The Influence of the Rate of Rigor State Development on Its Tension in Single Muscle Fibre

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Abstract. The rigor tension and stiffness of glycerinated fibres from rabbit psoas muscle were found to vary markedly in dependence on the rate of substitution of the solutions in the experimental chamber. The maximum value of rigor tension, which is close to that activated by Ca^{2+} with pCa4, was obtained at the slow development of rigor in the absence of Ca^{2+} ions. The observed dependence is assumed to be due to the different degrees of removal of the 'slack' in fibres, which may be contributed by compliant ends of the preparation. A new method allowing to obtain rather reproducible values of rigor tension is proposed.

Key words: Rigor tension — Glycerinated fibres — Rabbit psoas muscle

Introduction

The skinned muscle goes into rigor state when, due to hydrolysis and/or diffusion of the substrate into the external solution the ATP concentration inside the fiber approaches zero. The interest in rigor stems from the idea that this state can be represented as the end-stage of the power stroke in the cross-bridge cycle. The increase of fibre stiffness in rigor is believed to be the result of the formation of actomyosin (AM) complexes with well defined unique rigor-type configuration, frequently termed 45°-configuration. However, the uniqueness of structural and mechanical features of AM complexes in the rigor state has been challenged by the results of Kawai and Brandt (1976). Single skinned fibre from crayfish muscle in rigor induced by Ca^{2+}-activated contraction ('high rigor') exhibited higher tension and stronger stiffness than if the rigor state was induced in the absence of Ca^{2+} ions ('low rigor'). This experimental result prompted the suggestion that there may be two different kinds of rigor AM complexes. At the same time, Moss and Haworth (1984), taking into account the considerable scattering of rigor tension values, which can reach 100%, suggested that the difference in mechanical features of 'high' and 'low' rigor states may, at least in

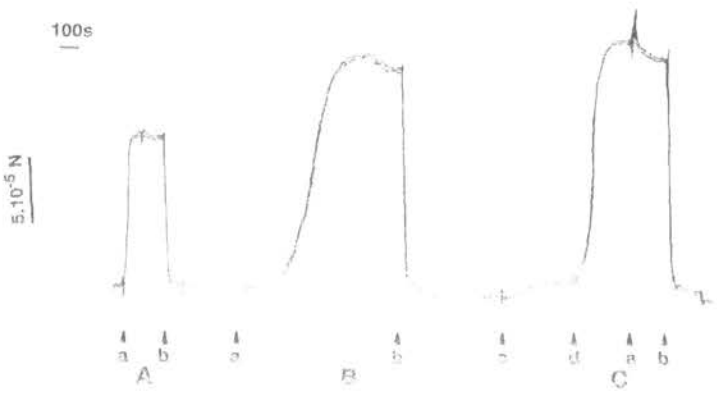

Fig. 1. Records of tension, developed by the same single fibre (65 μm in diameter) in rigor (*A*, *B*) and Ca^{2+}-activating solutions (*C*). The rates of the solution replacement were 500 μl s (*A*, *C*) and 5 μl s (*B*). The arrows indicate times, at which the injection of the following solutions: *a* — rigor; *b* — relaxing; *c* — Ca^{2+}-activating, pCa 5; *d* — Ca^{2+}-activating, pCa 4.

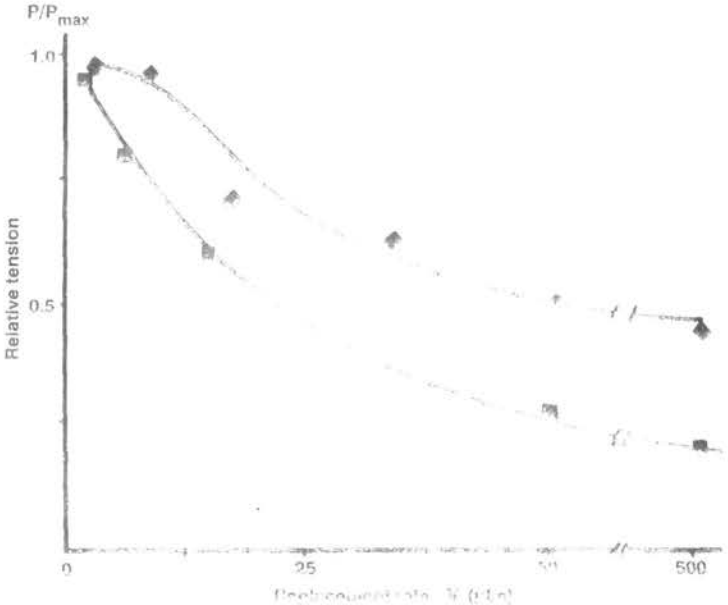

Fig. 2. Dependence of the relative rigor tension (P/P_{max}) on the rate of the solution replacement, V_{rep}, obtained for single fibres 65 μm (diamonds) and 45 μm (squares) in diameter. P_{max} is the rigor tension developed by 65 μm fibre at V_{rep} 3.3 μl s.

part, reflect the variations in the amount of end compliance in different preparations. On the other hand, since the rate of ATP depletion is of great importance for the development of rigor (White 1970) also conditions determining the substrate diffusion may be expected to affect the mechanical properties of the preparation in rigor state. To our knowledge, no quantitative analysis of this kind has been performed so far. The present study was undertaken in order to accurately characterize the dependences of rigor tension and stiffness in a single glycerinated fibre from rabbit psoas muscle both on variations in the rate of solution replacement during rigor development and on the fibre diameter.

