

Thermal Dependence of Force – Velocity Relation of Lamprey Live Striated Muscle Fibres

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Abstract. The thermal dependence of the force-velocity relation ($P - V$) in thin (20–40 fibres) live twitch muscle bundles from suction apparatus of lamprey by force-clamp method was investigated. The $P - V$ relation was hyperbolic and Hill's constants were as follows: a/P_0 was 0.13 ± 0.01 and 0.08 ± 0.01 (mean \pm S.E.M.), b was 0.46 ± 0.02 and 0.65 ± 0.03 at 8°C and 18°C , respectively. The maximal isometric tension (P_0) was about 100 mN/mm^2 at 8°C , 18°C and 22°C . After the temperature was switched from 8°C to 18°C , the dependence of P_0 on incubation time was observed. The maximal power output determined from $P - V$ relation using Hill's equation was 0.062 ± 0.002 and 0.056 ± 0.001 at 8°C and 18°C , respectively. The maximal velocities of shortening (V_0) were $3.9 \pm 0.1 L_0/s$ and $7.2 \pm 0.2 L_0/s$ at 8°C and 18°C , respectively. Q_{10} for V_0 in this range of temperatures was 1.86. a/P_0 and power output were about 2 times lower than those reported in literature for other animals. In general, the thermal dependence of the parameters studied was similar to those reported for fish muscles and skinned lamprey muscles, P_0 being relatively independent, V_0 highly dependent, and a/P_0 inversely dependent on temperature.

Key words: Lamprey muscle contraction — Force — Velocity relation — Thermal dependence of contraction

Introduction

The regular relationship between force (P) and velocity (V) of shorting is a fundamental property of muscle activity. Different characteristics of muscles, their adaptation features, thermolability, etc., are reflected in $P - V$ relation (Close

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1972; Bennett 1984; Langfeld et al. 1989; Johnson and Johnston 1991). Therefore the determination of the $P - V$ relation in muscles which perform varied motor and posture functions in animals of different evolutionary levels and ecological adaptations is an important challenge of comparative and evolutionary physiology of muscle function.

In previous experiments where the thermal dependence of the $P - V$ relation had been studied it was shown that in amphibian, reptilian and mammalian muscles a/P_0 , determining a curvature of $P - V$ relation, did not depend on temperature (Lännergren 1978; Marsh and Bennett 1986; John-Alder and Bennett 1987; Edman 1988) or was increased with increasing temperature (Cechi et al. 1978; Ranatunga 1982). The $P - V$ relation in fish muscles was also extensively studied mainly using skinned fibres which gave possibility to study the effects of temperature on the mechanical properties of the contractile proteins directly. Using skinned muscle fibres of the fish it was shown that, being inversely related to, a/P_0 significantly depended on temperature (Johnston 1985; Johnston and Altringham 1985). However, previous investigations have shown that a/P_0 was changed as the result of fibre skinning (Ferenczi et al. 1984; Sobol and Nasledov 1992). Only few reports were published where the thermal dependence of the $P - V$ relation in live fish muscle fibres had been studied (Langfeld et al. 1989).

We have undertaken this study to investigate the thermal dependence of the $P - V$ relation in live fast striated muscle fibres of lamprey, a representative of the lowest class of the vertebrates – the Cyclostomata.

Materials and Methods

Preparation. Lampreys, *Lampetra fluviatilis*, were caught in river in autumn and kept in a tank with aerated water at 3–5°C for up to 5 months before use. Animals 27 ± 3 cm in length were used. After the lamprey was killed by destruction of the spinal cord, the muscle hyomandibularis glossus (4–7 cm in length), which belongs to the suction apparatus, was removed and immersed in Ringer's solution containing (in mmol/l): NaCl – 115, KCl – 2.5, CaCl₂ – 1.8, NaH₂PO₄ – 2.15, Na₂HPO₄ – 0.85 (pH 7.1). Fibre bundles, 150–250 µm diameter and consisting of 20–40 fibres, were dissected at 18°C. After measurements of largest (a) and smallest (b) diameters at two places and calculation of cross-section area (as $a.b/4$), aluminium T-clips were attached to the tendons and bundles mounted horizontally in the experimental trough having a volume 0.6 ml. The length of muscle fibres (L_0) was adjusted to give a maximum tetanic tension. Temperature was maintained by heat-stabilized water circulating through double walls of the trough.

