ELSEVIER

Comparative Biochemistry and Physiology Part C 128 (2001) 391–399

CBP

www.elsevier.com/locate/cbpc

The effect of hydrogen peroxide on isolated body wall of the lugworm *Arenicola marina* (L.) at different extracellular pH levels

Daniela Storch*, Doris Abele, Hans-Otto Pörtner

Biologie I / Benthische Ökosysteme, Alfred-Wegener-Institut für Polar-und Meeresforschung, Columbusstrasse, Bremerhaven D-27568, Germany

Received 29 July 2000; received in revised form 3 December 2000; accepted 4 December 2000

Abstract

The effect of hydrogen peroxide on the rate of tissue oxygen consumption, on intracellular pH (pH_i) and on malondialdehyde (MDA) accumulation was studied in isolated body wall tissue of the lugworm *Arenicola marina* (L.). H_2O_2 effects were investigated at various levels of pH_i by changing medium pH (pH_e). The largest decrease of tissue oxygen consumption (by 17% below controls), as well as the highest degree of MDA accumulation (four-fold compared to control values) after H_2O_2 exposure were found at acidic pH_e of 6.4. This was attributed to the higher redox potential of H_2O_2 in acidic solutions. Oxygen consumption at alkaline pH_e (8.5) was not affected by H_2O_2. MDA accumulation in the tissue was considerably lower than at pH_e 7.4 or 6.4. Despite pH dependent alterations of H_2O_2 redox potential, we observed more or less constant pH_e independent acidification of the tissue upon exposure to H_2O_2. We attributed the acidification to an inhibition of ATP consuming proton equivalent ion transport across the cellular membrane. Inactivation of carrier proteins is discussed to be responsible for the decrease in tissue oxygen consumption. However, with a larger effect on oxygen consumption at acidic pH_e values, the latter may not be the only explanation, but additional impairment of other energy demanding processes may be involved.

Keywords: *Arenicola marina*; Acid-base status; Extracellular pH; Hydrogen peroxide; Intracellular acidification; Intracellular pH; Isolated body wall tissue; Malondialdehyde; Metabolic depression; Oxygen consumption

1. Introduction

Hydrogen peroxide is a non-radical reactive oxygen species and the most stable intermediate in the four electron reduction of O_2 to water. In aquatic environments, H_2O_2 predominantly derives from UV-driven photoactivation of dissolved organic material (DOM) (Cooper and Zika, 1983; Zika et al., 1985). During low tide in summer, H_2O_2 was found to accumulate to micromolar concentrations in shallow intertidal pools on the

* Corresponding author. Tel.: +49-471-483-110; fax: +49-471-483-111-49.

E-mail address: dstorch@awi-bremerhaven.de (D. Storch).

1532-0456/01/$ - see front matter
PII: S1532-0456(00)00209-X

German Wadden Sea coast (Abele-Oeschger et al., 1997a). Elevated concentrations of accumulating H_2O_2 during spring and summer represent an ecological factor that marine invertebrates have to cope with on intertidal sandflats. One key response to external elevated H_2O_2 concentrations is a decrease in overall oxygen consumption (Abele-Oeschger et al., 1994, 1997b; Abele et al., 1998). The effect of environmental H_2O_2 is enhanced by cytotoxic oxygen derivatives generated as by-products of various metabolic pathways. Internal H_2O_2 production increases at elevated temperature (Elstner, 1990) and with decreasing Po_2 and pH (Caughey and Watkins, 1985; Elstner, 1990).

In general, H_2O_2 is a strong oxidant in both acidic and alkaline solutions (redox potential in acidic solution: $\varepsilon_0 = +1{,}776$ V; in alkaline solution: $\varepsilon_0 = +0{,}878$ V, Hollemann, 1985). As an uncharged molecule H_2O_2 can cross cell membranes by diffusion (Halliwell and Gutteridge, 1986) and causes oxidative damage to lipids, proteins and nucleic acids (Gutteridge and Halliwell, 1990; Kurata et al., 1993). Lipid peroxidation results in reduced fluidity and permeability of membranes and thereby disturbs transmembrane metabolite and ion transport and reduces enzyme function (Jones, 1985; Kurata et al., 1993). Malondialdehyde (MDA) and lipofuscin accumulate as end products of lipid peroxidation in animal tissues (Slater, 1984; Halliwell and Gutteridge, 1986; Viarengo et al., 1991). MDA is the primary by-product and is usually measured as an indicator for membrane lesions caused by oxidative cell injury (Viarengo et al., 1991). An impairment of membrane transport systems resulting from oxidative insult will disturb cellular homeostasis, causing shifts of e.g. intracellular pH (Abele-Oeschger et al., 1997b). This can have a deactivating effect on major enzymes of aerobic metabolic pathways (Hyslop et al., 1988).

