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ACTIVITIES OF ENZYMES FOR GLUCOSE CATABOLISM IN THE SWIMBLADDER OF THE EUROPEAN EEL *ANGUILLA ANGUILLA*

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Summary

Gas secretion into the swimbladder of the eel relies on the production of CO_2 and lactic acid from glucose in the swimbladder epithelium. The activities of the enzymes involved in glucose catabolism have been measured and compared with those in the rete mirabile, the liver and white skeletal muscle to evaluate whether the pentose phosphate shunt may contribute to glucose metabolism in the swimbladder tissue. The activities of enzymes of the pentose phosphate shunt were higher in the swimbladder epithelium than in white muscle, and close to those in the liver. The activities of the enzymes of anaerobic glycolysis were 2–5 times higher in the swimbladder epithelium than in the rete mirabile, reaching or even exceeding the levels in liver and white muscle, whereas the activities of the enzymes of oxidative metabolism were extremely low. Compared to enzymes of the other tissues, swimbladder phosphofructokinase and glucose-6-phosphate dehydrogenase showed no special adaptation to low pH values. The results show that the swimbladder epithelium is equipped with enzymes that produce CO_2 from glucose without the removal of O_2, which is particularly advantageous for creating the high gas partial pressures needed for filling the swimbladder at great depth.

Introduction

The high oxygen partial pressures observed in the blood perfusing the swimbladder tissue are achieved by acidification of the blood with acid secreted from the swimbladder epithelium, or specialized parts of it called gas gland tissue, and by acid back-diffusion in the rete mirabile (Kobayashi *et al.* 1990). This acid then liberates oxygen from the haemoglobin mainly *via* the Root effect (Pelster *et al.* 1990; cf. Pelster and Weber, 1991).

The acid produced in, and secreted from, the gas gland tissue is mainly lactic acid, as shown by *in vitro* and *in vivo* studies (Deck, 1970; D'Auost, 1970; Steen, 1963; Kobayashi *et al.* 1989). Recently Pelster *et al.* (1989) presented evidence that CO_2 is also produced by the swimbladder epithelium in the European eel, and suggested that this CO_2 originated from the pentose phosphate shunt. If the

Key words: enzyme activities, energy metabolism, swimbladder, pentose phosphate shunt, *Anguilla anguilla*.

pentose phosphate shunt is indeed a source of CO_2, then its key enzymes should be present at significant activities in the secretory epithelium of the eel swimbladder. Relatively high activities of these enzymes have previously been measured in homogenates of cod whole swimbladders (Boström *et al.* 1972). In this preparation, however, the tissues of the gas gland and the rete mirabile were mixed, and Boström *et al.* (1972) did not try to determine enzyme activities in each tissue separately.

Extremely low pH values, down to pH 6.5, have been measured in blood-perfused swimbladder vessels (Steen, 1963; Kobayashi *et al.* 1990) and, although information about intracellular pH is not available, it is reasonable to assume that very low intracellular pH values pertain. Some of the enzymes, particularly phosphofructokinase, are known to be considerably less active at pH values below 7.5 (Bock and Frieden, 1976). As acid production is vital for swimbladder function, the enzymes of this tissue may be especially adapted to operate at low pH values. This study, therefore, reports enzyme activities for the glycolytic pathway, the citric acid cycle and the pentose phosphate shunt in the swimbladder epithelium of the European eel, and their dependence on pH.

Materials and methods

Tissue preparation

Specimens of the European eel (*Anguilla anguilla* L.; average body mass, 400–700 g) were purchased from a local supplier and kept in an aquarium at 12–14 °C. The animals were decerebrated and despinalized before samples were quickly taken from the liver and white skeletal muscle, and the swimbladder was dissected.

Both retia mirabilia (20–40 mg animal^{-1}) were separated from the swimbladder, and the outer two layers of the swimbladder wall were removed to obtain the secretory epithelium (40–80 mg animal^{-1}). The tissues were carefully rinsed in saline to remove most of the blood, blotted dry and frozen in liquid nitrogen. In some preparations the swimbladder was perfused with saline before dissection to remove essentially all blood cells. No difference, however, was noted between these two procedures, indicating that remaining blood cells did not bias the enzyme activities measured in homogenates from unperfused swimbladder preparations.

