Kinetic properties of myosin heavy chain isoforms in single fibers from human skeletal muscle

Karlheinz Hilber[a], Stefan Galler[a,*], Bärbel Gohlsch[b], Dirk Pette[b]

[a] *Institute of Zoology, University of Salzburg, Hellbrunnerstraße 34, A-5020 Salzburg, Austria*
[b] *Faculty of Biology, University of Konstanz, D-78457 Konstanz, Germany*

Received 3 June 1999; received in revised form 25 June 1999

Abstract The head portion of the myosin heavy chain is essential in force generation. As previously shown, Ca^{2+}-activated muscle fibers from rat and rabbit display a strong correlation between their myosin heavy chain isoform composition and the kinetics of stretch activation, corresponding to an order of velocity: myosin heavy chain Ib > myosin heavy chain IId(x) > myosin heavy chain IIa ≫ myosin heavy chain I. Here, we show a similar correlation for human muscle fibers (myosin heavy chain IIb > myosin heavy chain IIa ≫ myosin heavy chain I), suggesting isoform-specific differences between the kinetics of force-generating power strokes. The kinetics of myosin heavy chain I are similar in human and rodents. This holds also true for myosin heavy chain IIa, but human myosin heavy chain IIb is slower than rodent myosin heavy chain IIb. It is similar to rodent myosin heavy chain IId(x).

Key words: Muscle fiber type; Myosin heavy chain isoform; Human muscle; Order of velocity; Stretch activation

1. Introduction

To fulfill various functional demands, skeletal muscles are composed of different fiber types. This diversity relates to the existence of specific myofibrillar protein isoforms in different fiber types that are generally categorized according to their specific myosin heavy chain (MHC) isoforms (for review see [1]). Three fast fiber types have thus been delineated in limb muscles of rat and rabbit: types IIB, IID(X) and IIA, containing MHC isoforms MHCIIb, MHCIId(x) and MHCIIa, respectively. The slow type I fibers contain MHCIb corresponding to the β-cardiac heavy chain. Recent investigations suggest the existence of an additional, functionally different slow MHC isoform, named MHCIa and shown to co-exist with MHCIb in slow fibers of the rabbit plantaris muscle [2]. In human skeletal muscle, only three MHC isoforms (MHCIIb, MHCIIa and MHCI) corresponding to three fiber types (type IIB, type IIA and type I) have been characterized to date [3].

The head portion of the MHC represents the essential component of the molecular force-generating system of muscle [4,5]. As shown by single fiber studies, a clear correlation exists in rat and rabbit skeletal muscles between the kinetics of force responses following a quick stretch of maximally Ca^{2+}-activated muscle fibers (stretch activation) and their MHC isoforms [6–8]. This correlation probably points to different kinetic properties of the force-generating power strokes [9] of myosin heads from different isoforms.

Only little information is available with regard to this relationship in human muscle fibers [10]. The present study was, therefore, undertaken in order to record stretch activation kinetics of maximally Ca^{2+}-activated skinned fibers from human muscles, categorized according to their electrophoretically identified MHC composition. Our findings on the kinetic properties of the different human MHC isoforms appear to be most relevant with regard to molecular prerequisites for different functional properties of human fiber types.

2. Materials and methods

2.1. Muscle preparations

Muscle samples of a few mm in size were obtained from patients undergoing surgery. The patients were informed and gave their permission. The muscle samples originated from the deltoideus muscle of a 68 year old man, from the pectoralis major muscle of a 55 year old woman and from the rectus abdominis muscle of a 74 year old man. Immediately after excision, the samples were incubated in a cold relaxation solution containing 50% (v/v) glycerol (see below), washed and stored in this solution at −25°C for less than 6 weeks. A total of 110 fibers was analyzed.