The purpose of the present investigation was to determine the cellular effects of H_2O_2 in the lugworm *Arenicola marina*. This worm is exceptional among intertidal invertebrates with respect to the level of H_2O_2 in the body fluids. It has been shown in previous studies that the H_2O_2 concentration can reach up to 250 $\mu mol/l^{-1}$ in the blood of *A. marina* which is high compared to other invertebrates (Abele-Oeschger and Oeschger, 1995). The isolated body wall tissue was chosen because as the outer hull of the worm it is directly exposed to environmental H_2O_2. At the same time this tissue is well supplied with blood carrying high concentrations of H_2O_2 concomitantly, isolated body wall tissue displays long-term viability.

Oxygen consumption rates of the isolated tissue were determined under normoxia and at different H_2O_2 concentrations and varying extracellular pH values. Decreasing pH_e will cause intracellular pH to fall and augment the redox potential of H_2O_2. Therefore, experiments were designed to study a possible exacerbation of oxidative stress by experimentally induced intracellular acidification. The tissue concentration of MDA was measured as an indicator of acute oxidative stress.

2. Materials and methods

2.1. Specimens

Large individuals of *A. marina* (> 10 cm) were dug out in summer 1997 from the sandy intertidal flats of the North Sea coast near Bremerhaven. They were kept for several weeks in aquaria containing a bottom layer of sand (12 cm) from the worm's original habitat. The tanks were circulated with well aerated natural brackish sea water (24 PSU) and maintained at a temperature of 12 ± 2°C.

To obtain isolated body wall tissues, head and tail ends of the lugworms were quickly dissected, the body wall opened dorsally, entrails removed with tissue paper and gills cut off. The body wall musculature of one lugworm was cut longitudinally into two identical halves. One half was blotted dry and freeze clamped in liquid N_2 by means of a pre-cooled Wollenberger clamp (Wollenberger et al., 1960) (control tissue), the other half was immediately used for experimentation. The tissue preparation was carried out in sterile filtered (0.2-μm poresize) seawater (24 PSU, 12 ± 2°C), containing 4 mmol l^{-1} Hepes. The pH of the filtered seawater ($= pH_e$) was adjusted to 7.4, corresponding to the physiological extracellular pH of *A. marina* at 12°C (Sommer et al., 1997).

2.2. Experimental setup

Tissues were positioned between nylon gauzes (meshsize, 0.6 mm) to avoid curling up of the

body wall musculature. Then they were placed into a respiration tube ($\theta = 0.7$ cm, length = 10 cm, volume = 8 ml) and perfused at a constant flow rate of 20 ± 2 ml h^{-1}. Incubations were carried out in 100% air-saturated, thermostatted, filtered seawater (24 PSU, 12°C, poresize, 0.2 μm) buffered with 4 mmol l^{-1} Hepes. Oxygen consumption rates were determined in a flow-through respirometer (Eschweiler, Kiel, Germany) thermostatted to 12°C. Oxygen concentrations were recorded continuously at the outlet of the respiration chamber with a polarographic oxygen electrode connected to an oxymeter (M200, Eschweiler, Kiel, Germany) and a Linseis 2-channel chart recorder. The rate of oxygen consumption was calculated by averaging the data collected over intervals of 30 min.

In the respirometer each tissue was first subjected to 7 h of normoxic control conditions at pH_e 7.4. The first 1–3 h of control incubation were disregarded to allow the system to equilibrate. After 7 h the incubation water in the 500-ml storage-tank was replaced by seawater buffered at various values of extracellular pH (6.4, 6.9, 7.4, 8.2, 8.5) and oxygen consumption rates were recorded for another 9 h. Subsequently, tissues were either exposed to different H_2O_2 concentrations at a uniform pH_e of 7.4 or to a fixed H_2O_2 concentration of 600 μmol l^{-1} at various levels of pH_e for 6 h. H_2O_2 concentrations were monitored during exposure and readjusted to experimental levels when necessary. Oxygen consumption rates were not determined during H_2O_2 exposure owing to erroneous O_2 readings by the electrodes during spontaneous disintegration of H_2O_2 as well as H_2O_2 detoxification by catalase. After H_2O_2 exposure, recordings of oxygen consumption rates were restarted and continued for another 2 h. During this period pH was kept at the same value as during H_2O_2 exposure and oxygen consumption rates stabilized. At the end of the 24-h experiment, tissues were freeze-clamped and stored in liquid nitrogen for further analyses. Before and after each measurement, the rate of bacterial oxygen consumption was monitored in the respiration chamber. This value together with the electrode drift was below 5% of the total tissue oxygen consumption rate and was subtracted from the measured rate of oxygen depletion.

Additional tissue incubations were carried out as described above to obtain tissue samples for pH_i- and MDA-measurements after each incubation period.

