J. exp. Biol. ***175****, 195–209 (1993)*
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THE EFFECT OF THERMAL ACCLIMATION AND EXERCISE UPON THE BINDING OF GLYCOLYTIC ENZYMES IN MUSCLE OF THE GOLDFISH *CARASSIUS AURATUS*

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Accepted 2 September 1992

Summary

To examine whether the changes in metabolic organization induced by thermal acclimation modify glycolytic enzyme-binding, we acclimated goldfish, *Carassius auratus*, to 8 and 25 ˚C and measured the binding of phosphofructokinase (PFK), aldolase, pyruvate kinase and lactate dehydrogenase to subcellular particles in red and white muscle. When fish were sampled at rest at their acclimation temperature, only the percentage binding of PFK in red muscle was altered by thermal acclimation. By contrast, exhausting exercise at 15 ˚C led to markedly higher levels of binding of PFK in muscle of warm- than of cold-acclimated fish. This pattern was apparent in both red and white muscle. The specific activity of PFK in red muscle declined with exhausting exercise in warm- but not in cold-acclimated fish. In contrast, the units of PFK bound per gram of muscle did not differ in exhausted warm- and cold-acclimated fish. Cold- and warm-acclimated fish did not differ in their accumulation of lactate in white muscle at exhaustion. Furthermore, the PFK from white muscle of warm- and cold-acclimated fish did not differ in the pH dependence of binding to subcellular particles. These changes in the dynamics of PFK binding with temperature acclimation suggest that soluble PFK may be more susceptible to exercise-induced inhibition in warm- than in cold-acclimated goldfish. While the percentage binding and the specific activity of the other glycolytic enzymes were little affected by exhausting exercise, the units of aldolase bound per gram of white muscle decreased with exercise in warm-acclimated fish.

Introduction

For ectotherms, environmental temperature is a major determinant of cellular physiology. Given the detrimental effects of low temperature on cellular performance, cold acclimation leads many species of fish to enhance the aerobic capacity of their muscle (Guderley and Blier, 1988). In goldfish, *Carassius auratus*, as in other species of eurythermal temperate-zone fish, the percentage of red muscle and the mitochondrial volume density in red and white muscles rise markedly with cold acclimation (Tyler and Sidell, 1984; Johnston and Lucking, 1978; Sidell, 1980). In contrast to most eurythermal

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Key words: thermal acclimation, phosphofructokinase, fish, muscle, glycolysis, *Carassius auratus.*

fish, which show few changes in their contractile apparatus with thermal acclimation, cold acclimation of cyprinids, such as goldfish and carp, increases myofibrillar ATPase activity (Johnston *et al.* 1975; Heap *et al.* 1985), enhances power development and the maximum rate of shortening for fast and slow skinned fibers (Johnston *et al.* 1985) and modifies the contractile properties of isolated fin muscles (Heap *et al.* 1987) and intact slow muscle fiber bundles (Langfeld *et al.* 1991). In these cyprinids, cold acclimation changes the expression of myosin light chains (Crockford and Johnston, 1990; Langfeld *et al.* 1991), myosin heavy chains (Gerlach *et al.* 1990) and calcium regulatory proteins on the thin filaments (Johnston, 1979). Clearly, cold acclimation has pronounced effects upon the cellular physiology and architecture of cyprinid muscle.

It has been suggested that the binding of enzymes to subcellular structures acts as a means of metabolic regulation facilitating flux in multi-enzyme sequences (Srere, 1987; Somero and Hand, 1990). Modification of enzyme binding during changes in tissue physiology supports this concept. Thus, glycolytic enzyme binding is increased during ischemia in mammalian heart (Clarke *et al.* 1984; Choate *et al.* 1985) and during electrical stimulation in mammalian muscle (Clarke *et al.* 1980), is modified by exhausting exercise in frog and fish muscle (Guderley *et al.* 1989; Brooks and Storey, 1988*a*,*b*), is decreased by extended anoxia in molluscs (Plaxton and Storey, 1986; Lazou *et al.* 1989; Brooks and Storey, 1990, 1991) and is decreased in fish muscle during starvation (Lowery *et al.* 1987). Given the marked changes in the physiology and structure of fish muscle with thermal acclimation, enzyme binding to subcellular structures may be modified by thermal acclimation. Cyprinids, such as the goldfish, are the organisms of choice to study this phenomenon because of the extensive modifications of their metabolic and contractile apparatus during thermal acclimation.

