Differential Induction of Glutathione S-Transferases in the Clam *Ruditapes Decussatus* Exposed to Organic Compounds

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(Rceived 19 December 1999; Accepted 5 July 2000)

Abstract—Studies of glutathione S-transferase (GST) induction were performed in the Mediterranean clam *Ruditapes decussatus* after controlled exposure to organics in holding tanks. Clams were treated with phenobarbital (PB), benzo[a]pyrene (BaP), and 2,2'-bis-(p-chlorophenyl)-1,1-dichlorethylene (p,p'-DDE). Three different substrates, i.e., 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid (ETHA), and paranitrobenzenes chloride (PNBC), were used to determine GST activities in order to distinguish the isoenzymes induced by contamination. The isoforms conjugating ETHA were significantly induced by treatment with PB and BaP, whereas exposure to p,p'-DDE induced isoforms conjugating CDNB and ETHA. An antibody against affinity-purified GSTs from *R. decussatus* was prepared by injection into rabbit. The serum containing the antibody gave a positive reaction with both the purified GSTs from *R. decussatus* and the low molecular weight GSTs from rat. Subcellular fractions from both control and treated animals were analyzed by Western blot. Cytosolic extracts from clams contaminated with PB and p,p'-DDE showed a 24-kDa band in addition to the 26-kDa band recognized by the antibody. Results of these studies suggest that, in *R. decussatus*, organics may induce GSTs belonging to the π class.

Keywords—Glutathione S-transferases Clam Benzo[a]pyrene Phenobarbital 2,2'-Bis-(p-chlorophenyl)-1,1-dichlorethylene

INTRODUCTION

Mollusk bivalves are widely used in biomonitoring programs of chemical pollution in the aquatic environment (e.g., Mussel Watch [1]). The use of biotransformation enzymes as biomarkers of exposure to organic xenobiotics, in particular those catalyzing phase I transformations, is increasing [2]. The P450-dependent enzyme ethoxyresorufin-O-deethylase is routinely measured in the liver of several species of fish within the context of national and international biomonitoring programs. Nevertheless, ethoxyresorufin-O-deethylase, as an exposure biomarker of polycyclic aromatic hydrocarbons and organochlorine compounds, does not give satisfactory responses in invertebrates, especially in mollusks [3]. This article examines the possibility of using enzymes other than the P450-dependent enzymes as biomarkers of exposure in mollusks because these organisms are valuable biomonitors due to their sedentary life and nutritional status (filter feeders). Phase II enzymes catalyze the conjugation between xenobiotic compounds containing electrophilic sites and endogenous molecules such as glutathione (for glutathione S-transferases) and sulfate (for sulfo-transferases). Glutathione S-transferases, sulfo-transferases, and other phase II enzymes allow the excretion of metabolites from phase I biotransformations, which take place in the liver of organisms through the biliary pathway in the mammalian systems [4]. Such phase II enzymes can also directly metabolize molecules bearing –OH groups such as phenol, toluene, or trichlorethanol. Glutathione S-transferases are a multiple-enzyme family. Some investigators [5] have reported that at least 13 isoforms are present in rat liver and that these conjugate 1-chloro-2,4-dinitrobenzene. These isoforms have an overlapping specificity for other substrates; they are dimers, the majority of which are highly basic, that consist of subunits of 25 to 30 kDa. The subunits can be classified into three groups, i.e., α, μ, and π classes, on the basis of their structural relationships and ability to hybridize with one another. The activity of glutathione S-transferases (GSTs) has been shown to increase in organisms as a function of xenobiotic concentration in the medium. Induction has been shown in vivo in the rainbow trout *Oncorhyncus mykiss* [6], in mollusks [7], and in other invertebrates such as crabs [8]. Moreover, GST induction has been confirmed in the field by authors working on either organisms collected from polluted areas [8–11] or caged organisms [12]. Nevertheless, other studies have demonstrated the lack of GST induction in both the fish *Parophryus vetulus* in contact with polycyclic aromatic hydrocarbons and polychlorinated biphenyls [13] and the eel *Anguilla anguilla* exposed to xenobiotics [14]. Certain authors have reported that GSTs are biomarkers of toxicity, i.e., GST activity is often elevated in animals in which carcinogenesis has already occurred [15]. According to Foureman [16], induction could be caused by diseases rather than as a direct consequence of exposure to pollutants.