2.2. Mechanical measurements

The set-up and methods for mechanical measurement have been described in detail previously [11]. The attachment points of the muscle fiber ends to the mechanical apparatus were two vertically orientated epoxy carbon fibers of a 100 μm tip diameter. They were glued on silicon plates of force transducer elements (AE 801, Sensonor, Norway). One element, the force sensor (resonance frequency 8.3 kHz), was mechanically connected to a micrometer screw and electrically to a force bridge amplifier. The other element was a dummy, glued on the lever arm of a stepping motor. Rapid changes of the fiber length (≤ 0.5 ms) were carried out by a feedback-controlled stepping motor based on a Ling vibrator. The ability to make rapid solution changes (≤ 0.2 s) was provided by a specially designed cuvette transporting system. Laser diffractometry (He-Ne laser, 632.8 nm, 4 mW) was used to measure the sarcomere length of the myofibrillar bundles before, during and after activation of the fibers.

The solutions contained 60 mM HEPES, 8 mM Na_2H_2adenosine 5′-triphosphate (ATP), 10 mM sodium creatine phosphate, 40 g/l dextran T-500 and 1 mM free Mg^{2+}. The relaxation solution (negative logarithm of free Ca^{2+} concentration (pCa) > 9) additionally contained 50 mM ethylene-bis(oxy-ethylenenitrilo)tetraacetic acid (EGTA). The activation solution (pCa 4.7) contained 50 mM Ca-EGTA and the pre-activation solution (low Ca^{2+}-buffering capacity, pCa 7) 50 mM hexamethylenediamine-*N,N,N′,N′*-tetraacetic acid. The pH at 22°C was adjusted to 7.10 in all solutions. The ionic strength of the solutions was 0.25 M. The pCa ($-\log [Ca^{2+}_{free}]$) of the solutions was determined with a Ca^{2+}-selective electrode. Creatine phosphokinase (20 U/ml) was added to the solutions immediately before starting the measurements. Control experiments on fast rat muscle fibers comparing the maximal shortening velocity over a wide range of creatine

*Corresponding author. Fax: (43) (662) 8044 5698.
E-mail: stefan.galler@sbg.ac.at

Abbreviations: ATP, adenosine 5′-triphosphate; EGTA, ethylene-bis-(oxy-ethylenenitrilo)tetraacetic acid; MHC, myosin heavy chain; pCa, negative logarithm of free Ca^{2+} concentration

0014-5793/99/$20.00
PII: S0014-5793(99)00903-5

kinase concentrations have shown that 20 U/ml is sufficient for a satisfactory re-phosphorylation of adenosine diphosphate in our experimental system [12]. A relaxation solution containing 50% (v/v) glycerol (pH 6.9 at 22°C) was used for storing the muscle fibers at −25°C.

After attachment of the skinned muscle fibers with the tissue glue Vetseal (Braun, Melsungen, Germany), the fiber ends were fixed by superfusion (3–5 s) with a fine, rapidly downward flowing stream of stained glutaraldehyde solution (8% glutaraldehyde, 5% toluidine blue, fixative). For this purpose, the fiber was bathed in a low ionic strength rigor solution (10 mM imidazole, 2.5 mM EGTA, 7.5 mM EDTA, 134 mM potassium propionate, pH 6.8) with a lower specific mass than the fixative. This procedure created a sharp boundary between the functional part of the muscle fiber and the fixed fiber ends. Control experiments have shown that this method considerably improves the maintenance of the sarcomere order and the stability of the mechanical properties during prolonged activations [13].

Before the measurements, the fibers were bathed for 15 min at 22°C in a relaxation solution containing 1% (v/v) Triton X-100. Subsequently, the length of the preparations was adjusted to exactly the slack position (final active fiber length, 3.2 ± 0.4 mm) in relaxation solution and both the fiber width (50 ± 14 μm) and sarcomere length (2.3 ± 0.2 μm) were recorded. After transferring the fiber from the pre-activation solution to the activation solution, a series of quick (≤ 0.5 ms) stretches (range, from 0.5 to about 4 nm per half sarcomere) was applied to detect the time course of the resulting force transients. Measurements were only evaluated if the sarcomere length during and after maximal activation was not markedly changed (less than 5%) and if the signal was of a comparable quality as under relaxed conditions.

All experiments were performed at 22°C. Results were expressed as means ± S.D. and analyzed using two-tailed Student's *t*-tests and Mann-Whitney-U statistics.

