Fructose 2,6-bisphosphate and anaerobic metabolism in marine molluscs

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Fructose-2,6-bisphosphate contents of tissues of three species of marine molluscs were measured over a time course of environmental anoxia. During anaerobiosis fructose-2,6-P_2 levels in soft tissues (mantle, gill, hepatopancreas) decreased rapidly (to levels of 2.5–4% of control in oyster tissues). Content in adductor muscles of bivalves remained constant, however, while fructose-2,6-P_2 in gastropod foot muscle increased. The drop in fructose-2,6-P_2 in soft tissues may be part of the overall metabolic depression occurring during anaerobiosis and may function to limit the use of glycolytic carbon for purposes other than energy production.

Fructose 2,6-bisphosphate *Marine mollusc anaerobiosis* *Anaerobic metabolism regulation* Ostrea edule Mytilus edulis Littorina littorea

1. INTRODUCTION

Over the last four years the major role of fructose-2,6-bisphosphate in controlling 6-phosphofructo-1-kinase (PFK) and regulating glycolytic rate has been recognized [1]. Concentrations of the activator in mammalian tissues are modulated by hormones, starvation, diabetes, the relative availability of gluconeogenic substrates, anoxia and muscle work. The function of fructose-2,6-P_2 appears to be to concentrate signals from various metabolic processes at one point of control, PFK, the limiting reaction of glycolysis [2]. The role of fructose-2,6-P_2 in the metabolism of invertebrate animals is only beginning to be investigated. The compound activates PFK from insect flight muscle [3] and foot muscle of the marine whelk [4] and levels of fructose-2,6-P_2 rise during flight initiation in the cockroach [3].

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An ability to survive long periods of anoxia is a major requirement for the many species of marine invertebrates which inhabit the intertidal zone. Two adaptations are key. First, during aerial exposure species dramatically depress metabolic rate to levels of only 5–10% of the rate during immersion [5,6]. Secondly, anaerobic energy production is enhanced (over the glycogen to lactate fermentation typical of vertebrates) by the coupled oxidation of glycogen and aspartic acid as substrates, the accumulation of alternative end products (alanine, succinate, propionate), and substrate level phosphorylations of ADP linked to organic acid synthesis. Obviously, control over glycolytic rate is a prime requirement for both of these adaptations. The present study examines tissue contents of fructose-2,6-P_2 in three species of marine molluscs over a course of environmental anoxia and aerobic recovery. The data suggest that a suppression of fructose-2,6-P_2 content in soft tissues may be involved in limiting the use of carbohydrate for purposes other than energy production during anaerobiosis.

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2. MATERIALS AND METHODS

Marine molluscs were purchased from a seafood retailer and were held in aerated seawater (1000 mOsmol Instant Ocean) at room temperature (approx. 22°C) for 1 week before use. To impose anoxia animals were given aerial exposure under a N_2 gas atmosphere in sealed containers. Animals were sampled at varying times over the course of anoxia. Some animals were returned to the aerated seawater tank after anoxia and were sampled after 12 or 24 h of aerobic recovery. Individuals were rapidly dissected open and tissues were removed and frozen in liquid nitrogen. For *Mytilus edulis* and *Littorina littorea* specific tissues were first removed and remaining tissues were then pooled as the 'remaining body'.

Frozen tissues were rapidly weighed and then immediately homogenized in 10 or 12 vol of hot (80°C) 50 mM NaOH followed by incubation at 80°C for 10 min. After centrifugation aliquots of the supernatant (1–10 μl) were assayed for fructose-2,6-P_2 content using the method of Van Schaftingen [7] which relies upon the ability of fructose-2,6-P_2 to activate potato tuber pyrophosphate-linked PFK. That enzyme activation was due to fructose-2,6-P_2 content alone was confirmed by acid treatment of samples; when fructose-2,6-P_2 was destroyed samples had no effect on PP_i-PFK activity. A slight inhibitory effect of the high salt content of tissue extracts from marine invertebrates was compensated for by doubling the amount of PP_i-PFK used per assay.

