J. Exp. Mar. Biol. Ecol., 170 (1993) 213–226

JEMBE 01995

Impact of anoxia and hydrogen sulphide on the metabolism of *Arctica islandica* L. (Bivalvia)

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(Received 21 February 1991; revision received 9 March 1993; accepted 26 March 1993)

Abstract: The effects of environmental anoxia and anoxic sulphide exposure on metabolism are measured in tissues of the clam, *Arctica islandica*. Under anoxia the total activity of glycogen phosphorylase and the percentage of the enzyme in the active *a* form are significantly reduced. Alterations in pyruvate kinase kinetics produce slightly increased V_{max} values, strongly increased $S_{0.5}$ PEP, slightly increased $S_{0.5}$ ADP in the muscular tissues and mantle, and strongly reduced I_{50} for alanine (up to 90-fold increased sensitivity). Anoxia also stimulates a reduction of fructose-2,6-bisphosphate levels, an effector of phosphofructokinase, in all tissues tested. These effects are consistent with enzyme modifications induced by phosphorylation to produce a restricted activity. Anoxic sulphide exposure produced similar effects on the glycogenolytic enzyme glycogen phosphorylase (GP), as does anoxia alone. In the course of the experiments, mitochondrial energy metabolism is not affected by sulphide. The accumulation of the anaerobic indicator metabolite succinate is almost identical in adductor and foot under both stresses. The data suggest a similar coordinated metabolic rate control under environmental anoxia and anoxic sulphide exposure, i.e. H_2S has no distinctly different effects on the parameters tested. This study provides evidence that while being burrowed in anoxic sediments, the clams are able to withstand the detrimental effects of sulphide for a substantial period of time.

Key words: Anaerobiosis; Hydrogen sulphide; *Arctica islan dica*

INTRODUCTION

A well-developed ability to tolerate lack of oxygen characterizes various marine invertebrates. *Arctica islandica*, a sublittoral bivalve mollusc, is one such species. This species is confined to parts of the Western and the boreal North Atlantic Ocean, including parts of the Baltic. The animals have some notable characteristics, including a longevity estimated at greater than 100 years for some individuals (Thompson et al., 1980). These bivalves are found on silty or muddy sediments and in this kind of biotope may experience frequent exposure to oxygen deficiency, a common phenomenon of the deeper mud areas of the Western Baltic (Babenerd, 1991). *A. islandica* is among the few species encountered in those areas (Weigelt & Rumohr, 1986; Weigelt, 1991). This fact, as well as its annual production in Kiel Bight (Brey et al., 1990), stresses the ecological importance of those animals. An extraordinary high survival under anoxia

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(Theede et al., 1969) is achieved by a well-adapted anaerobic metabolism (Oeschger, 1990). Moreover, the animals exhibit another peculiarity as they undergo self-induced anaerobiosis without any apparent exterior triggering factor to be seen (Taylor, 1976; Oeschger, 1990). Marine invertebrates inhabiting muddy bottoms may be exposed to high levels of hydrogen sulphide. Minimal amounts of hydrogen sulphide are immensely toxic to most aerobic organisms, one of the toxic effects being inhibitory interactions with various enzymes, e.g. cytochrome *c* oxidase (National Research Council, 1979). In the Western Baltic *A. islandica* is often found on the same stations where the sulphide resistant priapulid species *Halicryptus spinulosus* lives. There the sulphide concentration in the pore water of the upper sediment layers easily exceeds 200 μM (Oeschger & Vetter, 1992).

An extensive literature deals with the anaerobiosis of molluscs (e.g. de Zwaan, 1977; Siebenaller, 1979; Holwerda et al., 1983; Shick et al., 1983; Gäde & Grieshaber, 1986; Storey, 1988b; Storey & Storey, 1990). A crucial adaptive strategy supporting facultative anaerobiosis of marine invertebrates is a profound depression of metabolic rate, reducing ATP demand to a level that can be sustained by less efficient fermentative catabolic pathways and so support long-term survival. For example, during prolonged anoxia, energy release in *A. islandica* is decreased to less than 1% of aerobic rates (Oeschger, 1990). Control of glycolysis is obviously a prerequisite for anaerobic survival, and is achieved by a flux control at key regulatory enzymes, such as pyruvate kinase (PK). Recent studies have shown the importance of covalent modification in enzyme control during anaerobiosis; via anoxia-induced protein phosphorylation or dephosphorylation reactions, large changes in the activity states of enzymes are made during anaerobiosis (Storey, 1984, 1988a).