2.3. Biochemical analysis

After completion of the mechanical measurements, the unfixed part of the muscle fiber was cut from the apparatus and used for biochemical analysis. The fiber fragment was dissolved in 11 μl of a sodium dodecyl sulfate (SDS) buffer (62 mM Tris-HCl, pH 6.8, 10% glycerol (v/v), 2.3% SDS (w/v), 5% β-mercaptoethanol (v/v), 19% sucrose (w/v)) and heated for 15 min at 65°C. A 2.5 μl aliquot of this extract was applied for electrophoresis on a 5–8% polyacrylamide gradient gel [14]. After electrophoresis, the gels were silver-stained [15]. Relative amounts of MHC isoforms in hybrid fibers were evaluated by integrating densitometry.

3. Results

3.1. Myosin heavy chain isoforms in human fibers

Three distinct MHC isoforms were separated by density gradient gel electrophoresis, the fastest migrating band designated MHCI, the slowest migrating band designated MHCIIb and MHCIIa slightly faster migrating than MHCIIb (Fig. 1). These isoforms have previously been assigned to the myofibrillar ATPase-based fast fiber types IIB and IIA and to the slow fiber type I [1]. Fibers containing only one MHC isoform ('pure' fibers) as well as fibers containing two MHC isoforms (hybrid fibers) were observed. Among the 110 fibers which were analyzed successfully, 26 were of type IIB, 18 of type IIA and 48 of type I. Eight fibers contained more MHCIIb than MHCIIa (type IIBA) and seven fibers contained more MHCIIa than MHCIIb (type IIAB). Two fibers contained more MHCIIa than MHCI (type IIC) and one fiber contained more MHCI than MHCIIa (type IC).

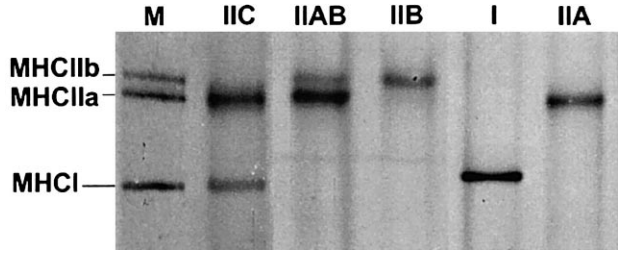

Fig. 1. Silver-stained polyacrylamide gradient gel electrophoreses of myosin heavy chain isoforms from single human skeletal muscle fibers for which mechanical properties were previously measured. The label at the top of each lane represents the fiber type. The first lane from the left is an electrophoresis of a total extract from a human pectoralis muscle and serves as a marker (M).

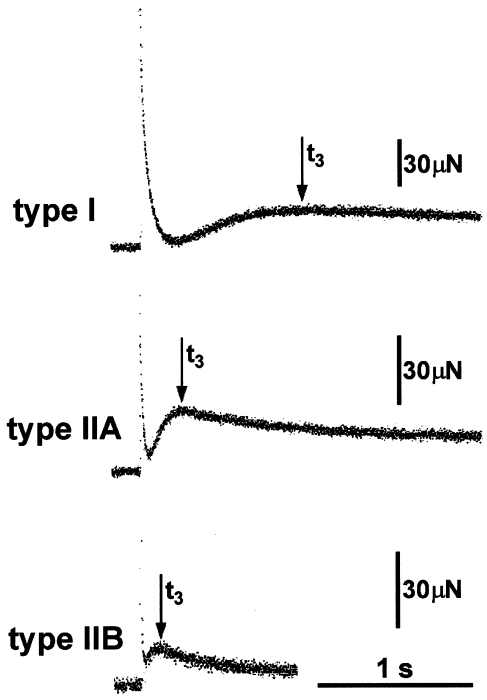

Fig. 2. Original recordings of force during quick stretch experiments of about 2–3 nm per half sarcomere on maximally Ca^{2+}-activated skinned human skeletal muscle fibers of different types. The fiber diameters and isometric tensions were 60, 75, 50 μm and 227, 197, 103 kN/m^2 for the type I, type IIA and type IIB fiber, respectively.

