Bull. Environ. Contam. Toxicol. (1999) 62:623-629

Bulletin of Environmental Contamination and Toxicology

Characterization of Glutathione S-Transferases (GST) Activities in *Perna perna* and *Mytilus galloprovincialis* Used as a Biomarker of Pollution in the Agadir Marine Bay (South of Morocco)

A. Kaaya,[1] S. Najimi,[1] D. Ribera,[2] J. F. Narbonne,[2] A. Moukrim[1]

[1] Laboratory of Water and Environment, Department of Biology, Faculty of Sciences, University of Ibn Zohr, BP 28/S, 80 000 Agadir, Morocco
[2] Laboratory of Physico and Toxicochemistry UPRES-A-CNRS 5472, University of Bordeaux I, 33 405 Talence, France

Received: 7 September 1998/Accepted: 3 March I999

Chemical pollution of marine ecosystems has led to the development of analytical techniques able to detect overall pollutants, but during the last decade an increasing interest has been focused on the study and use of biochemical parameters, called biomarkers, as indicators of water quality (Narbonne et al. 1991; Livingstone 1993). Such diagnostic and prognostic early-warning tests offer the potential of specificity, sensitivity and application to a wide range of organisms and for discriminating water contamination over broad geographic regions. Many biomarkers are actually extensively used in international programs of pollution monitoring.

In order to evaluate and control the health state of the Agadir marine bay (well known for their fisheries resources and touristic beaches), we established a research program focused on the study of biomarkers of pollution in two species of mussels, *Perna perna* and *Mytilus galloprovincialis,* that cohabit along the coast of Morocco. We have previously studied several biochemical parameters, such as acetylcholinesterase, catalase activities or malondialdehyde content in these organisms for biomarker validation (Moukrim et al. 1997; Najimi et al. 1997).

The aim of this work is to study and test, as a biomarker of pollution, glutathione S-transferases (GST) activities in the mussels *Perna perna* and *Mytilus galloprovincialis.* These enzymes are quantitatively an ubiquitous family of phase II detoxification enzymes (Beckett and Hayes 1993) and are influenced by exposure to various foreign compounds like PAH, PCB and phenobarbital (Buhler and Williams 1988). GST was thus proposed as biomarkers of pollution exposure and/or effects by several authors (Suteau et al. 1988; Fitzpatrick et al. 1997). This paper presents some characteristics of *Perna perna* and *Mytilus galloprovincialis* GST activities (kinetic parameters and organ distribution), their seasonal variations (during two years) and their response to field exposure in polluted and unpolluted sites.

MATERIALS AND METHODS

The study was conducted on adult mussels (40 to 60 mm) collected in two types of sites (Figure 1) representative of the Agadir bay: i) a reference site, Cap Ghir, located far from any human activity, and ii) a polluted site (Anza) that receives the industrial and domestic untreated waste water of Anza zone.

Correspondence to: A. Moukrim

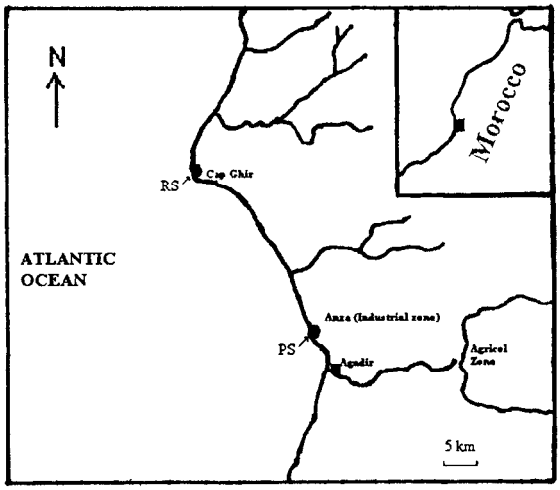

Figure 1. Sampling sites in Agadir bay (RS: Reference Site; PS: Polluted Site)

For seasonal variations and impact of pollution on GST. mussels were collected monthly during two annual cycles (February 95 to February 97). All the preparation procedures were conducted at 4°C. In order to limit an eventual sex related variability in measurement, males and females were pooled to form a sample. The sex of the animals was determined by examination of mantle color.