Preparation of homogenates

The frozen tissue was ground into powder and then homogenized under ice in 3–4 vols of the appropriate homogenisation medium. For determination of the activities of hexokinase (HK), glyceraldehyde phosphate dehydrogenase (GAPDH), pyruvate kinase (PK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH) and phosphofructokinase (PFK), media described by Zammit and Newsholme (1976) were used. For determination of

citrate synthase (CS) activity the tissue was homogenized according to Alp *et al.* (1976). The homogenate was centrifuged at 12000 *g* for 5 min, and the supernatant used for further analysis. The homogenates used to analyse enzyme kinetics were filtered through a Sephadex G-25 column, and the resulting filtrate was used for the determination of enzyme activity.

Enzyme assays

All chemicals used were of the highest available purity. Enzymes and coenzymes were purchased either from Boehringer (Mannheim, FRG) or from Sigma Chemicals (St Louis, MO, USA).

The assays were performed at 25 °C in a thermostatted spectrophotometer (Uvicon 860, Kontron, Düsseldorf, FRG) with 0.3 ml cuvettes. The measurements of enzyme activities followed the procedures given by the following authors: hexokinase (HK, EC 2.7.1.1) and phosphofructokinase (PFK, EC 2.7.1.11), Zammit and Newsholme (1976); glyceraldehyde phosphate dehydrogenase (GAPDH, EC 1.2.1.12), Bergmeyer *et al.* (1974); pyruvate kinase (PK, EC 2.7.1.40), Zammit *et al.* (1978); malate dehydrogenase (MDH, EC 1.1.1.37), Driedzic and Stewart (1982); citrate synthase (CS, EC 4.1.3.7), Alp *et al.* (1976); glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), Boström *et al.* (1972). The test medium for lactate dehydrogenase (LDH, EC 1.1.1.27) contained 50 mmol l^{-1} TRA/HCl (triethanolamine hydrochloride) at pH 7.4, 2.5 mmol l^{-1} pyruvate and 0.15 mmol l^{-1} $NADH+H^+$ (Pelster *et al.* 1988).

The pH dependence of the activities of the enzymes PFK and G-6-PDH was analysed in BisTris buffers (pH 6.5–7.5) and Tris buffers (pH 7.5–8.2).

Results

Enzyme activities at pH 7.4 or above

A comparison of enzyme activities of the glycolytic pathway, the citric acid cycle and the pentose phosphate shunt measured in homogenates of swimbladder epithelium, rete mirabile, white muscle and liver is shown in Table 1. High activities of the glycolytic enzymes (comparable to those in white skeletal muscle) were measured in the swimbladder epithelium. Much lower activities were found in the rete. The activities of enzymes of the citric acid cycle, in contrast, were very low in the swimbladder epithelium, even lower than in white skeletal muscle tissue.

The activity of G-6-PDH, a key enzyme of the pentose phosphate shunt, was more than 10 times higher in the swimbladder epithelium than in white skeletal muscle, and almost reached the levels measured in liver homogenates.

pH dependence of enzymes

The gas gland cell PFK activity showed a similar pH dependence to that of the white muscle enzyme. At pH 6.5, the activity was reduced to almost 35 % of its

Table 1. *Activities of representative enzymes of anaerobic glycolysis, the citric acid cycle and the pentose phosphate shunt in eel tissues*

Enzyme	Tissue			
	Swimbladder epithelium	Rete mirabile	Skeletal muscle	Liver
Glycolytic pathway				
Hexokinase (HK)	1.2±0.3 (3)	0.6±0.2 (4)	0.4±0.3 (3)	–
Phosphofructokinase (PFK)	10.1±3.6 (8)	1.9±0.4 (4)	9.6±6.0 (8)	1.5±0.2 (4)
Glyceraldehydephosphate dehydrogenase (GAPDH)	79.8±2.9 (3)	47.5±9.9 (4)	331±55 (3)	159±26 (4)
Pyruvate kinase (PK)	123±50 (5)	49.9±12.1 (4)	65.0±21.2 (4)	10.0±2.0 (4)
Lactate dehydrogenase (LDH)	190±113 (5)	43.1±10.9 (3)	395±177 (5)	19.7±14.0 (6)
Citric acid cycle				
Malate dehydrogenase (MDH)	51.7±8.8 (5)	92.1±12.4 (4)	68.4±46.1 (3)	773±197 (4)
Citrate synthase (CS)	1.1±0.5 (5)	1.2±0.2 (5)	1.7±0.6 (4)	6.7±1.8 (5)
Pentose phosphate shunt				
Glucose-6-phosphate dehydrogenase (G-6-PDH)	2.7±1.3 (6)	1.4±0.3 (4)	0.2±0.1 (5)	3.4±0.9 (6)
6-Phosphogluconate dehydrogenase (6-PGDH)	0.7±0.4 (6)	0.7±0.4 (4)	0.2±0.1 (3)	4.5±1.8 (6)