However, although much is known about the biochemical response to anoxia by marine invertebrates, there is only scanty information on the impact of hydrogen sulphide on bivalves. The present study seeks to determine the effect of anoxia and hydrogen sulphide on the metabolism of *A. islandica* which often is confronted with the lack of oxygen and the occurrence of toxic sulphide in a stressful environment in the deeper parts of the Baltic Sea.

Materials and Methods

Arctica islandica were dredged on several occasions with a rectangle dredge from a muddy station below 20 m water depth in Kiel Bight (Western Baltic Sea). In July 1989, some individuals were transported alive in a cooler to Ottawa. They were kept unfed in aerated seawater at 9.5 ± 0.5 °C and 22‰ salinity. Same conditions were applied during all experimental set-ups.

Anoxic experiments were performed in August 1989. Anoxia was achieved by bubbling pure N_2 through 1–2 l of seawater for at least 2–4 h prior to incubation. Before adding the animals into the flasks without sediments (6–12 specimens, according to

the volume of the incubation vessels), the pH of the seawater was adjusted to about 7.5 with 1 N HCl. After 6 days, anoxic seawater was renewed to avoid selfpollution. For anoxic sulphide incubation experiments in August 1992, a stock solution of hydrogen sulphide (10 mM) was prepared from washed crystals of $Na_2S \cdot 9\ H_2O$ in degassed deionised water. From this solution appropriate amounts were added to anoxic seawater to a given final concentration of 200 μM. During the experiments sulphide concentration was monitored daily several times using the spectrophotometric method of Cline (1969) and adjusted when necessary. During these anoxic experiments with and without sulphide, seawater was changed every 3 days. A possible bacterial production of H_2S in anoxic control experiments could be ruled out, since no sulphide was measurable in these incubation flasks. Hydrogen sulphide may occur in different ionic forms, depending on the pH of the medium, and this study refers to " sulphide" as the sum of H_2S, HS^- and S^{2-}. Upon the end of the experiments, after exposure to anoxia or anoxia and hydrogen sulphide, the animals were tested for life signs. Valve closure reflex served as an index.

The tissues were then quickly dissected out, blotted, immediately frozen in liquid nitrogen and stored at $-75\,^{\circ}C$ and kept frozen until analysis. Biochemicals and coupling enzymes were obtained from Sigma Chemical Co. and Boehringer. All other chemicals were of reagent grade and Milli-Q purified water was used throughout.

ENZYME PREPARATIONS

For glycogen phosphorylase, frozen tissue samples (≈ 100 mg of each tissue from individual specimens) were homogenised (1:4 W/V) in ice-cold imidazole-HCl buffer (50 mM, pH 7.0), containing 100 mM NaF, 5 mM EDTA, 5 mM EGTA, 100 mM NaCl, 2 mM DTT and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) with an Ultra Turrax tissue homogenizer. This homogenate was not centrifuged since glycogen phosphorylase is often associated with glycogen particles which may precipitate under centrifugation. Instead, particulate matter in the homogenate was allowed to settle on ice without centrifugation for at least 30 min, and then enzyme activity was measured in the supernatant. For pyruvate kinase (PK), tissue samples were extracted in imidazole HCl-buffer (50 mM, pH 7.0) 100 mM NaF, 5 mM EDTA, 5 mM EGTA, 30 mM 2-mercaptoethanol and 0.1 mM PMSF. Homogenates were centrifuged at 27000 $\times g$ at $4\,^{\circ}C$ for 20 min. The supernatants were desalted by centrifugation through a Sephadex G-25 column, previously equilibrated in homogenisation buffer excluding PMSF.

ENZYME ASSAYS

For glycogen phosphorylase *a* optimal assay conditions were 50 mM potassium phosphate buffer (pH 7.0), 2 or 6 $mg \cdot ml^{-1}$ glycogen (previously dialysed), 0.4 mM NADP, 10 μM glucose-1,6-bisphosphate ($G16P_2$), 0.25 mM EDTA, 15 mM $MgCl_2$,

with excess dialysed phosphoglucomutase (PGM) and glucose-6-phosphate-dehydrogenase (G6PDH).

