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Molecular cloning and expression study of pi-class glutathione *S*-transferase (pi-GST) and selenium-dependent glutathione peroxidase (Se-GPx) transcripts in the freshwater bivalve *Dreissena polymorpha*

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Abstract

Glutathione *S*-transferases (GST) and glutathione peroxidases (GPx) are essential components of cellular detoxification systems. We identified GST and GPx transcripts in the freshwater bivalve *Dreissena polymorpha*, their full-length coding sequences were obtained by reverse-transcription PCR using degenerated primers followed by 5′ and 3′ RACE-PCR (rapid amplification of cDNA ends-PCR). The cDNA identified encoded proteins of 205 and 243 amino acids corresponding respectively to a pi-class GST and a selenium-dependent GPx. The comparison of the deduced amino acid sequences with GST and GPx from other species showed that the residues essential to the enzymatic function of these two proteins are highly conserved. We studied their expression pattern in the digestive gland, the gills and the excretory system of *D. polymorpha*. The results showed that pi-GST mRNA expression is higher in the digestive gland than in the gills or the excretory system. Se-GPx transcripts are expressed at high, medium and very low levels in the digestive gland, the excretory system and the gills, respectively.

Keywords: *Dreissena polymorpha*; Bivalvia mollusc; Pi-class GST; Selenium-dependent GPx; Coding sequences; Expression pattern

1. Introduction

Glutathione *S*-transferases (GST) are enzymes belonging to the most important phase II biotransformation system. They have been purified for the first time in 1974 in mammals (Habig et al., 1974). They catalyse the conjugation of glutathione (GSH: Glu-Cys-Gly) to electrophilic compounds in order to facilitate their excretion (Ketterer et al., 1983). Seven major classes of cytosolic isoenzymes can be distinguished in mammals (alpha, mu, pi, theta, sigma, zeta and omega) based on their substrate specificity, immunological properties and protein sequence homologies (Sheehan et al., 2001). GST isoenzymes belong to the same class if they show at least 40% identities in their primary structures (Armstrong, 1997). Among freshwater mussels, GST activity has been demonstrated in a few species namely, *Anodonta cygnea* (Robillard et al., 2003), *Corbicula fluminea* (Vidal et al., 2001), *Sphaerium corneum* (Boryslawsky et al., 1988), *Unio tumidus* (Petushok et al., 2002) and *Dreissena polymorpha* (Rocher et al., 2006). Several GST sequences belonging to different classes have been described in molluscs and are available in GenBank (accession numbers are indicated in parenthesis). The omega-class GST was identified in the oyster *Crassostrea gigas* (AJ557141). The mu-class was also described in this bivalve (AJ558252), in the scallop *Chlamys islandica* (AM279651) and in the gastropod *Haliotus discus discus* (DQ530212). The sigma-class GST was identified in *C. gigas* (AJ577243), *H. discus discus* (DQ530213) and in the cephalopod *Octopus vulgaris* (X65544). The pi-class GST, which is the isoform exhibiting the highest expression level, was described in the cephalopod *Omnastrephes sloani* (LO2053), the bivalve *Ruditapes philipparum* (EF520700) and in four mussels species: *C. fluminea* (AY885667), *Mytilus edulis* (AY557404), *Mytilus galloprovincialis* (AF527010) and *U. tumidus* (AY885666). The pi-class GST can inactivate lipoperoxidation products, lipid hydroperoxides and their derivatives; they also directly inactivate reactive oxygen species via their SH group.

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Among the enzymatic antioxidant system, the glutathione peroxidases (GPx) belong to the first line of defense against peroxides, superoxide anion and hydrogen peroxide. They can be divided into two types of enzymes: the selenium-dependent GPx (Se-GPx) and the selenium-independent GPx (non-Se-GPx). GPx activity was described for the first time in 1957 (Mills, 1957); the enzyme was characterized as a selenoprotein in 1973 (Flohé et al., 1973; Rotruck et al., 1973). Se-GPx catalyse the reduction of organic and inorganic peroxides like hydrogen peroxide (H_2O_2) while non-Se-GPx reduce only organic peroxide (Almar et al., 1998). Se-GPx and non-Se-GPx activities have been studied in two freshwater mussels, *U. tumidus* (Cossu et al., 1997) and *D. polymorpha* (Rocher et al., 2006). To date few GPx coding sequences of molluscs are available in GenBank. The complete sequence of a non-Se-GPx was identified in the gastropod *Aplysia californica* (AF510851). A partial Se-GPx sequence was described in the cephalopod *Euprymna scolopes* (AY675197). We identified previously two freshwater mussel Se-GPx sequences: a partial sequence in *C. fluminea* (EF547366) and a complete sequence in *U. tumidus* (DQ830766).