Activities are given in μmol min^{-1} g^{-1} fresh mass.
Values are mean±S.D.; the number of preparations is given in parentheses.

maximum value, measured at pH 8.2 (Fig. 1A). The G-6-PDH activity was pH dependent as well (Fig. 1B), although the effect was less pronounced. At pH 6.5 about 80% of the maximum activity was retained, and similar results were obtained with the liver enzyme preparations (Fig. 1B).

A Lineweaver–Burke plot (Fig. 2) demonstrates that a higher proton concentration reduced the maximum velocity of the reaction of PFK with its substrate, fructose-6-phosphate (F-6-P), and also slightly increased the K_m value for the substrate F-6-P. Covariance analysis showed the regression lines obtained for pH 6.5 and 7.3 to be significantly different ($P<0.01$, Biostat I), giving K_m values of 0.68 mmol l^{-1} for pH 7.3 and 0.80 mmol l^{-1} for pH 6.5.

The activity of the swimbladder PFK was also sensitive to ATP, showing a 50% decrease in activity at an ATP concentration of 5 mmol l^{-1} (data not shown).

Discussion

A comparative analysis of enzyme activities in eel tissues demonstrates that eel gas gland cells, like liver cells, are equipped for metabolizing glucose by the pentose phosphate shunt. High activities of pentose phosphate shunt enzymes

have also been reported for the cod swimbladder (Boström *et al.* 1972). CO_2 arising from the activity of 6-PGDH may thus contribute to the high ratio of CO_2 production to O_2 consumption of about 4.5 observed in the perfused eel swimbladder preparation (Pelster *et al.* 1989).

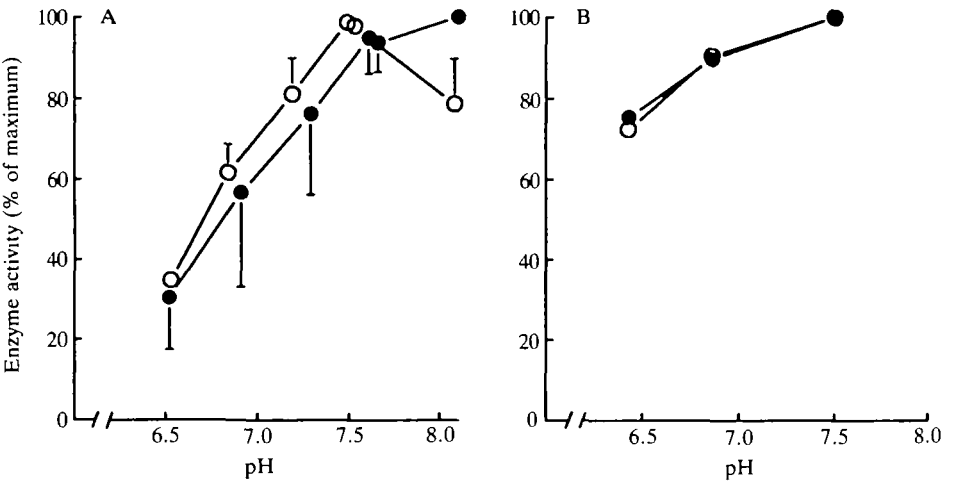

Fig. 1. (A) The effect of pH on the activity of phosphofructokinase in the swimbladder epithelium (●) and in white muscle tissue (○). ATP, 1 mmol l^{-1}; fructose-6-phosphate, 3 mmol l^{-1}; N=4 (mean±S.D.). (B) The effect of pH on the activity of glucose-6-phosphate dehydrogenase of the swimbladder epithelium (●) and liver (○) in two separate preparations of each tissue.