Soft tissues (whole animal or organs) were collected, thawed, washed with cold 100 mM Tris buffer (pH 7.4) weighed and homogenised in three volumes (w/v) of the same buffer using a Potter Elvehjem homogenizer tube. Homogenates were centrifuged at 9000g for 30 min and the resulting supernatant (post-mitochondria1 fraction or S9) were frozen (-30°C) until use.

GST activity was measured according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. This latter substrate is often prefered choice when total GST is being measured and is recommended for determination of GST activities and inhibition patterns (Habig et al 1974). Assays were performed in a reaction mixture containing 1.05 mL 100 mM Tris buffer (pH 7.4), 50 µL CDNB (1 mM in the assay), 50 µL reduced glutathione (GSH 1 mM in the assay) and 50 µL tissue homogenate. Before use, the GSH requiered for assay was dissolved in Tris buffer. The CDNB was dissolved in ethanol. In all cases the final concentration of ethanol in the assay mixture did not exceed 5% (v/v). Blanks were achieved in the same conditions, but replacing the sample with Tris buffer. Enzyme activity was determined by monitoring changes in absorbency at 340 nm (which translate the rate of CDNB conjugation with GSH (E_{340}CDNB-GSH conjugation = 9.6 $mM^{-1}c\,m^{-1}$) for 2 min at constant temperature with a Varian DMS-80 spectrophotometer. This GST activities are expressed as nmoles/min/mg of S9 protein (mg P).

Protein concentration was measured according to the method of Lowry et al. (1951). The statistical significance of the differences between samples was evaluated by the "t" test using the Statistica software (Release 4.5A Statsoft Inc. Ed. 1993). A p value of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

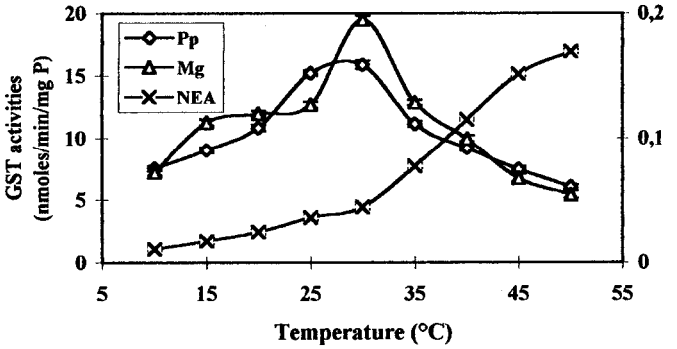

Figure 2. Effect of temperature incubation on GST activities of *Mytilus galloprovincialis* (*Mg*) and *Perna perna* (*Pp*) and on non-enzymatic activity (NEA). Enzyme activity was determined in S9 of whole animal at pH 7.4 for 2 min. The values indicate mean ±SD, (n=6).

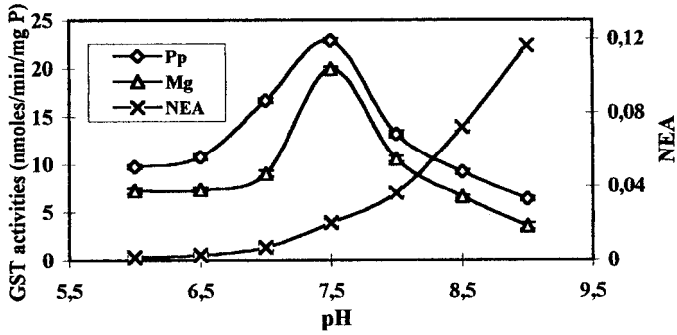

Figure 3. Effect of pH of incubation mixture on GST activities of *Mytilus galloprovincialis* (*Mg*) and *Perna perna* (*Pp*) and on non-enzymatic activity (NEA). Enzyme activity was determined in S9 of whole animal at 30°C for 2 min. The values indicate mean ±SD, (n=6).

