Elastic and Inelastic Behaviour of Resting Frog Muscle Fibres

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Abstract. Dynamic and static elasticity of the resting skeletal muscle of the frog have been studied as a function of the sarcomere length. Isolated intact fibres as well as glycerol extracted fibres show a resting tension starting at 2.05 μm sarcomere length and increasing approximately exponentially to 2·10^4 N/m^2 at 3.0 μm sarcomere length. Differences between the two types of preparation were seen in the dynamic experiments. The dynamic Young's modulus of intact fibres (recorded at 1 Hz and small amplitudes) increased from 2·10^5 N/m^2 at 2.1 μm sarcomere length to 2.5·10^6 N/m^2 at 2.9 μm while the static modulus varied from 5·10^3 N/m^2 to 3·10^5 N/m^2, the dynamic modulus at small amplitudes was equivalent to the modulus of the short range elasticity (SRE). The range of the SRE did not depend on the sarcomere length and amounted to about 5 nm per sarcomere. The dynamic modulus strongly depended on the amplitude: at large amplitudes the muscle became less stiff by a factor of 10 to 20. This tendency levelled off at about 10 Hz by a strain-induced relaxation process. The dynamic modulus of the glycerol extracted fibres was nearly of the same magnitude as the static modulus, there was neither evidence for an SRE nor for a significant amplitude-dependence of the dynamic modulus. For interpreting the results we propose to further develop the meander model of muscle (Pechhold et al. 1973 b).

Key words: Skeletal muscle — Resting tension — Dynamic Young's modulus — Short range elasticity

Introduction

In a stretched resting vertebrate muscle a static mechanical tension can be measured, the "resting tension". If stretching starts from a state of equilibrium, the muscle responds with a dynamic short range elasticity (SRE) (Hill 1968). The resting tension could be caused by the sarcoplasmic reticulum (Sandow 1966; Herbst 1975), connexin (Matsubara and Maruyama 1977), the sarcolemma (Rapoport 1972, 1973; Matsubara 1975) and the myofilaments (Hill 1968; Herbst 1974). Hill (1968) suggested long-lived cross-bridges between actin and myosin as source of the SRE. In order to be able to analyse the mechanical properties of the resting muscle it is important to be able to compare static and dynamic measurements at different sarcomere lengths. Therefore in the present work the resting tension, the static Young's modulus, the modulus of the SRE and the dynamic Young's modulus have been investigated as a function of the sarcomere length. These experiments were carried out on isolated intact fibres and glycerol extracted fibres of the frog.

Methods

Preparation. Experiments were performed on frogs (Rana temporaria). The isolated intact fibres of semitendinosus with long pieces of tendon attached were dissected in Ringer's solution. For the glycerol extraction bundles of fibres (semitendinosus) were treated in 50/50 glycerol-water solution for 48 h at 0°C and for measurements single fibres were dissected from the glycerinated bundles.

Experimental Apparatus. The muscle chamber was mounted in a machine (Zwick model 1442) for testing materials (Fig. 1) specifically designed for making dynamic measurements during the application of forced vibrations and constant velocity stretches.

Mechanical Parts. In the dynamic experiments an electrodynamic vibrator was used for excitation of the muscle (Ling, model 202). The displacement of the vibrator was measured by an inductive transducer (Schaevitz, model 010-M-LT). The muscle chamber was kept at constant temperature by a circuit of water, glass windows in the chamber allowed the passage of laser light for the measurements of the sarcomere length. The force transducer (unbonded strain gage, Toyo, model T7-8-240) had a rated capacity of 8·10^-2 N, an elasticity constant of 2.6·10^2 N/m and a loaded natural frequency of 64 Hz.
was possible to detect $10^{-6}$ N change in force. For experiments on whole muscle a force transducer with the characteristics (5.5 N; 1.24 $10^3$ N/m; 800 Hz; $10^{-4}$ N) was used. It was possible to produce a vertical movement of the machine for testing materials at a constant speed $L (5 \cdot 10^{-6} \text{ m/s} < L < 2 \cdot 10^{-2} \text{ m/s})$ and so change the initial stress and the sarcomere length respectively.

**Electrical Circuit.** The signal of displacement and force were compared with reference to amplitude and phase by a digital frequency response analyzer (Solartron, model 1170). The analyzer generated sine-wave-voltages with frequencies from $10^{-4}$ Hz to $9.999 \cdot 10^3$ Hz, which excited the vibrator via a power amplifier. The output signals of the displacement and force transducer were amplified by 5kHz carrier frequency amplifiers (Hottinger-Balwin, model KWS/3S-5) and analyzed by the analyzer or observed directly on the oscilloscope. A time recorder was used to investigate the development of force on stretching with constant speed [5.10$^{-6}$ m/s < L < 2.10$^2$ m/s] and so change the initial stress and the sarcomere length respectively.

**Solutions.** Solution 1 (Ringer's solution): 2.5 mM KCl; 1.8 mM CaCl$_2$; 115 mM NaCl; 0.85 mM NaH$_2$PO$_4$; 2.15 mM Na$_2$HPO$_4$; 9 mg/l tubo curarine, pH 7.0. This solution was used during the preparation and the analysis of single intact fibres. Solution 2: 50% glycerol (v/v); 10 mM Na$_2$HPO$_4$; 10 mM Na$_2$; pH 7.0. Muscle bundles for glycerol extraction were pretreated in this solution. Solution 3: 120 mM KCl; 5 mM EGTA; 10 mM MgCl$_2$; 10 mM ATP; 10 mM NaH$_2$PO$_4$; 5 mM NaN$_3$; pH 7.0. This solution was used in experiments on glycerol extracted fibres.

**Procedure.** Figure 2 lower graph shows the experimental procedure carried out on all muscle fibres. First the slack fibre was stretched until the beginning of force-development could be measured. The corresponding fibre length was called $L_0$. Then the fibre was stretched at constant speed $L$ from $L_0$ to the length $L_0 + \Delta L$, where relaxation could occur isometrically. "Relaxation" is used here for the transition into an equilibrium state, not for the transition from active contracting to resting state of muscle). After measurement of the dynamic modulus at 1 Hz and small amplitude a second stretch was begun with $\Delta L$. The upper graph is a sketch of the development of force with an initial steep slope and the sudden bend at a critical elongation $\Delta L_c$, as first described by D. K. Hill. He called the elasticity within the range $\Delta L_c$ short range elasticity (SRE) (Hill 1968). Using this procedure the following values could be calculated as a function of the sarcomere length:

- the static tension $\sigma_{stat}$
- the dynamic modulus at 1 Hz and small amplitudes $E_{1Hz}$
- the critical values of the SRE: the elongation $\Delta L_c$ and the corresponding tension $\sigma_c$
- the modulus of the SRE $E_{SRE} = \frac{\sigma_c}{\Delta L_c} \cdot L$

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**Fig. 1**

**Apparatus for mechanical measurements on muscle fibres**

**Fig. 2.** Procedure performed on all muscle fibres. Lower trace: program for changing the fibre length on time scale. Upper trace: tension response. In the stretching experiment the strain rate was about $2 \cdot 10^{-4}$ s$^{-1}$, the elongation range about 0.1 μm per sarcomere. The relaxed values of tension $\sigma_{stat}$ had been obtained at small sarcomere lengths a few minutes after the stretching ceased, and at greater sarcomere lengths after about 1 h. The oscillating traces indicate the measurement of the dynamic modulus at 1 Hz and small amplitudes. $\sigma_c$ and $\Delta L_c$ are the critical tension and the critical elongation of the short range elasticity (SRE).