Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres

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1. This study aims to assess the role of myosin heavy chain (MHC) and alkali myosin light chain (MLC) isoforms in determining maximum velocity of shortening in fast skeletal muscle fibres.

2. The maximum velocity of shortening as determined by the slack test (V_0) was tested for its relationship with MHC composition and with alkali MLC isoform ratio of fast fibres of known MHC composition.

3. MHC isoform composition was determined using sodium dodecyl sulphate– polyacrylamide gel electrophoresis (SDS–PAGE) and monoclonal antibodies against MHCs, and combining the results obtained using the two methods. Three groups of fast fibres containing only one MHC isoform were identified: IIA, IIX and IIB fibres containing respectively IIA MHC, IIX MHC and IIB MHC. Fibres containing more than one MHC isoform were discarded.

4. The mean V_0 value of IIA fibres was $2{\cdot}33 \pm 0{\cdot}29$ muscle lengths per second ($L\,s^{-1}$; mean ± S.D.), this was significantly lower than that for IIX fibres ($3{\cdot}07 \pm 0{\cdot}70\ L\,s^{-1}$) which in turn had a mean V_0 value significantly lower than that for IIB fibres ($3{\cdot}69 \pm 1{\cdot}01\ L\,s^{-1}$).

5. The relative proportion of alkali MLC isoforms (MLC_{3f}, MLC_{1f}) was determined by means of electrophoretic separation and densitometric quantification and was expressed as MLC_{3f}/MLC_{2f} with reference to the dithio-nitrobenzoic acid (DTNB) light chain (MLC_{2f}). The mean value of the MLC_{3f}/MLC_{2f} ratio was significantly lower in IIA than in IIX and IIB fibres.

6. V_0 was found to be proportional to the relative content of MLC_{3f} in IIA, IIX and IIB fibres. The slope of the regression line of V_0 *versus* MLC_{3f}/MLC_{2f} was significantly higher for fibres containing IIB MHC than for fibres containing IIX MHC.

7. These results show that both MHC content and the proportion of MLC_{3f} are important determinants of unloaded shortening velocity among fast fibres. The sensitivity of V_0 to variations in alkali MLC ratio depends on which MHC isoform is present. Variations in alkali MLC isoforms have much greater effects on V_0 in IIB than in IIX fibres.

It seems well established that mammalian skeletal muscle fibres containing the slow myosin heavy chain (I MHC) isoform have lower maximum velocity of shortening than fibres that contain any of the three known (IIA, IIB, IIX) fast MHC isoforms (Reiser, Moss, Giulian & Greaser, 1985; Greaser, Moss & Reiser, 1988; Sweeney, Kushmerick, Mabuchi, Sreter & Gergely, 1988; Bottinelli, Schiaffino & Reggiani, 1991). It is less well understood whether the large differences in maximum velocity of shortening among fast fibres are due to the presence of different fast MHCs, or on a different ratio between the two alkali myosin light chain isoforms (MLC_{1f} and MLC_{3f}), or on both.

Fibres containing IIA MHC have been reported to have lower mean values of unloaded shortening velocity evaluated by slack-test manoeuvres (V_0) than fibres containing IIB MHC (Eddinger & Moss, 1987; Sweeney *et al.* 1988; Rome, Sosnicki & Goble, 1990). A more recent study (Bottinelli *et al.* 1991) has for the first time also determined contractile properties of IIX MHC-containing fibres, and has shown that IIB fibres have mean maximum shortening

velocities, evaluated by extrapolating force–velocity curves to zero load, that are higher than II A and II X fibres.

Several studies suggest that alkali MLCs have a role in determining maximum velocity of shortening. Eddinger & Moss (1987) and Sweeney *et al.* (1988) have reported that V_0 is higher in fibres that contain larger amounts of MLC_{3f}. V_0 has been found to be proportional to MLC_{3f} content in a group of single fast fibres studied by Greaser *et al.* (1988). A possible modulation of V_0 based on the proportion of the slow (MLC_{2s}) and fast (MLC_{2f}) isoforms of dithio-nitrobenzoic acid (DTNB) MLC seems unlikely as fast fibres have been found to contain only one DTNB isoform, MLC_{2f} (Staron & Pette, 1987*b*; Wada & Pette, 1993).