Apparatus. Full details of the technique used have been described previously (Sobol and Nasledov 1992). Briefly, the fibre bundle was mounted between a force transducer (natural frequency – 1.2 kHz; sensitivity – 5 mV/mN; compliance – 2 µm/mN) (Sobol 1991), and a length transducer, an isotonic bamboo lever (40 cm) which was attached to a moving coil of a galvanometer. The isotonic afterload was generated by passing current through the coil. Tension and length were recorded on a dual beam oscilloscope photographically.

Stimulation. Muscle fibre bundles were stimulated via parallel platinum plate electrodes (5 ms duration, stimulus frequency 40 Hz at 8°C, 60 Hz at 18°C and 22°C). Stimulation lasted for 1–2 s with 5 min intervals.

General procedure. Fibre bundles were placed in the trough at one of the three temperatures (8°C, 18°C or 22°C) and series of isotonic quick-release were carried out for about 1.5 h. In 10 min after the temperature had been switched, the next series of measurements were performed. The $P - V$ relations were determined by the force-clamp method (Julian et al. 1986). Shortening velocity was measured 20–40 ms after the isotonic release. Maximum isometric force (P_0) was determined before each of the isotonic releases. To determine the maximum velocity of shortening (V_0), the slack step method was used (Edman 1979). Record of each fibre bundle were performed under one or three temperatures.

Analysis of data. Force-velocity curves obtained could be fitted to Hill's equation (Hill 1938):

$$(P + a)V = b(P_0 - P).$$

Force-velocity data have been linearized by plotting $(1 - P/P_0)/V$ against P/P_0 and a mean line was determined by the least squares method. a/P_0 was given by the intercept with the x axis and $1/b$ by the gradient. Lines were fitted to the force-velocity curves by computer using the constants a and b derived from the above analyses.

Statistics. Unless otherwise indicated, the results were expressed as the mean \pm the standard error of the mean and significance was analyzed of using Student's t -test. A value of $p < 0.01$ was considered to be statistically significant.

Results

The steady-state tetanic $P - V$ relation

Superimposed records of force and length during tetanus for two temperatures (8°C and 22°C) are shown in Fig. 1. Releases were performed during the plateau of maximum isometric tetanic contraction, 600 ms after initiation of stimulation.

$P - V$ relations for the two temperatures (8°C and 18°C) are shown in Fig. 2. Hyperbolic functions were fitted to the results for each fibre bundle. Hill's constants were determined from linearized equation (see Materials and Methods). Two curves were considerably different at $P/P_0 < 0.3$ where the curvature of the $P - V$ relation at 18°C was more pronounced than that at 8°C (Fig. 2). There were no significant temperature differences at the intermediate and the high forces. At 22°C the results were similar to those obtained at 18°C. Mean values of mechanical characteristics of fibre bundles at three temperatures are presented in Table 1.

Maximum velocity of shortening and maximum power output

Typical slack test responses recorded from one fibre bundle for two temperatures are shown in Fig. 3. The steps were initiated from a fixed initial length (L_0). Usually, 3 to 5 slack releases of different length were applied during the force plateau. V_0

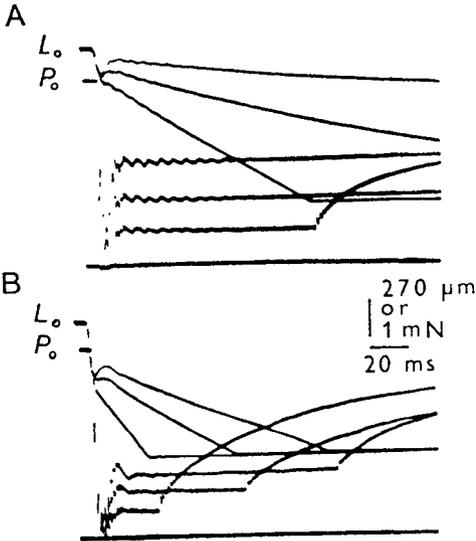


Figure 1. Three isotonic quick releases performed on a lamprey live fibre bundle. *A* - at 8°C and *B* - at 22°C. Upper traces: length; middle traces: corresponding tension; bottom line: resting tension. The velocities of shortening were: 1.02, 0.51 and 0.33 L_0/s at 8°C, and 2.25, 1.21 and 0.72 L_0/s at 22°C. $L_0 = 8.0$ mm, $P_0 = 4.1$ mN at 8°C and 4.5 mN at 22°C.