Bivalves GST and GPx were essentially described by their enzymatic activities. The aim of our work was to identify pi-GST and Se-GPx transcripts from *D. polymorpha* and to study their transcriptional expression levels in several tissues (digestive gland, gills and excretory system). Bivalve molluscs are appropriate species to study the quality of the aquatic environment. They are present in several aquatic systems, are sedentary and filter large amounts of water to cope with nutritional and respiratory needs.

In a first step, we performed RT-PCR (reverse-transcription polymerase chain reaction) on the digestive gland RNA using degenerated primers deduced from the comparison of GST and GPx conserved sequences in different organisms. In a second step, in order to obtain the full-length coding sequences, we carried out RACE-PCR (rapid amplification of cDNA ends-PCR) with specific primers designed according to the pi-GST and Se-GPx partial coding sequences identified. Then, we studied their mRNA levels performing RT-PCR on the transcripts of three tissues: digestive gland, gills and excretory system.

2. Materials and methods

2.1. Experimental animals

Fifteen mature *D. polymorpha*, sizing between 20 and 25 mm, were sampled from a site located in the Meuse River close to Commercy (France) in May 2007; this area has been used as a reference site in the case of previous studies (Guerlet et al., 2007). The tissues (digestive gland, gills, excretory system) were dissected immediately after collection, placed in 4 M guanidium isothiocyanate solution (Fermentas Life sciences, Vilnius, Lithuania) and conserved in liquid nitrogen to avoid RNA degradation.

2.2. RNA isolation

The fifteen mussels were randomly divided into three groups of five animals. Tissues of each group were pooled in order to have enough material for RNA isolation. The extractions were carried out using the RNeasy Mini Kit (Qiagen, Maryland, USA) following the manufacturer's instructions. An electrophoresis on 1.5% agarose gel in TAE buffer (TRIS 40 mM, acetic acid 1 mM, EDTA 40 mM) was performed in order to check the amount and integrity of RNA. The gel was stained with ethidium bromide and RNA was visualized under UV light.

2.3. Primer design and cDNA synthesis

Primers were obtained from Invitrogen (Carlsbad, USA), the sequences are given in Table 1. One μg of total RNA was reverse-transcribed using MuLV reverse transcriptase according to the instructions of the manufacturer (Fermentas Life Sciences, Vilnius, Lithuania). PCR reactions were conducted on one tenth (2 μL) of each cDNA.

2.4. GST and GPx cDNA amplification using degenerated primers

cDNA corresponding to the digestive gland was used to identify GST and GPx partial coding sequences. GST and GPx degenerated primers (GST.F1d, GST.R1d and GPX.F1d, GPX.R1d) were chosen in cDNA conserved regions of GST and GPx coding sequences from different species (*A. californica*, *Caenorhabditis elegans*, *Danio rerio*, *Dirofilaria immitis*, *Drosophila melanogaster*, *Gallus gallus*, *Mus musculus*, *M. edulis* and *Xenopus laevis*).

The conditions of amplification tested were as follows: 200 μM of each dNTP (2′-deoxyribonucleotide 5′-triphosphate), 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1, 1.5 or 2 mM $MgCl_2$, 1 μM of each degenerated primer and 1 unit of *Taq* DNA Polymerase (Fermentas Life Sciences). We tested different cycling parameters: a 2-min denaturation at 94 °C, 40 cycles of heat denaturation at 94 °C for 30 s, annealing at 44, 48 or 52 °C for 30 s or 1 min, polymerisation at 72 °C for 30 s, 1 min or 2 min, and a 2-min final extension at 72 °C.

Table 1
Sequences of primers used for amplification of *D. polymorpha* cDNA

Primers	Sequences[a] (5′–3′)
GST.F1d	ASMMTSATCTAYMMWAACTATGA
GST.R1d	YTAYTGTTTDCCRTTKCCRTT
GST.DF2	TGACTTGATCAAGGACGCGA
GST.DF3	GAGACGCTGATGTCCGGATG
GST.DR3	GGAGTGACGGTGTTTCCTTTATG
GST.DR5	GATACGACGCACAGGATGTC
GPX.F1d	TSCTSGSHTTYCCVTGYAAYCARTTC
GPX.R1d	KAYHARRAAYTTHKYRAAGTTCCA
GPX.DF2	GAGCCTGCCTGGAACGGCGT
GPX.DR2	CACTTGACGTCCTCGGTGTA
ACT.DF	GGATGATATGGAGAAGATCTGG
ACT.DR	CCTGCTTGCTGATCCACATCTG
Oligo-dG anchor	GACCACGCGTATCGATGTCGAC$(G)_{15}$H
Oligo-dT anchor	GACCACGCGTATCGATGTCGAC$(T)_{15}$V
Anchor	GACCACGCGTATCGATGTCGAC

[a] R: A/G, Y: C/T, S: C/G, W: A/T, K: G/T, M: A/C, D: A/G/T, H: A/C/T, V: A/C/G.