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TEMPERATURE ACCLIMATION IN THE COMMON CARP: FORCE–VELOCITY CHARACTERISTICS AND MYOSIN SUBUNIT COMPOSITION OF SLOW MUSCLE FIBRES

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Summary

Live slow fibre bundles were isolated from the superficial region of the pectoral fin abductor superficialis muscle of common carp (*Cyprinus carpio* L.) acclimated to either 8 or 20°C.

The maximum tetanic tension (P_0) of fibre bundles was similar when measured at the acclimation temperature of each group. However, at 8°C, P_0 was significantly higher in 8°C- than in 20°C-acclimated fish (202±8 *versus* 153±4 kN m^{-2}, respectively). For isometric tetani at 8°C, the times to 50 % peak force and from peak force to 50 % relaxation were 15–20 % faster in preparations from cold- than from warm-acclimated carp. Force–velocity (P–V) curves were fitted using a hyperbolic–linear equation. The curvature of the P–V relationship was found to be independent of acclimation temperature. Unloaded contraction velocity (V_{max}) was 17 % higher at 8°C in fibres from fish acclimated to 8°C than in fish acclimated to 20°C (1.18±0.04 and 0.98±0.04 muscle lengths s^{-1}, respectively). Calculated values for maximum power output at 8°C were 26.5 W kg^{-1} for cold-acclimated and 18.0 W kg^{-1} for warm-acclimated fish.

Native myosin was purified from isolated fibre bundles using sodium pyrophosphate gel electrophoresis. The mobility of myosin heavy chains on 8 % SDS–PAGE gels was similar for both acclimation groups. Myosin light chain subunits were separated on 15 % SDS–PAGE gels. Fibre bundles from warm-acclimated fish contained almost exclusively slow myosin light chains ($LC1_s$ and $LC2_s$). Preparations from cold-acclimated fish contained a significant proportion of fast myosin light chains ($LC1_f$ and $LC2_f$) in addition to $LC1_s$ and $LC2_s$. Histochemical studies revealed no differences in the fibre composition of preparations from warm- and cold-acclimated fish: both contained an average of 3 % fast oxidative fibres in addition to slow fibres.

It is concluded that cold-acclimation results in modest improvements in the contractile performance of slow muscle fibres at low temperatures. The mechanism may involve the expression of myosin light chain isoforms normally associated with faster-contracting fibre types.

Key words: slow muscle fibres, force–velocity relationship, myosin, temperature acclimation, common carp, *Cyprinus carpio*.

Introduction

The maximum cruising speed of some freshwater fish is increased at low temperatures and decreased at high temperatures after several weeks of cold acclimation (Fry and Hart, 1948; Heap and Goldspink, 1986). The mechanisms underlying this plasticity in swimming performance are complex and include changes in the relative proportions of different muscle fibre types (Johnston and Lucking, 1978; Jones and Sidell, 1982), altered patterns of muscle fibre recruitment (Rome *et al.* 1985) and adaptations in the properties of membranes and nerves (Harper *et al.* 1989). In the goldfish (*Carassius auratus* L.) the ATPase activity of fast muscle myofibrils is around three times higher at 1°C in fish acclimated to 1°C than in those acclimated to 26°C (Johnston *et al.* 1975). Similar increases in ATPase activity with cold-acclimation occur in other cyprinids, including common carp (*Cyprinus carpio* L.) and roach (*Rutilus rutilus* L.) (Heap *et al.* 1985), but may not be widespread among teleosts (Walesby and Johnston, 1981; Jones and Sidell, 1982; Sidell and Johnston, 1985; Johnston and Dunn, 1987).

Skinned fibres have been used to investigate the force–velocity characteristics of fast and slow myotomal muscles in common carp acclimated to either 7 or 23°C (Johnston *et al.* 1985). At 7°C, maximum tension (P_0) and unloaded contraction velocity (V_{max}) were about 1.5–2.0 times higher in cold- than in warm-acclimated fish. Recent studies, however, have shown differences in the mechanical properties of skinned and live fish muscle fibres. In particular, skinned fibres have lower tensions and shortening speeds, making them unsuitable for quantitative calculations of muscle power output (Altringham and Johnston, 1988; Curtin and Woledge, 1988). Unfortunately, all attempts to obtain a suitable live fibre preparation from fast myotomal muscle in the carp have proved unsuccessful (see also Rome *et al.* 1988). However, stable intact preparations can be isolated from the superficial slow fibre region of the pectoral fin abductor superficialis (Ab.s) muscle. In the present study we have used this preparation to calculate muscle power output from the force–velocity relationship in carp acclimated to either 8 or 20°C. The effect of temperature acclimation on the subunit composition of slow muscle myosin has also been investigated.