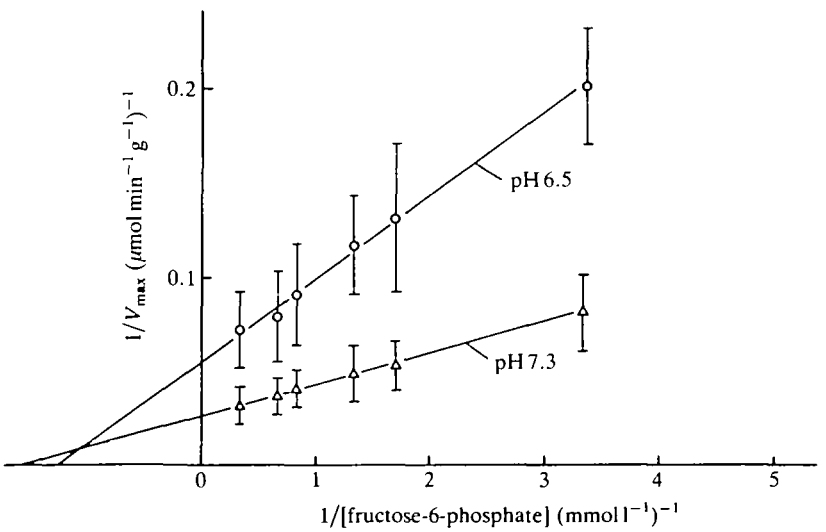

Fig. 2. The effect of proton concentration on substrate (fructose-6-phosphate) affinity of swimbladder epithelium phosphofructokinase, in a Lineweaver–Burke plot. ATP, 1 mmol l^{-1}; N=4 (mean±S.E.; regression lines, pH 6.5: $y=0.044x+0.055$, $P<0.01$; pH 7.3: $y=0.017x+0.025$, $P<0.01$).

Swimbladder epithelial cells also have a high potential for anaerobic metabolism, comparable to that of white skeletal muscle. The production of lactic acid and of CO_2, and their release into the capillary blood, is essential for the liberation of oxygen from the haemoglobin by reducing the haemoglobin oxygen-carrying capacity (Fänge, 1983; Pelster and Weber, 1991). CO_2 may be even more efficient than lactic acid in this regard because of its high diffusability. The activities of the enzymes of the (aerobic) citric acid cycle, in contrast, are extremely low, which is in line with the very low oxygen consumption of the tissue (Pelster *et al.* 1989) and the presence of only very few mitochondria (Dorn, 1961). This metabolic design prevents the oxygen from being consumed by swimbladder epithelial cells instead of entering the swimbladder gas phase.

The pH sensitivities of the swimbladder epithelial PFK and G-6-PDH were not different from those of the white skeletal muscle enzyme and the liver enzyme. A similar pH dependence has been described for the cod white muscle enzyme (Leaver and Burt, 1981). Our results therefore present no evidence for special adaptation of the swimbladder epithelial enzymes to the acidic pH values that are to be expected in the swimbladder tissue. The pentose phosphate shunt, however, may gain importance with decreasing pH, as the ratio of the activity of PFK to that of G-6-PDH drops from 3.7 at alkaline pH to 1.7 at pH 6.5.

A prediction of *in vivo* reactions based on *in vitro* measurements of enzyme activities is difficult, especially as phosphofructokinase is influenced by different cofactors and the inhibitory influence of one of them can be compensated for by that of others (Dobson *et al.* 1986). Our results, however, show that the metabolic design of the swimbladder epithelial cells does indeed allow for CO_2 production *via* the pentose phosphate shunt.

The activity of the pentose phosphate shunt requires reactions to reoxidize the NADPH that is formed (cf. Pelster *et al.* 1989). Several authors have shown that some of the oxygen radicals, which usually arise under hyperbaric oxygen pressures, are detoxified by oxidation of NADPH (Thierney *et al.* 1973; Basset and Fisher, 1979). Swimbladder tissue probably faces the highest oxygen pressures in nature and, therefore, the redox equivalents produced in the pentose phosphate shunt might well be necessary to prevent the tissue from oxygen damage. Preliminary measurements indicate significant activity of the enzyme glutathione reductase, which requires NADPH, in the swimbladder epithelium.

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