Recent studies carried out on the blue mussel *Mytilus edalis* have shown that different GST isoforms are induced in this animal as a function of the nature of pollutants [10,17]. In this work, various cosubstrates and chemical pollutants were used in order to determine whether different isoforms of GSTs are present in the Mediterranean clam *Ruditapes decussatus*. This animal has already been the object of biomarker studies. Indeed, biomarkers other than GSTs, such as metallothioneins (considered to be biomarkers of metal exposure [18,19]) and acetylcholinesterase (a biomarker of pesticide toxicity [20]), have been examined. The cosubstrates used to examine GST induction in the present study were 1-chloro-2,4-dinitrobenzo-
zene, ethacrynic acid, and paranitrobenzene chloride. These substrates have been reported to have different affinity constants toward GSTs [21]. *Ruditapes decussatus* was exposed for 48 h to phenobarbital, a well-known inducer of cytochromes P450 (CYP2B), and to benz[a]pyrene (BaP), which is a planar polycyclic aromatic hydrocarbon. Another possible GST inducer examined was *p,p*-DDE (2,2-bis-(p-chlorophenyl)-1,1-dichlorethylene), which is a metabolite of DDT. Glutathione S-transferase activities were measured in treated and control animals after exposure to the above-mentioned substrates. As the interpretation of total specific activity measurements is complicated by the fact that several GST isoenzymes are present [22], a more detailed understanding of these individual GSTs is required to allow interpretation of the results. For this purpose, Western blotting techniques were carried out on cytosolic fractions of *R. decussatus*.

**MATERIALS AND METHODS**

**Collection of animals and maintenance in aquariums**

Wild Mediterranean clams were collected from the Thau Lagoon (Mediterranean) and placed on ice for transport back to the laboratory. Animals were then transferred to four 200-L aquariums filled with natural aerated seawater at 18 ± 1°C (photoperiod 12:12). After one week, five animals from each aquarium were sacrificed in order to evaluate basal GST activities in the presence of different substrates. Contamination with organic compounds was then performed. In the first series of experiments, clams were exposed to phenobarbital (PB) and BaP; in the second experiment, conducted two weeks later, clams were exposed to *p,p*-DDE. For practical reasons, animals exposed to *p,p*-DDE were therefore submitted to a longer fasting period. Each contamination was performed for 48 h in aquariums containing 10 clams in 4 L of seawater, toxic compounds being dissolved in dimethylsulfoxide (DMSO). Controls were simultaneously kept in seawater containing 0.016% DMSO, a concentration similar to that introduced with the contaminants. The mean clam soft body wet weight was ~4 g. The contamination doses were 2 and 4 μg/L wet weight for PB, 125 and 250 μg/L for BaP, and 1.5 and 3 μg/L for *p,p*-DDE. For each treatment, the lower concentration was called C1 and the higher one C2. The doses of contaminants were chosen by comparison of experiments reported in the literature. Petrivalsky et al. [6] conducted 48-h rainbow trout exposures by intraperitoneal (i.p.) injection of 2 to 200 μg *p,p*-DDE/g and 80 μg PB/g to induce GST activities in the fish livers. In the same manner, Stien et al. [23] i.p. injected 20 μg BaP/g to induce ethoxyresorufin-O-deethylation activity over 24 h in the livers of the sea bass *Dicentrarchus labrax*. Since very few data exist on organic contamination in the medium where mollusks are under experiment, doses were arbitrarily chosen by increasing the nominal concentrations used for i.p. injection.

**Cellular fractionation**

All procedures were carried out at 4°C. A 20% w/v homogenate (from soft body mass) was prepared in 10 mM N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid, 0.25 mM sucrose, 1 mM ethylene diaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol (DTT), pH 7.4 using an ultraturrax. After preliminary centrifugation at 9,000 g for 25 min to remove large particulate material, a high-speed centrifugation (100,000 g for 90 min) was performed to sediment microsomes and obtain the supernatant, which contained the cytosol and on which all analyses were performed.