Materials and methods

Fish

Common carp (*Cyprinus carpio* L.) were obtained from Humberside Fisheries, Driffield, England. The total length and body mass of the fish studied was 31.0±2.5 cm and 735±10 g (mean±S.D.; N=24). Fish were held in tanks of partially recirculated filtered fresh water at either 8 or 20°C for 6–12 weeks (12 h light:12 h dark). Trout pellets were fed daily.

Isolation of muscle fibre bundles

Carp were killed by a blow to the head followed by decapitation and pithing.

The intact pectoral fin and associated skeleton were removed from the fish, the cleithrum being severed at the level of the lateral line. The assembly was pinned out on a silicone elastomer base (Sylgard 184, Dow Corning), with the abductor superficialis muscle (Ab.s) face down. The preparation was covered with Ringer's solution (in mmol l^{-1}): NaCl, 119.0; sodium pyruvate, 10; KCl, 2.7; $MgCl_2$, 1; $CaCl_2$, 1.8; $NaHCO_3$, 2.5; pH 7.4 at 5°C). The other muscles of the pectoral assembly were removed and the bone reduced as far as possible without damaging the attachments of the Ab.s. The preparation was repinned *via* the skin and the layer of fast fibres removed from the ventral surface. Finally, the slow fibre region was pared down to a bundle of 60–100 fibres (see Fig. 1). Aluminium foil clips were attached as close to the bone as possible (200–300 μm), and the bone was further reduced. Dissection was performed on a cooled plate (8°C) and the Ringer was changed frequently.

Measurement of contractile properties

The apparatus consisted of a Perspex chamber through which aerated Ringer was circulated at constant temperature (±0.1°C). Force was measured using a silicon beam strain gauge (AE 801, AME, Horten, Norway). Muscle length was measured and controlled *via* a servo motor (MFE model R4–077, Emerson Electronics, Bourne End, Bucks) and control unit built in-house (Altringham and Johnston, 1988). Sarcomere length was measured by laser diffraction and set to 2.3 μm. This resting sarcomere length was selected on the basis of preliminary experiments on the length–tension relationship of fibres. The average length of fibre bundles was measured using a binocular microscope. Fibres were stimulated *via* two platinum wire electrodes lying on either side, using 1.5 ms pulses at 1.2× threshold voltage. The stimulus strength and frequency required to produce maximum tetanic contractions were determined at 8 and 20°C for each preparation. Data were collected and analysed on a Nicolet 3091 digital oscilloscope, and stored on a BBC microcomputer.

The isometric properties of fibre bundles from both acclimation groups were measured at 8 and 20°C. The effects of temperature were reversible so several cycles of heating and cooling were performed on each preparation. Contraction velocity was measured at various loads using isovelocity releases (see Fig. 2A). During the plateau phase of tetanus (2 s, 25 Hz) the preparations were given an initial 2 ms release, of varying magnitudes, to lower the tension. A second, slower release was adjusted to hold the tension constant after the step (Altringham and Johnston, 1988). Force–velocity (P–V) curves were constructed by plotting velocity against the relative tension over the first 10 ms interval after release. P–V data for individual fibres were fitted to a hyperbolic–linear curve described by Marsh and Bennett (1986):

$$V = [B(1 - P/P_0)/(A + P/P_0)] + C(1 - P/P_0),$$

where B and C have dimensions of velocity and A is dimensionless. Data were iteratively fitted to the equation without constraining the curve to go through P_0.

Starting with a wide range of values for each constant, and stepping through them with progressively smaller ranges and increment sizes, the values yielding the minimum mean squared differences between observed and predicted data were calculated. The standard error of the estimate (S.E.E.) for the curve-fitting procedure was determined for each fibre from the equation:

$$\text{S.E.E.} = \sqrt{\text{RSS}/(N-2)},$$

where RSS is the residual sum of squares and N is the number of data points.

At the end of experiments preparations were frozen at their resting lengths using isopentane cooled to its melting point in liquid nitrogen (−159°C). Frozen fibre bundles were inserted into a block of liver tissue mounted on a cryostat chuck and re-frozen. Frozen sections (10 μm) were cut and stained for myofibrillar ATPase and succinate dehydrogenase activity (Johnston *et al.* 1974). The cross-sectional area of individual muscle fibres was determined by digital plannimetry using a microscope drawing arm. The total cross-sectional area of muscle fibres, excluding connective tissue and space, was used to calculate the tension.