Both quantitative and qualitative modifications could change the binding of glycolytic enzymes in muscle during thermal acclimation of goldfish. The opposite changes in the volume density of myofibrils and of mitochondria (Johnston and Maitland, 1980; Egginton and Sidell, 1989) should modify the availability of binding sites for glycolytic enzymes. In red muscle of *Carassius carassius*, cold acclimation decreases myofibrillar volume density from 57 % to 43 % and increases mitochondrial volume density from 14 % to 25 % (Johnston and Maitland, 1980). In goldfish red muscle, mitochondrial volume density rises from 5.6 % to 20 % with cold acclimation (Tyler and Sidell, 1984). The qualitative alterations of myofibrillar proteins could modify their interactions with glycolytic enzymes. Thus, we have examined whether the association of glycolytic enzymes with subcellular particles in goldfish red and white muscle is modified by thermal acclimation. For an initial comparison, we sampled resting fish at their respective acclimation temperatures (8 and 25 ˚C). We predicted that glycolytic enzymes would be more strongly associated with subcellular particles in muscle from warm- than from cold-acclimated fish. We next examined whether the modifications in metabolic organization induced by thermal acclimation change enzyme binding after exhausting exercise. To facilitate this comparison, thermally acclimated fish were exhausted at an intermediate temperature, 15 ˚C, to ensure equivalent degrees of exercise. To evaluate whether glycolysis had been activated to an equivalent extent in exhausted cold- and warm-acclimated goldfish, the accumulation of lactate in white muscle was compared. Finally,

given the qualitative changes in myofibrillar proteins during thermal acclimation, we investigated whether the pH sensitivity of phosphofructokinase (PFK) binding to subcellular particles (Roberts *et al.* 1988) differed between cold- and warm-acclimated goldfish.

Materials and methods

Experimental conditions

Common goldfish (*Carassius auratus* L., length: 7–9 cm) were purchased from local suppliers and acclimated to 8 ˚C and 25 ˚C for a minimum of 6 weeks. They were fed daily *ad libitum* and kept at a photoperiod of 14 h:10 h L:D. Resting fish were quickly netted, removed from the aquarium and killed by a blow on the head. If a fish struggled during this procedure it was not used as a rested fish. To exhaust fish, we chased individual fish around a well-aerated aquarium (60 l) maintained at 15 ˚C until they did not move when lifted out of the water. This required 30–60 min for both cold- and warm-acclimated fish.

Determination of enzyme binding

The dead fish was immediately placed on ice and the red muscle was dissected from both sides using a binocular microscope. White muscle was taken dorsally. The dissection was carried out in a cold room and was finished after at most 10 min. The muscle samples were immediately weighed and homogenized with a Polytron (Brinkmann) (3×5 s) in 5 volumes of ice-cold extraction buffer (0.25 mol l^{-1} sucrose, 1 mmol l^{-1} dithioerythreitol, DTT) following the method of Clarke *et al.* (1984). This method of evaluating the association of glycolytic enzymes with subcellular particles is fast and reproducible. While this method tends to overestimate binding, it provides conclusions that are similar to those obtained by other methods (Brooks and Storey, 1991). A sample of the homogenate was diluted in 4 volumes of cold stabilization buffer (0.1 mol l^{-1} potassium phosphate, 1 mmol l^{-1} EDTA (disodium ethylene diamine tetra-acetate), 2 mmol l^{-1} DTT, 0.1 mmol l^{-1} fructose 1,6-bisphosphate (FBP), 0.1 mmol l^{-1} ATP, pH 7.5) and kept on ice to measure total enzyme activities and to check the recovery of activity in the soluble and pellet fractions. Another sample was immediately centrifuged for 4 min (23 000 ***g***, 4 ˚C). The supernatant was diluted in 4 volumes of stabilization buffer. The pellet was extracted three times with stabilization buffer to maximize recovery of the activity. Homogenate, supernatant and pellet were assayed to verify the distribution of glycolytic enzyme activities (aldolase, lactate dehydrogenase, phosphofructokinase, pyruvate kinase) and only values of supernatant plus pellet which were within 80–120 % of the corresponding homogenate activity were retained.

Enzyme activity measurements

The conditions for the spectrophotometric determination of maximal enzyme activity were established in preliminary studies, using the assay approaches of Bergmeyer (1983). As the optimal conditions were virtually identical for red and white muscle enzymes, we

used the same conditions for enzymes from both tissues. The assays were run at 340 nm using a Varian Cary 210 UV/Vis spectrophotometer. Cuvette temperature was controlled at 20 ˚C using a thermostatted cell holder and a Haake circulating water bath; pH values were adjusted at 20 ˚C. The enzymes were assayed in the following order using the conditions given below.

Phosphofructokinase (PFK)

50 mmol l^{-1} Tris–HCl, 10 mmol l^{-1} $MgCl_2$, 330 mmol l^{-1} KCl, 1 mmol l^{-1} KCN, 2 mmol l^{-1} DTT, 0.26 mmol l^{-1} NADH, 3 mmol l^{-1} fructose 6-phosphate, 1 mmol l^{-1} ATP, pH 8.2, excess levels of aldolase, triosephosphate isomerase and 3-glycerophosphate dehydrogenase.

Pyruvate kinase (PK)

50 mmol l^{-1} imidazole–HCl, 10 mmol l^{-1} $MgCl_2$, 100 mmol l^{-1} KCl, 0.20 mmol l^{-1} NADH, 2 mmol l^{-1} ADP, 0.1 mmol l^{-1} FBP, 2.0 mmol l^{-1} phosphoenolpyruvate, pH 7.0, excess levels of lactate dehydrogenase.

Aldolase

50 mmol l^{-1} triethanolamine–HCl, 0.15 mmol l^{-1} NADH, 20 mmol l^{-1} FBP, pH 7.6, excess levels of triosephosphate isomerase and 3-glycerophosphate dehydrogenase.