For measurements of total phosphorylase, 1.6 mM AMP proved to be optimal for activating phosphorylase *b* and was added to the assay mixture. For PK, conditions for maximal enzyme activity were 50 mM imidazole-HCl buffer, pH 7.0, 10 mM phosphoenolpyruvate (PEP), 3, 1, or 0.75 mM ADP (depending on tissue, c.f. Results section), 50 mM KCl, 5 mM $MgCl_2$, 0.2 mM NADH and 1 U lactate dehydrogenase (LDH) per ml assay buffer. One unit of enzyme activity is defined either as the amount producing 1 μmol glucose-1-phosphate (G1P) (for phosphorylase), or utilising 1 μmol PEP (for PK) per minute at 23 °C. Kinetic constants were determined as follows: $S_{0.5}$ values for PEP and ADP were determined from Hill plots using experimentally determined V_{max} values, I_{50} values for L-alanine were estimated after Job et al. (1978) from plots of v vs. [inhibitor] at subsaturating PEP concentration (4 mM), values for K_a from plots of v vs. [activator] at subsaturating PEP concentration (0.25 mM). Assays were performed at 23 °C using a Gilson or a Kontron Uvikon 941 recording UV-spectrophotometer.

FRUCTOSE-2,6-BISPHOSPHATE DETERMINATION

Frozen tissue samples (50–100 mg) were homogenised 1:10 W/V in hot 50 mM NaOH (80 °C) followed by incubation for 10 min at 80 °C. Homogenates were centrifuged at 12000 × *g* and the supernatant used for determination of fructose-2,6-bisphosphate ($F26P_2$). Samples were assayed for $F26P_2$ content by the method of van Schaftingen (1984), which relies on the ability of $F26P_2$ to activate potato tuber pyrophosphate-linked phosphofructokinase (PPi-PFK).

SUCCINATE

Succinate was measured using the standard enzymatic method after Beutler (1985).

RESULTS

MODIFICATION OF GLYCOGEN PHOSPORYLASE

The effect of 10-day exposure to anoxia on activity of glycogen phosphorylase (GP) in *A. islandica* from July 1989 is given in Table I for adductor, foot, mantle, and remaining body (consisting of gills, mid-gut gland, and guts). In another experiment during August 1992 the clams were additionally exposed to 200 μM hydrogen sulphide. Figure 1 shows the effect of anoxia with and without sulphide on the activity of GP in different tissues.

During the course of both experiments the total activity of glycogen phosphorylase

TABLE I

Arctica islandica. Effect of long-term exposure to anoxia on glycogen phosphorylase in different tissues. Animals were kept unfed ≈2 months before incubated in August 1989 at 9.5 ± 0.5 °C, 22‰ salinity. Values are means ± SEM, $n = 4$ determinations on tissues from separate animals. Optimal [glycogen] for adductor assays: 6 mg · ml^{-1}, all other tissues: 2 mg·ml^{-1}. Activity of total phosphorylase is μmol glucose-1-P produced· min^{-1}·g^{-1} fresh mass determined in the presence of 1.6 mM AMP. * Significantly different from corresponding aerobic control value by the Student's *t*-test, $p < 0.05$.

		Total phosphorylase (U·g^{-1} fresh mass)	Phosphorylase-*a* (%)
Adductor	Control	3.6 ± 0.47	58.5
	Anoxia 10 days	2.44 ± 0.08*	30.6*
Foot	Control	2.85 ± 0.26	53.1
	Anoxia 10 days	1.29 ± 0.12*	23.3*
Mantle	Control	0.61 ± 0.07	62.1
	Anoxia 10 days	0.33 ± 0.02*	33.6*
Remaining body	Control	1.27 ± 0.05	59.9
	Anoxia 10 days	0.71 ± 0.04*	25.5*

(*a* + *b*) was significantly reduced compared to control animals. Values for total phosphorylase in anoxic animals decreased to 45–71% of the corresponding controls. Anoxic hydrogen sulphide exposure had a similar effect in decreasing GP activity to 70–82% of control animals. Only the foot of sulphide exposed clams had a significant higher GP activity than animals incubated in anoxia alone.