Electrophoresis

Sample preparation

The myosins from seven warm-acclimated and six cold-acclimated carp were studied electrophoretically. Muscle fibre bundles (5–18 mg) were placed in a test tube containing 25 vols of 40 mmol l^{-1} NaCl, 3 mmol l^{-1} phosphate buffer pH 7.0 at 2°C and crushed with a glass rod. The buffer was removed and replaced with 25 vols of a solution containing (mmol l^{-1}): $Na_4P_2O_7$, 50; EGTA, 2.5; β-mercaptoethanol, 1; 50 % glycerol, pH 8.8 at 2°C. The sample was homogenised using a hand-held pestle, and left to extract for 60 min on ice. The myosin extract was centrifuged at 40 000 *g* for 60 min at 1°C, and stored at −20°C.

Polyacrylamide gel electrophoresis

Sodium pyrophosphate gels contained: 25 mmol l^{-1} $Na_4P_2O_7$ (pH 8.8 at 1°C), 3.8 % acrylamide, 0.2 % *N*,*N*′-methylene bisacrylamide (BIS), 10 % glycerol, 0.1 % *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and 0.05 % ammonium persulphate. The electrode buffer, which contained 25 mmol l^{-1} $Na_4P_2O_7$ (pH 8.8 at 1°C) and 10 % glycerol, was recirculated at 700 ml min^{-1}. Electrophoresis was carried out at 1–2°C. Samples (20 μl for analytical and 60 μl for preparative gels) were loaded and run on to the gel for 30 min at 35 V; the voltage was then increased to 100 V and run for 18 h. The myosin bands were cut out from preparative gels, rapidly stained with Coomassie Blue, and incubated for 30 min at 30°C in an equal volume of 125 mmol l^{-1} Tris–HCl pH 6.75, 4 % SDS, 5 % β-mercaptoethanol, 20 % glycerol and 0.004 % Bromophenol Blue. The subunit composition of myosins was examined using 8 % and 15 % SDS–PAGE gels (Laemmli, 1970).

Staining

Gels were stained for 2 h at 30 °C in 0.1 % Coomassie Blue, 50 % methanol, 7 % acetic acid, and destained in 50 % methanol, 7 % acetic acid. A rapid method was used for preparative gels. The gel was placed in the same staining solution as above until the bands became visible (about 5 min) and washed for 10 min in five changes of ultrapure water (Milli-Q). The bands were then ready for cutting out for SDS–PAGE. SDS–PAGE gels of the myosin light chains were silver-stained using the Sigma silver stain kit. Protein bands were characterised using myosin light chain markers from carp myotomal muscles (Crockford and Johnston, 1990; Johnston *et al.* 1990) and proteins of known relative molecular mass (14×10^3–200×10^3) (Sigma). Gels were scanned at 650 nm using a Shimadsu CS 9000 densitometer.

Statistical analysis

Data for the two acclimation groups were compared using an unpaired *t*-test.

