FOLIA MALACOLOGICA
Vol. 9(1): 51–55
ISSN 1506-7629
The Association of Polish Malacologists
& Faculty of Biology, Adam Mickiewicz University
Poznań 2001

A COMPARATIVE STUDY OF ANTIOXIDANT ENZYME ACTIVITIES IN TISSUES OF *HELIX ASPERSA* (O. F. MÜLL.) AND *POMACEA BRIDGESI* (REEVE)

DARIA DZIEWULSKA-SZWAJKOWSKA, MAŁGORZATA ŁOZIŃSKA-GABSKA, JAN WOJTASZEK, KAMILA MIĄŻEK, ANDRZEJ DŻUGAJ

Department of Animal Physiology, Institute of Zoology, University of Wrocław, Cybulskiego 30, 50-205 Wrocław, Poland (e-mail: dzugajan@biol.uni.wroc.pl)

ABSTRACT: Antioxidant enzymes: catalase (CAT) [EC 1.11.1.6], peroxidase [EC 1.11.1.7], glutathione reductase (GSSGR) [EC 1.6.4.2] and glucose-6-phosphate dehydrogenase (G6PDH) [EC 1.1.1.49] were investigated in terrestrial *Helix aspersa* (O.F. Müll.) and aquatic *Pomacea bridgesi* (Reeve). The activity was determined in the heamolymph and homogenates of hepatopancreas and foot muscle. No CAT and peroxidase activity was detected in the hepatopancreas and foot muscle of *P. bridgesi,* and its haemolymph displayed a very low CAT activity (0.005 U/mg of protein). In *H. aspersa* the highest activities (U/g) observed for CAT and GSSGR in the hepatopancreas were 0.40, and 1.05, respectively; for peroxidase and G6PDH in the foot muscle the respective values were 1.22 and 0.22. The activities of antioxidant enzymes determined in both snail species are much lower than the corresponding values for mammalian tissues.

KEY WORDS: antioxidant enzymes, catalase, peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, *Helix aspersa, Pomacea bridgesi*

INTRODUCTION

Paradoxically, the life-giving oxygen can also be toxic. Its toxic effect is caused by reactive oxygen species (ROS), such as superoxide radical anion, hydrogen peroxide and the most reactive – hydroxyl radical. ROS are harmful to living cells, since they can modify membrane lipids, proteins, DNA and disturb oxidative phosphorylation (HERMES-LIMA et al. 1995, 1998, HONG & JOHNSON 1995, PANNUNZIO & STOREY 1998, HALLIWELL 1999a). Cells are protected against ROS by antioxidants of two kinds: low molecular effectors (ascorbate, vitamin A, vitamin E) and enzymes (HALLIWELL 1995, 1999b, HALLIWELL et al. 1995). Superoxide dismutase (SOD), catalase (CAT), peroxidase, glutathione peroxidase and glutathione reductase (GSSGR) have been recognized as antioxidant enzymes.

Antioxidant enzymes (AOE) catalyze reactions leading to decrease of ROS concentration. SOD synthesizes hydrogen peroxide from superoxide radical anion, decreasing the concentration of the latter. Decomposition of hydrogen peroxide by CAT results in water and oxygen. Peroxidases catalyze oxidation of several compounds, thanks to reduction of hydrogen peroxide. Glutathione oxidation by hydrogen peroxide is catalyzed by glutathione peroxidase. Glutathione reductase catalyzes reduction of oxidized glutathione with NADPH. Redox capacity of the cell depends on the GSSG/GSH ratio which, in turn, depends on the NADPH/$NADP^+$ ratio. Therefore glucose-6-phosphate dehydrogenase (G6PDH), catalyzing reduction of $NADP^+$ should be also regarded as an antioxidant enzyme (CHENG et al. 2000, O'BRIEN et al. 2000). Antioxidant enzymes have been investigated in vertebrate and invertebrate tissues, most studies dealing with mammalian antioxidant enzymes (BRYAN & JENKINSON 1987, MCELROY et al. 1992, KURATA et al. 1993, PEREZ-CAMPO et al. 1994, MCINTOSH et al. 1998, ANDERSON & PHILLIPS 1999,