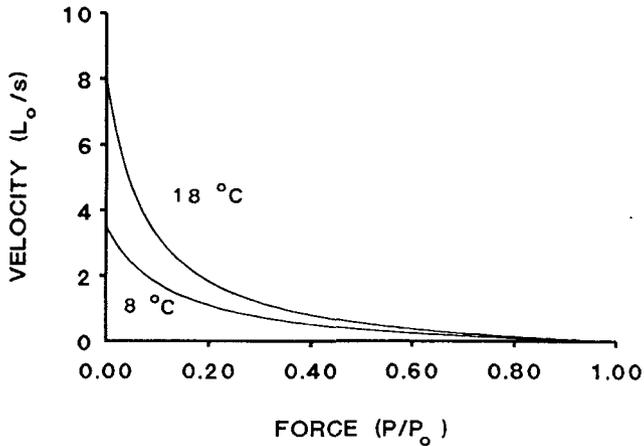


Figure 2. Force-velocity curves for lamprey fibre bundles at 8°C and 18°C. The shortening velocity (L_0/s) is plotted as a function of the fractional load (P/P_0) for that velocity. Results represent the determination on sixteen muscle fibre bundles at 8°C and on nine at 18°C. The continuous line was computed from constant a and b which are given in Table 1.

was expressed in units of L_0/s . The values of V_0 for three temperatures are given in Table 1. Its value is significantly greater at 18°C or 22°C than at 8°C. Q_{10}

Table 1. The contractile characteristics of lamprey muscle fibre bundles at different temperatures.

Parameter	mean \pm S.E.M.		
	8°C (<i>n</i> = 16)	18°C (<i>n</i> = 9)	22°C (<i>n</i> = 5)
Maximum velocity of shortening, V_0 (L_0/s)	3.9 \pm 0.1*	7.2 \pm 0.2	7.1 \pm 0.2
Q_{10} for V_0	—	1.86	
Maximum isometric force, P_0^{**} (mN)	2.9 \pm 0.2	3.3 \pm 0.4	3.0 \pm 0.3
(mN/mm ²)	82 \pm 31	94 \pm 12	89 \pm 10
Maximum power ($P \cdot V/P_0 V_0$)	0.062 \pm 0.002	0.056 \pm 0.001	0.056 \pm 0.001
a/P_0	0.13 \pm 0.01*	0.08 \pm 0.01	0.09 \pm 0.01
b , (L_0/s)	0.46 \pm 0.02*	0.65 \pm 0.03	0.67 \pm 0.04

* - $p < 0.01$

** - data from 4 experiments concerning dependence of P_0 on time of incubation were not included.

calculated for V_0 was 1.86 and was comparable to that reported earlier for lamprey muscle (Nasledov et al. 1989).

The normalized mechanical power output ($P \cdot V/P_0 \cdot V_0$) calculated from the force-velocity curve was 0.062 (Table 1). It was observed that the load at which maximal normalized power output was calculated using Hill's equation was increased from $P = 0.22 \cdot P_0$ at 18°C to $P = 0.31 \cdot P_0$ at 8°C. A similar tendency for live fish fibres was reported by Langfeld et al. (1989). The decrease of the power output with increasing temperature from 8°C to 18°C is a consequence of greater increase of V_0 (1.86 times) than that observed for absolute power output ($P \cdot V$, 1.61 times, as calculated from Fig. 2.).