Results

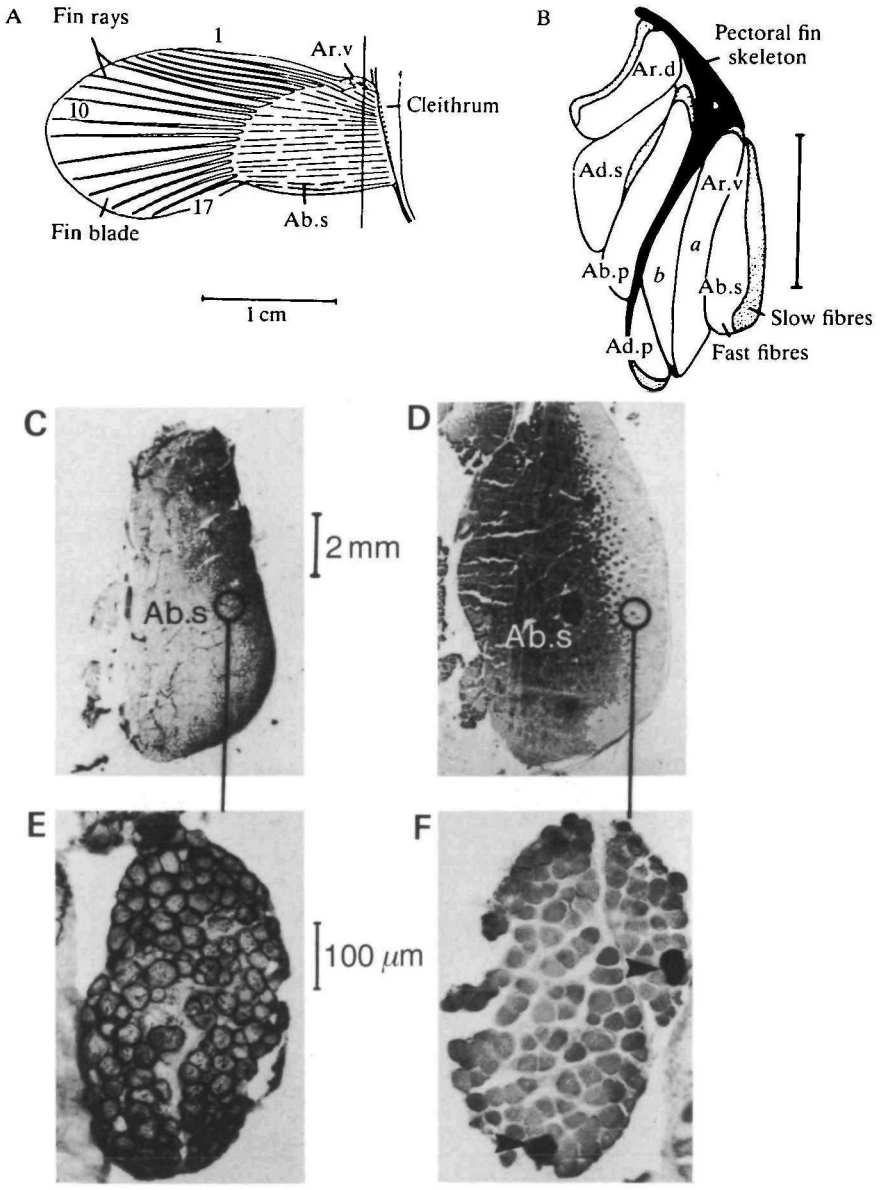
Histochemistry

The arrangement of pectoral fin muscles in the carp is shown in Fig. 1. Isolated fibre bundles were fibre-typed using serial frozen sections stained for myofibrillar ATPase and succinate dehydrogenase (SDH) activity (Fig. 1). The preparations were mostly composed of slow fibres which have a high SDH activity and a low myofibrillar ATPase activity which was readily inactivated at pH 10.4 (Johnston *et al.* 1974). Some preparations also contained a small percentage of fibres which stained intensely for myofibrillar ATPase activity following alkaline preincubation (Fig. 1). These correspond to fast oxidative or 'intermediate' muscle fibres (Johnston *et al.* 1974). The percentages of fast oxidative fibres in the preparations were 3.3±3.1 for from 8 °C-acclimated and 2.8±2.7 for 20 °C-acclimated fish (mean±S.D.).

Isometric properties

Isometric contractile properties showed a range of thermal sensitivities and varied with acclimation temperature (Table 1). R_{10} values for maximum tetanic tension (a number analogous to Q_{10} for non-rate variables, Bennett, 1984) were 1.10 and 1.34 for fish acclimated to 8 and 20 °C, respectively. At 8 °C, maximum tetanic tension was 32 % higher in fibres from 8 °C- than in those from 20 °C-acclimated fish ($P<0.01$). Values for tetanic tension measured at the acclimation temperature of each group were similar (Table 1).

The times to 50 % peak force ($T_{0.5a}$) and from peak force to 50 % relaxation ($T_{0.5r}$) were measured (Fig. 2B; Table 1). For isometric twitches $T_{0.5r}$ was much more temperature dependent than $T_{0.5a}$ (Fig. 2B). Q_{10} values were 2.53 and 2.13 for $T_{0.5r}$ and 1.21 and 1.28 for $T_{0.5a}$ in 8 °C- and 20 °C-acclimated fish, respectively. The corresponding Q_{10} values for tetanic contractions were more similar: the Q_{10}



values for $T_{0.5a}$ and $T_{0.5r}$ were, respectively, 2.14 and 2.17 in 8°C-acclimated fish and 1.69 and 1.86 in 20°C-acclimated fish. For both twitches and tetani, $T_{0.5a}$ was 15 % higher and $T_{0.5r}$ was 20 % higher at 8°C for fibres from 8°C- than for those from 20°C-acclimated fish (Table 1, $P<0.01$). Measured at the respective acclimation temperatures, $T_{0.5a}$ was 1.6 times and $T_{0.5r}$ was 1.7 times faster in 20°C- than in 8°C-acclimated fish. Thus, the rates of tension development and relaxation exhibited capacity adaptations in the region of 13–17 % following cold acclimation.