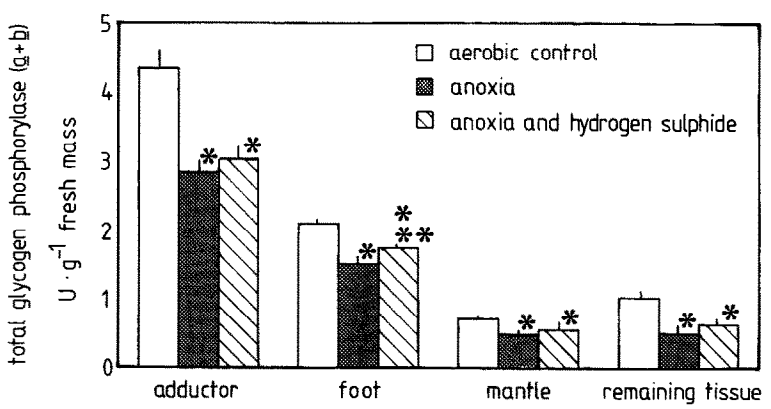

Fig. 1. *Arctica islandica*. Effect of 10-day exposure to anoxia with and without 200 μM sulphide on glycogen phosphorylase in different tissues in animals from August 1992: adductor, foot, mantle and remaining body (gills, mid-gut gland, gut). Clams were subjected to experimental conditions within one week after catch. Values are means ± SEM, $n = 4$. * All values for incubated clams with and without sulphide are significantly different from aerobic controls (Student's *t*-test, $p < 0.05$). ** GP activity significantly different between clams incubated with and without sulphide. For further details see Table I.

The percentage of phosphorylase in the active *a* form was reduced by anoxia during the experiments in 1989 (Table I). In another experiment in August 1992 with clams incubated within one week after catch, exposure to anoxia resulted only in a reduction of the active *a* form in foot tissue (Fig. 2). There was no significant difference between the effects of the two stresses, anoxia vs. hydrogen sulphide exposure, on the% phosphorylase *a* in all tissues analysed.

A concentration of 5 mM caffeine, as well as concentrations of 25 mM propionate, succinate, acetate, and alanine (anaerobic metabolites produced by the clams) added to assays did not produce any effect on phosphorylase activity.

KINETIC PROPERTIES OF PYRUVATE KINASE

Kinetic properties of PK from aerobic vs. 10 day anoxic exposed clams are shown in Table II for adductor, foot, mantle, and remaining body (consisting of gills, mid-gut gland, and gut). The kinetic properties of PK were considerably altered as a result of the experimental condition. In all tissues, affinity for substrate PEP was reduced for the enzyme from anoxia exposed tissues compared to the aerobic form. $S_{0.5}$ for PEP increased by a factor of 1.3 to 5.6 under anoxia. In the two muscular tissues, adductor and foot, affinity for ADP was also affected by the experimental condition, the $S_{0.5}$ for ADP decreased between 6 and 35% during anoxic incubation. $S_{0.5}$ ADP remained unchanged in the two other tissues studied. Anoxia exposure had the greatest effect on enzyme inhibition by L-alanine. I_{50} alanine was dramatically reduced by as much as 40 to 90-fold. In all organs I_{50} alanine dropped from an extremely high value (75 to >100 mM) of no or little physiological relevance to the enzyme to a value of about 1 to 3 mM that could bring the enzyme under the influence of cellular levels of alanine.

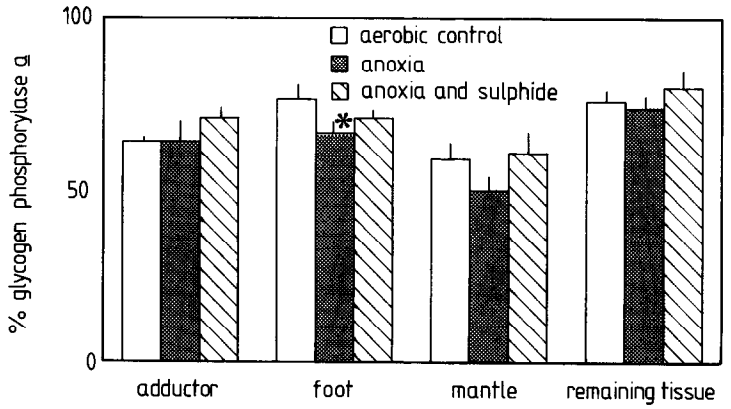

Fig. 2. *Arctica islandica*. Effect of 10-day exposure to anoxia with and without 200 μM sulphide on the percentage of phosphorylase in the active *a* form in different tissues. Values are means $\pm$ SEM, $n = 4$. * Significantly different from aerobic controls. For further details see Table I.

