**Journal Title:** **Journal of Marine Biotechnology**

**Volume:** 4 **Issue:** 3
**Month/Year:** 1996
**Pages:** 150-154

**Article Author:** Ge J, Singh BR

**Article Title:** Physicochemical characterization of glutathione S-transferase purified from oyster, Crassostrea
virginica

**ISSN:** 0941-2905

**English only!**

**Notes/Alternate Delivery:**

☐ Paged to SZ ☐ Paged to HS ☐ Emailed Loc

J Mar Biotechnol (1996) 4:150–154

Journal of Marine Biotechnology



# Physicochemical characterization of glutathione S-transferase purified from oyster, *Crassostrea virginica*

**Jie Ge and Bal Ram Singh**

Department of Chemistry, University of Massachusetts Dartmouth, 285 Old Westport Road, North Dartmouth, Massachusetts 02747, USA

Received: 14 August 1995/Accepted: 2 November 1995

**Abstract.** Glutathione S-transferase (GST) was purified from oyster by ammonium sulfate precipitation and affinity chromatography. The purified enzyme had a specific activity of 4.9 μmol/min/mg protein with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Kinetic analysis revealed the $K_m$ and $V_{max}$ values of the purified GST to be 0.75 mM and 6.9 μmol/min/mg, respectively. The inhibition pattern of oyster GST by tetrapyrroles was investigated both in crude extract and in purified forms. Inhibition studies of rat liver GST by tetrapyrroles was also carried out to reveal the differences between oyster and mammalian enzymes. Results indicate similar patterns of oyster and mammalian GST inhibition by tetrapyrroles both qualitatively and quantitatively, implying their similarity in structure and function. SDS-PAGE analysis suggested two major subunits with MWs of 27 and 30 kDa. IEF analysis indicated the existence of two major π class isoenzymes with pI of 4.7–5.1.

Glutathione S-transferases (GSTs, EC 2.5.1.18), also called ligandins, are a family of multifunctional enzymes found in almost all living organisms examined. GST catalyzes the conjugation of glutathione (GSH, γ-glutamylcysteinylglycine) to electrophilic compounds (e.g., benzene epoxide, aflatoxin $B_1$-8,9-oxide, and benzopyrene-7,8-dihydrodiol-9, 10-oxide) (Goldstein and Faletto 1993) so that they become more soluble and more easily excreted. Thus, this enzyme plays an important role in the detoxification and removal of xenobiotics.

Most glutathione S-transferases in animals exist in dimeric forms with a wide range of isoelectric points (i.e., pI 4.7–9.8). Based on these differences in pI, glutathione S-transferases are conveniently divided into three major classes (Mannervik et al. 1985): π (acidic), α (basic), and μ (near neutral). Each of these classes represents a separate multigene family. Typical subunit molecular weights are heterogeneous and range from 23,000 (π) to 25,000 (α) to 26,700 (μ) (Singh et al. 1986; Taniguchi and Pyrein 1989; Johnson et al. 1990). Additionally, many isoforms have been reported to exist within each class. Recently, another new, θ, class of glutathione S-transferase was identified, with a unique N-terminal amino acid sequence (Meyer et al. 1991).

The level of GSTs in animals has been shown to increase when exposed to environmental pollution (Boryslawskyj 1988; Lee 1988; Suteau 1988). For example, GST activity was elevated drastically in mussels and crabs from polluted sites. It is also reported that GST synthesis in primary cultured rat liver cells was induced upon exposure to the potent carcinogens, polychlorinated biphenyl (PCB) congeners (Aoki et al. 1992), which are known to be the major environmental problem in the New Bedford, MA, USA, harbor and its surrounding areas.

Estimation of the enzymatic level of glutathione S-transferase in marine organisms can be carried out to monitor the effect of environmental pollution (Vande Waa et al. 1993; Orser et al. 1993; Poirier and Singh 1994), leading to the use of such a detection system in the local marine industry. As a first step, we studied the enzyme activity, kinetics, and inhibition characteristics of glutathione S-transferase from oyster, a typical marine animal from the New Bedford local area.