2.3. Analyses

Intracellular pH was measured according to Pörtner et al. (1991). Samples were ground under liquid N_2, using mortar and pestle. Exposure of samples, tissue powder and liquid N_2 to air was minimized to prevent acidification of the sample due to CO_2 condensation. The tissue powder was transferred to Eppendorf cups prepared with 250 μl of ice-cold medium (2 mmol l^{-1} nitrilotriacetic acid and 160 mmol l^{-1} potassium fluoride). Cups were filled with medium, closed air bubble free and ultrasonicated for 1 min in a water bath at −7°C. Subsequently, samples were centrifuged at 0°C and $20\,000 \times g$ for 15 s, to remove insoluble tissue debris. The pH of the supernatant was measured repeatedly at 12°C, using a temperature-controlled capillary pH electrode (Radiometer, Copenhagen G297/G2).

MDA concentrations were measured according to Uchiyama and Mihara (1978). Samples were kept in liquid nitrogen for up to 3 months prior to analysis. Tissues were ground in liquid nitrogen. The tissue powder was homogenized in 0.2% phosphoric acid (1:5 tissue/acid) by use of an Ultra-Turrax. A second volume of 2% phosphoric acid was then added to the mixture resulting in a final concentration of 1.1%. To 0.4 ml of the homogenate 0.4 ml of 1% aqueous thiobarbituric acid solution (TBA) were added. The TBA was dissolved in 50 ml of 50 mmol l^{-1} NaOH containing 0.5 ml of 10 mol l^{-1} butylated hydroxytoluene (BHT) and 0.2 ml of 7% phosphoric acid. Individual blanks were prepared by replacing the TBA solution with 3 mmol l^{-1} HCl. The pH values of all samples were adjusted to 1.6 with either 10 mol l^{-1} NaOH or 12 mol l^{-1} HCl. Subsequently, samples were heated to 100°C for 15 min. After cooling to room temperature, 1.5 ml butanol was added to samples and blanks and mixed vigorously for 40 s. The butanol phase was separated by centrifugation for 5 min at $1000 \times g$. The supernatant was centrifuged again for 5 min at $14\,000 \times g$ and optical density was determined in the butanol phase at 532 and 600 nm. The difference between the TBA value (absorption at 600 nm) and the absorbance of thiobarbituric acid reactive substances was quantified using the

TBA-MDA extinction coefficient ($\varepsilon = 156$ cm^2 μmol^{-1}).

2.4. Calculations

Oxygen consumption rates are expressed as a percentage of the respective control value at pH_e 7.4 in order to facilitate comparison between different treatments. All data were checked for outliers beyond the $r(95)$ limits of an r-distribution [$r_A > r(95)$] using Nalimov's test (Noak, 1980). Statistical significance of differences was tested at the $p < 0.01$ (highly significant) and at the $p < 0.05$ (significant) levels using analysis of variance (ANOVA) or covariance (ANCOVA), followed by a post-hoc test. The Bonferroni/Dunn (Control) post-hoc test was used when treatments were compared to the control treatment. The Student–Newman–Keuls test was taken for comparing treatments other than the control treatment.

3. Results

3.1. Dependence of oxygen consumption rates on H_2O_2 and extracellular pH (pH_e)

Fig. 1 shows the oxygen consumption rate of isolated body wall tissues as a function of the applied H_2O_2 concentration. Exposure to H_2O_2 concentrations below 180 μmol l^{-1} caused tissue oxygen consumption to increase to a value 17% above control levels at a concentration of 42 ± 8 μmol l^{-1} H_2O_2. At H_2O_2 concentrations above 180 μmol l^{-1}, oxygen consumption rates became significantly depressed to 83 ± 0% of the control level at 328 ± 40 μmol l^{-1} H_2O_2 and to 91 ± 4% of the control level at 664 ± 36 μmol l^{-1} H_2O_2. There was no significant difference between oxygen consumption rates at 328 ± 40 μmol l^{-1} H_2O_2 and 664 ± 36 μmol l^{-1} H_2O_2. For a maximum effect on pH_i and MDA accumulation hydrogen peroxide concentration of 600 μmol l^{-1} H_2O_2 was chosen in further experiments. This concentration is approximately 2 times above the highest H_2O_2 level ever measured in blood of *A. marina* (Abele-Oeschger and Oeschger, 1995).