Fig. 1. Isolation of muscle fibre bundles from the abductor superficialis muscle of the common carp (*Cyprinus carpio* L.). (A) Ventral surface of the pectoral fin assembly. The numbers correspond to the fin rays. (B) Transverse section through the pectoral fin muscles. Information on the distribution of muscle fibre types was obtained by staining frozen sections for myofibrillar ATPase and succinic dehydrogenase activities (see text for details). The nomenclature used follows Winterbottom (1974). Abductor superficialis, Ab.s; arrector ventralis, Ar.v; arrector dorsalis (this muscle contains two distinct lobes, *a* and *b*, with the fibres having different orientations), Ar.d; abductor profundis, Ab.p; adductor profundis, Ad.p; adductor superficialis, Ad.s. (C) Transverse sections of the whole Ab.s stained for succinic dehydrogenase activity. The circle illustrates the area of muscle used to dissect fibre bundles. (D) Transverse sections of the whole Ab.s stained for myofibrillar ATPase activity. The circle illustrates the area of muscle used to dissect fibre bundles. (E) A transverse section of a typical muscle fibre bundle stained for succinic dehydrogenase activity. (F) A transverse section of a typical muscle fibre bundle stained for myofibrillar ATPase activity. The muscle fibre bundles in E and F were both isolated from an 8°C-acclimated fish. Fast oxidative fibres are marked by arrowheads (F).

Table 1. *Contractile properties of fibre bundles isolated from the pectoral fin superior abductor muscle*

	8°C-acclimated fish		20°C-acclimated fish	
Parameter	At 8°C	At 20°C	At 8°C	At 20°C
Maximum isometric tension ($kN\,m^{-2}$)	202±8	226±19	153±4**	218±8
Twitch half-rise time (ms)	46.2±1.6	36.6±2.9	53.1±1.8*	39.6±0.7
Twitch half-relaxation time (ms)	141.3±3.1	46.2±1.8	169.2±2.9**	68.4±2.8**
Tetanus half-rise time (ms)	125.4±4.3	50.2±2.7	143.9±3.6*	76.4±4.6**
Tetanus half-relaxation time (ms)	156.6±4.1	61.9±4.4	189.3±8.5*	89.8±3.6*

Values are mean±S.E. (N=6). The results for each acclimation group were compared at 8 and 20°C.

* $P<0.05$ and ** $P<0.01$ denotes a significant difference relative to 8°C-acclimated fish.

Force–velocity relationship

The P–V relationship of fibres was studied at 8°C. Representative P–V curves from cold- and warm-acclimated carp are shown in Fig. 3, and all the data are summarised in Table 2. The unloaded contraction velocity (V_{max}) of fibres was 17% higher in 8°C- than in 20°C-acclimated fish ($P<0.05$). $W_{max}/(V_{max}P_0)$, which provides a measure of the curvature of the force–velocity relationship (Marsh and Bennett, 1986), was not affected by acclimation temperature. The calculated maximum mechanical power output was 47% higher at 8°C in 8°C-

than in 20°C-acclimated fish, largely due to greater force production in the cold-acclimated fish (Table 2).

Electrophoresis

On sodium pyrophosphate gels myosins from the slow region of the Ab.s co-

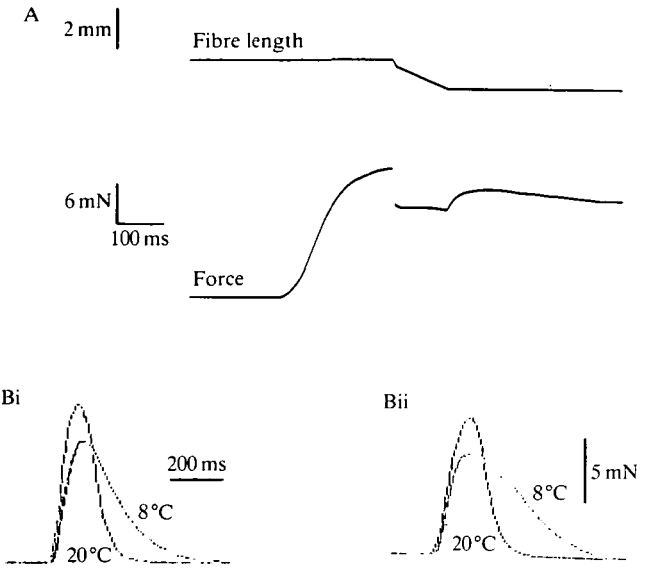


Fig. 2. (A) A typical isovelocity release of carp slow muscle fibres to illustrate the stability of force records during shortening. (B) Isometric twitch contractions of preparations from (i) 8°C-acclimated and (ii) 20°C-acclimated carp at 8 and 20°C.