SKAŁECKI et al. 1999). Less attention in this respect has been paid to lower vertebrates (PEREZ-CAMPO et al. 1994, BAINY et al. 1996, STOREY 1996, RUDNEVA 1997, 1999, WU & SQUIRES 1997) and still less to invertebrates (ARUN & SUBRAMANIAN 1998, SHEEHAN & POWER 1999). Only few papers are available on snail antioxidant enzymes (PUGH et al. 1979, HERMES-LIMA & STOREY 1995, HERMES-LIMA et al. 1998). HERMES-LIMA et al. (1998) investigated activities of antioxidant enzymes during estivation and activity. No comparative studies have been reported on land and freshwater snails. Since freshwater snails are exposed to lower and variable oxygen concentration compared to terrestrial snails, exposed to atmospheric oxygen, values of antioxidant enzyme activity in both groups could be expected to differ. This assumption prompted us to investigate the activity of CAT, peroxidase, glutathione reductase and glucose-6--phosphate dehydrogenase in the foot muscle and hepatopancreas of terrestrial (*H. aspersa*) and freshwater (*P. bridgesi*) snails. In this paper we present the results of our investigations and discuss their possible physiological significance.

MATERIAL AND METHODS

All the biochemicals were purchased from Sigma (St. Louis, MO, U.S.A.). Distilled, deionized water was used in all the experiments.

Adult individuals of two snail species – a terrestrial pulmonate *Helix aspersa* (O.F. Müll.), and a freshwater prosobranch *Pomacea bridgesi* (Reeve) – were used. Fifteen hibernating specimens of *H. aspersa* were obtained from the Institute of Animal Husbandry, Kraków/Balice. Prior to sampling tissues they were restored to activity, kept in terrarium at a temperature of 10 ± 1°C and not fed. Twenty individuals of *P. bridgesi*, coming from the laboratory culture of the Department of Animal Physiology, Wrocław University, were kept in an aquarium, water temperature 25 ± 1°C, and fed with vegetable fodder Vitabin (Tetra, Germany), superVit and *Tubifex*. The snails were weighed and their shells measured. The haemolymph was sampled with a cannula from the heart. The hepatopancreas and foot muscle were removed immediately, cleaned of adhering fragments of other tissues, washed and weighed. The haemolymph samples were centrifuged at 2,000 g during 10 min at 4°C and the supernatant was used for antioxidant activity determination. The tissues were homogenized as 1:2 w/v (*P. bridgesi*) and 1:5 w/v (*H. aspersa*) during 60 s at 13,000 r.p.m. in a buffer containing 20 mM Tris, 0.25 M saccharose, 0.5 mM EDTA pH =7.4 at 4°C, in the Ultra Turax homogenizer (Janke & Kunkel IKA Labortechnik, Germany). The homogenate was centrifuged at 14,000 g during 20 min at 4°C and the resulting supernatant was used to determine the activity of antioxidant enzymes.

Catalase (CAT) [EC 1.11.1.6] activity was determined spectrophotometrically, measuring H_2O_2 decomposition at 240 nm (AEBI 1983) using 39.5 $M^{-1}cm^{-1}$ as the molar absorption coefficient. The assay (1 ml) contained 50 mM phosphate buffer and 10 mM H_2O_2; pH 7.0. The substrate was added to start the reaction.

Peroxidase [EC 1.11.1.7] activity was measured as described by PÜTTER & BECKER (1983) determining formation of the oxidized form of ABTS [2,2'--Azinobis (3-ethylbenzthiazoline-sulfonic acid)] at 420 nm (using 18 600 $M^{-1}cm^{-1}$ as the molar absorption coefficient). The assay solution contained: 67 mM phosphate buffer, 1.7 mM ABTS and 10 mM H_2O_2; pH 6.0. The reaction was initiated by addition of the homogenate.

Glutathione reductase (GSSGR) [EC 1.6.4.2] activity was assayed following the absorbance decrease at 340 nm due to oxidation of NADP (GOLDBERG & SPOONER 1983). The assay mixture contained 100 mM phosphate buffer, 0.5 mM EDTA, 0.5 mM NADPH and 2.2 mM GSSG; pH 7.2. The substrate was added to start the reaction.

Glucose-6-phosphate dehydrogenase (G6PDH) [EC 1.1.1.49] activity was assayed as described by DEUTSCH (1983). The assay mixture (1 ml) consisted of 50 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, 0.4 mM NADP and 2 mM G6P; pH 8.0. The substrate was added to start the reaction.

Protein concentration determination followed LOWRY et al. (1951), with bovine serum albumin as a standard.

Spectrophotometric determinations were performed with an HP 8452 Diode Array Spectrophotometer at 25°C. One unit of activity was the amount of the enzyme that produced 1 µmol product per minute.

RESULTS AND DISCUSSION

The results of our investigation are presented in Table 1. The activity of AOE was found to be lower in the freshwater *P. bridgesi,* than in the terrestrial *H. aspersa*. No CAT or peroxidase activity was detected in the hepatopancreas and foot muscle, and no G6PDH in the haemolymph of *P. bridgesi*.