**Affinity purification procedure**

All chromatographic separations were performed using a low-pressure chromatography system (Econo System®; BIO RAD, Richmond, CA, USA). Approximately 50 ml of cytosol were passed through a glutathione-agarose affinity column (Sigma, St. Louis, MO, USA) pre-equilibrated with loading buffer composed of 10 mM tris[(hydroxymethyl)aminomethane (TRIS), pH 7.2, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, and 1 mM EDTA. The column was washed once with buffer (10 mM TRIS, pH 7.2, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, 1 mM EDTA, 0.2 M NaCl) and GSTs eluted at 1 ml/min with 100 mM TRIS, pH 9, 20 mM glutathione, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, and 1 mM EDTA. Wash buffer was again used to rinse the column. Fractions (3 ml) were collected for analysis of protein content at 280 nm and of enzyme activity.

**GST-specific activity determination**

Glutathione S-transferase activities of the cytosolic fraction with the various substrates (1-chloro-2,4-dinitrobenzene [CDNB], paranitrobenzene chloride [PNBC], ethacrynic acid [ETHA]) were measured spectrophotometrically at 37°C by following conjugation of the acceptor substrate with glutathione as described in [21]. Results are expressed as the formed conjugate per minute and per milligram of protein.

Proteins were determined according to [24] using a microplate reader.

**SDS-PAGE electrophoresis**

Electrophoresis was performed on cytosolic fractions from control and organic-treated animals (at both concentrations). Three samples containing between 15 and 20 μg protein from each treatment were applied to 12% sodium dodecyl sulfate (SDS) polyacrylamide gel for SDS-PAGE electrophoresis [25]. The resulting protein bands were electrophotoregly transferred onto nitrocellulose membrane.

**Immunological methods**

The first antibody was obtained from rabbit serum, following two months of injections of 30 μg affinity-purified GSTs from *R. decussatus* (injections were performed every three weeks). Before the first injection, a blood sample was taken (termed preimmune). The first injection was performed with 50% complete Freund’s adjuvant, whereas the following injections were performed using 50% incomplete Freund’s adjuvant. Fifty milliliters of rabbit blood were taken. The serum was collected by low-speed centrifugation after blood coagulation. The serum was aliquoted and stored at −20°C. Preimmune serum and sera obtained one (S1) and two months (S2) after the first injection were compared by immunoblot reaction against affinity-purified GSTs from rat (Sigma) and from *Ruditapes decussatus*.

The blots were incubated 1 h at room temperature in TRIS-HCl 50 mM, NaCl 150 mM, pH 7.4, 0.1% Tween 20 (TBS-T), and 1% bovine serum albumin. They were then incubated with the first antibody (dilution 1:2,000) in the same buffer for 1 h at room temperature and with gentle agitation. The blots were rinsed twice for 5 min in TBS-T, followed by 1 h incubation with antirabbit IgG (H + L)/phosphatase com-
plex (SIGMA, dilution 1:2,500) in TBS-T containing 1% bovine serum albumin. Subsequently, the blots were rinsed two times for 5 min in TBS-T. Phosphatase enzymatic activity was visualized by adding 0.005% 5-bromo-4-chloro-3-indolyl phosphate and 0.005% nitroblue tetrazolium in 100 mM TRIS-HCl, pH 9.

Result analyses

Quantification of the GST bands revealed by Western blot was performed using National Institution of Health 5.1® Software, which gave the intensity (pixel) of bands as a function of surface area.

Statistical comparison of the results was made using a non-parametric Mann–Whitney U test with \( n = 5 \) in each case.

**RESULTS**

**GST activities in Ruditapes decussatus**

Table 1 shows the GST activities recorded in control clams at different times throughout the experiment. When the animals were collected, GST initial activities in the cytosolic fraction varied depending on the conjugated substrate examined. The highest activity was found with CDNB (Table 1), whereas the activities measured with ETHA and PNBC were ~20 and ~40 times lower than with CDNB, respectively (Table 1). These activities were not modified when clams were maintained 2 d in seawater containing 0.016% DMSO. At the end of two weeks, the level of GST-CDNB activity was significantly elevated, whereas GST-ETHA and GST-PNBC activities were unchanged in comparison with initial activities (Table 1).