The effect of temperature was studied at temperatures between 10°C and 50°C (Figure 2). The weakest activities were observed for temperatures below 20°C and above 35°C. The highest activities were found at 30°C for the two species of mussels. This value is in the range temperature (20°C and 30°C) described for GST activities of other species (Suteau et al. 1988). Because the non-enzymatic activity was low at 30°C, this temperature was chosen as optimum for the following assays.

The relationships between GST activities and pH (6 to 9) of the incubation mixture are shown in Figure 3. In both species, the activity is very sensitive to pH variation. The maximum activity occurred at pH 7.5 with a very low non-enzymatic activity. These results appeared to be similar to those obtained with other marine species (James et al. 1979; Fitzpatrick and Sheehan 1993).

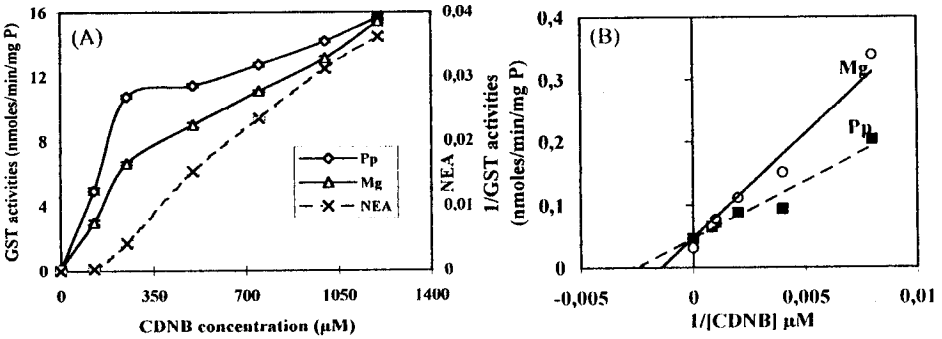

Figure 4. Effect of CDNB concentration in the incubation mixture, on GST activities of *Mytilus galloprovincialis* (*Mg*) and *Perna perna* (*Pp*) and non-enzymatic activity (NEA). (A) Michaelis-Menten representation; (B) Lineweaver-Burke representation. Enzyme activity was determinated in S9 of whole animal at 30°C pH 7.4 for 2 min. The values indicate mean ±SD, (n=6).

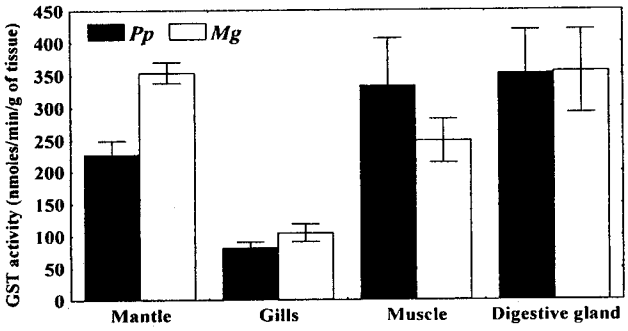

Figure 5. Organ distribution of GST activities in *Mytilus galloprovincialis* (*Mg*) and *Perna perna* (*Pp*). Enzyme activity was determinated in S9 of each organ at 30°C pH 7.4 for 2 min,The values indicate mean ±SD, (n=6).

Figure 4 shows the Michaelis-Menten (A) and Lineweaver-Burke (B) representation of the kinetic of GST activity in *Mytilus galloprovincialis* and *Perna perna*. The maximum activity appeared at 1200 µM CDNB in the incubation mixture, but not obviously because of the limit of this substrate solubility. The linear regression of Lineweaver-Burke representation exhibited a significant correlation coefficient (r=0.98 for the two mussels). The apparent Km value was 702.38 and 408.38 µM and the apparent Vmax was 22.2 and 21.2 nmoles/min/mg P, respectively for *Mytilus galloprovincialis* and *Perna perna*. The different values obtained for Vmax and Km can translate the difference of GST affinity to CDNB in these two bivalves. This type of specific difference was previously described by James et al. (1979).