Table 1. Tissue distributon of activities of antioxidant enzymes in *Helix aspersa* and *Pomacea bridgesi*

Enzyme	Tissues	Activity [U/g wet wt]		Specific activity [U/mg protein]	
		Helix aspersa (n=15)	*Pomacea bridgesi* (n=20)	*Helix aspersa* (n=15)	*Pomacea bridgesi* (n=20)
catalase	hepatopancreas	0.404 ± 0.201	nd	0.049 ± 0.027	nd
	foot muscle	0.150 ± 0.095	nd	0.013 ± 0.009	nd
	haemolymph*	0.088 ± 0.079	0.080 ± 0.064	0.012 ± 0.011	0.005 ± 0.004
peroxidase	hepatopancreas	0.373 ± 0.170	nd	0.043 ± 0.021	nd
	foot muscle	1.225 ± 0.767	nd	0.110 ± 0.067	nd
	haemolymph*	0.246 ± 0.186	0.248 ± 0.060	0.040 ± 0.035	0.013 ± 0.002
glutathione reductase	hepatopancreas	1.050 ± 0.514	0.754 ± 0.800	0.129 ± 0.078	0.037 ± 0.032
	foot muscle	0.398 ± 0.141	0.499 ± 0.286	0.036 ± 0.015	0.031 ± 0.017
	haemolymph*	nd	0.312 ± 0.285	nd	0.022 ± 0.016
glucose-6--phosphate dehydrogenase	hepatopancreas	0.192 ± 0.115	0.095 ± 0.042	0.024 ± 0.020	0.012 ± 0.024
	foot muscle	0.225 ± 0.181	0.230 ± 0.139	0.021 ± 0.017	0.028 ± 0.045
	haemolymph*	nd	nd	nd	nd

Values are expressed as mean ± SD; * enzyme activity in haemolymph is expressed as U/ml; nd – not detected

In the hepatopancreas of *H. aspersa* the activity of CAT and GR was three times higher, while that of peroxidase three times lower, compared to the values for these enzymes in the foot muscle. The activities of antioxidant enzymes determined either in land or in freshwater snail tissues are much lower than the corresponding values for mammalian tissues (HONG & JOHNSON 1995, MCDERMOTT et al. 1995, SKAŁECKI et al. 1999). Based on the studies carried out in our laboratory (SKAŁECKI et al. 1999), with the use of the same methods, only the activity of GR in the hepatopancreas and muscle, and G6PDH in human muscle are comparable to the values obtained for both snail species. The observed lower activities in our studies are in accordance with the results obtained for marine invertebrates: two bivalve and two crustacean species, a snail and a starfish (GAMBLE et al. 1995, ARUN & SUBRAMANIAN 1998, PANNUNZIO & STOREY 1998).

AOE are inducible enzymes. In vertebrates and invertebrates various factors affect changes in their activity, for example: diet (GAMBLE et al.1995, CAO et al. 1997, 1999), ecological conditions, feeding behavior, natural and pollutant pro-oxidant xenobiotics (WINSTON & DI GULIO 1991). Production of oxy radicals can be increased by changing oxygen pressure (WINSTON et al. 1990). In marine invertebrates of intertidal zone, exposed to variable oxygen conditions (hypoxia and anoxia), the activity of AOE is higher (GAMBLE et al. 1995). HERMES-LIMA & STOREY (1995) and HERMES-LIMA et al. (1998) have investigated activity of AOE in tissues of a terrestrial pulmonate *Otala lactea* (O. F. Müller) during estivation and activity. They found a higher CAT activity in the foot muscle of estivating snails. In the hepatopancreas of a marine prosobranch *Littorina littorea* L. the maximum activities of antioxidant enzymes clearly responded to changes in oxygen availability. The activity of CAT and GSSGR was suppressed (to 70% and 60% control, respectively) during anoxia exposure (PANNUNZIO & STOREY 1998).

The snails studied by us came from a culture and it can be supposed that, compared to *Littorina littorea* and *Otala lactea* collected in their natural habitats, they were exposed to much lower concentrations of toxic substances, which may explain the decreased activity of AOE. The absence of CAT in the hepatopancreas of *P. bridgesi* may indicate a lower enzymatic antioxidant potential which may be compensated for by other non-enzymatic antioxidants, as observed in some elasmobranch fishes (RUDNEVA 1997, 1999).