The above observations may have some limitations due to: (1) the possible presence of undetected MHC isoforms; (2) the coexistence of different MHCs in the same fibre; and (3) preferential association between MHCs and alkali MLCs. Only one group (Bottinelli *et al.* 1991) has also studied the functional properties of the novel fast MHC, II X or II D (Schiaffino, Saggin, Viel, Ausoni, Sartore & Gorza, 1986; Bar & Pette, 1988; Schiaffino *et al.* 1989). In all other works, only two fast MHCs (II A and II B), at the most, have been identified, and fibres containing II X MHC have very probably been pooled together either with II A or with II B fibres according to the method of identification used (Larsson, Edstrom, Lindergren, Gorza & Schiaffino, 1991). None of the work on the functional role of MHC isoforms has reported the presence of more than one fast MHC in the same fibre, with the exception of a very recent paper by Larsson & Moss (1993) on human skeletal fibres, in which, however, only two (II A and II B) of the three fast MHC isoforms have been identified. MHC coexistence in mammalian muscle fibres has been shown to be very frequent (Lutz, Weber, Billiter & Jenny, 1979; Danieli-Betto, Zerbato & Betto, 1986; Staron & Pette, 1987*a*,*b*; Termin, Staron & Pette, 1989; De Nardi *et al.* 1993). Therefore all attempts, to date, to relate functional properties to MHC content might bear some uncertainty due to undetected coexistence of MHCs in the same fibre. Further difficulties in establishing a relation between V_0 and myosin isoforms have been caused by the preferential association between MLC_{3f} and II B MHC and between MLC_{1f} and II A MHC (Dalla Libera, Sartore, Pierobon-Bormioli & Schiaffino, 1980; Mabuchi, Szvetko, Pinter & Sreter, 1982; Salviati, Betto & Danieli-Betto, 1982; Wada & Pette, 1993). II B fibres, in fact, could be faster than II A fibres, not because they contained II B MHC, but because they contained larger amounts of MLC_{3f} and vice versa. To address this problem it is necessary to relate maximum shortening velocity to alkali MLC ratio in single fibres containing only one known MHC isoform. The only paper that has very recently followed this approach (Larsson & Moss, 1993) had to deal not only with the inability of separating all three fast MHC isoforms, but also with the problem that the human fast fibres used showed coexistence of the two isoforms of DTNB MLC, MLC_{2f} and MLC_{2s}. Under these circumstances, no relationship between V_0 and alkali MLC ratio was found either in II A or in II B fibres. Undetected MHC coexistence and variations in alkali MLC isoform ratio might be the basis of the large variability of maximum shortening velocity among fast fibres presumed to contain the same fast MHC isoform (Sweeney *et al.* 1988; Bottinelli *et al.* 1991; Larsson & Moss, 1993).

The aim of this work was to clarify the role of MHC and alkali MLC isoforms in determining V_0 in fast skeletal fibres. To reach this goal it was necessary to identify all three fast MHCs, and to address both the problem of MHC coexistence and that of preferential association between MHC and alkali MLC isoforms. MHC composition was analysed by combining electrophoretic and immunocytochemical methods. In this way not only could all four MHC isoforms be identified, but also the presence of an MHC isoform coexisting with a predominant one could be detected. This allowed us to discard data from fibres containing more than one MHC and to consider and compare fibres containing only one fast MHC. Moreover, V_0, MHC composition and alkali MLC ratio was determined in the same fibres, so that V_0 could be related to alkali MLC isoform ratio in single fibres containing only one known MHC isoform, either II A or II X or II B.

A preliminary report of this work has been published in abstract form (Bottinelli, Betto & Reggiani, 1993).

METHODS

Solutions

Skinning, relaxing, preactivating and activating solutions were prepared according to Bottinelli *et al.* (1991) with some modifications (Table 1). The Triton X-100 concentration (BDH Chemicals, Poole, Dorset, UK) was raised from 0·5 to 10 μl ml^{-1}. No caffeine was used, as the higher concentration of Triton X-100 was sufficient to disrupt the sarcoplasmic reticulum and prevent tension oscillations during activation.

Fibre dissection

Male Wistar rats (3–5 months old) were killed under anaesthesia induced by ether. Soleus, plantaris and tibialis anterior muscles were dissected and immersed in skinning solution at 12–15 °C. Only the superficial white part of the tibialis anterior muscle was used for experiments. Single fibres were isolated from the whole muscles while immersed in skinning solution under a stereomicroscope (Wild ×10–40). After 2–4 h, the solution was replaced for 1 h with the skinning solution containing Triton X-100. From each fibre two adjacent segments about 2 mm long were cut. One was used for mechanical measurements and thereafter characterized by monoclonal antibodies. The other segment was immersed in 20 μl of a solution of the following composition: 62·5 mM Tris-HCl, 2·3 % sodium dodecyl sulphate (SDS; w/v), 10 % glycerol (w/v), 5 % mercaptoethanol (Laemmli, 1970); it was stored at −20 °C and then characterized by SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

Table 1. Composition of the solutions (mM unless otherwise indicated)

Solution	Potassium propionate	KH_2PO_4	Magnesium acetate	Na_2ATP	EGTA	$CaCl_2$	CrP	CPK*
Skinning	150	5	5	3	5	—	—	—
	KCl	Imidazole	$MgCl_2$	Na_2ATP	EGTA	$CaCL_2$	CrP	CPK*
Relaxing	100	20	5	5	5	—	—	—
Preactivating	100	20	5	5	5	—	25	300
Activating	100	20	5	5	5	5	25	300

A skinning solution of the same composition as above, but containing in addition Triton X-100 (10 μl ml^{-1}) was also prepared. DTT (dithiothreitol, 1 mM) was present in all the solutions. The pH of all the solutions was set at 7·0. In the activating solution: pCa = 4·45; pMgATP = 2·41; pMg = 3·14. *Composition given in U ml^{-1}, where one unit transfers 1·0 μmol of phosphate from CrP to ADP per minute at pH 7·4 and 30 °C. Abbreviations: CrP, creatine phosphate; CPK, creatine phosphokinase.

Experimental set-up

The details of the experimental set-up have been previously described (Bottinelli *et al.* 1991). Three chambers milled in an aluminium plate were filled with relaxing, preactivating and activating solutions, respectively; the aluminium plate could be lowered, moved and raised to immerse the fibre quickly in any of the three chambers. The fibres were attached by aluminium foil clips to a force transducer (AE 801 Aksjeselskapet Mikroelektronikk, Horten, Norway; resonance frequency in water, 2 kHz) at one end and to an electromagnetic puller (model 101 vibrator, Ling Dynamic System, Royston, UK) at the other end. The electromagnetic puller was equipped with an inductance position transducer and driven by a feedback circuit. The electromagnetic puller could apply quick releases of different amplitudes completed in 1 ms. The set-up was placed on the stage of an inverted microscope (Axiovert 10, Zeiss, Germany). Sarcomere length was measured at ×320 magnification.