Maximum isometric force

We calculated both the absolute force and the force per cross-section area of muscle bundles (Table 1). P_0 attained its value within 10 min after the temperature had been changed. However, after the temperature switch from 8°C to 18°C a gradual increase of P_0 was observed in the case when the experiments had been started and lasted for about 2 h at 8°C ($n = 4$, Fig. 4, data not shown in Table 1). At the same time a/P_0 and V_0 remained unchanged. Under other condition, at any of the

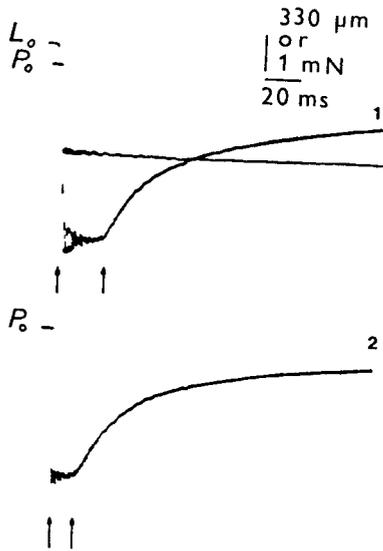


Figure 3. Examples of measurements of the maximum velocity (V_0) by the slack test method. Traces labelled 1 and 2 are the output of the force transducer for the same muscle fibre bundle as in Fig. 1 stimulated isometrically at 8°C and 22°C, respectively. Release amplitude was 1.0 mm in both cases. Arrows indicate the determination of the "slack time". V_0 was 3.5 L_0/s at 8°C and 6.9 L_0/s at 22°C, respectively.

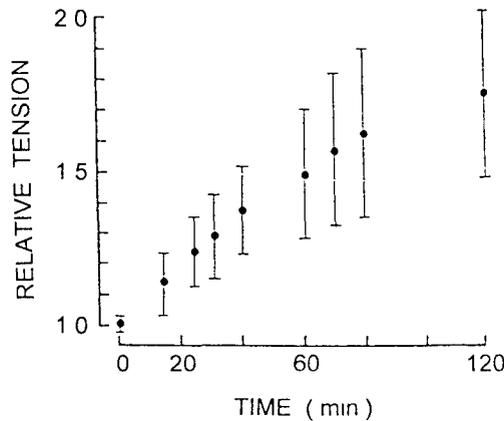


Figure 4. The dependence of relative tension on time of incubation at 18°C after the experiment was started at 8°C. Ordinate: maximum tension at 18°C normalized to tetanus at 8°C. Abscissa: time of incubation (10 min after the temperature was switched from 8°C to 18°C the time scale was taken to be equal to zero). Mean \pm S.D.M.

three temperatures used. P_0 did not vary more than by 7% during the whole time of registration.

Discussion

In this study the $P - V$ relation of intact lamprey muscle fibre bundles was found to become more curved, i.e., a/P_0 decreased with increasing temperature from 8°C to 18°C. It can be explained so that in the region where the velocities are close to V_0 the force-velocity curve changed with temperature more than in the region at $P/P_0 > 0.3$ where this curve changed with temperature very little. An analysis of the Huxley (1957) model by Eisenberg et al. (1980) has shown that the most part of the force-velocity curve, from P_0 to approximately $P_0/4$, is practically determined by the rate of attachment of the cross-bridge and only for velocities which were closed to V_0 the shape of the curve was affected by the detachment rate. Thus, for lamprey muscle the rate of attachment of the cross-bridge changed very little with temperature in contrast to the detachment rate. This feature of temperature dependence of $P - V$ relation appears to be also typical of fish muscle fibres and may account for inverse dependence of a/P_0 on temperature. In contrast, in amphibians a/P_0 has been shown to be independent of temperature (Lännergren 1978; Edman 1988) or to be slightly increased with increasing temperature (Cechi et al. 1978). An inverse temperature dependence of a/P_0 is considered to increase power output at low temperature and may reflect evolutionary adaptation for cold adapted fish species (Johnston and Altringham 1985). The inverse temperature dependence of a/P_0 permits also to increase both fish motility and the efficiency in converting free energy into work on increasing the temperature. This can be explained by the correlation of V_0 with tailbeat frequency (Rome et al. 1988) which in turn is a major factor limiting maximum length specific swim speed (Johnston and Altringham 1988; Rome et al. 1988). As for efficiency, Woledge (1968) argued a relationship between efficiency of energy conversion by a muscle and the curvature of its force-velocity curve, i.e., the more the $P - V$ relation was curved, the more efficient the muscle worked. For example, one of reasons for efficiency of fish muscle to be increased on increasing the temperature may be deficiency in oxygen supply. In respect to lamprey, Hardisty and Rovainen (1982) reported that adult lampreys could swim effectively over short distances.