**Effects of contaminants on GST activities**

The effects of the two concentrations of each contaminant on GST activities were examined as a function of the conjugated substrate. After 48 h of contamination treatment with PB, no significant difference was found between activities measured in control and PB-treated animals at either concentration (Fig. 1). After 72 h of PB treatment, GST-ETHA activity was significantly higher than that of controls at both contaminant concentrations (65 ± 0.5 and 74 ± 0.2 nmol/min/mg protein \( n = 5 \)) at C1 and C2, respectively [results not shown in Fig. 1). A significant increase in GST-ETHA activity was observed after treatment with BaP at the highest concentration tested (61 ± 0.6 nmol/min/mg protein \( n = 5 \); Fig. 1). Exposure to \( p,p’ \)-DDE significantly increased GST-CDNB and GST-ETHA activities at both concentrations, although the activity in the controls was elevated in the case of GST-CDNB (Fig. 1 and Table 1).

**Specificity of antibody raised against R. decussatus GSTs**

The specificity of the antibody raised against \( R. \) decussatus GST was assayed using affinity-purified GST from \( R. \) decussatus cytosol in comparison with rat GSTs. Figure 2 (left part) shows that affinity-purified GST from \( R. \) decussatus presented a wide band of molecular weight, ranging from 24 to 27 kDa.

![Image](312x552)

**Table 1. Mean cytosolic glutathione S-transferase (GST) activities (± standard deviation) in control clams Ruditapes decussatus measured before contamination (\( t_0 \)) and in parallel with the contamination procedure occurring at \( t_0 + 2 \) d and two weeks later, at \( t_0 + 17 \) d\(^*\)**

<table>
<thead>
<tr>
<th>Activities (μmol/min/mg)</th>
<th>GST-CDNB</th>
<th>GST-PNBC</th>
<th>GST-ETHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (( t_0 ))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n = 8 )</td>
<td>0.847 ± 0.054</td>
<td>0.030 ± 0.008</td>
<td>0.042 ± 0.010</td>
</tr>
<tr>
<td>Control first series ( t_0 + 2 )</td>
<td>0.840 ± 0.081</td>
<td>0.024 ± 0.006</td>
<td>0.047 ± 0.004</td>
</tr>
<tr>
<td>( n = 8 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control second series ( t_0 + 17 )</td>
<td>1.610 ± 0.110(^*)</td>
<td>0.038 ± 0.010</td>
<td>0.056 ± 0.006</td>
</tr>
<tr>
<td>( n = 8 )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) CDNB = 1-chloro-2,4-dinitrobenzene; PNBC = paranitrobenzene chloride; ETHA = ethacrynic acid.

\(^b\) Comparison with \( t_0 \) at \( p < 0.01 \).

Fig. 1. Mean (\( n = 5 ± SD \)) glutathione S-transferase (GST)-specific activities (toward 1-chloro-2,4-dinitrobenzene [CDNB], paranitrobenzene chloride [PNBC], and ethacrynic acid [ETHA]) in the cytosolic extract from Ruditapes decussatus treated for 48 h with phenobarbital (PB), benz(a)pyrene (BaP), or 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene (\( p,p’ \)-DDE). Results are expressed in μmol/min/mg protein for GST-CDNB and nmol/min/mg protein for GST-PNBC and GST-ETHA. Values that differ significantly from control values are noted as *, \( p < 0.05 \); **, \( p < 0.01 \). C = control samples; C1 = samples treated with the lower dose of contaminant, and C2 = samples treated with the higher dose. The treatment with PB after the 72-h exposure is not shown (see Results).
Fig. 2. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and Western blot analysis of purified glutathione S-transferase (GSTs) from *Ruditapes decussatus* and from rat. Left: SDS/PAGE of glutathione-agarose affinity-purified proteins from *R. decussatus* cytosolic extract (lane 1) and rat GSTs from Sigma (lane 2). Proteins were visualized using Coomassie blue reagent. (Right) Western blot: lanes 1, 3, and 5, *R. decussatus* GSTs; lanes 2, 4, and 6, rat GSTs (same proteins as for SDS/PAGE). Lanes 1 and 2, incubation with preimmune serum; lanes 3 and 4, incubation with S1; lanes 5 and 6, incubation with S2.

which is comparable to that of the SDS-PAGE of GSTs from rat (22 and 28 kDa). Western blot experiments (Fig. 2, right part) with S1 and S2 sera containing the antibody raised against GST from *R. decussatus* gave a positive reaction with purified GST from *R. decussatus* and from rat. In this last case, two low molecular weight (<24 kDa) GSTs cross-reacted. These results demonstrate that the clam primary antibody recognized GSTs from *R. decussatus* and to a lesser degree from a rat.