Experimental plan

To determine maximum shortening velocity slack-test manoeuvres were performed on 109 fibres. After the mechanical experiments the fibres were removed from the apparatus and used for immunocytochemical determination of MHC isoform composition. A separate segment of each fibre was used for MHC identification by SDS–PAGE. Fibres containing only one fast MHC isoform (see below) were further processed by SDS–PAGE to determine the ratio between the two alkali MLC isoforms.

Experimental procedure

The fibre segment was mounted in the chamber containing relaxing solution. Sarcomere length was set at 2·7 μm. Segment length was measured using a stereomicroscope fitted over the apparatus at ×40 magnification. Fibre diameter at three different locations along the length of the fibre was measured at ×320 magnification. Following the procedure previously

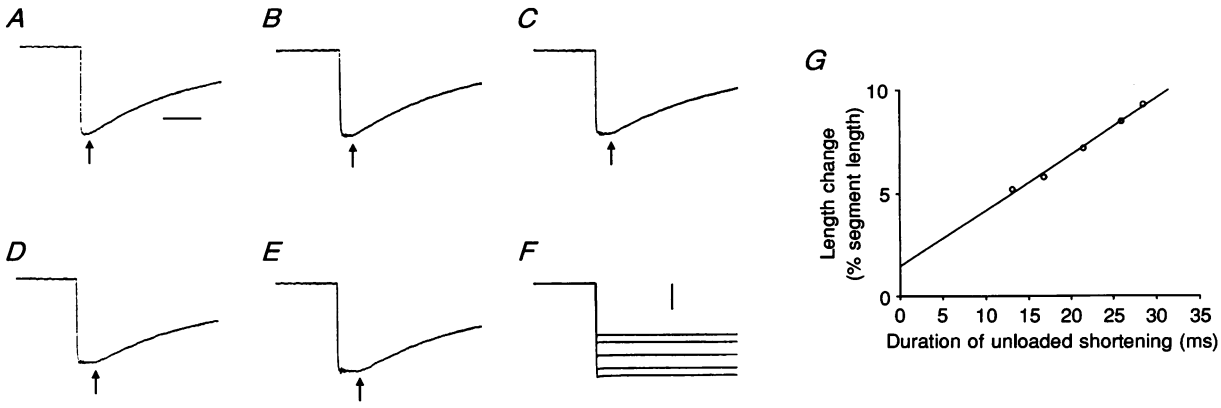

Figure 1. Determination of unloaded shortening velocity
Original force (*A–E*) and length (*F*) records of five releases of increasing amplitude (from *A* to *E*) are shown. The releases were performed during subsequent activations in the order (*A*, *C*, *B*, *E* and *D*). All releases were sufficient to completely unload the fibre. An arrow indicates the time when tension redevelopment begins. In panel *G* the time to tension redevelopment is plotted *versus* the amplitude of the release, the slope of the regression line (2·708 L s^{-1}) is the velocity of unloaded shortening. Calibrations: time, 50 ms (panel *A*); length, 50 μm (panel *F*); fibre segment length, 1·857 mm.

described (Bottinelli *et al.* 1991), activation was obtained by transferring the fibres quickly from the chamber containing preactivating solution (Ca^{2+} free) to the chamber containing activating solution (pCa, 4·45). To determine V_0 the fibre segment was maximally activated at 12 °C. When the tension had reached a steady value, the fibre was unloaded by applying a sudden release of amplitude between 5 and 12 % of initial fibre length (Fig. 1*A–F*). The movement was completed in 1 ms. Force was allowed to redevelop at the final length and then the fibre was relaxed. After relaxation the segment was elongated to the initial length. The whole procedure was repeated 5–6 times. Each activation lasted 1 min.

At the end of all mechanical experiments the fibre was removed from the apparatus, embedded in gelatine and frozen in liquid nitrogen for subsequent immunocytochemical analysis.

Data recording and analysis

The outputs of the force and position transducers were fed to a storage oscilloscope (model 5113, Tektronix, Beaverton, OR, USA) to a digital oscilloscope (Nicolet 4094 C, Madison, WI, USA) and to a chart recorder (Graphtec WR 3701, Tokyo, Japan). Analysis was performed on disk recordings. In the experimental conditions used, the imposed releases of 5–12 % fibre length in 1 ms made the fibre go slack before redeveloping force at the shorter length. Five or six releases of different amplitude were used. Examples of original experimental records are given in Fig. 1*A–F*. V_0 was obtained from the slope of the linear regression between the time necessary to take up the slack (*X*-axis) and the amount of shortening imposed (*Y*-axis; Fig. 1*G*) (Edman, 1979). The intercept of the regression line with the *Y*-axis gives an indication of the series elasticity.

Statistical analysis

Data were expressed as means and standard deviations. Statistical significance of the differences between means was assessed by variance analysis followed by the Student–Newman–Keuls test. A probability of less than 5 % was considered to be statistically significant. The statistical package 'Primer in Biostatistics' (by S. A. Glantz, released by McGraw & Hill, 1989) was used. Covariance analysis was performed to assess statistical significance of the differences between regression lines. Slopes and intercepts with the *Y*-axis were compared in sequence. The procedure described by Snedecor & Cochran (1967) was followed.