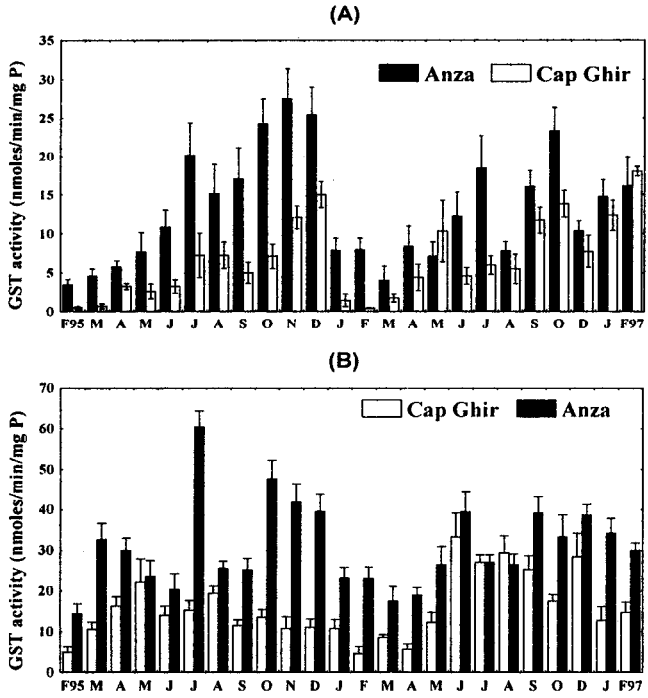

Figure 6. Seasonal variation of GST activities of *Mytilus galloprovincialis* (A) and *Perna perna* (B) sampled in the polluted site (Anza) and reference site (Cap Ghir). Enzyme activity was determinated in S9 of whole animal at 30°C pH 7.4 for 2 min. The values indicate mean ±SD, (n=6).

Results about organ distribution of GST activities in *Mytilus galloprovincialis* and *Perna perna* are shown in Figure 5. In these two species, the highest activity is noticed in the digestive gland followed by the mantle in *Mytilus galloprovincialis* and the muscle in *Perna perna.* The abundance of GST activity in the digestive gland could be explained by the role of this organ in conjugation detoxifying metabolism (James et al. 1979; Fitzpatrick and Sheehan 1993).

Seasonal variations of GST are presented in Figure 6. In the two sites studied the GST activities in *Mytilus galloprovincialis* and *Perna perna*, generally present the same seasonal profile: the highest levels of activity were observed in summer and autumn and the lowest levels in winter and spring. These variations could be linked to the mussels reproduction cycle already described in our laboratory by Id Halla et al. (1997). High levels of GST activity coincide with restoration of reserves and gametogenisis periods and lower values coincide with spawning periods. Seasonal variations of GST activities were also reported in *Mytilus edulis* by Power and Sheehan (1996). Recently in our laboratory, we have shown the existence of a seasonal profile of lipid peroxidation and acetycholinesterase activities in *Mytilus galloprovincialis* and *Perna perna* in the Agadir bay (Moukrim et al. 1997; Najimi et al. 1997). Some authors attribute the seasonal variations of biomarker activities to the seasonal nature of the metabolism

described in mussels (Ahmad et al. 1979) and the complex interactions between exogenous and endogenous factors (Gabbott 1983).

The GST activities were more important, in both species, in the polluted site (Anza) as compared to the reference site (Cap Ghir). This difference is probably linked to the state of pollution in the Anza site which receives industrial and domestic untreated waste water. Data on pollutants nature in the Agadir bay are rare, except preliminary results indicating important levels of heavy metals and PAHs either accumulated by mussels or in sediment in Anza site (Id Halla 1997; Najimi 1997). Similar results coming from field studies were also described in *Mytilus edulis* and *Mytilus galloprovincialis* (Suteau et al. 1988; Fitzpatrick et al. 1997).