Lactate dehydrogenase (LDH)

100 mmol l^{-1} potassium phosphate, 0.15 mmol l^{-1} NADH, 0.33 mmol l^{-1} sodium pyruvate, pH 7.4.

Lactate measurements

For these experiments, fish were acclimated to 5 and 25 ˚C for 6 weeks and exhausted at 15 ˚C as described above. After the fish had been killed, the white muscle was rapidly sampled, directly under the dorsal fin and freeze-clamped in less than 1 min after removing the fish from the water. These samples of white muscle were ground to a fine powder under liquid nitrogen, weighed in pre-tared, liquid-nitrogen-cooled tubes, diluted fivefold in ice-cold perchloric acid (6 %) and immediately homogenized (two 15 s bursts separated by a 30 s cooling period) using a Polytron. The extract was centrifuged at 27000 ***g*** for 15 min at 4 ˚C and the supernatant was neutralized with 5 mol l^{-1} K_2CO_3 (final pH approximately 6.8) and recentrifuged at 27 000 ***g*** for 5 min at 4 ˚C. Lactate was measured spectrophotometrically in the supernatant following the procedure outlined by Bergmeyer (1983).

pH sensitivity of PFK binding

Resting goldfish were quickly netted from the acclimation aquaria. White muscle was dissected from directly under the dorsal fin and homogenized with a Polytron (3×15 s) at full speed in 10 volumes of ice-cold extraction buffer. Using weak acid and base solutions, samples of 2.5 ml of the homogenate were adjusted to pH values of 6.5, 6.9, 7.3, 7.7 and 8.1 with constant stirring while being incubated in a water bath at 15 ˚C. This

procedure required approximately 15 min. Of each sample, 400 μl was placed in 3 volumes of stabilization buffer and the remainder was immediately centrifuged as described for the determination of enzyme binding. The supernatant was then diluted in 0.5 volumes of stabilization buffer and the pellet was resuspended in 10 volumes of the same buffer. These fractions were assayed to determine the distribution of PFK activity between the supernatant and pellet fractions as well as the recovery of the PFK activity. Only recoveries between 80 and 120 % were retained.

Biochemicals

Biochemicals were from Sigma (St Louis, USA) or Boehringer Mannheim Canada (Montréal, Canada). All other reagents were analytical grade.

Statistical analysis

The enzyme binding data (percentage binding and bound specific activities) and the specific activities were compared using analysis of variance. A Student–Newman–Keuls *a posteriori* test was used to determine which differences were significant (Superanova, Abacus Concepts, Berkeley California). Enzyme activities are expressed in international enzyme units (μmol substrate converted to product per minute). The pH sensitivity of PFK binding in extracts of muscle from warm- and cold-acclimated fish and the lactate levels in white muscle of exhausted cold- and warm-acclimated fish were compared using the Mann–Whitney *U*-test (Statview, Brainpower, Calabassas, California).

Results

Binding of glycolytic enzymes to subcellular particles

In both thermal acclimation groups and muscle types, phosphofructokinase consistently showed a markedly higher percentage binding to subcellular elements than did aldolase, pyruvate kinase or lactate dehydrogenase (Figs 1 and 2). At rest up to 79 % of PFK activity was associated with the pellet. This value rose to 93 % in white muscle of warm-acclimated goldfish after exhausting exercise. In white muscle, approximately 40–60 % of the activity of PK, LDH and aldolase was bound, while in red muscle 10–30 % of their activity was bound to subcellular elements.

The percentage binding of these enzymes was affected by the experimental treatment, i.e. acclimation temperature, muscle type and exercise status (ANOVA, P=0.0001 for each enzyme). Temperature acclimation influenced the percentage binding of PFK in red muscle of resting fish: as predicted, binding was stronger in warm- than cold-acclimated fish (P<0.01) (Fig. 1). For fish exhausted at 15 ˚C, PFK binding was significantly higher in warm- than in cold-acclimated fish, in both red and white muscle (P<0.01) (Fig. 1). In warm-acclimated fish, PFK binding was significantly higher after exhausting exercise than at rest in both red and white muscle (P<0.01). In cold-acclimated fish PFK binding did not differ between fish sampled at rest or after exhausting exercise at 15 ˚C. Cold acclimation increased the binding of PK in white muscle of rested goldfish, while PK binding in red muscle was not significantly altered by acclimation or exercise status