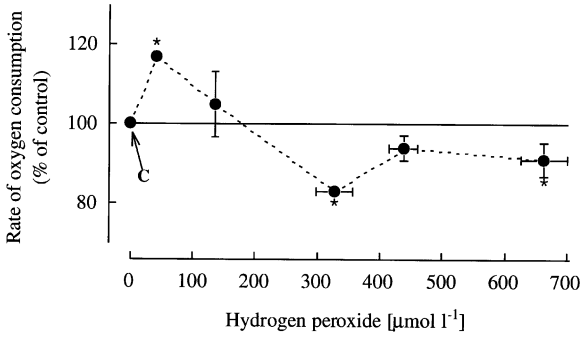

Fig. 1. Oxygen consumption of *Arenicola marina* isolated body wall musculature after 2 h of recovery from 6 h exposure to different H_2O_2 concentrations ($pH_e = 7.4$, $T = 12$°C, $S = 24$ PSU). Rates of oxygen consumption are expressed as a percentage of control value without H_2O_2. The horizontal line indicates the 100% level of O_2 consumption. C, control, * oxygen consumption significantly different from the control value, values are means ± S.D. ($n > 2$) or means ± deviation from the mean (MD) ($n = 2$) of oxygen consumption as well as H_2O_2 concentration (μmol l^{-1} H_2O_2), (1–100) ($n = 2$), (101–200) ($n = 2$), (301–400) ($n = 2$), (401–500) ($n = 2$), (601–700) ($n = 8$), * indicates a significant ($p < 0.05$) difference from the control value.

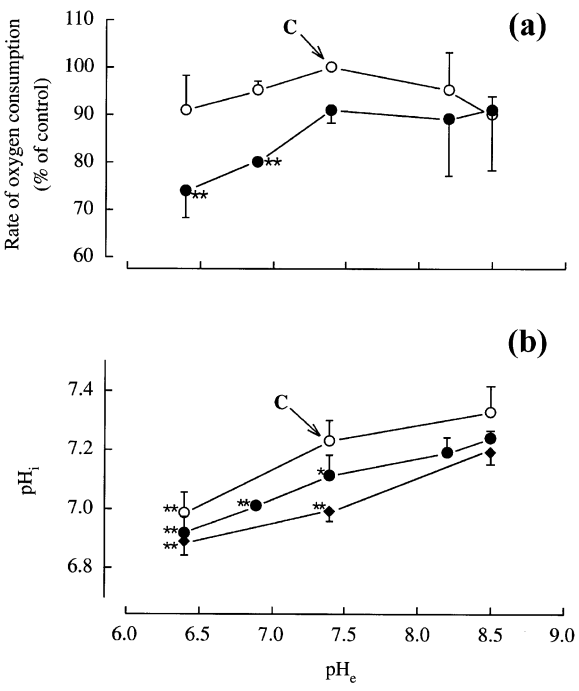

Fig. 2. (a) Oxygen consumption in *Arenicola marina* isolated body wall musculature at different levels of pH_e and (b) effect of pH_e on pH_i in *A. marina* isolated body wall musculature. Open circles: without H_2O_2; filled rhombs: 6 h under 600 μmol l^{-1} H_2O_2; filled circles: 2 h of recovery from 600 μmol l^{-1} H_2O_2 exposure. C, control at physiological pH, (7.4) without H_2O_2. Rates of oxygen consumption are expressed as % of control value at pH_e 7.4 without H_2O_2. Values are means ± S.D. ($n > 2$) or means ± MD ($n = 2$), (a) and (b) ($n = 4$–6) except for pH, 6.9 where ($n = 2$), * indicates a significant ($p < 0.05$) and a highly significant ($p < 0.0$ 1) difference from the respective control value.

Fig. 2a depicts the relationship between oxygen consumption rates of isolated tissues and pH_e before and after exposure to 600 μmol l^{-1} H_2O_2. At both low and high pH_e the reduction in oxygen consumption compared to control values at pH_e 7.4 remained insignificant (Fig. 2a). At pH_e 6.4 oxygen consumption fell by 9% and at pH_e 8.5 it was depressed by 10% below respiration in controls. H_2O_2 exposure at control pH_e (7.4) caused a drop in tissue oxygen consumption ty 9%. H_2O_2 exposure at acidic pH_e (pH_e 6.9 and 6.4) elicited a more pronounced decrease of the respiratory rate (by 20 and 26%, respectively). This drop was highly significant when compared to the oxygen consumption rate at pH_e 7.4 (without H_2O_2) and still significant compared to the oxygen consumption rate at pH_e 7.4 under H_2O_2. In contrast, peroxide exposure at alkaline pH_e did not induce a significant decrease in oxygen consumption.

3.2. Dependence of intracellular pH (pH_i) on pH_e and H_2O_2 (600 μmol l^{-1})

Further studies were conducted to elucidate how the changes in tissue oxygen consumption correlate with the acid-base status of the tissue. pH_i was measured in tissues exposed to different pH_e with H_2O_2, without H_2O_2, as well as after 2 h of recovery from H_2O_2 exposure. Results are summarized in Fig. 2b. Intracellular pH could be adjusted to specific values ('clamped') by choosing the appropriate level of extracellular pH. At control pH_e of 7.4, pH_i was 7.23 ± 0.07. pH_i was more or less linearly dependent on medium pH in the acidic as well as the alkaline range. An experimental reduction of pH_e from 7.4 to 6.4 resulted in a highly significant decrease of pH_i to 6.98 ± 0.07.