SDS–PAGE

MHC isoforms of a segment of each fibre were identified on 6 % polyacrylamide slab gels as previously described (Danieli-Betto *et al.* 1986; Danieli-Betto, Betto & Midrio, 1990). Gels were silver stained. Three bands were separated (Fig. 2). The three bands have been shown to correspond to I MHC, IIB MHC and IIA or IIX MHC (Danieli-Betto *et al.* 1986; Schiaffino *et al.* 1989). IIA and IIX MHC co-migrated, and their electrophoretic band was indicated as IIA/IIX. The electrophoretic runs *d–h* (Fig. 2) show coexistence of different MHCs in the same fibre. As a rule, the relative amount of co-existing MHC isoforms was not quantified, as fibres showing more than one electrophoretic band were discarded. However, some quantification was done to estimate the resolution of the gel electrophoretic system in detecting small amounts of an MHC isoform coexisting with a predominant one. Densitometric analysis of SDS–PAGE runs showing two MHC bands suggested that an MHC band was detectable when its staining intensity was around 1 % of that of the predominant band. This result is in agreement with the observation that silver staining can detect a 2 % 'contamination' of an MHC in a co-electrophoresis of a mixture of MHCs (Carraro & Catani, 1983). Taking into account the amount of myosin present in the segment of fibre used for SDS–PAGE, values of 1–2 % are in agreement with the observation that silver staining could detect 0·38 ng protein mm^{-2} in a gel (Switzer, Merril & Shifrin, 1979).

Immunocytochemistry

The procedure used to characterize the fibres according to MHC composition by immunocytochemistry and the three antibodies used (SC-71, RT-D9 and BF-35) have already been described (Schiaffino *et al.* 1989; Bottinelli *et al.* 1991). SC-71 reacts specifically with IIA MHC, RT-D9 with IIX and IIB MHC and BF-35 with all MHCs except IIX MHC. A camera (Digital Vision, Toulouse, France) was fitted over a microscope (Laborlux D, Leitz, Wetzlar, Germany) and

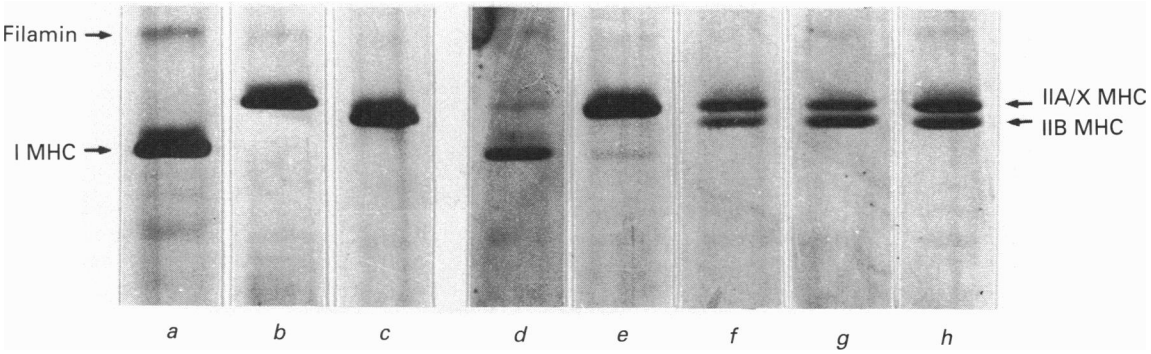

Figure 2. SDS–PAGE analysis of MHC isoforms of single skinned skeletal muscle fibres of the rat

Runs *a* and *d* refer to single fibre segments from soleus; runs *b*, *c* and *e–h* to single fibre segments from plantaris. The three bands which correspond, as indicated by arrows, to I MHC, IIA or IIX MHC (IIA/X band; IIA and IIX MHCs were not separated), and IIB MHC were separated. Fibres in lanes *a–c* contain only one MHC isoform, whereas fibres in lanes *d–h* contain more than one MHC. The band corresponding to filamin is indicated for reference.

connected to an analog–digital interface (Cyclope, Digital Vision, Toulouse, France). The interface was run by a personal computer using specially designed software (Sygma, Radio Tele Engineering (RTE), Monza, Italy). The whole system was supplied by RTE. The microscopic images of single fibre cross-sections at ×400 magnification were stored in the computer and simultaneously displayed on the same screen. Direct comparison was therefore possible.

Combination of immunocytochemistry and SDS–PAGE

All fibres were first characterized by SDS–PAGE. As SDS–PAGE, under the conditions used in this study, cannot easily separate II A from II X MHCs, further analyses were performed on fibres displaying only the II A/II X band to determine whether they contained II A or II X MHC. Serial sections of the single fibres were stained with the antibody that reacts specifically with II A MHC (SC-71), with the antibody that reacts with all MHCs, except II X MHC (BF-35), and with the antibody that reacts with II B and II X MHC, but not with II A MHC (RT-D9). Fibres strongly reactive with SC-71 and BF-35, and not reactive with RT-D9 were interpreted as fibres containing exclusively II A MHC, whereas fibres unreactive with both SC-71 and BF-35, and reactive with RT-D9 were interpreted as fibres containing exclusively II X MHC. The validity of this interpretation is confirmed by a recent study on the distribution of II A and II X MHC mRNAs in fibres characterized with respect to antibody staining (De Nardi *et al.* 1993). No further characterization was carried out on those fibres that were found to contain more than one MHC using SDS–PAGE.

