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Two functionally distinct myosin heavy chain isoforms in slow skeletal muscle fibres

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Abstract The head part of the myosin heavy chain (MHC) represents the essential component of the molecular force-generating system of muscle [1–3]. To date, three fast but only one slow MHC isoforms have been identified in adult mammalian limb muscles [4,5]. We show here two functionally different slow MHC isoforms, MHCIβ and MHCIa, coexisting in a considerable fraction of slow fibres of rabbit plantaris muscle. The two isoforms exhibit distinct electrophoretic mobilities and different kinetic properties. Thus, as it is known for the fast muscle, also the slow muscle seems to use different MHC isoforms in order to fulfil different functional demands.

Key words: Myosin heavy chain isoforms; Muscle fibre types; Slow skeletal muscle; Muscle mechanics

1. Introduction

Myosin contains the essential component of the molecular force-generating system of muscle [1–3]. In skeletal muscle it exists as distinct isoforms in a variety of fibre types best classified according to their myosin heavy chain (MHC) complement (for review ref. [6]). Accordingly, three fast fibre types have been identified in mammalian limb muscles: types IIB, IID(X), and IIA containing MHCIIb, MHCIId(x), and MHCIIa, respectively [4,5]. A clear correlation exists between the kinetics of force responses following stepwise length changes of maximally Ca^{2+}-activated muscle fibres and their MHC isoforms [7–9]. This correlation suggests distinct functional properties of the various MHC isoforms.

Only one slow fibre type (type I) has to date been characterized by its MHC complement in mammalian limb muscles. Its MHCI is thought to be identical to the β-cardiac MHC isoform [10]. Previous observations on the variable distribution of the two slow essential (alkali) light chains, LC1sa and LC1sb, in type I fibres pointed to the possibility that the type I fibres might not represent a homogeneous population [11,12]. This possibility is supported by recent findings on the electrophoretic separation of MHCI into two bands, i.e. the original MHCI, and MHCIa, a slightly faster moving band [13,14]. Because these two electrophoretically distinct protein bands might represent two slow MHC isoforms, we have tried in the present study to characterize their identity in a combined micromechanical and biochemical approach. For this purpose, the kinetics of force responses following stepwise length changes [2] were studied in skinned single type I fibres, electrophoretically identified by their MHC isoforms [5].

2. Materials and methods

2.1. Mechanical measurements

The methods of the mechanical experiments and the apparatus are more extensively described in one of our earlier publications [15]. The experiments were performed on glycerinated skinned fibres from rabbit plantaris muscle at 22°C. The temperature of the bath solutions was continuously checked using an ALMENO digital thermometer with a micro thermocouple IT-21. The attachment points for the muscle fibre ends on the mechanical apparatus were two vertically orientated epoxy carbon fibres of 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 10 ± 0.5 kHz), was connected mechanically 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 fibre 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 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 fibres.

The solutions contained 60 mM HEPES, 8 mM Na_2H_2ATP, 10 mM sodium creatine phosphate, 20 units/ml creatine phosphokinase, 40 g/l dextran T-500 and 1 mM free Mg^{2+}. In addition, the relaxation solution (pCa > 9) contained 50 mM EGTA. The activation solution (pCa 4.7) contained 50 mM Ca-EGTA, and the preactivation solution (low Ca^{2+} buffering capacity, pCa 7) 50 mM hexamethylenediamine-*N*,*N*,*N*′,*N*′- tetraacetic acid (HDTA). The pH was adjusted to 7.10 in all solutions at 22°C. The pCa ($-\log[Ca_{free}^{2+}]$) of the solutions was determined with a Ca^{2+}-selective electrode. The solution for storing the muscle fibres at −20°C was a relaxation solution containing 50% (v/v) glycerol (pH 6.9).

After attachment of the skinned muscle fibres with the tissue glue Vetseal (Braun, Melsungen, FRG), the fibre ends were fixed by superfusion (3–5 s) with a fine, rapidly downward flowing stream of pigmented glutaraldehyde solution (8% (v/v) glutaraldehyde and 5% (w/v) Toluidine blue, fixative). For this purpose, the fibre 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 lower specific mass than the fixative. This fixation procedure created a sharp boundary between the functional part of the muscle fibre and the fixed fibre ends. Control experiments ([15], Hilber and Galler, unpublished) have shown that this method considerably improves the maintenance of sarcomere order and the stability of the mechanical properties during prolonged activations.