3.2. Stretch activation kinetics

In order to detect differences related to the MHC isoform composition, quick (≤0.5 ms) stretches of 0.5–4 nm per half sarcomere were applied to maximally Ca^{2+}-activated skinned muscle fibers (189 ± 78 kN/m^2, $n = 110$). The stretch induced an instantaneous rise in force followed by a decay and a secondary (delayed) transient increase in force (i.e. stretch activation, Fig. 2). The time elapsed between the beginning of the stretch and the peak value of the delayed force increase (t_3) was chosen for evaluation. The t_3 values of pure fiber types differed significantly between each other ($P < 0.001$) and no overlaps of t_3 values were observed between fiber types. In contrast to fiber types IIB and IIA, type I fibers displayed a large scattering within the histogram shown in Fig. 3A and thus did not seem to represent a homogeneous population. Moreover, several type I fibers exhibited extraordinarily large t_3 values as compared to others.

The means (± S.D.) of the t_3 values from all fiber types are listed in Table 1 and their reciprocal values are summarized in the diagram shown in Fig. 3B. No significant differences ($P > 0.05$) in t_3 values existed within type IIB or type IIA fibers from the three different muscles investigated in the

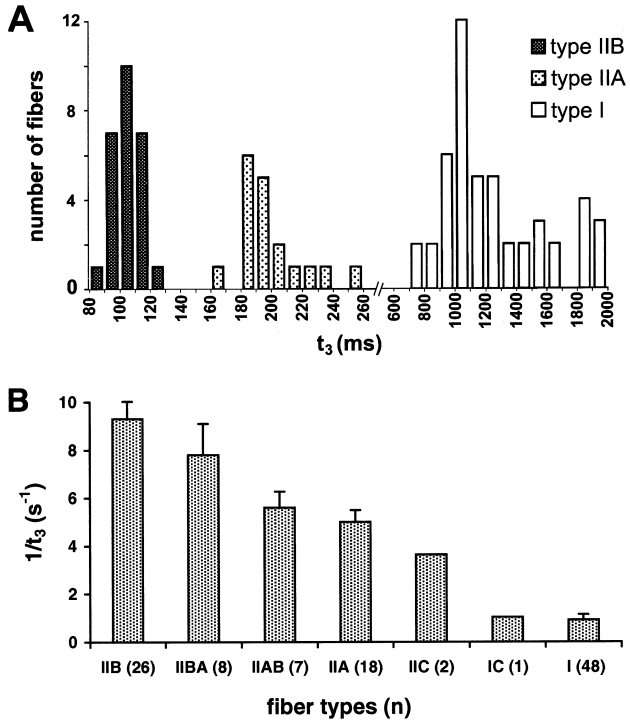

Fig. 3. (A) Histogram showing the distribution of t_3 values in pure fiber types of human skeletal muscle. (B) Reciprocal t_3 values (mean ± S.D.) of various fiber types of human skeletal muscle.

present study. However, the t_3 values of type I fibers from the three muscles displayed obvious differences. The type I fibers from the pectoralis muscle exhibited the largest t_3 values (1369 ± 350 ms, $n = 25$), followed by the rectus muscle (1025 ± 207 ms, $n = 18$) and last, the deltoid muscle (862 ± 156 ms, $n = 5$). Student t-tests proved that the differences were significant ($P < 0.01$) between pectoralis and rectus muscles and between the pectoralis and deltoid muscles.

4. Discussion

The present findings reveal a strong correlation between the kinetics of stretch activation (t_3) of maximally Ca^{2+}-activated human skeletal muscle fibers and their MHC composition. MHCIIb is shown to be the fastest isoform, MHCI the slowest and MHCIIa intermediate. This result is based on measurements of both pure and hybrid fibers from three different human muscles. The time to the peak level of the stretch-induced delayed force increase was chosen as a kinetic parameter for stretch activation. This value had been termed 't_2' in our earlier publications. However, for a better agreement with the corresponding phase 3 firstly described by Huxley and Simmons [16], we decided to use the designation 't_3'.