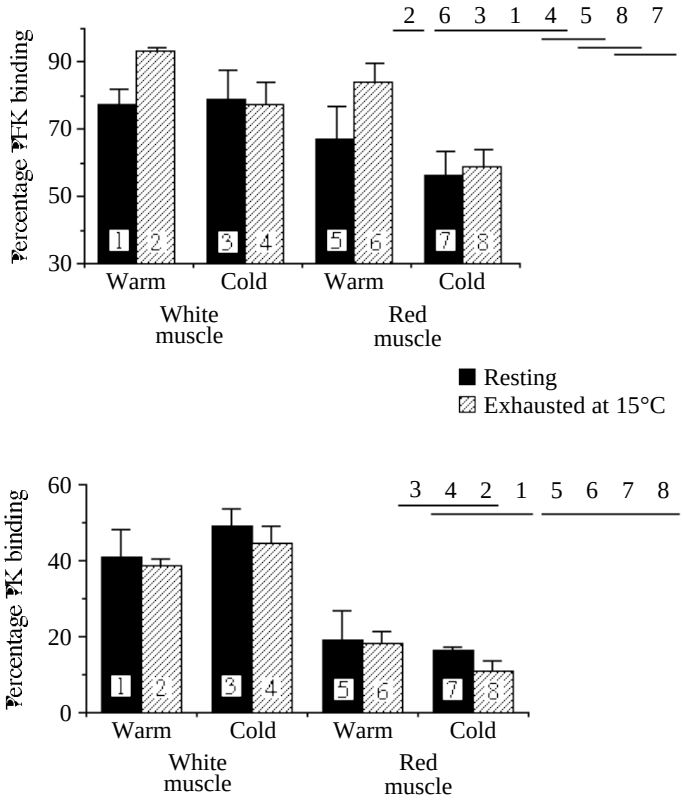

Fig. 1. Percentage binding of phosphofructokinase (PFK) and pyruvate kinase (PK) in white and red muscle of warm- (25 ˚C) and cold- (8 ˚C) acclimated goldfish (*Carassius auratus*) at rest at their acclimation temperature and after exhausting exercise at 15 ˚C. The values are shown as mean ± S.D. for 4–9 fish. Significant differences among the treatments (identified by a number in the bar) are noted in the upper right-hand corner of each figure. If the numbers are not joined by a line, the treatments differ significantly (ANOVA followed by a Student–Newman–Keuls *a posteriori* multiple comparisons test).

(P>0.05). Aldolase binding was higher in red muscle from exhausted warm- than cold-acclimated fish (P<0.01) (Fig. 2). Contrary to our prediction, the percentage binding of LDH in white muscle of resting fish was higher in cold-acclimated fish (P<0.05) (Fig. 2). Finally, glycolytic enzyme binding was consistently stronger in white than in red muscle (P<0.01).

Specific activities of glycolytic enzymes

The experimental treatment (in particular, muscle type and exercise status) significantly affected the specific activities of the enzymes studied (ANOVA, P<0.01). PFK, PK and LDH activities were consistently higher in red than in white muscle (P<0.05) (Figs 3 and 4). The specific activity of aldolase did not differ between muscle types. Acclimation temperature did not modify the specific activity of the enzymes in resting fish (Figs 3 and 4).

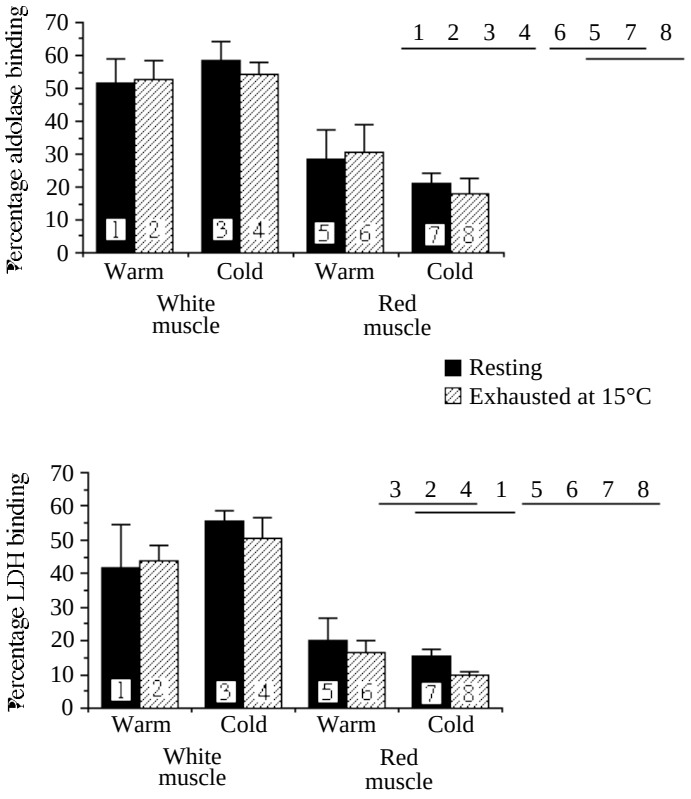

Fig. 2. Percentage binding of aldolase and lactate dehydrogenase (LDH) in white and red muscle of warm- (25 ˚C) and cold- (8 ˚C) acclimated goldfish (*Carassius auratus*) at rest at their acclimation temperature and after exhausting exercise at 15 ˚C. The values are shown as mean ± S.D. for 3–10 fish. Statistical differences are shown as in Fig. 1.

After exhaustion at 15 ˚C, warm-acclimated fish had a significantly lower specific activity of PFK in red muscle than cold-acclimated fish (Fig. 3) (P<0.05). The specific activity of PFK was lower in red muscle from exhausted warm-acclimated fish than from rested warm-acclimated fish (P<0.05). No such difference was observed between rested and exhausted cold-acclimated fish. Exercise did not modify the specific activity of the other glycolytic enzymes.