## Materials and methods

### *Crude extract preparation*

Freshly shucked oysters were purchased from Fairhaven Chowder House, Fairhaven, MA, USA. Crude extract of oyster was prepared according to Singh and Shaw (1988). The oyster tissues (454 g) were combined with 60 ml grinding buffer (100 mM sodium phosphate, 300 mM sucrose, 1 mM EDTA, pH 6.5) and 800 μl 7.1 mg/ml phenylmethylsulfonate (PMSF), then blended in a cold (4°C) Waring blender at low speed for 30 s. The resulting extract was centrifuged at 10,000 rpm for 20 min in a SS-34 rotor at 4°C using a Sorvall RC-5B centrifuge machine. The supernatant was collected and was further spun for 5 min in an Eppendorf centrifuge to remove any small particles. The supernatant was finally diluted 10-fold

*Correspondence to:* B.R. Singh. Fax: 508-999-8451.

Table 1. Purification of glutathione S-transferases from oyster.[a]

| | Volume (ml) | Total protein (mg) | Total activity (μmol/min) | Specific activity (μmol/min/mg) | Yield (%) | Purification (-fold) |
|---|---|---|---|---|---|---|
| Crude extract | 320 | $4.39 \times 10^3$ | 109 | 0.0247 | | 1 |
| Recovered protein[b] | 460 | $4.50 \times 10^3$ | 165 | 0.0366 | 100 | 1.5 |
| Purified GST | 22 | 7.06 | 29.6 | 4.20 | 18 | 170 |

[a] Amount of starting tissue was 454 g (1 lb).
[b] Redissolved protein from ammonium sulfate precipitation.

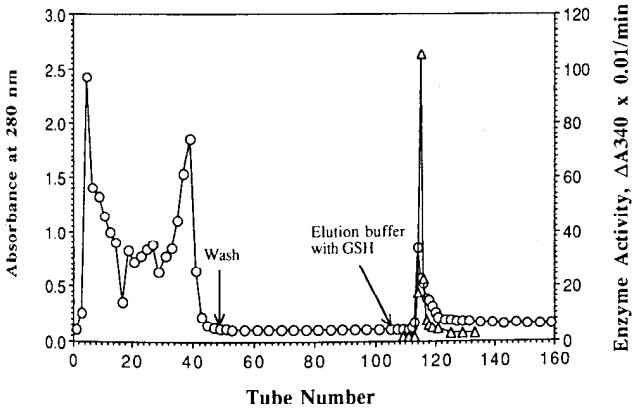


Fig. 1. A typical elution profile of glutathione affinity chromatography in terms of GST activity as indicated by a change in absorbance at 340 nm and protein content as indicated by absorbance at 280 nm. Arrows indicate application of the sample wash buffer and elution buffer. Each fraction collected contained 25 drops, ~1 ml.

with the assay buffer (22 mM sodium phosphate, pH 7.0) before the enzyme assay for GST activity.

## *Purification*

The crude extract was treated with 39.1 g ammonium sulfate salt/100 ml supernatant (final concentration 2.96 M). The protein precipitate was separated by centrifuging the mixture at 12,000 rpm for 20 min at 4°C using the SS-34 rotor. The supernatant was discarded, and the pale yellow precipitate was redissolved in the assay buffer. This solution was then centrifuged again at 12,000 rpm, 4°C, for 20 min to remove any particulate forms.

The redissolved protein sample was applied to a glutathione–agarose (Sigma Chemical Co., St. Louis, MO, USA) column (Simons and Vander Jagt 1977, 1981) equilibrated with the assay buffer, washed with four bed volumes of the assay buffer, and glutathione S-transferase was eluted with 0.05 M Tris buffer (pH 9.6) containing 5 mM glutathione. The fractions containing purified GST were then dialyzed against assay buffer prior to the enzyme activity assay. All procedures described above were carried out at 4°C.

## *Protein assay*

The total protein concentration of the crude extract, redissolved protein from ammonium sulfate precipitation, and purified GST were determined by the method of Bradford (1976), with the protein assay kit purchased from Bio-Rad (Hercules, CA, USA). Bovine serum albumin (BSA) served as standard.