TABLE II

Arctica islandica. Effect of 10-day exposure to anoxia on kinetic properties of pyruvate kinase from different organs in animals incubated in September 1989. Substrate concentrations for V_{max} were 10 mM PEP for all tissues and ADP at 3,1, and 0.75 mM for adductor, mantle and remaining body respectively; for foot optimal ADP was 3 mM for the control and 1 mM for the anoxic enzyme. Effector constants were determined at subsaturating PEP, 4 mM for I_{50} values and 0.25 mM for K_a values. Data are means ± SEM, $n = 3$–4 determination on tissues from separate animals. Values in parentheses refer to the fold activation by optimal levels of $F16P_2$. *Significantly different from aerobic value by the student's *t*-test, $p < 0.05$.

	Aerobic controls	Anoxic
Adductor		
V_{max} ($U \cdot g^{-1}$ fresh mass)	6.28 ± 1.79	9.28 ± 1.19
$S_{0.5}$ PEP (mM)	2.95 ± 0.55	4.87 ± 0.32*
$S_{0.5}$ ADP (mM)	0.18 ± 0.02	0.12 ± 0.05*
I_{50} L-alanine (mM)	> 70	1.89 ± 0.14*
K_a $F16P_2$ (mM)	1.14 ± 0.08	1.57 ± 0.06*
($F16P_2$: fold activation)	(3.44 ± 0.10)	(7.30 ± 0.23)*
Foot		
V_{max} ($U \cdot g^{-1}$ fresh mass)	9.89 ± 0.43	12.85 ± 1.22*
$S_{0.5}$ PEP (mM)	0.25 ± 0.01	1.41 ± 0.11*
$S_{0.5}$ ADP (mM)	0.16 ± 0.01	0.15 ± 0.01*
I_{50} L-alanine (mM)	> 100	1.11 ± 0.09*
K_a $F16P_2$ (mM)	0.33 ± 0.06	0.28 ± 0.06
($F16P_2$: fold activation)	(2.52 ± 0.26)	(5.54 ± 0.54)*
Mantle		
V_{max} ($U \cdot g^{-1}$ fresh mass)	2.19 ± 0.27	2.50 ± 0.38
$S_{0.5}$ PEP (mM)	0.53 ± 0.08	1.01 ± 0.05*
$S_{0.5}$ ADP (mM)	0.12 ± 0.01	0.14 ± 0.01
I_{50} L-alanine (mM)	> 100	1.57 ± 0.20*
K_a $F16P_2$ (mM)	0.47 ± 0.09	0.27 ± 0.05
($F16P_2$: fold activation)	(2.71 ± 0.14)	(4.58 ± 0.48)*
Remaining body (gills, mid-gut gland, gut)		
V_{max} ($U \cdot g^{-1}$ fresh mass)	3.40 ± 0.71	7.13 ± 0.27*
$S_{0.5}$ PEP (mM)	0.66 ± 0.04	0.83 ± 0.11*
$S_{0.5}$ ADP (mM)	0.10 ± 0.01	0.11 ± 0.01
I_{50} L-alanine (mM)	> 100	2.81 ± 0.28*
K_a $F16P_2$ (mM)	0.52 ± 0.09	0.28 ± 0.03*
($F16P_2$: fold activation)	(2.73 ± 0.17)	(3.45 ± 0.27)*

Other possible inhibitors of PK were tested in mantle and foot at concentrations of up to 50 mM, but had no effect on the enzyme in the anoxic exposed form; these included the metabolites produced by the animals during anaerobiosis, such as succinate, acetate and propionate.

Activation of PK by fructose-1,6-bisphosphate ($F16P_2$) was also assessed. The K_a value was not altered by anoxia in foot and mantle, but the fold activation of enzyme V_{max} by optimal levels of $F16P_2$ did increase significantly. The effect was greatest for the muscular enzymes representing a doubling of the fold activation.

Aspartate, an activator of PK in some other marine molluscs, produced no effect at all at concentrations of up to 50 mM.

