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Antioxidant Enzymes Activity in Subcellular Fraction of Freshwater Prawns *M. malcolmsonii* and *M. lamarrei lamarrei*

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ABSTRACT

Subcellular distribution of superoxide dismutase (SOD), catalase (CAT), selenium (SC) dependent glutathione peroxidase, and Se-independent glutathione peroxidase (GSH-Px) activities were detected in different tissues (hepatopancreas, muscle, and gill) of freshwater prawns *Macrobrachium malcolmsonii* and *Macrobrachium lamarrei lamarrei.* CAT and SOD were found almost equally between the mitochondrial and cytosolic fraction. Both Se-dependent and Se-independent GSH-Px activities were mainly found in cytosolic fraction.

Index Entries: Catalase; cytosol; glutathione peroxidase; microsomes; mitochondrial; *Macrobrachium malcolmsonii; Macrobrachium lamarrei lamarrei;* superoxide dismutase.

INTRODUCTION

Increasing attention is being given to antioxidant enzymes as biomarkers in monitoring the environmental pollution *(1).* Stepwise partial reduction of molecular oxygen and secondary reaction with protons, transition metals, or hydrated electrons and free radicals lead to the formation of potentially deleterious reactive oxygen species, called oxyradicals *(2).* Production of oxyradicals can be increased by intake of natural and pollutant pro-oxidant xenobiotics and by changing oxygen tension *(3,4).* These oxyradicals have been implicated in lipid peroxidation, nucleic acid dam-

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age, protein degradation *(5)*, and oxidative tissue damage *(6)*. Self-scavenging of these oxyradicals is carried out by specially adapted enzymes known as antioxidant enzymes. They include superoxide dismutase (SOD; EC 1.15.1.1), which reduces O_2^- via $2O_2^- + 2H^+ \rightleftarrows H_2O_2 + O_2$ and catalase (CAT; EC 1.11.1.6), which reduces H_2O_2 via $2H_2O_2 \rightleftarrows 2H_2O + O_2$ and glutathione peroxidase (GSH-Px, EC 1.11.1.9), which reduces H_2O_2 via 2GSH $\rightleftarrows$ GSSG (oxidized glutathione) + $2H_2O$.

Induction of these detoxifying enzymes in response to oxyradicals appears to be a general phenomenon *(7)* in invertebrates and vertebrates *(8)*. The protective antioxidant enzymes have been detected in *Mytilus edulis (9)*. In crustacea no quantitative data are available *(10)*. Since the information on the tissue and subcellular distribution of antioxidant enzyme activities in crustacea is sparse, to fill up the gap, a preliminary study has been carried out on the freshwater prawns *Macrobrachium malcolmsonii* and *Macrobrachium lamarrei lamarrei.*

MATERIALS AND METHODS

Samples

The freshwater prawns *M. malcolmsonii* and *M. lamarrei lamarrei* were collected from river Cauvery (Tiruchirappalli, India) and were transported in aerated polythene bags to the laboratory. The prawns were acclimated for 5 d before sacrifice of the animals for enzyme assay. The prawns were starved 1 d prior to sacrifice of the animals to have empty gut.

Preparation of Subcellular Fractions

Subcellular fractions were prepared from fresh tissues by the procedure of Livingstone and Farrar *(11)*. All preparation procedures were carried out at 4°C. Tissues of the prawns were put into ice-cold 0.25 *M* sucrose (1 g/4 mL), thawed, and then homogenized in a Potter-Elvehjem-type glass-Teflon homogenizer. The homogenates were centrifuged at 10,000*g* for 20 min to obtain mitochondrial fractions, 105,000 *g* for 60 min at 4°C to obtain microsomal fractons (pellet), and for the rest of the microsomal samples, the cytosol (supernatant) was stored at −80°C for further analysis.

Biochemical Analysis

CAT activity was assayed by the method of Caliborne *(12)*. The reaction mixture contained 50 m*M* of phosphate buffer, pH 7.0, and 50 m*M* H_2O_2. The rate of reaction was measured at 240 nm. One unit of CAT was defined as 1 μmol of H_2O_2 degraded/min/mg protein.