## *SDS-PAGE*

The molecular weight of the purified GST was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 8–25% gradient and 12.5% homogeneous gels on the PhastSystem (Pharmacia). Low-range molecular-weight standards (Bio-Rad) used were lysozyme (14.4 kDa), trypsin inhibitor (21.5 kDa), carbonic anhydrase (31.0 kDa), ovalbumin (45.0 kDa), serum albumin (66.2 kDa), and phosphorylase b (97.4 kDa).

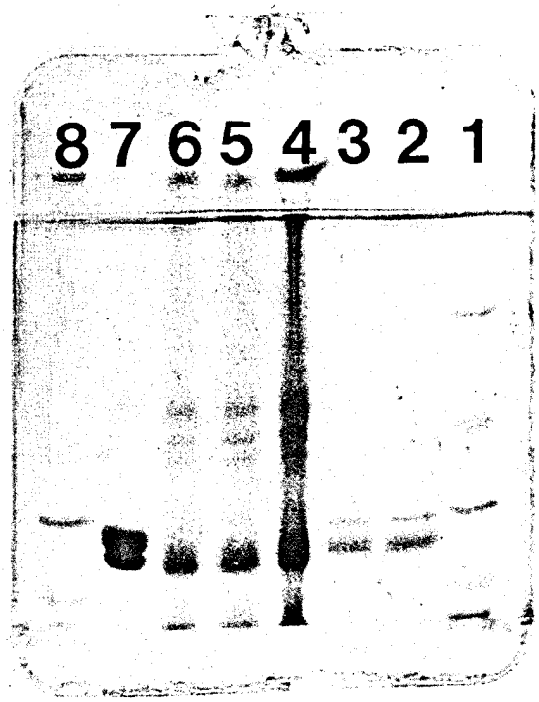


Fig. 2. SDS-PAGE homogeneous 12.5% polyacrylamide gel of the purified oyster GST with low-range molecular-weight standards (Bio-Rad). Lanes 1 and 8 show low-range molecular-weight standards: lysozyme (14.4 kDa), trypsin inhibitor (21.5 kDa), carbonic anhydrase (31.0 kDa), ovalbumin (45.0 kDa), serum albumin (66.2 kDa), and phosphorylase b (97.4 kDa). Lanes 2 and 3 are purified oyster GST. Lane 4 shows crude oyster extract. Lane 5 shows redissolved proteins from ammonium sulfate precipitation. Lane 6 shows washout portions from the GSH affinity column. Lane 7 shows rat liver GST purchased from Sigma.

## *Isoelectric focusing*

An isoelectric focusing (IEF) gel (pH 3–9) was run for the purified enzyme on the PhastSystem (Pharmacia) at 15°C. Broad pI calibration kits (Pharmacia) used were amyloglucosidase (pI 3.50), soybean trypsin inhibitor (pI 4.55), β-lactoglobulin A (pI 5.20), bovine carbonic anhydrase B (pI 5.85), human carbonic anhydrase B (pI 6.55), horse myoglobin-acidic band (pI 6.85), horse myoglobin-basic band (pI 7.35), lentil lectin-acidic band (pI 8.15), lentil lectin-middle band (pI 8.45), lentil lectin-basic band (pI 8.65), and trypsinogen (pI 9.30).


**Fig. 3.** IEF 3-9 gel of the purified oyster GST with broad pI calibration kit (Pharmacia). Lane 2 shows the purified oyster GST sample. Lanes 1 and 3 are standards containing amyloglucosidase (pI 3.50), soybean trypsin inhibitor (pI 4.55), β-lactoglobulin A (pI 5.20), bovine carbonic anhydrase B (pI 5.85), human carbonic anhydrase B (pI 6.55), horse myglobin-acidic band (pI 6.85), horse myoglobin-basic band (pI 7.35), lentil lectin-acidic band (pI 8.15), lentil lectin-middle band (pI 8.45), lentil lectin-basic band (pI 8.65), and trypsinogen (pI 9.30).