CHANGES IN CONCENTRATIONS OF FRUCTOSE-2,6-BISPHOSPHATE

Besides the changes in kinetic parameters of GP and PK changes in concentration of the PFK activator $F26P_2$ were analysed to assess the consequences of anoxic exposure on the metabolism in different tissues of *A. islandica*. Levels of $F26P_2$ in aerobic vs. experimental animals are shown in Fig. 3. Content of $F26P_2$ decreased significantly during anoxia in all organs to values 29–70% of corresponding aerobic values.

EFFECT OF ANOXIA AND HYDROGEN SULPHIDE ON SUCCINATE ACCUMULATION

In Fig. 4, the effect of anoxia with (200 μM) and without sulphide on the succinate accumulation in two muscular tissues is shown. During the course of the 10 d experiments under both stresses there was no difference in the accumulation of this anaerobic marker metabolite in adductor (Fig. 4a) and foot (Fig. 4b).

Discussion

EFFECT OF ANOXIA

For facultative anaerobic marine invertebrates, a general strategy of metabolic response to lack of oxygen is emerging. A major requirement for survival during long-term anoxia is a profound metabolic rate depression, exemplified in the case of *A. islandica* (Oeschger, 1990), as well as other marine invertebrate species (e.g. de Zwaan, 1977,1991; Pamatmat, 1979; Shick et al., 1983; Storey & Storey, 1990; Brooks et al. 1991). Glycolysis is the primary pathway of ATP synthesis during anoxia. A new homeostasis between ATP synthesis and ATP utilisation must be achieved by coordinated lowering of the rates of both types of processes including targeting ATP synthesis by lowering glycolytic rate. Key mechanisms for coordinated control of glycolytic rate depression have been revealed in gastropod molluscs, including changing the state of regulatory enzymes via phosphorylation/dephosphorylation reactions and regulating PFK via dramatic changes in the level of its potent activator, $F26P_2$ (e.g. Plaxton & Storey, 1984; Michaelidis et al., 1988; Storey, 1988 a,b). The data in the present study indicate that covalent modification of both GP and PK forms an important part of the response to anoxia by tissues of *A. islandica*. Total activity of GP ($a + b$) drops substantially compared with the initial values in all tissues. These findings are consistent with an overriding influence of metabolic rate depression by reducing glycogenolysis during anoxia.

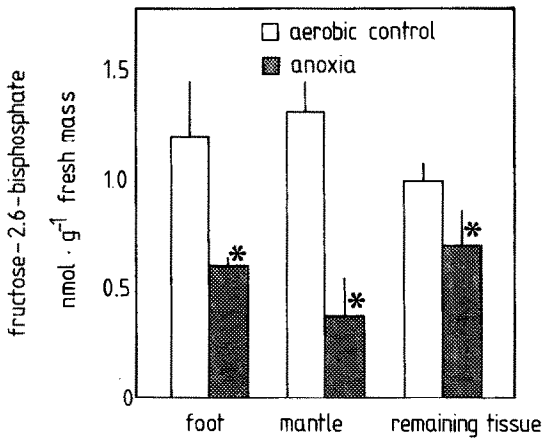

Fig. 3. *Arctica islandica*. Effect of 10-day exposure to anoxia on fructose-2,6-P_2 in different tissues in clams incubated in September 1989: foot, mantle and remaining body (gill, mid-gut gland, guts). Values are in nmol·g^{-1}·fresh mass, means ± SEM, $n = 3$–4 determinations on tissues from separate animals. * Significantly different from aerobic control value by the Student's t-test, $p < 0.05$

The data also supply strong evidence for anoxia-induced phosphorylation of PK in the tissues of *A. islandica*. The enzyme shows two major changes in kinetic properties: increased $S_{0.5}$ (PEP) and a strongly reduced I_{50} (alanine). These anoxia-induced modifications will greatly reduce the activity of the enzyme expressed in vivo. In particular, the change in I_{50} for alanine is outstanding. Alanine is an extremely poor inhibitor of the enzyme form in aerobic tissues, the I_{50} values being far above the normal range of alanine concentrations in vivo (Taylor, 1976).

However, the anoxic enzyme form is modified, by probable protein phosphorylation, to be highly sensitive to alanine inhibition (I_{50} 1 to 3 mM), at a time when cellular levels of alanine, an anaerobic end-product, typically increase several fold in molluscs (de Zwaan et al., 1982). The same focus of anoxia-induced phosphorylation in dramatically changing alanine inhibition of the enzyme is also seen in other marine invertebrates (Plaxton & Storey, 1984; Storey, 1988a; Oeschger & Storey, 1990). The principle involved is a very effective one; i.e. to greatly reduce PK activity in anoxia, adaptive forces have targeted enzyme sensitivity to the metabolite whose levels most specifically represent anaerobic metabolism.