MLC determination

MLC isoforms were separated on 10–20 % linear polyacrylamide gradient slab gels (Fig. 3*A*, *B* and *D*) as described by Salviati *et al.* (1982). Gels were silver stained and scanned with a computer-assisted densitometer (GS300, Hoefer Scientific Instruments, San Francisco, CA, USA). From densitometric scans of the stained gels the ratio between MLC_{3f} and MLC_{2f} was determined (Fig. 3*C* and *E*). The ratio was corrected by dividing it by the molar weights of individual MLCs. On the assumption of a molar equivalence between the amount of MLC_2 and the sum of the amounts of MLC_{1f} and MLC_{3f}, the MLC_{3f}/MLC_{2f} ratio is a good index of the ratio between the two alkali MLC isoforms, MLC_{3f}/MLC_{1f} (Salviati *et al.* 1982). The MLC_{3f}/MLC_{2f} ratio is free from the uncertainties in determining densitometric area of MLC_{1f} which, in the rat, co-migrates with the fast isoform of troponin I (R. Betto, unpublished observations).

The MLC_{3f}/MLC_{2f} ratios of thirty-four fibres of known V_o value were successfully determined. For several reasons, about 44 % of the fast fibres of known V_o and containing only one MHC ($n = 59$) could not be characterized for MLC content. The

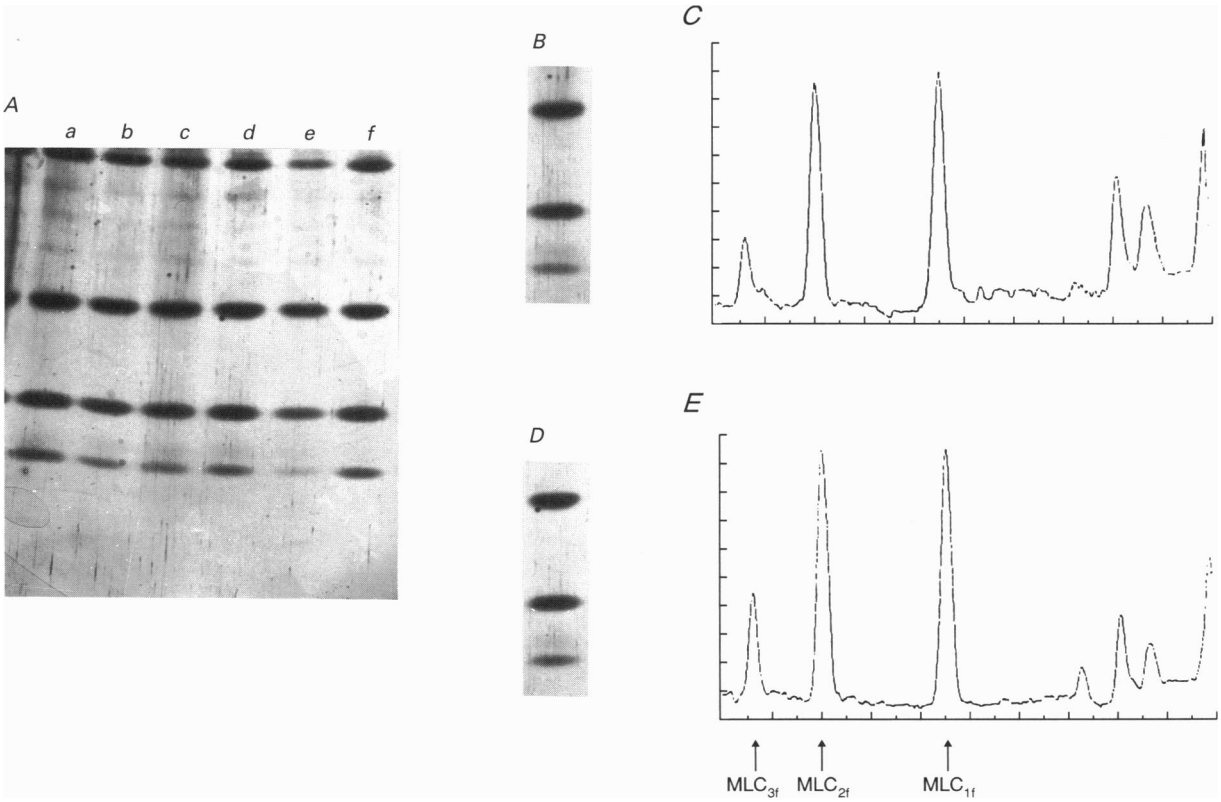

Figure 3. Electrophoretic separation of myosin light chains and their densitometric analysis
The electrophoretic patterns of myosin light chains of six fast fibres are shown in *A*. The gel was a 10–20 % polyacrylamide linear gradient and was stained with silver. Two lanes (*c* and *d*) are shown in detail (*B* and *D*, respectively) with their densitometric traces (*C* and *E*, respectively). The ratio between the MLC_{3f} isoform and the MLC_{2f} isoform of light chain taken as reference (MLC_{3f}/MLC_{2f}) was 0·267 for lane *c* and 0·397 for lane *d*.

highest priority was given to the study of MHC isoforms that sometimes had to be repeated. This decreased the amount of material available for MLC determination. MLC determination requires about 10 times more material than MHC determination. Sometimes gels were not clear enough to give reliable measurements of the MLC_{3f}/MLC_{2f} ratio.