SOD was measured using the Sun and Zigman *(13)* method. The inhibitory effect of SOD on the auto-oxidation of epinephrine (0.1 m*M*) in

50 m*M* sodium carbonate buffer, pH 10.0, was analyzed spectrophotometrically at 340 nm. One unit of SOD was defined as the amount of the enzyme inhibiting the oxidation of epinephrine by 50%.

GSH-Px was measured by the method as described by Lawrence and Burk *(14)*. The reaction mixture contained 100 m*M* phosphate buffer pH 7.5, 2 m*M* GSH, 1 m*M* sodium azide, 1 U GSH reductase, 0.12 m*M* NADPH, 2 m*M* H_2O_2, and 6 m*M* cumene hydroperoxide. The rate of reaction was measured by the decrease in NADPH at 340 nm, using H_2O_2 (selenium [Se] dependent activity) and cumene hydroperoxide (sum of Se-dependent and Se-independent activities) as substrate. One unit was defined as 1 μmol of GSH conjugated/min/mg protein.

Protein concentration was determined by the method of Lowry et al. *(15)*.

RESULTS AND DISCUSSION

The specific activities of the antioxidant enzymes in the subcellular fractions (mitochondrial, cytosol, and microsomal) of freshwater prawns *M. malcolmsonii* and *M. lamarrei lamarrei* are listed in Table 1. The SOD, CAT, and GSH-Px activities were found to be positive in all tissues (hepatopancreas, muscle, and gill) of freshwater prawns. Higher enzyme activities were exhibited by *M. malcolmsonii* than *M. lamarrei lamarrei*. It might be owing to the habitate, i.e., *M. malcolmsonii* prefer and live in a lotic water system where the availability of oxygen is greater. It would ultimately be reflected in higher enzyme production, and the larger size would also be a factor for the high level of enzymes recorded in *M. malcolmsonii* as reported by Arun and Subramanian *(16)*. In both *M. malcolmsonii* and *M. lamarrei lamarrei*, the highest activities of enzyme were observed in hepatopancreas followed by muscle and gill. The hepatopancreas is the major site for xenobiotic uptake and oxyradical-generating biotransformation enzymes *(17)*. Oxyradicals are produced variously in all subcellular compartments of the cell *(2)*. Subcellular sites for oxyradical generation have been identified in the digestive gland of the slug *Arion arter (18)*. Scavengers of these oxyradicals are SOD, CAT, and peroxidases, which are generally regarded to have complementary functions and complementary localization, i.e., SOD in mitochondria and cytosol eliminates O_2^-. CAT in peroxisomes scavenges H_2O_2, and GSH-Px mainly in cytosol removes H_2O_2 and other hydroperoxides *(19)*. SOD and CAT activities were measured almost equally between mitochondrial and cytosolic fractions. In microsomal fractions, a significant amount of SOD and CAT activities was measured almost in all tissues of *M. malcolmsonii* and *M. lamarrei lamarrei*. This could also have been owing to contamination from submitochondrial particels. Livingstone and Farrar *(11)* also reported the similar

Table 1
The Specific Activity of SOD, CAT, and GSH-Px (Se Dependent and Se Independent) in Subcellular Fractions of *M. malcolmsonii* and *M. lamarrei lamarrei*[a]