Rat and rabbit muscle fibers exhibit a similar type of correlation between the MHC isoform composition and stretch activation [8]. Isoforms of other myofibrillar proteins do not seem to be involved in this correlation [17]. Our findings on human muscle fibers, therefore, corroborate the assumption that a causal relationship exists between MHC isoforms and differences in the kinetics of stretch activation (t_3). In experiments where activated muscle fibers are exposed to sinusoidal length changes of varying frequencies, maximal work output occurs at a frequency which corresponds to the rate constant of the delayed force increase in the stretch experiment (e.g. [6,18,19]). This is in line with the suggestion that the stretch-induced delayed force increase is caused by a simultaneous power stroke of a group of stretch-synchronized myosin heads [20]. Consequently, the kinetics of stretch activation would be related to the kinetics of the power strokes of the myosin heads. Our finding of a strong correlation between the MHC isoform composition of a given fiber and its stretch activation kinetics is in support of this assumption. From this point of view, our data suggests the kinetics of power strokes of myosin heads from different MHC isoforms to vary by a factor of more than 10 in human skeletal muscle. In rat and rabbit skeletal muscles, these variations are larger, attaining differences of 30-fold or more. Most likely, these variations relate to the diversity of functional requirements in small animals like rat and rabbit and large animals like human. All animals need slow fibers for slow movements and tonic (anti-gravity) activity, but small animals need faster fibers, for quick locomotion, than large animals.

The mean t_3 values of type I (1187 ms) and type IIA (202 ms) fibers obtained in this study were not very different from those of rat (type I: 901 ms, type IIA: 157 ms) and rabbit (type I: 919 ms, type IIA: 154 ms) [8]. In addition, they were similar to the mean t_3 values of type I (956 ms) and type IIA fibers (164 ms) from a biopsy taken from the quadriceps muscle of a 31 year old male [10]. Because the value for t_3 is highly temperature-sensitive ($Q_{10} > 3$ for rabbit psoas fibers [18]), the slower values in the present investigation may have resulted from the slightly lower temperature (22°C) used as compared to that in previous studies (22–24°C). It appears thus that the MHCI and MHCIIa isoforms of rat, rabbit and human display similar kinetics of stretch activation. As suggested by different electrophoretic mobilities, MHC isoforms of fiber types homologous according to their myofibrillar ATPase histochemistry may not be identical in different mammalian species [21]. Their similar kinetics of stretch-induced force responses, however, indicate that this property is conserved.

The type IIB fibers in human and rodents display similar histochemical staining profiles for myofibrillar ATPase activity [1,22]. However, type IIB fibers of human muscle (mean t_3: 108 ms, present study, 22°C) are much slower than type IIB fibers of rodents (mean t_3: 27 ms, 22–24°C) [8]. The t_3 values of human type IIB fibers, thus, lie between type IID(X)

Table 1
Kinetics of stretch activation expressed by the time parameter t_3 (ms) of various human skeletal muscle fiber types

Fiber type	IIB	IIBA	IIAB	IIA	IIC	IC	I
Mean	108	131	181	202	275	970	1 188
S.D.	8	22	22	22	0		345
n	26	8	7	18	2	1	48

The t_3 values of all groups were significantly different from each other with $P < 0.05$ using two-tailed Student t-tests or non-parametric Mann-Whitney-U statistics (type IIB, type I).

(mean: 55 ms for rat and 54 ms for rabbit, 22–24°C) and type IIA fibers (mean: 157 ms for rat and 154 ms for rabbit, 22–24°C) of rodents. Taking into account differences in the temperature at which measurements were taken, human type IIB fibers exhibit kinetic properties that are more similar to type IID(X) than to type IIB fibers of rodent muscles. This is in line with findings from in situ hybridization and reverse transcriptase-PCR, showing that transcripts homologous to rat MHCIIx are abundant in human muscle fibers histochemically and electrophoretically classified as type IIB [23,24]. These and the present findings indicate that human muscle fibers formerly classified as type IIB are homologous to type IID(X) in rat and rabbit.