The length of the preparations was always adjusted to exactly the slack position (final active fibre length, 2.5–3.5 mm) and the sarcomere length was recorded (2.0–2.4 μm). Subsequently, the fibre was treated with 1% (v/v) Triton X-100 in a relaxation solution (15 min; 22°C). After transferring the fibre from the preactivation solution to the maximal activation solution, a series of quick (< 0.5 ms) stretches and releases (range 0.5 to 6 nm per half sarcomere) was applied to

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Abbreviations: ATP, adenosine 5′-triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid; EGTA, ethylene-bis(oxyethylenenitrilo)tetraacetic acid; MHC, myosin heavy chain; HDTA, hexamethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid; pCa, negative logarithm of free Ca^{2+} concentration

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detect the time course of resulting force transients. Measurements were only taken for evaluation if the sarcomere length signal during and after maximal activation was unchanged and of comparable quality as under relaxed conditions.

2.2. Biochemical analysis

After completion of the mechanical measurements, the unfixed part of the muscle fibre was cut from the apparatus for biochemical analysis. The fibre fragment was dissolved in 11 μl sodium dodecyl sulphate (SDS) lysis 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 at 65°C for 15 min. A 2.5 μl aliquot of this extract was applied to a 5–8% polyacrylamide gradient gel according to the method previously described [5]. Densitometric evaluation was used in order to quantify the ratio of different MHC isoforms in single fibres.

3. Results

Electrophoretic analyses on rabbit plantaris muscle fibres detected a considerable fraction of type I fibres (21 out of 37) displaying, in addition to MHCI, a band of slightly higher mobility (Fig. 1). According to [13] and [14], this band was designated as MHCIa. The isoform with the lower electrophoretic mobility (the former MHCI) was designated as MHCIβ. According to densitometric evaluation of the gels, MHCIβ represented the major isoform in all type I fibres investigated in plantaris muscle. The relative concentration of MHCIa maximally amounted to 30% of total MHC isoforms. Fibres containing only MHCIβ were termed type Iβ; fibres containing MHCIβ > MHCIa were designated as type Iβ/Ia.

Quick (< 0.5 ms) stretches and releases of 0.5–6 nm per half sarcomere were applied to maximally Ca^{2+}-activated skinned muscle fibres (187 ± 79 kN cm^{-2}, $n = 37$) in order to detect differences which could be correlated to their MHC isoform complement. As in studies on rat muscle fibres [7,8], the kinetics of force responses during fibre stretches correlated closely with the kinetics during fibre releases. However, only stretch response measurements are presented here because of the greater precision with which they can be detected. A stretch of the muscle fibres induced an instantaneous force rise followed by a decay and a secondary (delayed) transient force increase (i.e. stretch activation; Fig. 2A). For evaluation, the time from the beginning of the stretch to the peak value of the delayed force increase (t_2 value) was chosen. This parameter is representative for other kinetic parameters of force responses following stepwise length changes [8], and, because of its independence of the length change amplitude [7,16], it is one of the most useful parameters to distinguish

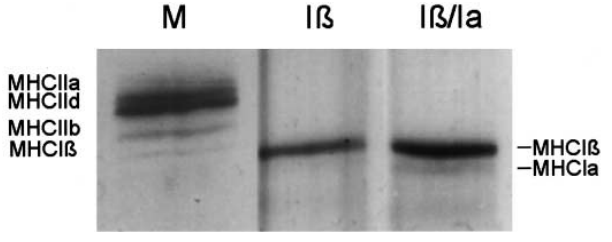

Fig. 1. Silver-stained polyacrylamide gradient gel electrophoreses of MHC isoforms from rabbit plantaris slow muscle fibres for which mechanical properties were previously measured. The label at the top of each lane represents the fibre type. The left lane is an electrophoresis of rabbit gastrocnemius muscle and serves as a marker (M).