Peroxide incubation at different pH_e values caused the same significant decrease of pH_i at all values of medium pH tested resulting in a parallel shift of the pH_i vs. pH_e relationship to lower pH_i values. Within 2 h of recovery from H_2O_2 stress, pH_i rose slightly at all applied pH_e values, but did not return to control levels.

3.3. Impact of pH_e and H_2O_2 exposure (600 μmol l^{-1}) on MDA accumulation in the tissues

Tissues stored in liquid nitrogen directly after animals had been killed were used as controls for MDA measurements. Fig. 3 shows the effect of pH_e in combination with H_2O_2 on MDA accumulation in body wall tissue.

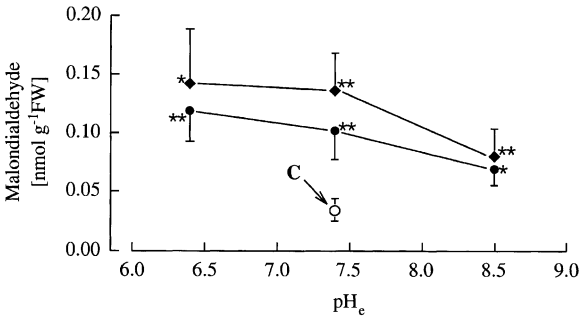

Fig. 3. Malondialdehyde concentration in *Arenicola marina* isolated tissue at different levels of pH_e. Open circle: without H_2O_2; filled rhombs: 6 h under 600 μmol l^{-1} H_2O_2; filled circles: 2 h of recovery from 600 μmol l^{-1} H_2O_2 exposure. FW, fresh weight, C, control at physiological pH, (7.4) without H_2O_2, open circles, ($n = 15$), filled rhombs, ($n = 6$), filled circles, ($n = 4$–5), * indicates a significant ($p < 0.05$) and * a highly significant ($p < 0.01$) difference from the control value.

Peroxide incubation caused a significant accumulation of MDA to levels four-fold above controls at pH_e 7.4 and 6.4 after 6 h of H_2O_2 exposure, whereas, at pH_e 8.5 MDA accumulated to 0.080 ± 0.023 nmol g^{-1} FG, about two-fold above controls. Within 2 h of recovery from 6 h of H_2O_2 exposure, a slight decrease of MDA-concentrations occurred at all values of pH_e but MDA still remained significantly above control levels.

4. Discussion

The objective of the present study was to assess the effects of elevated H_2O_2 concentrations on the cellular homeostasis of the body wall musculature of *Arenicola marina*. The impact of H_2O_2 was studied under different pH_e conditions to evaluate the significance of the acid-base status in a given tissue for oxidative injury phenomena.

To our knowledge, this is the first report documenting a significant increase of oxygen consumption rates upon H_2O_2 exposure (Fig. 1) at concentrations of 42 ± 8 μmol l^{-1} H_2O_2 and even at a concentration as high as 135 ± 1 μmol l^{-1} H_2O_2 oxygen consumption rates were slightly elevated above controls. At higher concentrations (300–600 μmol l^{-1}) H_2O_2 elicited a significant decrease in oxygen consumption compared to non-exposed control tissues. All organisms stud-

ied so far like the Antarctic intertidal limpet *Nacella concinna* (Abele et al., 1998), the shrimp *Crangon crangon* (Abele-Oeschger et al., 1997b) and the polychaete *Hediste diversicolor* (Abele-Oeschger et al., 1994) responded to much lower H_2O_2-concentrations (4–20 μmol l^{-1} H_2O_2) with a decrease in aerobic metabolic rates. These data underline the exceptional situation in *A. marina* compared to other intertidal invertebrates. High H_2O_2 levels of 154 ± 42 μmol l^{-1} in the blood of *A. marina* under normoxic conditions compared to 15.3 ± 4.6 μmol l^{-1} H_2O_2 in haemolymph of the intertidal bivalve *Astarte borealis* substantiate this difference (Abele-Oeschger and Oeschger, 1995). The background of such high H_2O_2 levels in *A. marina* remains unexplained. Even higher H_2O_2 concentrations in *Arenicola* blood result under stressful conditions, e.g. hypoxia and H_2S exposure (Abele-Oeschger and Oeschger, 1995).

The change in oxygen consumption in response to H_2O_2 exposure could only be studied after 2 h of recovery. Therefore, metabolic depression was likely higher during the actual period of H_2O_2 exposure. This assumption is strengthened by pH_i and MDA measurements. Both parameters show a maximum effect during H_2O_2 exposure and partial recovery 2 h after H_2O_2 incubation.