### *Enzyme assay and tetrapyrroles inhibition*

The enzyme activity was measured by adding 50 μl of 10-fold diluted crude extract, 50 μl of 20 mM 1-chloro-3,5-dinitrobenzene (CDNB), and 50 μl of 20 mM reduced glutathione into a 1-ml reaction mixture and by monitoring the absorbance change at 340 nm (Singh and Shaw 1988). In order to determine the inhibition of GST activity by tetrapyrroles, 50 μl of 0.1 mg/ml solution of each tetrapyrrole (bilirubin, biliverdin, chlorophyllin, and hemin) was added to the reaction mixture before the addition of reduced GSH.

CDNB, GSH, and the four tetrapyrroles were purchased from Sigma Chemical Co. All the solutions were freshly prepared immediately before the enzyme activity assay.

### *Enzyme kinetics*

The initial rate kinetics of oyster GST with respect to glutathione was studied by measuring the initial reaction rate of the conjugation reaction while varying the GSH concentration and keeping CDNB concentration constant. A control experiment was run using the same concentration of CDNB and GSH in the absence of GST, and the initial rate in the presence of GST was corrected for the control. A Lineweaver-Burk plot was generated by plotting $1/V$ vs. 1/[GSH], then $K_m$ and $V_{max}$ values were determined from the slope ($K_m/V_{max}$) and $y$-intercept ($1/V_{max}$) of the plot.

## Results and Discussion

Each gram of wet oyster tissue contains 9.7 mg total protein showing a specific activity of 0.025 μmol/min/mg. The GST

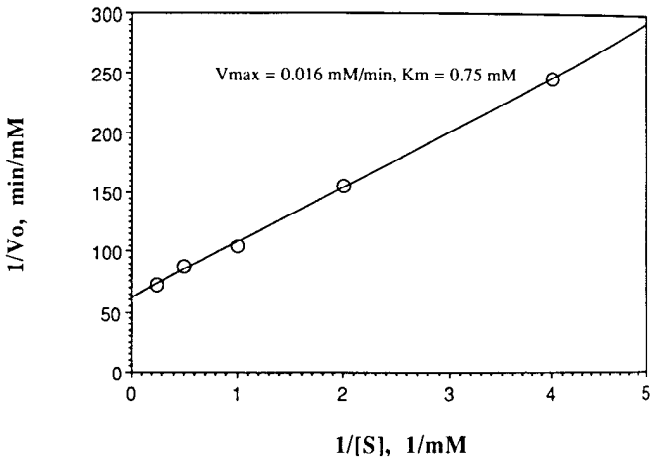


**Fig. 4.** Lineweaver-Burk plot of $1/v$ vs. 1/[GSH] for purified oyster GST. Initial reaction rates ($V_0$) were determined from the slope of $\Delta A_{340}$ vs. time plots at different concentrations of GSH. Experiments were carried out at room temperature (25°C).

was first purified 1.5-fold by ammonium sulfate precipitation and then 170-fold by affinity chromatography with total activity recovery of 18%. A typical protocol for purification of oyster GST is presented in Table 1.

Since oyster GST possesses high affinity with the GSH-agarose column, it can be purified from the redissolved protein in a single chromatographic step. The elution profile of the enzyme on the affinity column is presented in Fig. 1. Unabsorbed bulk proteins were removed in the washing step. The bound GST was eluted with the Tris buffer at pH 9.6 containing 5 mM glutathione, and activity appeared as a single sharp peak. However, there was a significant amount of GST activity recovered in the unabsorbed proteins. We are currently investigating this observation. Kinetic and inhibition studies presented in this article mostly deal with the enzyme preparation that binds preferably to the affinity column.

The purified enzyme showed a specific activity of 4.0 μmol/min/mg protein towards CDNB (1 mM CDNB and 1 mM GSH in 1 ml reaction mixture). This activity is low compared to glutathione transferases from other marine animals: 142 μmol/min/mg protein for octopus hepatopancreatic GST (Tang et al. 1994); 140 μmol/min/mg for GST isoenzyme I, 35.3 μmol/min/mg for GST isoenzyme II from shrimp eye (Lin and Chuang 1993); 820 μmol/min/mg for GST from squid (Tomarev et al. 1993); and 222 μmol/min/mg for GST isoenzyme I and 182 μmol/min/mg for GST isoenzyme II from blue crab hepatopancreas (Keeran and Lee 1987). Specific activity of rat liver GST (Sigma Chemical Co.) was determined to be 17.1 μmol/min/mg protein.