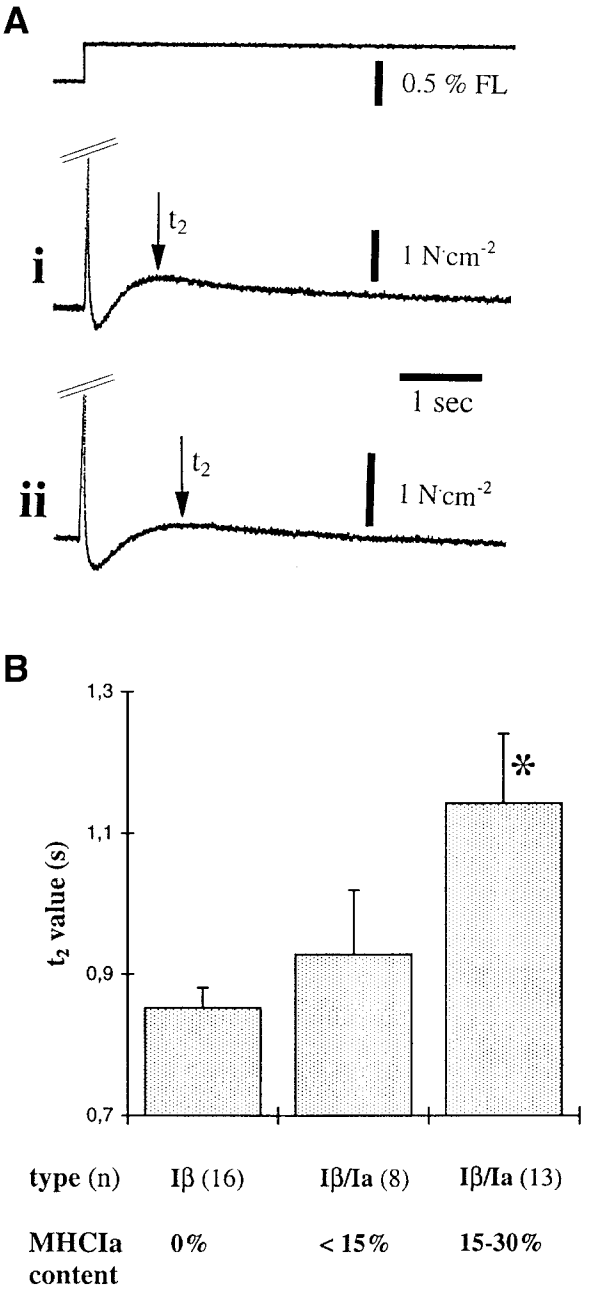

Fig. 2. (A) Original recordings of fibre length (upper panel) and force (lower panels) during quick stretch experiments (2 nm per half-sarcomere) on maximally Ca^{2+}-activated skinned rabbit muscle fibres. (i) represents a fibre containing only the MHCIβ isoform (former MHCI); (ii) represents a fibre which contains both the MHCIβ and the MHCIa isoform. (B) t_2 values (mean ± SE) of type Iβ and Iβ/Ia fibres. The type Iβ/Ia fibres are divided into two groups depending on their MHCIa content. * means significantly different from type Iβ fibres ($p < 0.01$, unpaired t-test).

between fibres with different MHC isoforms. Type Iβ fibres (852 ± 29 ms, SE, $n = 16$) exhibited significantly ($p < 0.01$, unpaired t-test) smaller t_2 values than type Iβ/Ia fibres (1067 ± 75 ms, $n = 21$). This difference is remarkable since the mean of the relative MHCIa content in type Iβ/Ia fibres was rather small (15% of total MHC content). The differences of the t_2

values are further displayed in Fig. 2B where the type Iβ/Ia fibres were divided into two groups depending on their MHCIa content.

4. Discussion

Subtypes of fast muscle fibres display a strong correlation between the MHC isoform complement and the kinetics of force responses following stepwise length changes. This is most conspicuous with regard to the t_2 value of stretch activation [7,8,16]. In hybrid fibres containing two fast MHC isoforms, the t_2 values depend on the relative proportion of each isoform [7,16]. The present results indicate that a similar relationship exists in slow fibres exhibiting both the MHCIβ and MHCIa bands. It appears, therefore, that the MHCIa band truly represents an additional slow myosin heavy chain isoform associated with specific functional properties. This novel finding indicates that a considerable fraction of slow muscle fibres contains two functionally distinct MHC isoforms.

The MHCIa isoform characterised in the present study appears to be distinct from the α-cardiac MHC isoform because the latter has a lower electrophoretic mobility than the β-cardiac isoform. As judged from its electrophoretic mobility, the MHCIa also seems to differ from the α-cardiac-like MHC isoform detected in the diaphragm and chronically stimulated fast-twitch muscles of rabbit [17]. Furthermore, the β-cardiac MHC isoform (predominantly present in cardiac ventricle) displays slower functional characteristics than the α-cardiac MHC isoform [18]. Taken together, our data suggest that MHCIa is a new MHC isoform with slower properties than MHCIβ (or the β-cardiac MHC).

Force responses following stepwise length changes may be related to the elementary process of force generation [2,19–21]. According to this interpretation, our results suggest that the two slow MHC isoforms exhibit different kinetic properties during the force-generating process. Thus, mammalian slow muscle fibres represent a heterogeneous population composed of fibres characterised by the expression of one or two MHC isoforms and the kinetic properties associated with each isoform. This presumably enables slow muscles to fulfil differing functional demands.

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