3. RESULTS

Table 1 shows the levels of fructose-2,6-P_2 in tissues of the oyster, *Ostrea edule*, over the course of anoxia and after 24 h of aerobic recovery. In the control situation levels of fructose-2,6-P_2 were much higher in soft tissues (mantle, gill, hepatopancreas) than in muscles. This relationship is also typical of mammalian tissues (liver, brain > skeletal muscle) although absolute content of fructose-2,6-P_2 is significantly higher overall in mammalian tissues than in mollusc tissues [8]. Content of fructose-2,6-P_2 in mantle, gill and hepatopancreas decreased dramatically when animals were exposed to a nitrogen gas atmosphere; after 3 h of anoxia content had dropped to 10–19% of control values with a continuing decrease over time to 2.5–4% of control values by 24 h. After long term anoxia (96 h), however, content of the hexose bisphosphate increased somewhat. When oysters were reimmersed in aerated seawater control levels of fructose-2,6-P_2 in soft tissues were re-established within the 24-h recovery period. Fructose-2,6-P_2 content of adductor muscles responded differently to anoxia. Over the first 24 h of anoxia content remained constant in both phasic and catch adductors. By 96 h, however, fructose-2,6-P_2 in phasic adductor had increased somewhat while the content in catch adductor decreased. After 24 h of recovery fructose-2,6-P_2 content of the phasic adductor had returned to control levels but catch adductor showed an elevated content, 2-fold higher than control.

Table 1

Levels of fructose-2,6-P_2 (pmol/g wet wt) in tissues of the oyster, *Ostrea edule*, over the course of anoxia and aerobic recovery

	Control	Anoxia				Recovery (24 h)
		3 h	6 h	24 h	96 h	
Mantle	433 ± 62	83 ± 8	46 ± 6	12 ± 4	61 ± 2	423 ± 53
Gill	696 ± 75	73 ± 6	76 ± 11	27 ± 12	75 ± 3	610 ± 17
Hepatopancreas	806 ± 97	110 ± 6	50 ± 2	20 ± 2	73 ± 7	667 ± 99
Phasic adductor	75 ± 3	60 ± 8	56 ± 7	70 ± 8	98 ± 10	75 ± 7
Catch adductor	47 ± 3	47 ± 3	50 ± 6	53 ± 9	20 ± 5	93 ± 7

Data are means ± SE, $n = 5$

Table 2

Levels of fructose-2,6-P_2 (pmol/g wet wt) in tissues of the sea mussel, *M. edulis* and the periwinkle, *L. littorea*, over the course of anoxia and aerobic recovery

Mytilus edulis	Control	Anoxia		Recovery (12 h)
		3 h	6 h	
Gill	150 ± 11	48 ± 10	35 ± 5	147 ± 18
Foot muscle	110 ± 9	45 ± 6	32 ± 6	112 ± 10
Adductor muscle	135 ± 15	135 ± 26	132 ± 30	139 ± 18
Remaining body	206 ± 22	44 ± 1	29 ± 4	196 ± 21

Littorina littorea	Control	Anoxia			Recovery (12 h)
		3 h	6 h	12 h	
Foot muscle	266 ± 7	313 ± 48	850 ± 29	1000 ± 105	320 ± 46
Remaining body	497 ± 62	447 ± 51	220 ± 25	260 ± 31	647 ± 78

Data are means ± SE, $n = 5$

The effects of environmental anoxia on fructose-2,6-P_2 levels in tissues of the sea mussel, *M. edulis*, are shown in table 2. Although animals were treated to a shorter course of anoxia, the effects of anoxia were similar to those seen in the oyster. Thus, the content of fructose-2,6-P_2 in adductor muscle (posterior and anterior muscles combined) remained constant throughout anoxia and aerobic recovery. Fructose-2,6-P_2 content in gill, combined remaining soft tissues (including mantle, hepatopancreas, kidney and gut) and foot muscle decreased sharply within 3 h of the onset of anoxia, was further lowered after 6 h of anoxia but rebounded to control levels within 12 h of the return to aerated seawater.