Analysis of the purified oyster GST on SDS-PAGE (Fig. 2) suggested two major subunits with molecular weights of 27 and 30 kDa, which are consistent with molecular weights for other glutathione S-transferases (22–30 kDa) (Aoki 1992; Orser et al. 1993; Lin and Chuang 1993; Tomarev et al. 1993).

Isoelectric focusing gel (Fig. 3) showed two major bands and a minor band with pI ranging between 4.7 and 5.1. All of these isoenzymes are acidic and should be classified into the π class. These isoforms probably arose from posttransla-

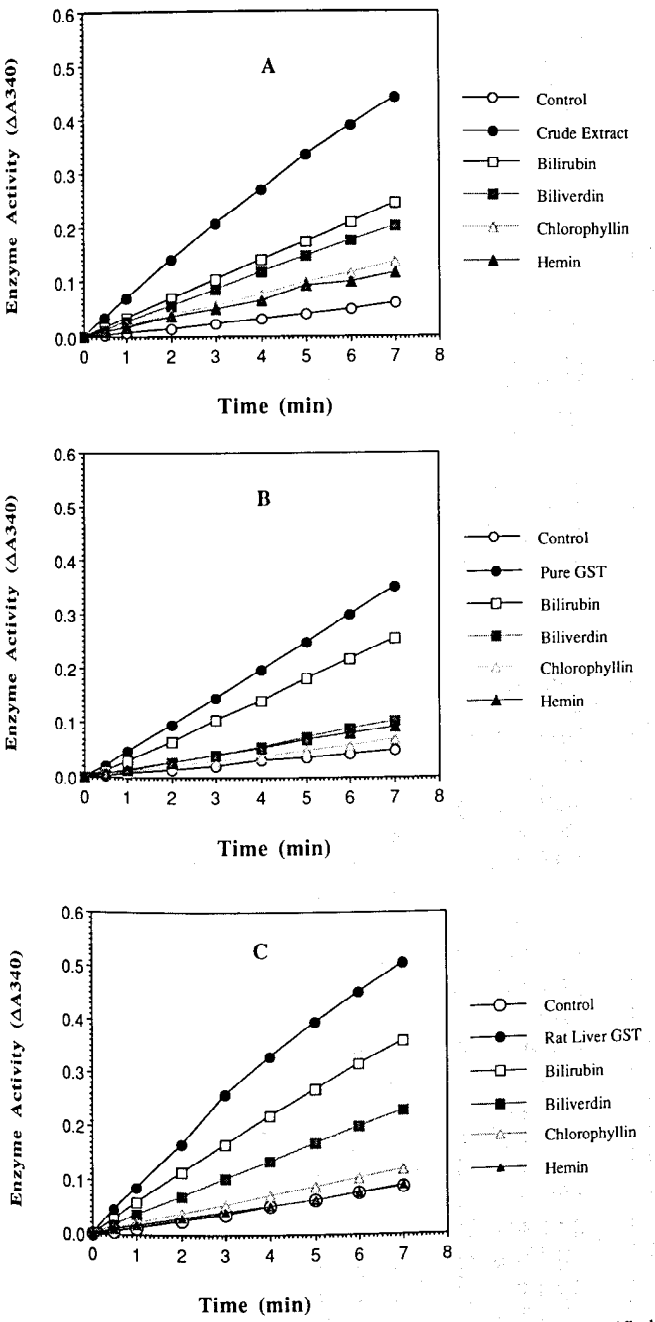


**Fig. 5.** Inhibition of enzyme activity of (**A**) oyster crude extract, (**B**) purified oyster GST, and (**C**) rat liver GST by tetrapyrroles. For the purified oyster and rat liver GSTs, the final inhibitor to enzyme ratio was 21 (w/w). All the experiments were carried out at room temperature (25°C).

tional modifications such as phosphorylation (Taniguchi and Pyrein 1989).

