fibres of one type which are going to be transformed into the opposite type. In the experimental animals the transition is obtained by cross-innervation or by chronic electric stimulation (Sreter *et al.*, 1976; Gauthier *et al.*, 1983; Pette & Schnez, 1977). In human muscle, several mechanisms have been proposed to produce the same effect. For example, in elderly subjects, Jennekens (1971) has suggested an increased incidence of denervation and reinnervation processes, whereas Borg (1981) reported an increased proportion of motor neurons with electrical characteristics intermediate between those of fast and slow motor neurons.

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