Another interesting observation concerns the large scattering of type I fibers. Several type I fibers are characterized by extraordinarily slow stretch activation kinetics with t_3 values between 1.3 and 1.9 s. In the rabbit plantaris muscle, type I fibers with such large t_3 values exhibited in addition to MHCIβ low amounts of a second MHC isoform, tentatively designated as MHCIa [2]. Such an isoform could not be separated in the present study. However, the failure to electrophoretically detect additional slow MHC isoforms does not exclude their existence, especially in view of studies with monoclonal antibodies, suggesting the existence of at least three slow MHC isoforms in developing rat and human muscles. Moreover, the assumption that slow fiber subtypes exist in human muscle has recently been supported by in situ hybridization studies in which histochemically defined type I fibers differed with regard to specific slow myosin light chain compositions [25]. The observed spectrum of t_3 values in type I fibers may, therefore, relate to the presence of more than one slow MHC isoform in human muscle. This would be in line with the suggestion that the t_3 values of type I fibers from the three different muscles under study relate to different, as yet not identified MHCI isoforms.

Acknowledgements: Supported by grants from the FWF, the National Bank of Austria, by a grant of the Rector of the University of Salzburg and the Deutsche Forschungsgemeinschaft.

References

[1] Pette, D. and Staron, R.S. (1990) Rev. Physiol. Biochem. Pharmacol. 116, 1–76.
[2] Galler, S., Hilber, K., Gohlsch, B. and Pette, D. (1997) FEBS Lett. 410, 150–152.
[3] Staron, R.S. (1997) Can. J. Appl. Physiol. 22, 307–327.
[4] Huxley, H.E. (1969) Science 164, 1356–1366.
[5] Rayment, I., Holden, H., Whittaker, M.M., Yohn, C.B., Lorenz, M., Holmes, K.C. and Milligan, R.A. (1993) Science 261, 58–65.
[6] Kawai, M. and Schachat, F.H. (1984) Biophys. J. 45, 1145–1151.
[7] Galler, S., Schmitt, T. and Pette, D. (1994) J. Physiol. (Lond.) 478, 523–531.
[8] Galler, S., Hilber, K. and Pette, D. (1997) J. Muscle Res. Cell Motil. 18, 441–448.
[9] Kawai, M. and Zhao, Y. (1993) Biophys. J. 65, 638–651.
[10] Hilber, K. and Galler, S. (1997) Pflüg. Arch. 434, 551–558.
[11] Galler, S. and Hilber, K. (1994) J. Muscle Res. Cell Motil. 15, 400–412.
[12] Hilber, K. and Galler, S. (1998) J. Muscle Res. Cell Motil. 19, 143–155.
[13] Hilber, K. and Galler, S. (1998) J. Muscle Res. Cell Motil. 19, 365–372.
[14] Termin, A., Staron, R.S. and Pette, D. (1989) Histochemistry 92, 453–457.
[15] Oakley, B.R., Kirsch, D.R. and Morris, N.R. (1980) Anal. Biochem. 105, 361–363.
[16] Huxley, A.F. and Simmons, R.M. (1971) Nature 233, 533–538.
[17] Galler, S., Schmitt, T.L., Hilber, K. and Pette, D. (1997) J. Muscle Res. Cell Motil. 18, 555–561.
[18] Zhao, Y. and Kawai, M. (1994) Biophys. J. 67, 1655–1668.
[19] Kawai, M. and Brandt, P.W. (1980) J. Muscle Res. Cell Motil. 1, 279–303.
[20] Kawai, M. and Halvorson, H.R. (1991) Biophys. J. 59, 329–342.
[21] Hämäläinen, N. and Pette, D. (1995) Microsc. Res. Tech. 30, 381–389.
[22] Staron, R.S. and Johnson, P. (1993) Comp. Biochem. Physiol. B 106, 463–475.
[23] Smerdu, V., Karsch-Mizrachi, I., Campione, M., Leinwand, L. and Schiaffino, S. (1994) Am. J. Physiol. 267, C1723–C1728.
[24] Ennion, S., Pereira, J.S., Sargeant, A.J., Young, A. and Goldspink, G. (1995) J. Muscle Res. Cell Motil. 16, 35–43.
[25] Jostarndt, K., Puntschart, A., Hoppeler, H. and Billeter, R. (1996) J. Histochem. Cytochem. 44, 1141–1152.