Varying pH_e without H_2O_2 exposure already alters pH_i in isolated pieces of tissues. Acidification as well as alkalization caused a reduction of tissue oxygen consumption. pH_i is thought to be a main factor controlling metabolic rate in invertebrate tissues (e.g. Hand and Gnaiger, 1988) and a decrease of aerobic metabolism upon reduction of pH_i has been described for *A. marina* (Kamp and Juretschke, 1989). Alternatively, pH_e was shown to effectively reduce the rate of oxygen consumption in isolated Sipunculid muscle (Reipschläger and Pörtner, 1996). H_2O_2 exposure exacerbated the drop in tissue oxygen consumption only at acidic pH_e (17% at pH_e 6.4) in *A. marina* body wall, while a comparable H_2O_2 effect was not observed at alkaline pH (Fig. 4). Thus, peroxide induced intracellular acidification (see below) may enhance the reduction of metabolic rate only at acidic pH_i. However, over the whole range of pH_i, oxygen consumption of H_2O_2 exposed tissues was lower compared to non-H_2O_2 exposed tissues at the same pH_i (Fig. 5). Obviously oxygen consumption is reduced by H_2O_2 exposure beyond the effect on intracellular pH. The degree of reduction may depend on the

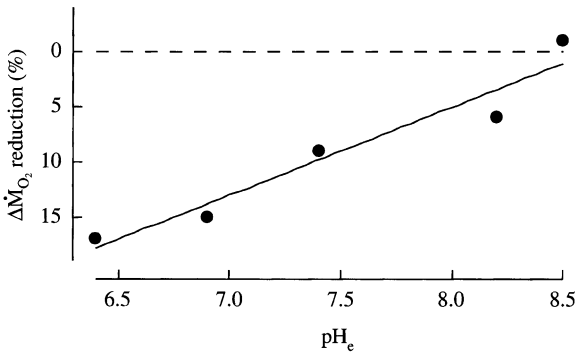

Fig. 4. H_2O_2-dependent reduction of oxygen consumption [$\Delta \dot{M}O_2$ (%)] in *Arenicola marina* isolated body wall musculature at different levels of medium pH. Data were calculated by deducting the oxygen consumption value at different pH_e and 600 μmol l^{-1} H_2O_2 from the oxygen consumption value at the respective pH_e without H_2O_2 (data taken from Fig. 2b, rates of oxygen consumption with and without H_2O_2 are expressed as % of control value at pH_e 7.4 without H_2O_2).

extracellular pH and on the pH dependent redox potential of H_2O_2 (Fig. 4).

H_2O_2 induced an intracellular acidification of the isolated tissues by 0.15–0.2 pH-units independent of pH_e. A similar decrease of pH_i upon peroxide exposure has been reported for the abdominal muscle of the mudshrimp *Crangon crangon* (Abele-Oeschger et al., 1997b) and was attributed to a reduction of energy-dependent proton equivalent ion exchange. Transmembrane ion transport mechanisms depending on Na/K-ATPase play a central role in maintaining intracellular pH above equilibrium values in animal tissues (Roos and Boron, 1981). Na/K-ATPase in rabbit kidney has been found to be susceptible to

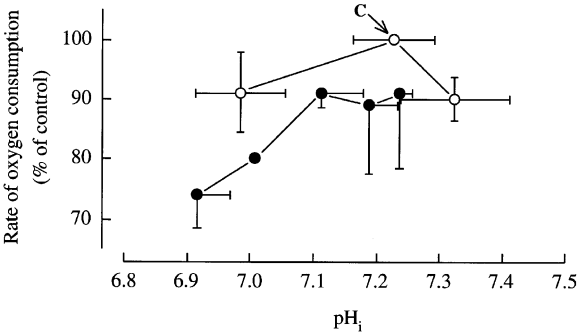

Fig. 5. Oxygen consumption of *Arenicola marina* isolated body wall musculature at different pH_i values. C, control rate measured at $pH_e = 7.4$ without H_2O_2. Open circles: without H_2O_2; filled circles: 2 h of recovery from 6 h of 600 μmol l^{-1} H_2O_2 exposure. (Oxygen consumption data see Fig. 2a, pH_i data see Fig. 2b).

free radical damage (Mense et al., 1997), indicating that oxidative injuries can result in a loss of transporter activity. An inactivation of ATP-consuming ion exchange mechanisms caused by H_2O_2 will reduce cellular energy consumption and could be responsible for the observed reduction in the oxygen consumption rates. Active ion exchange mechanisms contribute significantly to bulk ATP consumption of a given tissue (Na/K-ATPase in skeletal muscle of mammals, 35–40% Siems et al., 1984; Kelly and Mc Bride, 1990; Na/K-ATPase in fish: 12–28%, Suzuki et al., 1994; Krumschnabel et al., 1997).

In summary, H_2O_2 is concluded to have a direct inhibiting effect on ion transport systems which on the one hand results in reduced ATP demand while on the other hand it causes a slowing down of net proton export and intracellular acidification. This intracellular acidification by itself may support metabolic depression resulting in additional reduction in ATP turnover (Hand et al., 1996).