The effects of environmental anoxia on tissue levels of fructose-2,6-P_2 in the gastropod mollusc, the periwinkle *L. littorea*, are also shown in table 2. As in the bivalves the soft tissues showed a decline in fructose-2,6-P_2 content throughout anoxia although the decrease, at 50%, was considerably less than that found in the bivalve tissues. Gastropod foot muscle differed from the bivalve adductor muscles, however, in showing a progressive increase in fructose-2,6-P_2 content over the course of anoxia with levels, after 12 h of anoxia, 4-fold greater than those of control muscle. Within the 12 h recovery period content returned to levels similar to control.

4. DISCUSSION

Overall metabolic rate of intertidal molluscs drops dramatically during environmental anoxia. This is in part due to the cessation of physical activities such as feeding and pumping water over the gills and in part to a specific slowdown of non-essential metabolic activities when oxygen is limiting. The reduction in metabolic rate is apparently the reason why no Pasteur effect is observed and why glycolytic rate is apparently inhibited during the aerobic/anaerobic transition despite the fact that the anaerobic fermentation of glycogen and aspartate must supply the full ATP requirements of the anoxic animal. Anaerobiosis in marine molluscs is initiated with no change in the percentage of phosphorylase *a* [9], with phosphorylation and inactivation of pyruvate kinase and PFK [4,10] and with changes in fructose-6-P and fructose-1,6-P_2 contents indicative of an inhibition of PFK [11].

The drop in fructose-2,6-P_2 content which occurs in the soft tissues of these molluscs during anoxia is probably part of the overall mechanism of metabolic shutdown. In mammalian tissues fructose-2,6-P_2 is a signal for the abundance of glucose and potentiates the use of carbohydrate for anabolic purposes [1,2,12]. Fructose-2,6-P_2 content decreases during anoxia in hepatocytes and

during electrical stimulation in muscle [8]; both of these are situations in which glycolytic rate is activated to increase energy production. However, the use of carbohydrate for other purposes should be restricted so fructose-2,6-P_2 activation of PFK is withdrawn and glycolytic rae becomes energy linked via AMP activation of PFK. The situation in marine molluscs is apparently similar. The rapid reduction in fructose-2,6-P_2 content in soft tissues is probably important in the initial rapid reduction in metabolic rate. This would depress carbohydrate availability as energy demand declines and carbohydrate-utilizing anabolic processes are shut down. A slow rise in AMP content of tissues over hours or days of anaerobiosis, may then become the chief modulator keying glycolytic rate to energy requirements.

The situation in muscle tissues is somewhat different. These maintain a function during anaerobiosis: adductor muscles hold the valves closed and control gaping movements of the valves during anoxia in bivalves while an initial response of the periwinkle to anoxia is often to move using the foot muscle in an attempt to avoid hypoxic or anoxic conditions. Fructose-2,6-P_2 content in these muscles may be retained or even increased to promote muscle function during anoxia. Although opposite to the mammalian situation, muscle function in another invertebrate muscle, the insect flight muscle, is also accompanied by an increase in fructose-2,6-P_2 content [3]. However, an overriding mechanism for overall metabolic depression during anoxia appears to be protein phosphorylation. Anoxia induces the phosphorylation of both PFK and pyruvate kinase as well as a number of other cellular proteins [4,10]. The anoxic (phosphorylated) form of PFK from whelk foot muscle has a reduced sensitivity to fructose-2,6-P_2 activation (K_a is 3.5-fold higher than that of the aerobic enzyme) and an increased sensitivity to ATP inhibition [4]. Thus, despite constant or even increasing fructose-2,6-P_2 content in muscles, the influence of this metabolite on PFK may actually decline during anoxia. If covalent modification also applies to the soft tissues then PFK activity could be depressed via two mechanisms, enzyme phosphorylation and decreased concentrations of the activator.

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