Kinetic studies (Fig. 4) indicated a $K_m$ value of 0.75 mM and a $V_{max}$ value of 6.9 μmol/min/mg for the purified oyster GST. From the literature, a $K_m$ value for GST isoenzyme I from shrimp eye (Lin and Chuang 1993) is reported to be 0.19 mM, and that for GST isoenzyme II is 0.17 mM. Hepatopancreatic GST isoenzyme I from blue crab (Keeran and Lee 1987) has a $K_m$ of 0.40 mM, GST isoenzyme II has a $K_m$ of 0.43 mM, all of which are lower than but comparable to the $K_m$ value for oyster GST. However, the $V_{max}$ value of oyster GST (6.8 μmol/min/mg) is lower than that of GSTs from shrimp eye (176 μmol/min/mg for isoenzyme I, 41.2 μmol/min/mg for isoenzyme II) and from blue crab hepatopancreas (243 μmol/min/mg for isoenzyme I, 208 μmol/min/mg for isoenzyme II). The turnover number $k_{cat}$ for oyster GST is 186 $min^{-1}$.

**Table 2.** Comparison of inhibition patterns.

| Condition | Relative ratio |
|---|---|
| Crude extract | 1.00 |
| +bilirubin | 0.90 |
| +biliverdin | 0.29 |
| +chlorophyllin | 0.20 |
| +hemin | 0.17 |
| Purified oyster GST | 1.00 |
| +bilirubin | 0.69 |
| +biliverdin | 0.18 |
| +chlorophyllin | 0.06 |
| +hemin | 0.14 |
| Rat liver GST | 1.00 |
| +bilirubin | 0.58 |
| +biliverdin | 0.30 |
| +chlorophyllin | 0.06 |
| +hemin | 0.00 |

Inhibition of enzyme activity of oyster crude extract, purified GST, and rat liver GST by four tetrapyrroles was performed. Inhibition patterns were compared in order to understand the structural and functional properties of this ubiquitous family of enzymes. From Table 2, inhibition patterns for the three GST preparations appear similar qualitatively, being inhibited by closed-chain tetrapyrroles (chlorophyllin and hemin) more effectively than by open-chain tetrapyrroles (bilirubin and biliverdin). This indicated a similarity in structure between oyster GST and rat liver GST and that they have binding site(s) for both open-chain and closed-chain tetrapyrroles, with a preference for the latter.

However, the ratios of inhibition are quantitatively different for oyster and mammalian GSTs. While GST from oyster is inhibited 31% by bilirubin, 82% by biliverdin, and 86% by hemin, the enzyme from rat liver shows inhibition of 42% by bilirubin, 70% by biliverdin, and 100% by hemin. The fact that oyster GST is more inhibited by biliverdin and less by bilirubin and hemin compared to rat liver GST may be related to the distinct biological distribution of tetrapyrrole compounds in those two organisms. In oat plants, GST is known to be inhibited by chlorophyllin and hemin but not significantly by bilirubin and biliverdin (Singh and Shaw 1988), perhaps reflecting the physiological presence of chlorophyll and heme in the plant cell. Differential binding and inhibition patterns of GSTs by tetrapyrroles have been used in the past to distinguish GSTs from different mammalian tissues (Caccuri et al. 1990; Vander Jagt et al. 1985).

In summary, it is found that glutathione S-transferase exists in oysters and has similar structural characteristics to mammalian GST. This provides the basis for future studies, including detailed structural investigation, feasibility of some environmental pollutants as substrates of this enzyme, and correlation between GST level and marine organisms health conditions.

*Acknowledgments.* This research project was supported by the 1994 James Flack Norris Summer Scholarship from the Northeastern Section of the American Chemical Society, and by the Center for Marine Science and Technology, UMass Dartmouth.