MDA is considered to be one of the main products of the peroxidation of membrane lipids and its accumulation in tissues is reasonably indicative of oxidative stress (Viarengo et al., 1991). The higher MDA accumulation after H_2O_2 exposure at pH_e 6.4 and 7.4, compared to alkaline pH_e, concurs with the highest depression of oxygen consumption in the tissues (Fig. 2). Since H_2O_2 has a much higher redox potential in acidic solutions ($\varepsilon_0 = +1{,}776$ V) than in alkaline solutions ($\varepsilon_0 = +0{,}878$ V), the greater oxidative strength of H_2O_2 could account for the stronger effect at acidic pH_e. The uncharged H_2O_2 molecules can easily cross cell membranes as discussed by Mueller et al. (1997). As a consequence, intracellular membranes can be damaged by H_2O_2 and this will support MDA accumulation at acidic pH values. However, it seems unlikely that lipid peroxidation in itself explains the intracellular acidification as it decreases membrane fluidity and increases membrane leakiness (Jones, 1985) This would have led to metabolic stimulation, whereas, depression was observed. We conclude that lipid peroxidation is not involved in metabolic depression under H_2O_2.

Repair mechanisms are likely responsible for the partial reversibility of MDA accumulation during 2 h of recovery from H_2O_2. Lipid peroxidation has been shown to activate secondary antioxidant defense mechanisms including a cytosolic phospholipase, which mitigates membrane damage (Rashba-Step et al., 1997). This agrees with the finding that MDA is a reactive and transient intermediate itself and any free MDA that is formed in the cell will be rapidly metabolized (Gutteridge and Halliwell, 1990). In addition, MDA may decrease by diffusion out of the tissue (McAnulty and Waller, 1999).

Extracellular H_2O_2 will attack independent of intracellular antioxidant protection, so that transmembrane ion transport mechanisms can be inflicted also at alkaline pH values. Hence, we observed a pH_e independent acidification of the tissue which was attributed to an H_2O_2 induced impairment of H^+-transporters. At first sight, the impact on the transmembrane ion and proton exchangers seems unrelated to pH_e, in contrast to the effect on oxygen consumption. However, turnover rates of acid-base transporters may vary depending on pH leading to variable changes in oxygen consumption. In addition the pH-dependent redox potential of H_2O_2 may support the stronger effect of H_2O_2 on tissue oxygen consumption under acidic pH_e conditions. Consequently, the decrease in oxygen consumption rates at acidic pH_e's under H_2O_2 exposure may not only be due to inactivation of transmembrane carrier proteins, but may also relate to the impairment of other energy demanding processes like, e.g. protein synthesis. More in depth studies are needed to assess the contribution of these processes to metabolic depression upon tissue exposure to elevated H_2O_2 concentrations.

References

Abele, D., Burlando, B., Viarengo, A., Pörtner, H.O., 1998. Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*. Comp. Biochem. Physiol. 120B, 425–435.

Abele-Oeschger, D., Oeschger, R., 1995. Hypoxia-induced autoxidation of haemoglobin in the benthic invertebrates *A. marina* (Polychaeta) and *Astarte borealis* (Bivalvia) and the possible effects of sulfide. J. Exp. Mar. Biol. 187, 63–80.

Abele-Oeschger, D., Oeschger, R., Theede, H., 1994. Biochemical adaptations of *Nereis diversicolor* (Poly-

chaeta) to temporarily increased hydrogen peroxide levels in intertidal sandflats. Mar. Ecol. Prog. Ser. 106, 101–110.

Abele-Oeschger, D., Röttgers, R., Tüg, H., 1997a. Dynamics of UV-driven hydrogen peroxide formation on an intertidal sandflat. Limnol. Oceanogr. 42, 1406–1415.

Abele-Oeschger, D., Sartoris, F.J., Pörtner, H.O., 1997b. Hydrogen peroxide causes a decrease in aerobic metabolic rate and in intracellular pH in the shrimp *Crangon crangon*. Comp. Biochem. Physiol. 117C, 123–129.

Caughey, W.S., Watkins, J.A., 1985. Oxyradical and peroxide formation by haemoglobin and myoglobin. In: Greenwald, R.A. (Ed.), CRC handbook of methods for oxygen radical research. CRC Press, Inc., Florida, pp. 95–104.

Cooper, J.C., Zika, R.G., 1983. Photochemical formation of hydrogen peroxide in surface and ground waters exposed to sunlight. Science 220, 711–712.

Elstner, E.F., 1990. Der Sauerstoff. Wissenschaftsverlag, Mannheim/Wien/Zürich, pp. 1–529.

Gutteridge, J.M.C., Halliwell, B., 1990. The measurement and mechanism of lipid peroxidation in biological systems. Trends. Biochem. Sci. 15, 129–135.

Halliwell, B., Gutteridge, J.M.C., 1986. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch. Biochem. Biophys. 246, 501–514.

Hand, S.C., Gnaiger, E., 1988. Anaerobic dormancy quantified in Artemia embryos: a calorimetric test of the control mechanism. Science 239, 1425–1427.