Table 2. *Force–velocity characteristics of fibre bundles isolated from the pectoral fin superior abductor muscle*

	Acclimation temperature	
Parameter	8°C	20°C
V_{max} ($L\,s^{-1}$)	1.18±0.04	0.98±0.04*
W_{max} ($W\,kg^{-1}$)	26.5	18.0*
Load for maximum power output	$0.48P_0$	$0.46P_0$
A	0.040±0.006	0.046±0.004
B ($L\,s^{-1}$)	0.032±0.005	0.031±0.003
C ($L\,s^{-1}$)	0.32±0.03	0.32±0.02
$W_{max}/(V_{max}P_0)$	0.11±0.007	0.12±0.007
r^2	0.98	0.98

All experiments were carried out at 8°C.

V_{max}, extrapolated maximum contraction velocity; W_{max}, maximum power output; A, B and C are constants from the hyperbolic–linear equation; $L\,s^{-1}$, muscle lengths per second; P_0, maximum tetanic tension.

Values are mean±S.E., where relevant, $N=6$.

* $P<0.05$ denotes a significant difference relative to 8°C-acclimated fish.

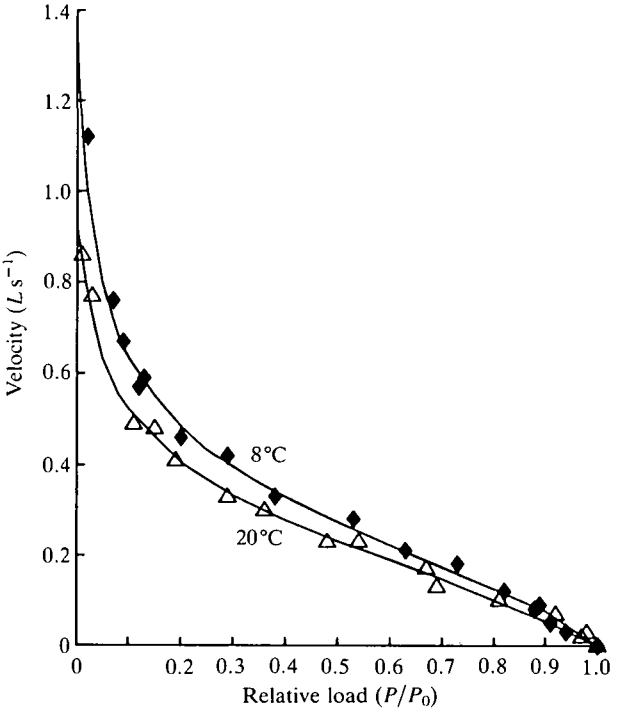


Fig. 3. Typical force–velocity curves for abductor superficialis preparations from 8°C-(△) and 20°C-acclimated (♦) carp. The data were fitted to the hyperbolic–linear equation as described in the text, where the abbreviations are also explained.

migrated with slow myotomal muscle myosin. No differences were observed in the relative mobilities of native myosins (pyrophosphate gels) or myosin heavy chains (8% SDS–PAGE gels) between cold- and warm-acclimated fish (not shown).

Electrophoretically purified native myosins were examined on 15% SDS–PAGE gels. The apparent relative molecular masses for the myosin light chains were; $LC1_f$, 27.5×10^3; $LC2_f$, 18.8×10^3; $LC1_s$, 26×10^3; $LC2_s$, 19.9×10^3. Fibre preparations from 20°C-acclimated carp contained almost exclusively slow muscle light chain isoforms ($LC1_s$ and $LC2_s$) (Figs 4, 5) with traces of fast muscle myosin light chains ($LC1_f$ and $LC2_f$). Preparations of Ab.s from 8°C-acclimated carp contained a much higher proportion of the two fast muscle myosin isoforms (Figs 4, 5). $LC3_f$ was either not present on the gels or it may have co-migrated with $LC2_s$, since both peptides have a similar apparent relative molecular mass in carp (Crockford and Johnston, 1990).