It may be expected that the highest activity of antioxidant enzymes should be present in cells of the highest ROS concentration. Several ways of ROS generation have been observed. Superoxide radical anion ($O_2^{\cdot -}$) may be generated by spontaneous reduction of dissolved oxygen by haeme, or in reaction catalyzed by some oxidases (xanthine oxidase), or else as a by-product of oxidative phosphorylation. Hydrogen peroxide (H_2O_2) may be generated from superoxide radical anion in reaction catalyzed by superoxide dismutase, but also from superoxide anion in reaction catalyzed by some oxidases (HALLIWELL 1995).

In mammals the highest antioxidant enzyme activities have been detected in the liver. Lower values have been found in the lung and erythrocytes (SKAŁECKI et al. 1999). Mammalian alveoli consist of two kinds of cells: pneumocytes I and II. Both these kinds are exposed to the same partial pressure of oxygen but antioxidant enzymes are present only in pneumocytes II. The decisive factor in generation of ROS seems to be the rate of metabolism. The higher the rate of metabolism, the higher ROS level. The

rate of metabolism of ectothermic animals depends on the ambient temperature. Though in our experiments *P. bridgesi* was kept at a much higher temperature than *H. aspersa,* the AOE activity in the latter species was somewhat higher. Therefore the slightly higher AOE activity in the terrestrial snail may result from exposure to a higher oxygen concentration. Another parameter which should be considered when comparing snails with mammals is the sensitivity of their cells to the toxic effect of oxygen. One may expect that during evolution the cells become more sensitive to oxygen toxicity which might explain the generally lower AOE activities in snail tissues.

Further studies on the problem should aim at determination of concentration of non-enzymatic antioxidants in the tissues, in order to estimate the total antioxidant potential.

REFERENCES

AEBI H. E. 1983. Catalase. In: Methods of enzymatic analysis (BERGMEYER H. U., ed.). Vol. III, pp. 273–282, Verlag Chemie, Weinheim.

ANDERSON D., PHILLIPS B. J. 1999. Comparative in vitro and in vivo effects of antioxidants. Fd Chem. Toxicol. 37: 1015–1025.

ARUN S., SUBRAMANIAN P. 1998. Antioxidant enzymes in freshwater prawn *Macrobrachium malcolmsonii* during embryonic and larval development. Comp. Biochem. Physiol. 121B: 273–277.

BAINY A. C. D., SAITO E., CARVALHO P. S. M., JUNQUEIRA V. B. C. 1996. Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia (*Oreochromis niloticus*) from a polluted site. Aquat. Toxicol. 34: 151–162.

BRYAN C. L., JENKINSON S. G. 1987. Species variation in lung antioxidant enzyme activities. J. Appl. Physiol. 63: 597–602.

CAO G., PRIOR R. L., CUTLER R. G., YU B. P. 1997. Effect of dietary restriction on serum antioxidant capacity in rats. Arch. Gerontol. Geriatr. 25: 245–253.

CAO G., SHUKITT-HALE B., BICKFORD P. C., JOSEPH J. A., MCEWEN J., PRIOR R. L. 1999. Hypoxia-induced changes in antioxidant capacity and the effect of dietary antioxidants. J. Appl. Physiol. 86: 1817–1822.

CHENG M. L., HO H. Y., LIANG C. M., CHOU Y. H., STERN A., LU F. J., CHIU D. T. 2000. Cellular glucose-6-phosphate dehydrogenase (G6PD) status modulates the effects of nitric oxide (NO) on human foreskin fibroblasts. FEBS Lett. 475: 257–262.

DEUTSCH J. 1983. Glucose-6-phosphate dehydrogenase. In: Methods of enzymatic analysis (BERGMEYER H. U., ed.), Vol. Ill, pp. 190–197, Verlag Chemie, Weinheim.

GAMBLE S. C., GOLDFARB P. S., PORTE C., LIVINGSTONE D. R. 1995. Glutathione peroxidase and other antioxidant enzyme function in marine invertebrates (*Mytilus edulis, Pecten maximus, Carcinus maenas* and *Asterias rubens*). Mar. Environ. Res. 39: 191–195.

GOLBERG D. M., SPOONER R. J. 1983. Glutathione reductase. In: Methods of enzymatic analysis (BERGMEYER H. U., ed.). Vol. Ill, pp. 258–266, Verlag Chemie, Weinheim.

HALLIWELL B. 1995. Antioxidant characterization. Biochem. Pharmacol. 49: 1341–1348.

HALLIWELL B. 1999a. Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanism and the effects of nutrition. Mutat. Res. 443: 37–52.

HALLIWELL B. 1999b. Vitamin C: poison, prophylactic or panacea? Trends Biochem. Sci. 24: 255–259.

HALLIWELL B., AESCHBACH R., LÖLIGER J., ARUOMA O. I. 1995. The characterization of antioxidants. Fd Chem. Toxicol. 33: 601–617.