	Mitochondrial	Cytosol	Microsomes
Protein[b]			
Hepatopancreas	78.25 ± 3.19[g]	17.28 ± 2.51	7.35 ± 1.74
	58.83 ± 4.43[h]	13.42 ± 2.95	6.45 ± 1.12
Muscle	48.42 ± 3.58[g]	8.64 ± 1.27	3.12 ± 1.25
	32.38 ± 3.49[h]	6.81 ± 1.34	2.85 ± 0.82
Gill	39.46 ± 2.87[g]	4.37 ± 0.89	4.75 ± 1.24
	28.58 ± 3.69[h]	3.85 ± 0.67	3.83 ± 0.37
SOD[c]			
Hepatopancreas	34.13 ± 1.71[g]	31.13 ± 0.86	16.43 ± 0.54
	27.32 ± 0.59[h]	24.83 ± 0.83	14.02 ± 0.50
Muscle	21.43 ± 0.76[g]	16.80 ± 0.81	7.23 ± 0.32
	15.36 ± 0.90[h]	12.73 ± 0.73	5.48 ± 0.36
Gill	28.32 ± 0.96[g]	20.72 ± 0.94	9.83 ± 0.64
	22.22 ± 0.60[h]	18.60 ± 0.84	7.43 ± 0.42
CAT[d]			
Hepatopancreas	56.32 ± 0.84[g]	49.29 ± 0.96	43.08 ± 0.95
	49.08 ± 0.76[h]	46.66 ± 0.94	40.12 ± 0.75
Muscle	44.83 ± 0.68[g]	38.28 ± 0.56	31.43 ± 0.55
	33.69 ± 0.91[h]	30.14 ± 0.64	27.12 ± 0.82
Gill	32.04 ± 0.43[g]	27.04 ± 0.75	24.38 ± 0.98
	26.31 ± 0.51[h]	23.91 ± 0.65	19.48 ± 0.86
SE- Independent GSH-Px[e]			
Hepatopancreas	3.02 ± 0.27[g]	9.37 ± 0.30	5.83 ± 0.62
	2.81 ± 0.62[h]	7.58 ± 0.54	3.46 ± 0.08
Muscle	2.48 ± 0.38[g]	6.58 ± 0.18	3.81 ± 0.72
	1.08 ± 0.16[h]	4.89 ± 0.13	2.63 ± 0.62
Gill	2.08 ± 0.09[g]	5.65 ± 0.43	3.21 ± 0.48
	0.98 ± 0.82[h]	3.28 ± 0.08	1.50 ± 0.66

(continued)

Table 1 *(continued)*

	Mitochondrial	Cytosol	Microsomes
SE- Dependent GSH-Px[f]			
Hepatopancreas	0.81 ± 0.035[g]	1.48 ± 0.066	1.43 ± 0.074
	0.64 ± 0.027[h]	1.21 ± 0.033	0.87 ± 0.038
Muscle	0.30 ± 0.048[g]	0.56 ± 0.026	0.48 ± 0.066
	0.17 ± 0.024[h]	0.31 ± 0.038	0.29 ± 0.042
Gill	0.26 ± 0.068[g]	0.38 ± 0.021	0.31 ± 0.059
	0.13 ± 0.051[h]	0.27 ± 0.042	0.24 ± 0.048

[a] Each value is mean ± SEM of 5 determination.
[b] mg protein/g tissue.
[c] U/min/mg protein.
[d] μmol/min/mg protein.
[e] nmoles/min/mg protein.
[f] μmol/min/mg protein.
[g] *M. malcolmsonii.*
[h] *M. lamarrei lamarrei.*

response in the common mussel *Mytilus edulis.* In general, O_2^- is predominantly found in lipophilic environments especially in the membranes. To combate this radicals, the concentration of the SOD is high in the cytosolic fraction of *M. malcolmsonii* and *M. lamarrei lamarrei.* The activity of SOD was high in gills when compared to muscles, which indicates the greater potential of O_2^- production in gills during respiration.

Se-dependent and Se-independent GSH-Px activities were measured in different tissues of *M. malcolmsonii* and *M. lamarrei lamarrei* with their subcellular fractions. Both Se-dependent and independent GSH-Px predominantly occurred in cytosol with a significant amount present in microsome. Livingstone et al. *(9)* also observed the same results in common mussel *M. edulis.* The GSH-Px activities were found to be low in the mitochondrial fraction when compared to microsomes. According to Ursini and Bindoli *(20),* GSH-Px occurs mainly in the cytosol, although specific forms exist in the mitochondria, nuclei, and microsomes. Furthermore, it is responsible for the decomposition of H_2O_2 in the cytoplasm and endoplasmic reticulum of the cell *(7,21).* Unlike catalase, very low levels of both Se-GSH-Px and Se-independent GSH-Px activities were detected in the tissues of *M. malcolmsonii* and *M. lamarrei lamarrei.* This would confirm the primacy of CAT over GSH-Px in prawn tissues. GSH-Px is considered to play a main role in the removal of H_2O_2 in mamalian

tissues *(21,22)*, and CAT catalase is thought to be more important in invertebrates *(23)*. The specific activity of GSH-Px was much lower in invertebrates than in vertebrates *(9)*. Thus, determination of these detoxifying enzymes in the subcellular fraction is prone to gathering the knowledge of pro-oxidant and antioxidant events occurring in the cell.

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