## References


Aoki Y, Satoh K, Sato K, Suzuki KT (1992) Induction of glutathione s-transferase P-form in primary cultured rat liver parenchymal cells by co-planar polychlorinated biphenyl congeners. Biochim J 281:539–543

Boryslawskyj M (1988) Elevation of glutathione-S-transferase activity as a stress response to organochloride compounds, in the freshwater mussel, *Sphaerium corneum*. Mar Environ Res 24:101–104

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Caccuri AM, Aceto A, Piemonte F, Dillio C, Rosato N, Federici G (1990) Interaction of hemin with placental glutathione transferase. Eur J Biochem 189:493–497

Goldstein JA, Faletto MB (1993) Advances in mechanisms of activation and deactivation of environmental chemicals. Environ Health Persp 100:169–176

Johnson JA, Neal TL, Collins JH, Siegel FL (1990) Characterization of methylation of rat liver cytosolic glutathione S-transferase by using reverse phase high performance liquid chromatography and chromatofocusing. Biochem J 270:483–489

Keeran WS, Lee RF (1987) The purification and characterization of glutathione S-transferase from hepatopancreas of the blue crab, *Caallinectes sapidus*. Arch Biochem Biophys 255(2):233–243

Lee RF (1988) Glutathione S-transferase in marine invertebrates from Langesundfjord. Mar Ecol 46:33–36

Lin D-S, Chuang N-N (1993) Anionic glutathione S-transferase in shrimp eyes. Comp Biochem Physiol 105B(1):151–156

Mannervik B, Ålin P, Guthenberg C, Jensson H, Tahir MK, Warholm M, Jörnvall H (1985) Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. Proc Natl Acad Sci USA 82:7202–7206

Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B (1991) Theta, a new class of glutathione transferase purified from rat and man. Biochem J 274:409–414

Orser CS, Dutton J, Lange C, Jabionski P, Xun L, Hargis M (1993) Characterization of a flavobacterium glutathione S-transferase from human liver: J Bacteriol 175(9):2640–2644

Poirier MA, Singh BR (1994) Selective inhibition of oat glutathione-S-transferase by tetrapyrroles. The Nucleus 72(7):14–17

Simons PC, Vander Jagt DL (1977) Purification of glutathione S-transferases from human liver by glutathione-affinity chromatography. Anal Biochem 82:334–341

Simons PC, Vander Jagt DL (1981) Purification of glutathione S-transferases from human liver by glutathione-affinity chromatography. Methods Enzymol 77:235–237

Singh BR, Shaw RW (1988) Enzymatic activity of glutathione-S-transferase in non-aqueous solvents. FEBS Lett 234(2):379–382

Singh SV, Ansari GAS, Awasthi YC (1986) Anion-exchange HPLC of glutathione s-transferases. Separation of the minor isoenzymes of human erythrocyte, heart and lung. J Chromatogr 361:337–345

Suteau P (1988) PAH-metabolizing enzymes in whole mussels as biochemical tests for chemical pollution monitoring. Mar Ecol 46:45–49

Tang S-S, Lin C-C, Chang G-G (1994) Isolation and characterization of octopus Hepatopancreatic glutathione S-transferase. Comparison of digestive gland enzyme with lens S-crystallin. J Protein Chem 13(7): 609–618

Taniguchi H, Pyrein W (1989) Glutathione S-transferase is an in vitro substrate of $Ca^{++}$-phospholipid-dependent protein kinase (protein kinase C). Biochem Biophys Res Commun 162:903–907

Tomarev SI, Zinovieva RD, Guo K, Piatigorsky J (1993) Squid glutathione S-transferase. J Biol Chem 268(6):4534–4542

Vender Jagt DL, Hunasker LA, Garcia KB, Royer RE (1985) Isolation and characterization of the multiple glutathione S-transferase from human liver. J Biol Chem 260:11603–11610

Vande Waa EA, Campbell CK, O'Leary KA, Tracy JW (1993) Induction of *Schistosoma mansoni* glutathione S-transferase by xenobiotics. Arch Biochem Biophys 303(1):15–21

# Journal of Marine Biotechnology


Volume 4, Number 3 1996

Springer

S24 JMBOEW 4 (3) 127-184 ISSN 0941-2905
PRINTED ON ACID-FREE PAPER