Covalent modification control over PK is only part of the regulation of glycolysis. The present study shows also a significant reduction of F26P_2 levels in different tissues. That diminution results in an additional slow down of glycolysis during anoxia by affecting PFK, one of its rate limiting enzymes, as demonstrated for various other species (e.g. Storey, 1986; 1988a,b).

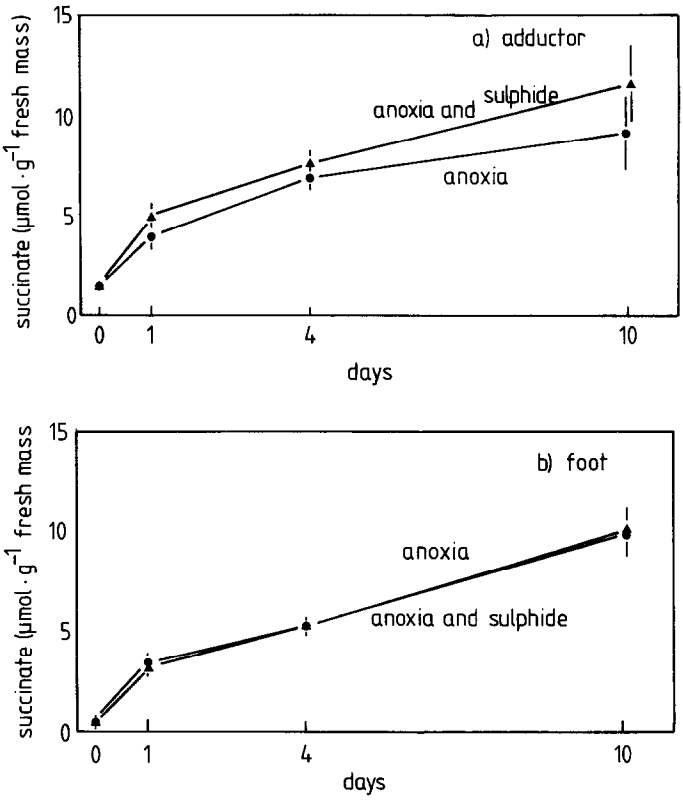

Fig. 4. *Arctica islandica*. Succinate accumulation in adductor (a) and foot (b) of clams exposed in August 1992 to anoxia with (200 μM) and without sulphide at 9.5 ± 0.5 °C and 22‰ salinity. Clams were subjected to experimental conditions within one week after catch. Values are in μmol succinate·g^{-1}·fresh mass ± SEM, $n = 5$.

EFFECT OF HYDROGEN SULPHIDE

In its habitat in the deeper parts of the Baltic Sea *A. islandica* is often exposed to hydrogen sulphide, a compound toxic to many aerobic organisms. *Arctica islandica*, however, has a remarkable ability to withstand prolonged anoxic incubation with H_2S, with extraordinarily high LT_{50}-values (Theede et al., 1969; Theede, 1973). Not only exogenous factors, like hydrographic or sediment conditions may be responsible for the animals' exposure to hydrogen sulphide. Also endogenous factors, like the animals' behaviour may be responsible for the risk of being confronted with that toxic substance, since *A. islandica* burrows into the anoxic sediment. Shell closure may serve as a passive exclusion mechanism against sulphide as a first short-term defence. But even bivalves are not able to exclude H_2S from the inner medium over prolonged periods. The clam *Scrobicularia plana*, e.g. is only able to keep internal sulphide concentration initially low,

while after 8 days of exposure to 200 μM, internal sulphide is in equilibrium with outer levels (Pedersen & Oeschger, in prep.). Moreover, *A. islandica* uses its foot for burrowing, an organ well-adapted for an effective glycolytic ATP production, as demonstrated by the high activities by opine dehydrogenases in this organ (Dando et al., 1981; Livingstone et al., 1983). The animals also perform protruding movements with the foot, not only during anoxia (Oeschger, 1990), but also during exposure to hydrogen sulphide. This implies, then, that at least some of the animals's tissues are direct in contact with hydrogen sulphide and raises the question about the impact of H_2S on metabolism and enzyme function. In this study that question is addressed at the glycogenolytic enzyme level (GP) and the level of mitochondrial energy production.