RESULTS

V_0 and MHC composition

A population of 109 fibres dissected from soleus, plantaris and tibialis anterior muscles of the rat was mechanically characterized and analysed for MHC composition. The combination of electrophoretic and immunocytochemical analysis enabled resolution of all four MHC isoforms. Fifty-nine fast fibres and twelve slow fibres contained one MHC isoform, and thirty-eight fibres more than one. Fibres containing more than one isoform were discarded. Mean values ($\pm$ S.D.) of unloaded shortening velocity (V_0) were (in $L\,s^{-1}$): $1{\cdot}05 \pm 0{\cdot}37$ for slow fibres ($n = 12$), $2{\cdot}33 \pm 0{\cdot}29$ for IIA fibres ($n = 8$), $3{\cdot}07 \pm 0{\cdot}70$ for IIX fibres ($n = 15$), and $3{\cdot}69 \pm 1{\cdot}01$ for IIB fibres ($n = 36$). All differences between mean V_0 values of the four fibre groups were statistically significant.

Figure 4 shows the distribution of V_0 values of slow, IIA, IIX and IIB fibres. It can be seen that the variability in V_0 values was very large within groups of fibres of homogeneous MHC content. Only IIA fibres showed a somewhat narrower range of variability in V_0 values than the other groups.

Association between fast MHC and alkali MLC isoforms

The MLC_{3f}/MLC_{2f} ratio of thirty-four fast fibres of known V_0 and containing only one MHC was successfully determined. The MLC_{3f}/MLC_{2f} ratio was lower in IIA ($0{\cdot}14 \pm 0{\cdot}12$, $n = 5$) than in IIX ($0{\cdot}29 \pm 0{\cdot}25$, $n = 10$) which in turn was lower than in IIB fibres ($0{\cdot}37 \pm 0{\cdot}12$, $n = 19$). Only the difference between IIA and IIB fibres was statistically significant. None of the fibres studied showed coexistence of the slow and fast isoform of DTNB MLC, MLC_{2s} and MLC_{2f} respectively. All fibres contained only MLC_{2f}.

V_0 and MLC composition in fast fibres

Figure 5 shows the relationship between the MLC_{3f}/MLC_{2f} ratio and V_0 for fibres containing IIX MHC and IIA MHC (panel *A*), and IIB MHC (panel *B*). A significant correlation between the amount of MLC_{3f} and V_0 was found in all fibre groups. The slopes of the linear regression were $1{\cdot}76 \pm 0{\cdot}17$ (S.E.M.) for IIA fibres, $1{\cdot}33 \pm 0{\cdot}24$ (S.E.M.) for IIX fibres, and $5{\cdot}80 \pm 1{\cdot}35$ (S.E.M.) for IIB fibres. Covariance analysis showed that the slopes of the linear regressions of IIX and IIB fibres and the intercepts with

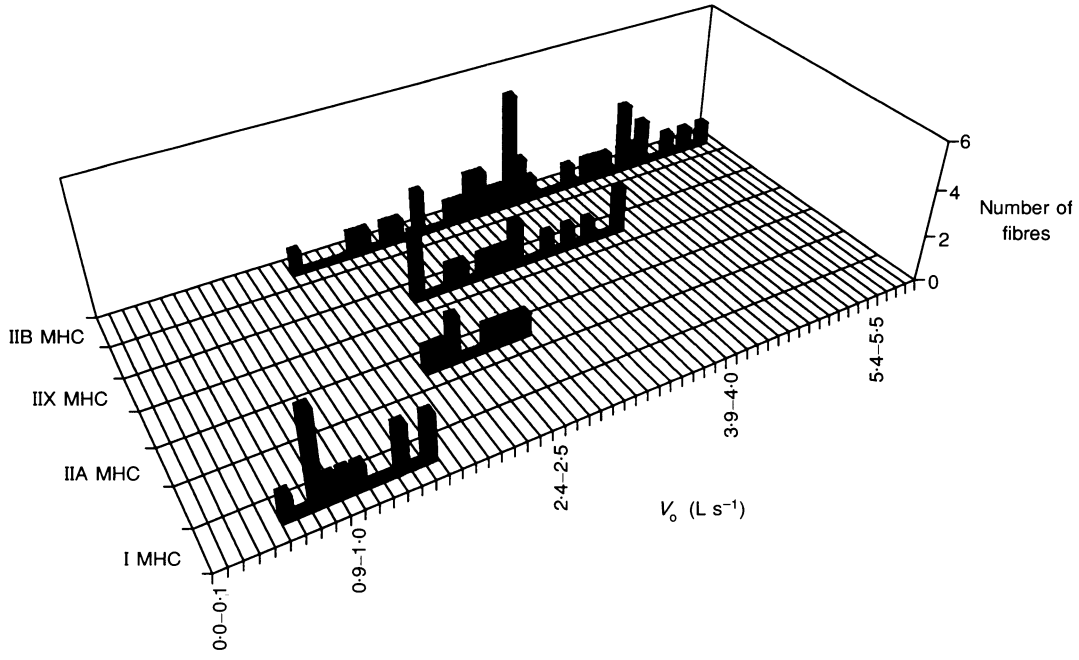

Figure 4. Distribution of unloaded shortening velocity determined by slack test (V_0) in the population of single fibres studied

Fibres are grouped on the basis of their composition of myosin heavy chain (MHC) isoforms, as determined by electrophoretic separation and staining with antibodies specific to MHC isoforms.

the *Y*-axis of the linear regressions of IIA and IIX fibres were significantly different. The difference in slope between IIA and IIB fibres did not reach statistical significance. Fibres containing IIB MHC were isolated either from plantaris or from the superficial part of tibialis anterior. The latter are represented in Fig. II*B* by filled circles. No difference is visible between the two groups.