**Immunoblot analysis of GST induction**

In order to characterize the induction of GSTs by contaminants, a cytosolic extract was analyzed by SDS-PAGE from treated and control clams followed by immunoblotting using the antibody prepared from rabbit serum (Fig. 3a, PB treatment; Fig. 3b, BaP treatment; and Fig. 3c, *p,p'*-DDE treatment). Immunoblots showed that, besides the 26-kDa band representing the antibody-responding GST (termed band a), a 24-kDa stripe (called band b) was increased in certain cases. The ratio b/(a + b), calculated from the intensity of each stripe after quantification, allows the detection of induced GST isoforms following organic contamination (Fig. 4).

In Figure 3a, it can be seen that, in clams treated by the lower PB concentration, the 24-kDa stripe (b) increased (approx two- to threefold) as well as the ratio b/(a + b) (Fig. 4) in comparison with controls, whereas when clams were treated with 4 µg/L PB, no induction was detected and the basal band (band a) was slightly reduced, placing the ratio b/ (a + b) above that of the controls. After clam contamination with BaP, no induction was detected (Fig. 3b); the ratio b/(a + b) (Fig. 4) showed a modest but not statistically significant increase with increasing contaminant concentration. Immunoblots of cytosolic extract from *p,p'*-DDE-treated clams showed a significant induction at the higher concentration used (3 µg/L, Fig. 3c), the intensity of band b as well as the ratio b/(a + b) being much higher than that of the controls (Fig. 4).

**DISCUSSION**

This work was a first approach to determine whether different isoforms were induced in the clam *R. decussatus*. That is why the whole tissues of the clams were considered. The contamination concentrations were arbitrarily chosen; some were above the solubility levels, which explain the lack of dose–response. However, the existence of different isoforms of GSTs was shown.

Exposure of *R. decussatus* to organic toxicants elicits different GST responses that vary depending on both the substrate used to measure enzymatic activities and the organic pollutant introduced into the medium. In a given organism, the specific GST activities on a range of substrates may be used as a criterion for distinguishing GST isoenzymes [26]. In the present study, GST activity measured with CDNB was markedly increased in the control animals after two weeks of acclimation. Conversely, the activities using the substrates PNBC and ETHA were not affected. Thus, it is likely that experimental conditions of storage induced isoenzymes having a strong ac-
GST in clams treated with organics

Fig. 4. Quantification of glutathione S-transferase (GST) isoform induction in the cytosolic extract after organic contamination of Ruditapes decussatus. Bars represent the relative amount of induced 24-kDa band (band b) expressed as a percent of the total GST (bands a + b). Values are the mean ± SE of three determinations.

Table 2. Classification of some isoforms of glutathione S-transferases (GSTs) reported in the literature a

<table>
<thead>
<tr>
<th>Isoforms</th>
<th>Molecular weight (kDa)</th>
<th>Substrate</th>
<th>Species</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-P with subunits</td>
<td></td>
<td></td>
<td>Rat</td>
<td>[29]</td>
</tr>
<tr>
<td>Ya</td>
<td>26.5</td>
<td>ETHA/CDNB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yp</td>
<td>26</td>
<td>ETHA/CDNB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Class with subunits</td>
<td></td>
<td></td>
<td>Rat</td>
<td>[28]</td>
</tr>
<tr>
<td>1</td>
<td>25.5</td>
<td>CDNB/PNBC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27.5</td>
<td>CDNB/ETHA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>ETHA/PNBC/CDNB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>π-Class isoforms</td>
<td></td>
<td></td>
<td>Vertebrates</td>
<td>[32–34]</td>
</tr>
<tr>
<td>GST I</td>
<td>24.5</td>
<td>ETHA/CDNB</td>
<td>Mussel</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDNB/ETHA</td>
<td>(Mytilus edulis)</td>
<td></td>
</tr>
<tr>
<td>Transferase A</td>
<td>27</td>
<td>CDNB</td>
<td>Plaice</td>
<td>[5]</td>
</tr>
<tr>
<td>Transferase B</td>
<td>25</td>
<td>CDNB/ETHA/PNBC</td>
<td>(Pleuronectes platessa)</td>
<td></td>
</tr>
<tr>
<td>Band a</td>
<td>26</td>
<td>CDNB/ETHA</td>
<td>Clam</td>
<td>This work</td>
</tr>
<tr>
<td>Band b</td>
<td>24</td>
<td>ETHA/CDNB</td>
<td>(Ruditapes decussatus)</td>
<td></td>
</tr>
</tbody>
</table>