Hand, S.C., Hardewig, I., 1996. Downregulation of cellular metabolism during environmental stress: mechanisms and implications. Annu. Rev. Physiol. 58, 539–563.

Hollemann, A.S., 1985. Lehrbuch der Anorganischen Chemic. Walter de Gruyter, Berlin, pp. 467–469.

Hyslop, P.A., Hinshaw, D.B., Halsey Jr., W.A. et al., 1988. Mechanisms of oxidant-mediated cell injury. J. Biol. Chem. 263, 1665–1670.

Jones, D.P., 1985. The role of oxygen concentration in oxidative stress: hypoxic and hyperoxic models. In: Sies, H. (Ed.), Oxidative Stress. Academic Press, London, Orlando, Florida, pp. 151–195.

Kamp, G., Juretschke, H.P., 1989. Hypercapnic and hypocapnic hypoxia in the lugworm *A. marina*: A ^{31}P NMR study. J. Exp. Zoo. 252, 219–227.

Kelly, J.M., Mc Bride, B.W., 1990. The sodium pump and other mechanisms of thermogenesis in selected tissues. Proc. Nutr. Soc. 49, 185–202.

Krumschnabel, G., Biasi, C., Scharzbaum, P.J., Wieser, W., 1997. Acute and chronic effects of temperature and of nutritional state, on ion homeostasis and energy metabolism in teleost hepatocytes. J. Comp. Physiol. B 167, 180–286.

Kurata, M., Suzuki, M., Agar, N.S., 1993. Antioxidant systems and erythrocyte lifespan in mammals. Comp. Biochem. Physiol. 106B, 447–487.

McAnulty, J.F., Waller, K., 1999. The effect of quinacrine on oxidative stress in kidney tissue stored at low temperature after warm ischemic injury. Cryobiology 39, 197–204.

Mense, M., Stark, G., Apell, H.J., 1997. Effects of free radicals on partial reactions of the Na,K-ATPase. J. Membrane Biol. 156, 63–71.

Mueller, S., Riedel, H.-D., Stremmel, W., 1997. Determination of catalase activity at physiological hydrogen peroxide concentrations. Anal. Biochem. 245, 55–60.

Noak, S., 1980. Statistische Auswertung von Mess und Versuchsdaten mit Taschenrechner und Tischcomputer. Walter de Gruyter, Berlin, New York, pp. 373–382.

Pörtner, H.O., Boutilier, R.G., Tang, Y., Toews, D.P., 1991. Determination of intracellular pH and pCO_2 after metabolic inhibition by flouride and nitrilotriacetic acid. Resp. Physiol. 81, 255–274.

Rashba-Step, J., Tatoyan, A., Duncan, R., Ann, D., Pushpa-Rehka, T.R., Sevanian, A., 1997. Phospholipid peroxidation induces cytosolic phospholipase A_2 activity: membrane effects vs. enzyme phosphorylation. Arch. Biochem. Biophys. 343, 44–54.

Reipschläger, A., Pörtner, H.O., 1996. Metabolic depression during environmental stress: the role of extracellular vs. intracellular pH in *Sipunculus nudus*. J. Exp. Biol. 199, 1801–1807.

Roos, A., Boron, W.F., 1981. Intracellular pH. Physiol. Rev. 61, 296–434.

Siems, W., Dubiel, W., Dumdey, R., Müller, R., Rapoport, S.M., 1984. Accounting for the ATP-consuming processes in rabbit reticulocytes. Eur. J. Biochem. 139, 101–107.

Slater, T.F., 1984. Free-radical mechanisms in tissue injury. J. Biochem. 222, 1–15.

Sommer, A., Klein, B., Pörtner, H.O., 1997. Temperature-induced anaerobiosis in two populations of the polychaete worm *A. marina*. J. Comp. Physiol. 167B, 25–35.

Suzuki, E.Y., Early, R.J., Patterson, P.H., 1994. Energy metabolism in isolated chick (*Gallus domesticus*) gastrocnemius and tilapia (*Tilapia mossambica*) expalial muscle at various temperatures in vitro. Comp. Biochem. Physiol. 109A, 139–150.

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271–278.

Viarengo, A., Canesi, L., Pertica, M., Livingstone, D.R.,

1991. Seasonal variations in the antioxidant defence systems and lipid peroxidation of the digestive gland of mussels. Comp. Biochem. Physiol. 100C, 187–190.
Wollenberger, A.O., Ristau, O., Schoffa, G., 1960. Eine einfache technik der extrem schnellen abkühlung grosser gewebestücke. Pfügers Arch 270, 399–412.
Zika, R.G., Moffet, J.W., Petasne, R.G., Cooper, W.J., Saltzman, E.S., 1985. Spatial and temporal variations of hydrogen peroxide in Gulf of Mexico waters. Geochim. cosmochim. Acta 49, 1173–1184.