DISCUSSION

In this work V_0, MHC and alkali MLC composition were determined in the same fibres. V_0 values of skeletal muscle fibres containing only a single MHC isoform, either IIA, or IIX or IIB MHC isoform, could be related to the ratio between alkali MLC isoforms. Under these conditions, the role of both MHC and alkali MLC isoforms in determining V_0 of fast fibres could be defined, and the impact of variations in alkali MLC isoform ratio on V_0 could be quantified. The procedure of analysis of MHC composition identified all known fast MHC isoforms, and detected MHC coexistence, provided the amount of the 'contaminating' MHC isoform was more than 1–2 % of the predominant one. Fibres containing only one fast MHC isoform could be compared, whereas fibres containing more than one MHC were excluded from analysis. The primary results of this study are that: (1) V_0 is highly sensitive to alkali MLC ratio; (2) the sensitivity of V_0 to changes in alkali MLC content depends on the MHC composition of the fibre, being much greater for IIB than for IIX fibres; (3) the effect on V_0 of changes in fast MHC content can be expected to depend on the ratio between the two alkali MLC isoforms.

V_0 and MHC composition

In this study the differences in V_0 not only between slow and fast fibres, but also among fast fibres were found to be related to MHC composition. This result confirms previous observations (Eddinger & Moss, 1987; Sweeney *et al.* 1988; Rome *et al.* 1990), and extends the conclusion to IIX fibres that were not previously analysed from this point of view.

Large variations in maximum shortening velocity within groups of fibres of presumed homogeneous MHC content have been previously reported (Sweeney *et al.* 1988; Bottinelli *et al.* 1991). Even though in this study only fibres containing one MHC were considered, the variability in V_0 values of IIX and IIB fibres was still very large. IIA fibres showed lower variability in V_0 than IIX and IIB fibres. It is unlikely that the error involved in the measurement of V_0 was sufficient to justify the scatter in the data (Greaser *et al.* 1988). It is more likely that some other factor, in addition to MHC, can contribute to V_0 determination. The analysis of V_0 and MHC content by itself cannot be sufficient to establish clearly whether MHCs have a role in determining V_0, and how important it is. In fact, the preferential association between MLC_{3f} and IIB MHC and

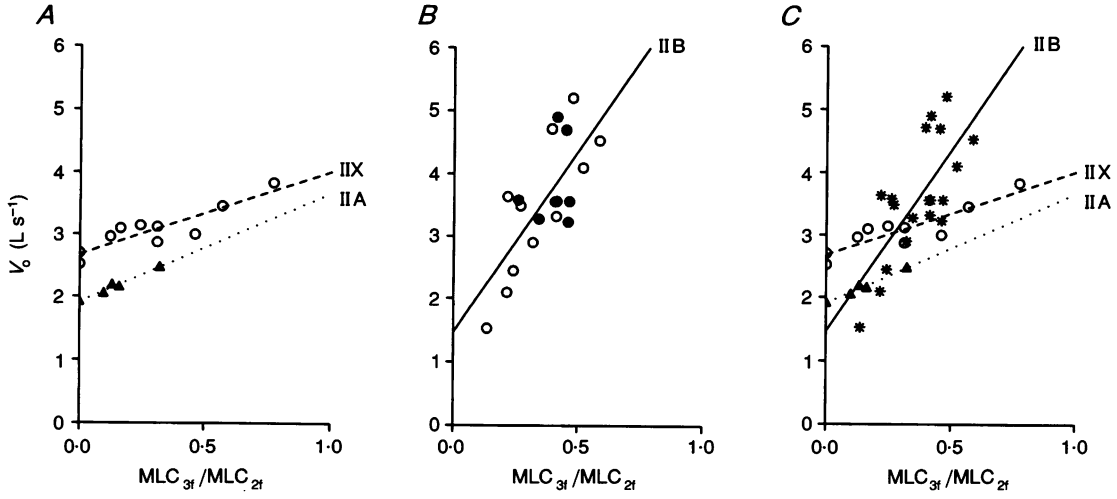

Figure 5. Relationship between unloaded shortening velocity (V_0) and the proportion of the MLC_{3f} isoform in fast fibres

A, fibres containing IIA MHC (▲) and IIX MHC (○). *B*, fibres containing IIB MHC (○, fibres from plantaris; ●, fibres from tibialis anterior). The proportion of the MLC_{3f} isoform is expressed with reference to the DTNB light chain MLC_{2f}, as the MLC_{3f}/MLC_{2f} ratio. The slopes of the linear regressions of IIA fibres (1·76 ± 0·17, $r = 0·986$), of IIX fibres (1·33 ± 0·24, $r = 0·894$) and of IIB fibres (5·80 ± 1·35, $r = 0·722$) are significantly different from zero. The intercepts of the regression lines with the *Y*-axis were: 1·90 ± 0·03 for IIA, 2·67 ± 0·09 for IIX, and 1·46 ± 0·52 for IIB fibres. Panels *A* and *B* are superimposed in panel *C* to show the different slopes of the relationships for IIA (▲), IIX (○), and IIB fibres (✱), and the intersection between the regression line for IIB fibres (continuous line) and those for IIA (dotted line) and IIX (dashed line) fibres.

between MLC_{1f} and IIA MHC shown in previous studies (Dalla Libera *et al.* 1980; Salviati *et al.* 1982; Eddinger & Moss, 1987; Sweeney *et al.* 1988; Wada & Pette, 1993) and confirmed in this work suggests that IIB fibres might be faster than IIA fibres just because they contain a larger amount of MLC_{3f}. Differences in MHC between fibres with different V_0 might be just incidental.