Discussion

Cold-acclimation produced modest increases in the rates of force development and relaxation in slow muscle fibres at low temperatures (Fig. 2; Table 1). Much

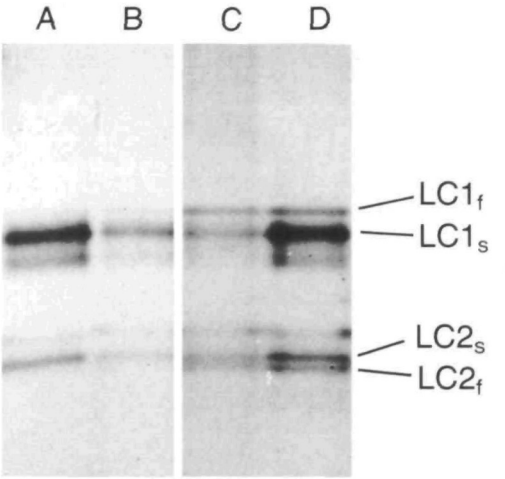


Fig. 4. A silver-stained 15% SDS–PAGE gel of electrophoretically purified myosin from abductor superficialis muscle fibre bundles. Lanes A and B were from 20°C-acclimated carp; lanes C and D were from 8°C-acclimated carp. $LC1_s$, myosin light chain 1 slow; $LC1_f$, myosin light chain 1 fast; $LC2_s$, myosin light chain 2 slow; $LC2_f$, myosin light chain 2 fast.

larger changes in twitch duration have been shown to occur in fast myotomal muscle fibres. For example, Fleming *et al.* (1990) studied a fast myotomal nerve–muscle preparation from common carp acclimated to either 8°C or 20°C. The half-times for twitch activation and relaxation were around half as long at 8°C in cold- as in warm-acclimated fish (Fleming *et al.* 1990). Similarly, twitch duration for the whole pectoral fin adductor muscle of the goldfish (*Carassius auratus*) at 5°C was half as long in 10°C- as in 28°C-acclimated fish (Heap *et al.* 1987). This

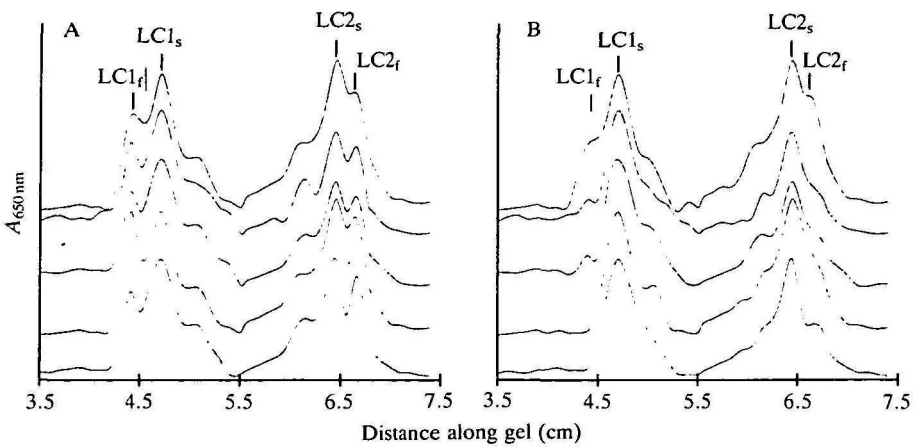


Fig. 5. Densitometric scans of myosin light chains from several individual fish acclimated to either (A) 8°C or (B) 20°C.

mixed muscle contains predominantly fast oxidative and fast glycolytic fibre types (Heap *et al.* 1987). Muscle fibres from cold-acclimated goldfish were found to contain a larger number of smaller-diameter myofibrils and to have a higher surface density of sarcoplasmic reticulum than fibres in warm-acclimated fish (Penney and Goldspink, 1980). These factors, together with the increase in myofibrillar ATPase activity (Johnston *et al.* 1975), probably account for the observed reductions in twitch duration in cold-acclimated individuals. Fleming *et al.* (1990) found that the pCa–tension relationship, parvalbumin content and the surface and volume density of sarcoplasmic reticulum (SR) in myotomal muscles were not altered by temperature acclimation in the common carp. In this case the faster relaxation of twitch tension in the fast myotomal muscle of cold-acclimated fish was associated with an increase in SR Ca^{2+}-ATPase activity (Fleming *et al.* 1990; Johnston *et al.* 1990).

Acclimation of common carp to 8 °C resulted in a perfect capacity adaptation of force production in slow fibres relative to 20 °C-acclimated fish (Table 1). Other studies have shown that maximum tension (P_0) does not vary continuously with acclimation temperature, but reaches upper and lower limits (Penney and Goldspink, 1981). For example, P_0 for fast myotomal fibres at 0 °C is similar in carp acclimated to 2–11 °C, but declines progressively at higher acclimation temperatures (Crockford and Johnston, 1990). The volume density of myofibrils is slightly higher in the muscles of warm- than in cold-acclimated crucian carp (Johnston and Maitland, 1980). Thus, differences in myofibril density cannot explain the changes in force production with temperature acclimation. The higher P_0 values in cold-acclimated fish at low temperatures probably reflect an increase in the number of attached cross bridges in the force-generating state and/or in the force generated by each cross bridge. Both these mechanisms are thought to contribute to increases in force with acute rises in temperature (Bressler, 1981; Stephenson and Williams, 1981; Brenner, 1986).