Bound specific activity of the glycolytic enzymes

The pattern of enzyme binding revealed by the bound specific activity (i.e. units bound to subcellular particles per gram of muscle) (Figs 5 and 6) differs from that provided by the percentage binding (Figs 1 and 2). PFK generally had the lowest bound specific activity, followed by aldolase, PK and finally LDH. Only in red muscle from exhausted cold-acclimated fish, did aldolase have a slightly lower bound specific activity than PFK.

The experimental treatments had fewer effects on the bound specific activity (Figs 5 and 6) than on the percentage binding. Only the bound specific activity of aldolase showed treatment effects (ANOVA, P<0.05). After exhaustion at 15 ˚C, warm-

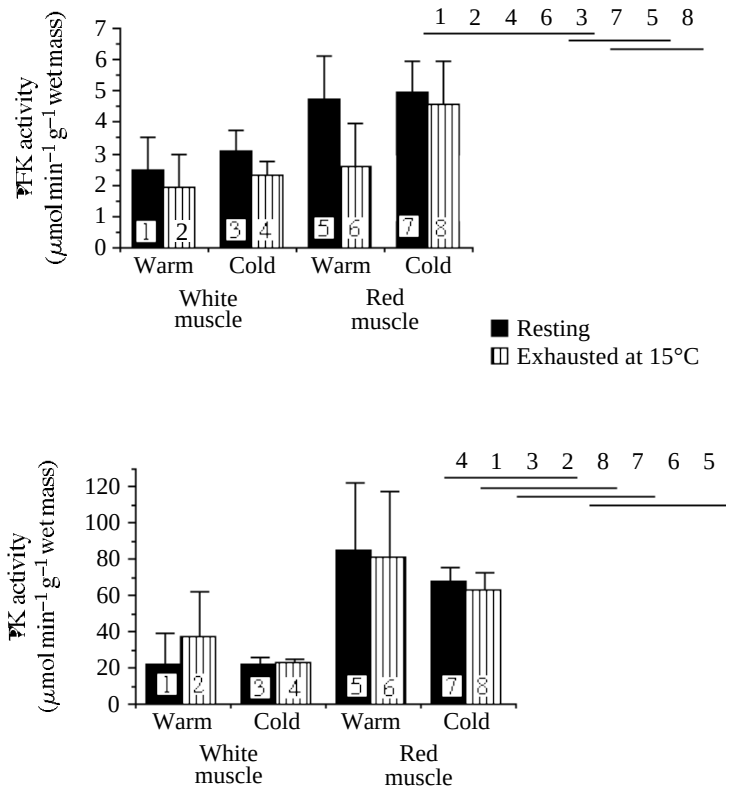

Fig. 3. Enzyme activities (μmol min^{-1} g^{-1} wet mass) of PFK and PK in white and red muscle of warm- (25 ˚C) and cold- (8 ˚C) acclimated goldfish (*Carassius auratus*) at rest at their acclimation temperature and after exhaustion at 15 ˚C. The values are shown as mean ± S.D. for 4–9 fish. Activities were determined in the homogenates prepared for the measurement of glycolytic enzyme binding as described in Materials and methods. Statistical differences are shown as in Fig. 1.

acclimated fish had higher bound specific activities of aldolase than cold-acclimated fish, in both white and red muscle (P<0.05). The bound specific activity of aldolase in white muscle was higher in exhausted than rested warm-acclimated fish. The lack of change in the number of units of PFK bound per gram of muscle suggests that the increased percentage binding of PFK in exhausted warm-acclimated goldfish may be due to a decrease in the activity of soluble PFK. In other words, the decrease in the specific activity of PFK with exhaustion (Fig. 3) primarily seems to affect soluble PFK.

Lactate accumulation

Since increases in the percentage of PFK binding are thought to reflect increases in glycolytic flux, we examined whether the differing responses of the percentage binding of PFK in cold- and warm-acclimated goldfish reflected differing degrees of lactate accumulation at exhaustion. Both cold- and warm-acclimated goldfish accumulated considerable quantities of lactate after exhaustive exercise at 15 ˚C. Cold-acclimated fish