V_0 and alkali MLC isoform content

V_0 values of fast fibres of homogeneous MHC composition were found to be proportional to their MLC_{3f} content (Fig. 5*A* and *B*). The slopes of the linear regression lines relative to fibres containing IIX and IIB MHC and the intercepts with the *Y*-axis of the regression lines of IIA and IIX fibres were significantly different. This can be regarded as the first direct demonstration that V_0 of fast fibres is determined both by MHC and alkali MLC composition. Moreover, this study is the first to establish a quantitative relationship between V_0 and alkali MLC isoform ratio in single skeletal fibres of known MHC isoform composition. In previous studies, due to the preferential association between IIB MHC and MLC_{3f} (Sweeney *et al.* 1988) or to difficulties in separating fast MHC isoforms (Greaser *et al.* 1988), and possibly to undetected coexistence of MHC isoforms, the specific contribution of MHC and MLC could not be assessed. V_0, MHC and MLC composition of single fibres has been determined in only one study, published very recently (Larsson & Moss, 1993). Fibres were obtained from biopsies of human skeletal muscles. No relationship between V_0 values of IIA and IIB fibres and alkali MLC isoform ratio was found. However, interpretation of the latter results is complicated by the frequent coexistence of MLC_{2f} and MLC_{2s} in the human fast fibres used and by the possible presence of additional MHC isoforms. *In situ* hybridization studies show that an MHC mRNA highly homologous to the rat IIX MHC transcript is present in a large population of human fast fibres (S. Schiaffino & L. Leinwand, unpublished observations). In this study all fast fibres contained only MLC_{2f}.

The interplay between MHC and alkali MLC content in determining V_0 seems to be rather complex. The impact of changes in the MLC_{3f}/MLC_{2f} ratio on V_0 is much larger in fibres containing IIB MHC than in fibres containing IIX MHC. A variation of 10 % in MLC_{3f} proportion induces a change in V_0 of 16 % in IIB, and of 4 % in IIX fibres. V_0 values of IIB fibres with very low (below 0·1) relative MLC_{3f} content can be lower than the V_0 of IIX, and IIA fibres with the same MLC_{3f} content (Fig. 5*C*). At intermediate values of MLC ratio, fibres containing different MHCs have similar V_0 values. When the slopes of the linear regression lines for IIA, IIX and IIB fibres are considered, it is apparent that changes in MLC_{3f}/MLC_{2f} ratio account for virtually the whole range of variability in V_0 shown in Fig. 4 for the different fibre types. However, it must be pointed out that the small number of IIA fibres studied, and the small variations in their V_0 and MLC_{3f}/MLC_{2f} values have not enabled us to specify the importance of alkali MLCs in determining V_0 of IIA fibres as satisfactorily as has been done for IIX and IIB fibres.

Alkali MLC isoforms have been found to have a minor effect on actin-activated ATPase activity of chicken skeletal muscle (Pastra-Landis, Huiatt & Lowey, 1983). The ATPase activity of hybrids of myosins containing different alkali MLC isoforms is essentially due to the heavy chains (Wagner & Giniger, 1981). On the other hand, an influence of alkali MLC ratio on actin–myosin interaction has been suggested by biochemical studies (Trayer & Trayer, 1985). Moreover, very recently alkali MLC isoforms have been shown to have small, but significant effects on actin-activated ATPase activity (Lowey, Waller & Trybus, 1993), and to be important for the movement of actin filaments on myosin heads in *in vitro* motility studies (Lowey *et al.* 1993).

Recent structural analyses (Rayment *et al.* 1993) have shown that both alkali and regulatory light chains are wrapped around the long α-helix which stretches from the globular part to the C-terminus of myosin subfragment 1 (S1), and transmits the internal movements of S1 to the backbone of the thick filament. MLCs probably give the α-helix the stiffness and the stability which is necessary to transmit and amplify the movement. One can speculate that MLC_{1f}, making contact with actin with its forty-one amino acid extension (Trayer, Trayer & Levine, 1987), might delay actin–myosin detachment that is supposed to be the rate-limiting step of unloaded shortening velocity. Alternatively, MLC_{1f} might stabilize the α-helix more effectively than MLC_{3f}, possibly reducing the extent of the working stroke. Differences in the physical characteristics of the α-helix or in the molecular motor inside the S1 globular part between IIA, IIX and IIB MHC isoforms might account for the different effect of alkali MLCs on V_0 values of IIB, IIX, and possibly IIA fibres. Additional structural studies and further information on the amino-acid sequence of the different MHC isoforms are needed in order to understand completely the role of the alkali MLC in actin–myosin interaction.

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