Materials and Methods

Rabbit psoas muscle was glycerinated by the method of Huxley (1963). Single fibre segments, 1–3 mm in length and 40–70 μm in diameter, were glued between hooks (tungsten wire, $\varnothing$ 100 μm) of a tension transducer and length modulator. Mechanical measurements were made using an apparatus similar to that described earlier (Lednev et al. 1982). Sinusoidal length change of peak-to-peak amplitude of 0.1%–0.2% at 150 Hz was applied to the preparation continuously throughout the experiment. The peak-to-peak tension excursions were a measure of the fibre stiffness. The experimental chamber volume was 650 + 10 μl. All measurements were made at solution temperature of 20°C. The sarcomere length was measured by the position of the first diffraction maxima obtained with the aid of a He-Ne laser, and was controlled throughout the experiment. The initial sarcomere length was set to 2.4–2.6 μm in relaxing solution (in mmol l: KCl 85; EGTA 5; $MgCl_2$ 6; Na_2-ATP 5; imidazole 15; pH 7.0) by adjusting the overall length of the fibre segment. The rigor state was induced by substituting the relaxing solution in the experimental chamber for the rigor solution (in mmol l: KCl 100; EGTA 5; $MgCl_2$ 1; imidazole 15; pH 7.0) using a peristaltic pump LKB 2132. The rate of the substitution was varied within 500 μl s^{-1}–3 μl s^{-1} to obtain different time course of the rigor development. The rigor state was considered as complete if no further changes in the isometric tension were observed. Preparations which during the rigor development showed shortening of the sarcomere exceeding 10% were excluded from the present analysis. The solution in the chamber was actively stirred throughout the experiment. In several instances Ca-activating solution (in mmol l: KCl 85; EGTA 5; Na_2-ATP 5; $MgCl_2$ 6; creatine-PO_4 10; $CaCl_2$ 2.69 for pCa4 or $CaCl_2$ 2.3 for pCa5; imidazole 15; pH 7.0) was used to obtain maximal tension. In this case the fibre was activated by rapid replacement of the whole volume of the relaxing solution with the Ca^{2+}-containing solution.

Results

Fig. 1 shows a record of the contraction-relaxation cycles for the same preparation obtained under different conditions. As can be seen, the rigor tension varies markedly with the changing rate of the solution replacement, V_{rep} (Fig. 1*A*, *B*). The strength of this influence was found to depend on the fibre diameter. A decrease in V_{rep} from 500 μl/s to 3 μl/s leads to almost 4-fold increase in tension

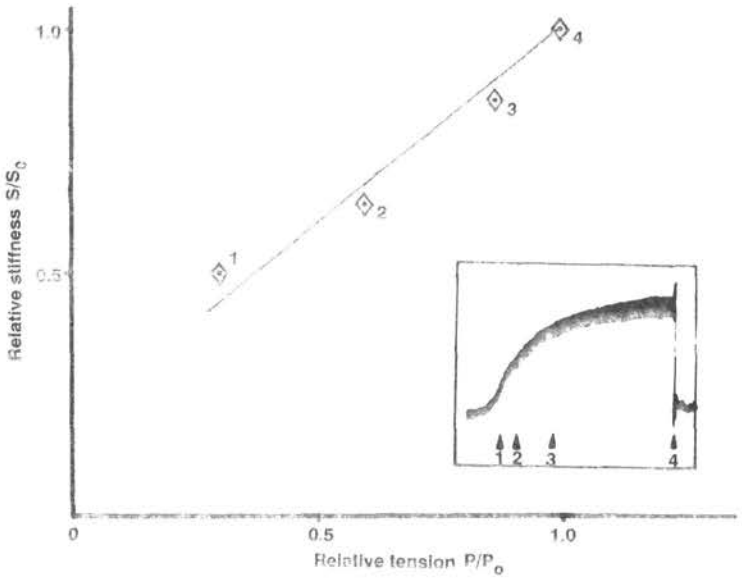

Fig. 3. Relationship between relative stiffness (S/S_{max}) and tension (P/P_{max}) during rigor development in single fibre, 65 μm in diameter. The inset shows the original record of the tension and stiffness growth. Oscillations (150 Hz) were superimposed. The arrows indicate measurement points of the characteristics.