*a CDNB = 1-chloro-2,4-dinitrobenzene; PNBC = paranitrobenzene chloride; ETHA = ethacrynic acid.
is likely that conjugation of ETHA is one of the characteristics of \( \pi \)-class GSTs [32–34] in vertebrates, although this has not been confirmed in invertebrates. The molecular weight found here for GST isoenzymes induced by PB was in the order of 24 kDa, a value generally ascribed to \( \pi \)-class isoenzymes (24.8 kDa) [4]. The affinity-purified extract of the blue mussel \( M. edulis \) contains four main proteins separable by ion-exchange chromatography [17]. The immunoblotting and amino acid sequencing studies of these proteins indicate that the major isoenzyme belongs to the \( \pi \) class of GSTs. Benzo[a]pyrene treatment increased GST activity with ETHA, but the immunoblot analysis showed a very modest increase in the b band. Benzo[a]pyrene, a planar molecule of 3-methylcholanthrene type, induces CYP1 enzymes, such as BaP-hydroxylase in mollusks [35], and the resulting compounds should be conjugated by GST and then excreted. If this is the case, then the GST response could be performed by transcriptional and post-transcriptional mechanisms. This biotransformation process of a planar molecule is often opposed to that of PB, a globular molecule, which induces other cytochromes such as CYP2. Nevertheless, the effects of PB and 3-methylcholanthrene could no be distinguished when inducing the GSTs in treated rats [36].

Treatment with the organochlorine compounds such as \( p,p' \)-DDE significantly induced GSTs when measured using CDNB as a substrate. Nevertheless, after a 48-h treatment, activity levels were higher at the lower concentration tested. This may be due to a toxic effect associated with high doses that could have reduced GST induction by interacting with cell metabolism. Another isozyme, conjugating ETHA, appeared to be induced by treatment with \( p,p' \)-DDE. Thus, a \( \pi \)-class isozyme may be induced in the same manner as seen for PB and BaP. No significant increased conjugation of GST activities with PB and \( p,p' \)-DDE as a substrate was measured following the three treatments. Certain authors [5] have found in noninduced placenta liver two GSTs (Table 2), called transferase A (homodimer of 27 kDa subunits) and transferase B (homodimer of 25 kDa subunits), both of which display activities toward CDNB and 1,2-dichloro-4-nitrobenzene. Transferase B, but not transferase A, was observed to possess the particularity of displaying activity, albeit low, with PBNC and ETHA as substrates.

In conclusion, different GST isoforms seem to be present in the clam \( R. decussatus \). They appear to be induced in particular by \( p,p' \)-DDE and phenobarbital. Induction of GST-ETHA activity and Western blot analysis of isozymes indicate that these isoforms may be suitable biochemical markers of exposure to compounds that do not induce phase I enzymes as cytochrome P450A1. It seems that the use of CDNB activity is less specific than that of ETHA from a toxicological point of view. Further research is needed, however. Indeed, the major GST isozymes from \( R. decussatus \) have yet to be identified and purified. It should be possible to distinguish between them based on their biochemical and immunochromatographic properties, such as their subunit compositions, charges, and affinity for different chromatography matrices.

Acknowledgement—Financial support was given to P. Hoarau through a fellowship contributed by Provence Alpes Côte d’Azur Region and the firm SAFEGE-CETIIS, Aix-en-Provence, France.

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