It is interesting to note that a/P_0 for lamprey muscle fibre is lower than that noticed in literature for all kinds of muscle except for dogfish skinned myotomal muscle fibres where a/P_0 was found to be equal to 0.06 at 8°C (Altringham and Johnston 1982). It may be accounted for that a/P_0 was decreased after skinning (Ferenczi et al. 1984). Thus, for the whole single muscle fibre of the frog, a/P_0 was recently shown to be decreased after skinning from 0.28 to 0.06 at 8°C (Sobol and Nasledov 1992). At last, Curtin and Woledge (1988) reported that in dogfish live myotomal muscle fibres a/P_0 was equal to 0.27 at 12°C. The low value of a/P_0 observed in our experiments can be supposed to be a consequence of lamprey swimming style, which differs from that of fishes, and/or of muscle location in

body of lamprey. Johnson and Johnston (1991) observed that in *Callionymus lyra* a/P_0 was lower for fast fibres isolated from the myotomes than from the adductor profundis muscle.

Skinning apparently does not influence the thermal dependence of a/P_0 . A value of 1.6, Q_{10} for a/P_0 in our experiments for live muscle is comparable to that for Pacific blue marlin skinned fast muscle fibres ($Q_{10} = 1.4$) and lower than for icefish skinned fast muscle fibres ($Q_{10} = 2.8$) (Johnston and Altringham 1985, values calculated from their Fig. 1).

In our experiments V_0 was found to be increased by 86% when the temperature was raised from 8°C to 18°C or 22°C. This value is similar to those for living (Langfeld et al. 1989) and skinned fish muscle fibres (Johnston and Altringham 1985) and comparable to many reported values of Q_{10} for V_0 for different animals (Bennett 1984).

The values of isometric force normalized for cross-section area of lamprey fibre bundles are about two times lower than those reported by other authors for fish living muscle fibres (Johnson and Johnston 1991). We cannot exclude the explanation that in our experiments damaged fibres which did not develop tension were involved in cross-section area calculation.

It was found that P_0 was almost independent of temperature in the range from 8°C to 22°C, which was in agreement to literature data of Q_{10} for P_0 (Bennett 1984). However, when the fibre bundles were incubated for a long time at 8°C and then the temperature was switched to 18°C, P_0 was increased by more than 70 per cent within 2 h after the incubation at 18°C. The nature of P_0 dependence on incubation time appears to be like “temperature-induced tension hysteresis” described by Johnson and Johnston (1991). In their experiments following exposure to low temperature the maximum value of tetanic tension was recovered at the optimum temperature only in 20–30 min. The mechanisms underlying this phenomenon remain unclear.

The normalized power output for lamprey muscle fibres is about two times lower than that reported in literature (Curtin and Woledge 1988; Langfeld et al. 1989) and lies between values reported for frog fast (0.096) and reptilian slow (0.042) muscle fibres (Woledge 1968). Such low value of normalized power output is accounted for the high curvature of the $P - V$ relationship.

In conclusion, the measurements of the contractile properties of lamprey striated muscle at different temperatures are in reasonable agreement with the results obtained by others in living and skinned fish muscle fibres. The force-velocity relation of fibre bundles becomes more curved at high temperature. a/P_0 and, as a consequence, the normalized power output are lower than that reported for all kinds of living fish muscle fibres. The maximum force depends on temperature weakly, i.e. it exhibits temperature compensation. In contrast, the maximum velocity of shortening depends on temperature significantly which means the absence of tem-

perature compensation. At appropriate conditions at 18°C P_0 has been increased in a "time dependent" manner.

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