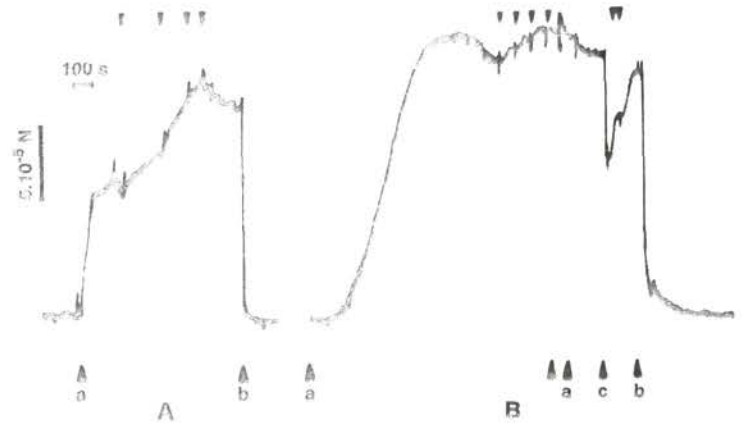

Fig. 4. The effect of the addition of MgATP to the preparation under rigor induced by rapid (*A*) and slow (*B*) replacement of the solutions. The rates of the solution replacement were 500 μl/s (*A*) and 5 μl/s (*B*). At *a* and *b* the rigor and the relaxing solution was injected respectively. At *c* the segment length was cut by 0.5 %. Arrows above the tension records indicate the additions of aliquots (10 μl) of the relaxing solution.

for 45 μm diameter fibre and only to 2-fold for 65 μm preparations (Fig. 2). In all experiments the tension growth was accompanied by increased stiffness (Fig. 3). The maximum rigor tension of 130 + 17 kN/m^2, which is close to that of Ca-activated at pCa4 (Fig. 1*C*) was obtained with V_{rep} not exceeding 5 μl/s. The 'high rigor' state was produced also following the method of Kawai and Brandt (1976). As shown in Fig. 1*C*, after Ca^{2+}-activated tension was produced with pCa4, Ca and ATP ions were removed by washing the preparation with Ca^{2+}-free rigor solution. The values of tension and stiffness for fibres introduced into 'high rigor' were 1.17 $\pm$ 0.1 and 1.04 $\pm$ 0.1 relative to their maximum values in Ca^{2+}-free rigor solution (Fig. 1*B*), respectively.

The effect of the addition of ATP in micromolar range to the preparations in rigor produced under different conditions was tested. As can be seen in Fig. 4, the fibre in rigor produced by fast replacement of bathing solutions exhibits a remarkable increase in tension (Fig. 4*A*) on the addition of small aliquots of the relaxing solution, while the fibre that has developed rigor at low rate of replacement shows only a small growth in tension. However, after the length of the preparation introduced into rigor 'slowly' had been manually shortened by about 0.5%, its response to the substrate addition became similar to that of 'rapid rigor' fibre (Fig. 4*B*).

Discussion

The data presented herein clearly demonstrate that the mechanical parameters of rigor muscle fibers critically depend on the rate of ATP diffusion from them. A possible explanation for this observation may be the following. When the rate of the solution replacement decreases the contraction phase during the rigor development is prolonged and the number of cycles performed by the cross-bridges can be expected to increase. The latter will produce a more complete removal of the 'slack', which may be contributed by such passive elements as compliant ends of the preparation (Tawada and Kimura 1984), and as a consequence, a higher value of rigor tension. The usual way to obtain the rigor state in skinned fibers is their rapid transfer from relaxing to the ATP-free solution (White 1970; Kawai and Brandt 1976; Moss and Haworth 1984; Tawada and Kimura 1984). During this procedure complete removal of the 'slack' may not be achieved, and a some rigor tension can develop. Thus it appears that changes in the time course of the rigor state development due to uncontrolled variations in the diameter of different preparations (even with the same procedure of rigor production), may be responsible for the considerable scattering of tension values (Moss and Haworth 1984). The procedure of rigor development described here, i.e. slow substitution of the solutions, provides the possibility of obtaining

rather reproducible values of rigor tension: the standart deviation of the maximum value did not exceed 13% ($n = 6$) for fibres with different diameters. The high reproducibility of characteristics of muscle preparations in rigor state is a prerequisite for studies of the dependence of rigor tension on physico-chemical parameters of the bathing solution such as pH, ionic strength, temperature, etc. Our data indicate that the maximal value of rigor tension may be achieved without Ca^{2+}-activating solution (Fig. 1*C*). As can be seen from Fig. 4*B*, the effect of MgATP on the fibre, which developed rigor at slow substitution of the solution in the absence of Ca^{2+} ions, is similar to that seen with the 'high rigor' preparation in the presence of Ca^{2+} ions (Fig. 4 in Kawai and Brandt 1976). We may conclude that the presence of Ca^{2+} ions is not nesesssary to produce the 'high rigor' state and that the differences in mechanical properties between 'high' and 'low' rigor states may be due to variations in the experimental procedures of rigor development, as suggested by Moss and Haworth (1984). It should be noted, however that the presented here results do not rule out the existence of structurally and functionally different actomyosin complexes in different muscles under rigor (Lednev 1983; Taylor et al. 1984; Ajtai and Burghardt 1986).

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