Total activity of GP decreased under anoxic H_2S incubation, and parallels the effect of anoxia. Only in foot is there a significant difference from the effect of anoxia, as GP activity is significantly higher under hydrogen sulphide vs. anoxia exposure. Anoxic exposed *A. islandica* with and without sulphide have the same percentage of the active *a* form without a significant difference between the two incubations. These experiments were conducted with animals used shortly after catch. Keeping the clams unfed for a longer period of time, obviously affects the depression of the active *a* form, as is seen in a series done in 1989, where a stronger reduction of the *a* form after anoxic exposure was observed (c.f. Table I).

Hydrogen sulphide exposure produced no additional effects on the energy release related with mitochondrial anaerobic pathways. This can be seen from the accumulation of the indicator marker metabolite succinate, which is produced in the mitochondria. The time course of succinate accumulation in the muscular tissues, adductor and foot, is almost identical under the experimental conditions with and without the presence of sulphide in these tissues.

Facultative glycolytic rate depression results from combined alterations of GP activity, variations in $F26P_2$ and changes in PK and phosphofructokinase (PFK) activity (Storey & Storey, 1990). Since in *Halicryptus spinulosus* anoxic sulphide exposure produces the same effects on GP and PK activity as does anoxia alone (Oeschger & Storey 1990), we assume analogous consequences for *A. islandica*. Comparing the overall effect of anoxia and exposure to sulphide, both stresses prove to be similar in their effects on the glycogenolytic enzyme GP and the anaerobic energy release in the presence of sulphide on the metabolism of *A. islandica*.

It seems, that hydrogen sulphide acts indirectly on the glycolytic enzymes even though it acts directly by binding other molecules. Mitochondrial membrane-bound enzymes, e.g. cytochrome *c* oxidase, are much more sensitive to the impact of hydrogen sulphide (National Research Council, 1979).

H_2S itself is probably not causing the change in kinetic properties of the tested enzymes. But as a result of the effect of H_2S on other systems, glycolytic enzyme function is affected. Possibly these changes are mediated by protein kinases or phosphatases or as a result of decreased energy charge alone, because H_2S inhibits oxygen-based metabolism.

For marine invertebrates the use of efficient anaerobic pathways is obviously a prerequisite to cope with the presence of sulphide in their environment during long-term exposure (Oeschger & Vetter 1992). The metabolic responses to anoxia and sulphide seem to be similar, because both stresses effectively cut off oxidative metabolism in the animals. The mechanisms of metabolic control that work for anoxia, are equally well to deal with H_2S stress. This seems also to hold true for *A. islandica* when evaluating mitochondrial energy production, which has been unaffected by sulphide during our experiments. For *A. islandica* sulphide is not such a menace as for other higher organisms. According to our results, burrowing into hydrogen sulphide containing anoxic sediment does not pose an additional threat to *A. islandica*. This applies for a substantial period of time, as our study reveals. However, sulphide tolerance is reduced when the animals are incubated for extended periods (50 days and longer at 10 °C, Theede et al., 1969). A possible explanation for this feature might be a disturbance of the regulatory machinery at crucial levels other than energetic enzyme functioning. Such an effect might indirectly lead to counteraction of the rate depression and thus finally to a decreased sulphide tolerance.

Whether it is possible for the animals to even exploit the oxidation energy of the H_2S molecule for oxidative phosphorylation, like the bivalve, *Solemya reidi*, is not known at this time (Powell & Somero, 1986; O'Brien & Vetter, 1990), and requires further research.

Acknowledgements

Part of the research was carried out during a visit by R.O. to the laboratory of K.B. Storey at Carleton University at Ottawa/Canada. Thanks are due to H. Theede (Universität Bremen) for support of this visit. The authors thank J.M. Storey for critical reading of the manuscript and J. Duncan and R. Whitwam for technical assistance. Travel funds from the Universität Bremen to R.O., operating grants from N.S.E.R.C. to K.B.S., and from the Bundesminister für Forschung und Technologie for studies on the sulphide effects on metabolism as part of the joint research programme DYSMON (03F0045A) to H. Theede are also gratefully acknowledged.

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