Our study constitutes an initial basic information on the functioning of GST in two species of mussels living in the Agadir bay (South of Morocco) and particularly in the African mussel (*Perna perna*), species less studied comparatively to the Mediterranean mussel (*Mytilus galloprovincialis*). GST activities seem to be of particular interest regarding their inducibility by pollution in *Mytilus galloprovincialis* and *Perna perna* in the Agadir bay. However, as their use as biomarkers, one must consider the nature of pollutants in this bay and the seasonal variations as well as the biological cycle in these sentinel organisms. This should permit one to obtain a better appreciation of this biomarker.

Acknowledgements: We thank the Ifs (Sweden), AUPELF-FICU (Canada) and Ministère des Affaires Etrangères (France) for their financial assistance.

REFERENCES

Ahmad TA, Chaplin AE (1979) Seasonal variation in the anaerobic metabolism of the mussel *Mytilus edulis (L.).* Comp Biochem Physiol 64B:351-356.

Beckett GJ, Hayes JD (1993) Glutathione S-transferases: Biomedical applications. Adv Clin Chem 30:281-380

Buhler DR, Williams DE (1988) The role of biotransformation in the toxicity of chemicals. Aqua Toxicol 11:19-28

Fitzpatrick PJ, O'Halloran J, Sheehan D, Walsh AR (1997) Assessment of a glutathione S-transferase and related proteins in the gill and digestive gland of *Mytilus edulis (L.),* as potential organic pollution biomarkers. Biomarkers 2:51-56

Fitzpatrick PJ, Sheehan D (1993) Separation of multiple forms of glutathione S-transferases from the blue mussel, *Mytilus edulis.* Xenobiotics 23:851-861

Gabbott PA (1983) Developmental and seasonal metabolic activity in marine molluscs. In: Hochachka PW (ed) The mollusca: their ecology and physiology, vol 2. Academic Press, London, 165-217.

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249 25:7130-7139

Id Halla M, Bouhaimi A, Zekhnini A, Narbonne JF, Mathieu M, Moukrim A (1997) Etude du cycle de reproduction de deux espèces de moules *Perna perna*

(Linné 1758) et *Mytilus galloprovincialis* (Lamarck 1819) dans la baie d'Agadir (Sud du Maroc). Haliotis 26:51-62

James MO, Bowen ER, Dansette PM, Bend JR (1979) Epoxide hydrase and glutathione S-transferase activities with selected alkene and arene oxides in several marine species. Chem Biol Interact 25:321-344

Livingstone DR (1993) Biotechnology and pollution monitoring: use of molecular biomarkers in the aquatic environment. J Chem Tech Biotechnol 57:195-211

Lowry OH, Rosenbrough NJ, Farr A, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275

Moukrim A, Bouhaimi A, Lagbouri A, Zekhnini A (1997) Health state of Agadir bay ecosystems evaluated by biomarkers of pollution. Proceedings of International Symposium on Environmental Pollution and Impact Assessment, 9-11 October 1996, Mohammedia, Morocco, 325-337

Najimi S, Bouhaimi A, Daubeze M, Zekhnini A, Pellerin J, Narbonne JF, Moukrim A (1997) Use of acetylcholinesterase in *Perna perna* and *Mytilus galloprovincialis* as a biomarker of pollution in Agadir Marine Bay (South of Morocco). Bull Environ Contam Toxicol 58:901-908

Narbonne JF, Ribera D, Michel X, Raoux C, Garrigues P, Monod JL, Lemaire P, Galgani F, Romeo M, Salaun JP, Lafaurie M (1991) Indicateurs biochimiques de contamination de l'environnement marin: Etude comparative en mer Mediterranée. Océanis 17:257-275

Power A, Sheehan D (1996) Seasonal variation in the antioxidant defense systems of gill and digestive gland of the blue mussel, *Mytilus edulis.* Comp Biochem Physiol 114C:99-103

Suteau PM, Daubeze M, Migaud ML, Narbonne JF (1988) PAH-metabolizing enzymes in whole mussels as biochemical tests for chemical pollution monitoring. Mar Ecol Prog Ser 46:45-49