HERMES-LIMA M., STOREY K. B. 1995. Antioxidant defenses and metabolic depression in a pulmonate land snail. Am. J. Physiol. 268: R1386–1393.

HERMES-LIMA M., WILLMORE W. G., STOREY K. B. 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. Free Radic. Biol. Med. 19: 271–280.

HERMES-LIMA M., STOREY J. M., STOREY K. B. 1998. Antioxidant defenses and metabolic depression. The hypothesis of preparation for oxidative stress in land snails. Comp. Biochem. Physiol. 120B: 437–448.

HONG H., JOHNSON P. 1995. Antioxidant enzyme activities and lipid peroxidation levels in exercised and hypertensive rat tissues. Int. J. Biochem. Cell Biol. 27: 923–931.

KURATA M., SUZUKI M., AGAR N. S. 1993. Antioxidant systems and erythrocyte life-span in mammals. Comp. Biochem. Physiol. 106B: 477–487.

LOWRY O. H., ROSENBROUGH N. J., FARR A., RANDALL R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

MCDERMOTT B. M., STRAIN J. J., FLATT P. R. 1995. Effects of dietary carbohydrate intake on antioxidant enzyme activity and copper status in the copper-deficient streptozotocin (STZ) diabetic rat. J. Nutr. Biochem. 6: 638–643.

MCELROY M. C., POSTLE A. D., KELLY F. J. 1992. Catalase, superoxide dismutase and glutathione peroxidase activities of lung and liver during human development. Biochem. Biophys. Acta 1117: 153–158.

MCINTOSH L. J., HONG K. E., SAPOLSKY R. M. 1998. Glucocorticoids may alter antioxidant enzyme capacity in the brain: baseline studies. Brain Res. 791: 209–214.

O'BRIEN P. J., SLAUGHTER M. R., SWAIN A., BIRMINGHAM J. M., GREENHILL R.W., ELCOCK F., BUGELSKI P. J. 2000. Repeated acetaminophen dosing in rats: adaptation of hepatic antioxidant system. Hum. Exp. Toxicol. 19: 277–283.

PANNUNZIO T. M, STOREY K. B. 1998. Antioxidant defenses and lipid peroxidation during anoxia stress and aerobic

recovery in the marine gastropod *Littorina littorea*. J. Exp. Mar. Biol. Ecol. 221: 277–292.

PEREZ-CAMPO R., LOPEZ-TORRES M., ROJAS C., CADENAS S., BARJA G. 1994. Longevity and antioxidant enzymes, non-enzymatic antioxidants and oxidative stress in the vertebrate lung: a comparative study. J. Comp. Physiol. 163B: 682–689.

PUGH C. R., ULTSCH G. R., LINDAHL R. 1979. Enzyme changes accompanying thermal acclimation in two species of pleurocerid snails. J. Exp. Zool. 209: 65–72.

PÜTTER J., BECKER R. 1983. Peroxidases. In: Methods of enzymatic analysis (BERGMEYER H. U., ed.), Vol. III, pp. 286–293, Verlag Chemie, Weinheim.

RUDNEVA I. I. 1997. Blood antioxidant system of Black Sea elasmobranch and teleost. Comp. Biochem. Physiol. 118C: 255–260.

RUDNEVA I. I. 1999. Antioxidant system of Black Sea animals in early development. Comp. Biochem. Physiol. 122C: 265–271.

SHEEHAN D., POWER A. 1999. Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve mollusks. Comp. Biochem. Physiol. 123C: 193–199.

SKAŁECKI K., RAKUS D., WIŚNIEWSKI J. R., KOŁODZIEJ J., DŻUGAJ A. 1999. cDNA sequence and kinetic properties of human lung fructose(1,6)bisphosphatase. Arch. Biochem. Biophys. 365: 1–9.

STOREY K. B. 1996. Oxidative stress: animal adaptations in nature. Braz. J. Med. Biol. Res. 29: 1715–1733.

WU J., SQUIRES E. J. 1997. The effect of dietary corn oil, vitamin E, and selenium on lipid peroxidation and hemorrhage in chicken liver. J. Nutr. Biochem. 8: 629–633.

WINSTON G. W., DI GULIO R. T. 1991. Prooxidant and antioxidant mechanism in aquatic organisms. Aquat. Toxicol. 19: 137–161.

WINSTON G. W., LIVINGSTONE D. R., LIPS F. 1990. Oxygen reduction metabolism by the digestive gland of the common *Mytilus edulis* L. J. Exp. Zool. 255, 296–308.

received: November 21st, 2000
accepted: January 15th, 2001