The calculated maximum power output of slow muscle fibres was around 50 % higher at 8 °C in cold- than in warm-acclimated carp (Table 2). This was due to a large increase in force production (Table 1) and a modest increase in contraction velocity (Table 2). In contrast, V_{max} for skinned fast muscle fibres increases by up to twofold following cold-acclimation (Johnston *et al.* 1985). It is therefore likely that muscle power output shows a more substantial capacity adaptation in fast than in slow muscle fibres.

The curvature of the force–velocity (P–V) relationship was not changed by temperature acclimation (Fig. 3). Langfeld *et al.* (1989) investigated the effects of acute temperature change on the P–V relationship of intact fast myotomal muscle fibres in the sculpin (*Myoxocephalus scorpius*). They found that the P–V relationship became progressively less curved at low temperatures. To quantify this effect the curves were normalised for P_0 and V_{max} at each temperature. The change in curvature between 8 and 1 °C was sufficient to increase the relative power output by around 15 % at 1 °C (Langfeld *et al.* 1989). Although the curvature of the P–V relationship does not vary with acclimation temperature in

carp, the above mechanism will serve to increase relative power output at low temperatures.

Fish myosin is composed of two heavy chains of M_r 200×10^3 and four light chains ranging in relative molecular mass from 17×10^3 to 29×10^3 (Focant *et al.* 1981; Rowlerson *et al.* 1985). Force production and shortening speed in vertebrate skeletal muscle are thought to be primarily determined by myosin heavy chain composition (Reiser *et al.* 1985; Lannergren, 1987). The myosin heavy chains (MHC) are a multi-gene family of proteins that are expressed in a developmental stage-specific and tissue-specific manner (Richter *et al.* 1987). Cloning studies indicate that common carp possess at least 28 different myosin chain genes (Gerlach *et al.* 1990), about twice the number reported in mammals (Leinwand *et al.* 1983). Gerlach *et al.* (1990) found that a 1.7 kb DNA probe specific for fast muscle MHC chains hybridized more strongly to RNA from warm-acclimated than to that from cold-acclimated common carp. This suggests that temperature acclimation may involve changes in MHC gene expression. The alkali myosin light chains only differ in sequence at the NH_2 terminus and are thought to be produced *via* an alternative splicing mechanism from a single gene (Nabeshima *et al.* 1984). Crockford and Johnston (1990) found evidence that the composition of the alkali light chains of myosin varies with acclimation temperature. Single fast muscle fibres from cold-acclimated carp contained an extra band on isoelectric focusing gels with a pI intermediate between those of $LC2_f$ and $LC3_f$ and an apparent relative molecular mass of 20×10^3. Densitometric scans showed that the ratio of ($LC1_f$+$LC3_f$+extra band):$LC2_f$ was approximately 1 in both cold- and warm-acclimated fish. This suggests that the extra band corresponds to an additional light chain ($LC3_{fc}$) which is only expressed in cold-acclimated fish. The total $LC3_f$ content of fast muscle fibres was also lower in cold- than in warm-acclimated individuals (Crockford and Johnston, 1990). Studies on single fibres from rabbit plantaris muscle have shown that V_{max} increases with the myosin LC3 content, suggesting a modulatory role for the alkali light chains (Greaser *et al.* 1988). Thus, the relatively large changes in contractile properties of fast muscle fibres with temperature acclimation probably result from changes in the expression of both myosin heavy and light chains. It is well established that fast and slow myosins can be expressed in the same fibre type, and that myosin heavy and light chain genes are independently regulated (Reiser *et al.* 1985; Greaser *et al.* 1988). In the present study fibre bundles from the superior abductor muscle of 8°C-acclimated carp contained a higher content of myosin light chain isoforms normally associated with faster-contracting fibre types (Figs 4, 5). Since the percentage of fast fibres in preparations did not vary with acclimation temperature, it would appear that $LC1_f$ and $LC2_f$ are co-expressed with $LC1_s$ and $LC2_s$ in the slow fibres of 8°C-acclimated fish. This may contribute to the higher values of force production and V_{max} observed following cold-acclimation.

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