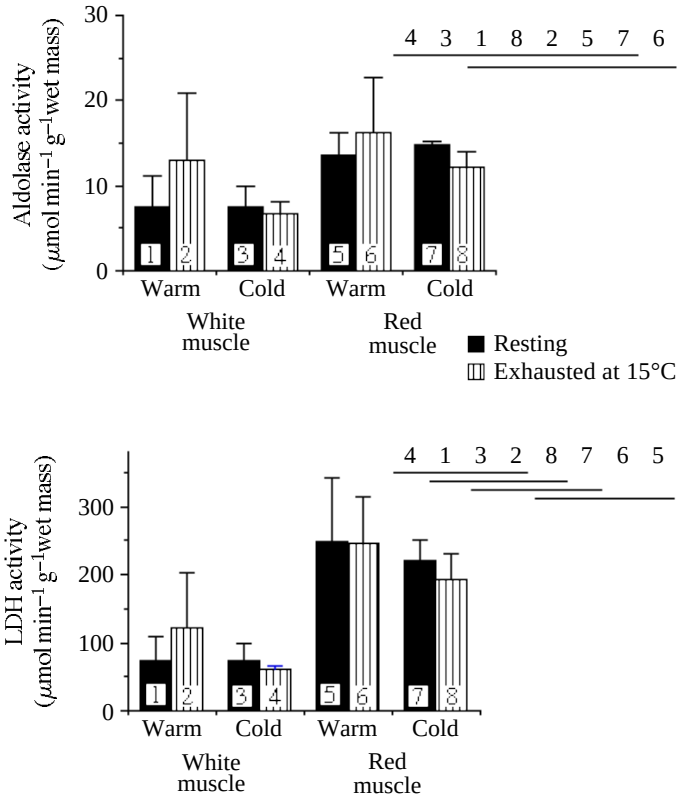

Fig. 4. Enzyme activities (μmol min^{-1} g^{-1} wet mass) of aldolase and LDH in white and red muscle of warm- (25 ˚C) and cold- (8 ˚C) acclimated goldfish (*Carassius auratus*) at rest at their acclimation temperature and after exhausting exercise at 15 ˚C. The values are shown as mean ± S.D. for 3–10 fish. Activities were determined in the homogenates prepared for the measurement of glycolytic enzyme binding as described in Materials and methods. Statistical differences are shown as in Fig. 1.

required on average 37 min of chasing before exhaustion and lactate levels reached 15.1±11.1 mmol l^{-1} (mean ± S.D., *N*=6). For warm-acclimated fish, somewhat more chasing was required for exhaustion (52 min), but lactate levels were not significantly higher (12.6±6.4 mmol l^{-1}, mean ± S.D., *N*=6). Considerable variability in swimming performance was observed in both groups and lactate levels varied accordingly. However, cold- and warm-acclimated goldfish clearly do not differ in their accumulation of lactate in white muscle at exhaustion.

PFK binding: the effect of pH and of thermal acclimation

As thermal acclimation modifies the expression of contractile proteins in cyprinids and since glycolysis was activated in both cold- and warm-acclimated goldfish, we examined whether the differing responses of the percentage binding of PFK between warm- and cold-acclimated fish reflected changes in the pH dependence of PFK binding to subcellular particles. For both cold- and warm-acclimated goldfish, the percentage of

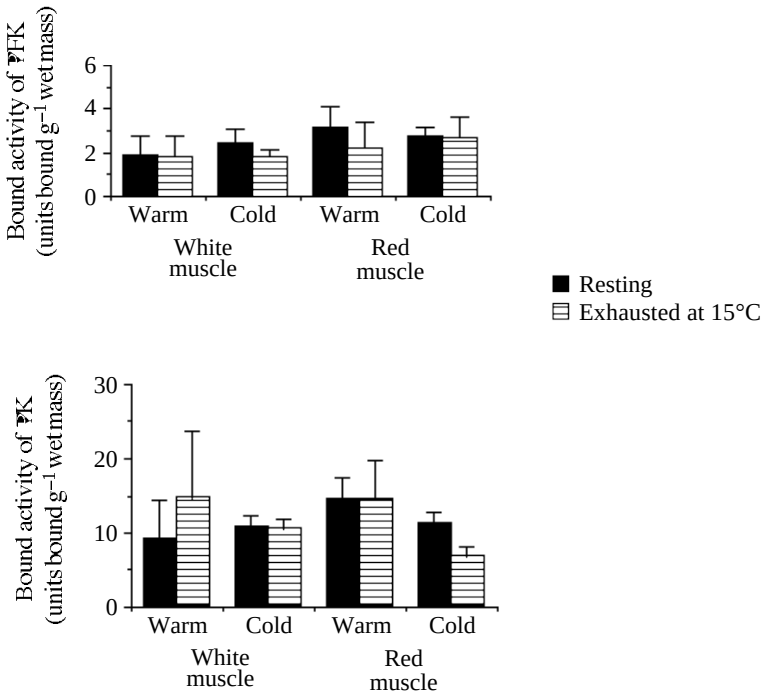

Fig. 5. Bound specific activities (units bound g^{-1} wet mass) of PFK and PK in white and red muscle of warm- (25 ˚C) and cold- (8 ˚C) acclimated goldfish (*Carassius auratus*) at rest at their acclimation temperature and after exhausting exercise at 15 ˚C. The values are shown as mean ± S.D. for 4–9 fish. These data were calculated using the percentage binding and specific activity data for individual fish. No differences were found among these values (ANOVA, $P > 0.05$).

bound PFK activity in extracts of white muscle incubated at 15 ˚C increased with decreasing pH. At pH 6.5, almost all the PFK activity was bound, in both warm- and cold-acclimated goldfish, while at pH 8.1, only 35 % of the activity remained bound (Fig. 7). The pH dependence of PFK binding did not differ between extracts of white muscle from cold- and warm-acclimated goldfish.

Discussion

The binding of phosphofructokinase to subcellular particles in muscle showed a different pattern in warm- and in cold-acclimated goldfish. Warm-acclimated fish showed a higher percentage binding of PFK after exhaustion at 15 ˚C than did cold-acclimated fish. In warm-acclimated goldfish, exhausting exercise at 15 ˚C increased the percentage PFK binding well above resting levels. By contrast, in cold-acclimated goldfish, the percentage of bound PFK was the same at rest at 8 ˚C and after exhaustion at 15 ˚C. A lack of change in the percentage binding of PFK with exhausting exercise of cold-acclimated goldfish was noted by Duncan and Storey (1991). As these authors did not

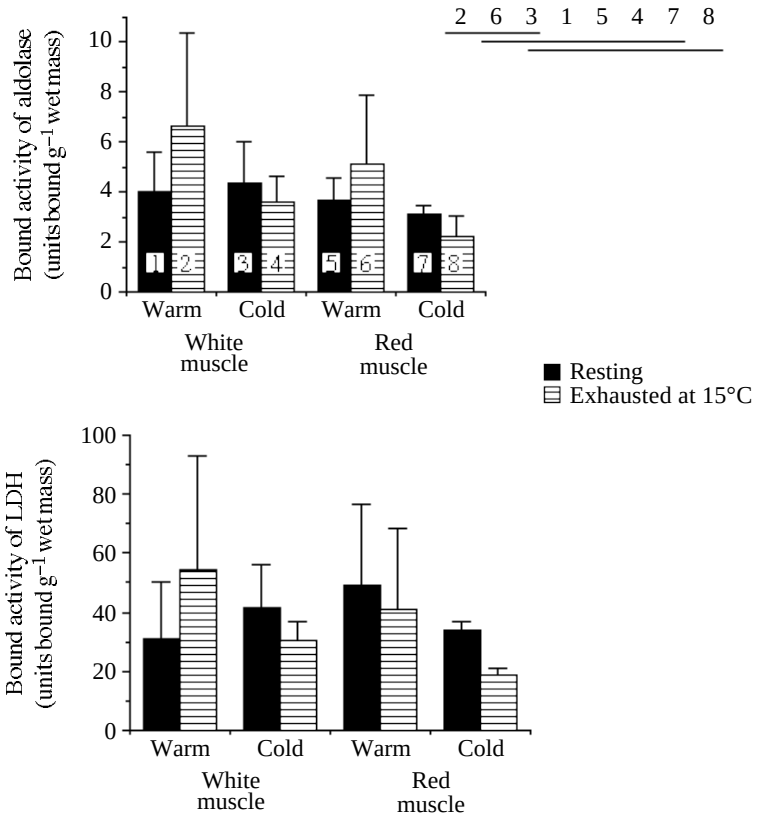

Fig. 6. Bound specific activities (units bound g^{-1} wet mass) of aldolase and LDH in white and red muscle of warm- (25 ˚C) and cold- (8 ˚C) acclimated goldfish (*Carassius auratus*) at rest at their acclimation temperature and after exhausting exercise at 15 ˚C. The values are shown as mean ± S.D. for 3–10 fish. These data were calculated using the percentage binding and specific activity data for individual fish. Statistical differences are shown as in Fig. 1.

examine warm-acclimated goldfish, they suggested that the lack of change in PFK binding with exhausting exercise reflected a fundamental property of goldfish muscle. The different responses of PFK binding in warm- and cold-acclimated goldfish do not reflect a lack of activation of glycolysis during exhausting exercise in cold-acclimated fish; warm- and cold-acclimated goldfish accumulated equivalent amounts of lactate in white muscle after exhausting exercise. Furthermore, these differences are not due to changes in the pH dependence of PFK binding to subcellular particles.

Could shifts in intracellular pH during thermal acclimation explain the responses of PFK binding to thermal acclimation? When cold-acclimated fish are transferred to a higher temperature, intracellular pH should drop (Cameron, 1984; Somero, 1986). Subsequent burst exercise would further decrease this value. As low pH favors PFK binding, these shifts should cause cold-acclimated fish exhausted at 15 ˚C to have a higher percentage PFK binding than rested fish sampled at 8 ˚C. By contrast, as transfer of warm-acclimated fish to 15 ˚C would increase intracellular pH, subsequent exhausting

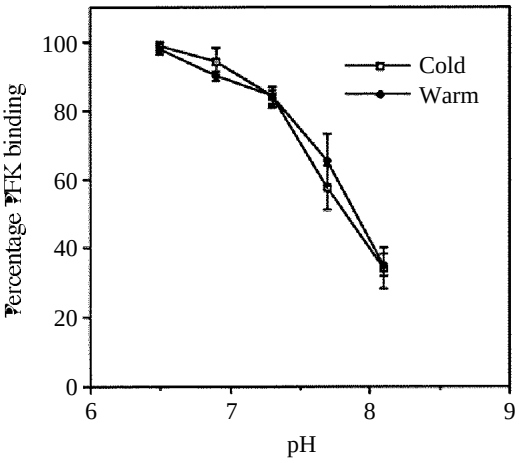

Fig. 7. pH dependence of the percentage binding of phosphofructokinase in extracts of white muscle of warm- (25 ˚C) and cold- (8 ˚C) acclimated goldfish, incubated at 15 ˚C, according to the description in Materials and methods. The values represent the mean ± S.D. of the data obtained for 4–6 fish. The percentage binding of phosphofructokinase from the warm- and cold-acclimated fish did not differ at any of the pH values (Mann–Whitney *U*-test, $P>0.05$).

exercise would lead to a smaller net decrease in pH than in cold-acclimated fish. Clearly, such pH changes do not explain the greater percentage PFK binding observed for exhausted warm- than cold-acclimated fish.

The different patterns provided by the percentage binding, the bound specific activity (units bound per gram muscle) and the specific activity (units per gram muscle) of PFK suggest a possible basis for the different responses of warm- and cold-acclimated goldfish. The units of PFK bound per gram muscle remained constant in our treatments. The specific activity of PFK declined with exhausting exercise, particularly in red muscle of warm-acclimated fish. This decrease in PFK activity with exhausting exercise is similar to changes reported for ischemic heart (Carpenter and Hand, 1986) as well as for fatigued frog muscle (Guderley *et al.* 1989) and may reflect changes in the polymerization or phosphorylation status of the enzyme (Somero and Hand, 1990; Hofer and Sörensen-Ziganke, 1979). We suggest that the greater percentage of PFK binding in the muscle of exhausted, warm-acclimated goldfish reflects an enhanced inhibition of cytosolic PFK. Either dimerization or increased phosphorylation could inhibit the soluble enzyme. The dissociation of PFK into inactive dimers (Carpenter and Hand, 1986) may well be stronger in the cytosol than in the particulate fraction. Liou and Anderson (1980) and Luther and Lee (1986) report that binding of PFK to actin activates the enzyme by reducing its sensitivity to ATP inhibition and by increasing its affinity for fructose 6-phosphate. These changes should reduce its tendency to dissociate into dimers. Phosphorylation increases the affinity of PFK for actin while reducing the enzyme's affinity for fructose 6-phosphate (Luther and Lee, 1986). Our data suggest that exercise decreases the activity of cytosolic PFK more readily in the muscle of warm- than of cold-acclimated goldfish.

Despite the major modifications of cellular architecture that accompany thermal acclimation in goldfish, the binding of glycolytic enzymes to subcellular particles in red and white muscle of resting fish was only slightly modified. Although warm-acclimation increases the myofibrillar volume density in red and white muscle (Johnston and Maitland, 1980; Egginton and Sidell, 1989), only the percentage binding of PFK in resting red muscle was enhanced. As thermal acclimation leads to larger changes in myofibrillar and mitochondrial volume density in red than in white muscle, it is logical that more modifications occur in red than in white muscle. However, thermal acclimation left the bound specific activities of these glycolytic enzymes unaltered.

The conservation of the levels of glycolytic enzyme-binding in rested animals at their acclimation temperature suggests that a given pattern of enzyme binding is required for optimal metabolic regulation. Can such a constant pattern be found? While comparisons among studies suggest that the exact extent of the percentage of enzyme binding is species- and muscle-specific, PFK consistently shows the highest percentage binding in skeletal muscles from lower vertebrates. In mammalian skeletal muscle, PFK and aldolase show the highest percentage binding. The percentage of PFK binding generally increases with exhausting exercise, except in cold-acclimated goldfish. Alternatively, the relative constancy of glycolytic enzyme-binding with thermal acclimation may indicate that the groups involved with the binding of glycolytic enzymes to subcellular particles conserve a constant ionization status at the different acclimation temperatures, given alphastat pH regulation (Somero, 1986).

Most studies concerning the binding of glycolytic enzymes express the binding in terms of changes in the percentage of enzyme activity associated with the particulate fraction. While this method is convenient, our analysis shows that it can be misleading: the units bound remained constant with exhausting exercise while the percentage bound increased markedly in muscle of warm-acclimated fish. When shifts in percentage binding accompany shifts in specific activity (this study; Guderley *et al.* 1989), percentage binding only gives a partial view of the situation. Brooks and Storey (1988*b*) provide an interesting analysis, which clearly illustrates that, despite its high percentage binding, PFK generally has the least bound activity, in agreement with its role in a reaction that controls flux through glycolysis. Unfortunately, the bound activities have rarely been reported.

Finally, comparison of the specific activities of glycolytic enzymes in red and white muscle shows that levels are generally higher in red muscle. Although in mammals fast glycolytic fibers have higher levels of glycolytic enzymes than fast oxidative fibers, the opposite trend has consistently been found for goldfish (van den Thillart and Smit, 1984; Tsukuda and Yamawaki, 1980; Tsukuda, 1982). This is in keeping with the goldfish's reliance upon glycogen rather than lipid during sustained swimming (Davison and Goldspink, 1984).

This study was supported by a grant from NSERC to H.G. M.H. received a scholarship from the Austrian Bundesministerium für Wissenschaft und Forschung. The excellent technical assistance of F. Houle is gratefully acknowledged. We thank several colleagues, in particular E. Lehoux and P. Blier, for helpful comments and discussion.

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