Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pesticide Biochemistry and Physiology 83 (2005) 29–36
PESTICIDE
Biochemistry & Physiology
www.elsevier.com/locate/ypest

# Molecular cloning, expression, and characterization of a phi-type glutathione *S*-transferase from *Oryza sativa*

Hyun-Young Cho, Kwang-Hoon Kong *

*Department of Chemistry, College of National Sciences, Chung-Ang University, 221, Huksuk-Dong, Dongjak-Ku, Seoul 156-756, Republic of Korea*

Received 6 January 2005; accepted 15 March 2005
Available online 20 April 2005

**Abstract**

The structural gene for glutathione *S*-transferase in *Oryza sativa* was successfully cloned from a cDNA library by the polymerase chain reaction method. The deduced amino acid sequence of this gene showed 44–66% similarity to the sequences of the class phi GSTs from *Arabidopsis thaliana* and *Zea mays*. This gene was expressed in *Escherichia coli* with the pET vector system and the gene product was purified to homogeneity by GSH–Sepharose affinity column chromatography. The expressed *Os*GSTF3-3 was a homo-dimer composed of 24 kDa subunit and its p*I* value was approximately 7.3. The *Os*GSTF3-3 was retained on GSH affinity column and its $K_m$ value for GSH was 0.28 mM. The *Os*GSTF3-3 displayed high activity toward 1-chloro-2,4-dinitrobenzene, a general GST substrate and also had high activities towards acetanilide herbicides, alachlor, and metolachlor. The *Os*GSTF3-3 was highly sensitive to inhibition by benastatin A and *S*-hexyl-GSH. From these results, the expressed *Os*GSTF3-3 is a phi class GST and seems to play an important role in the conjugation of the chloroacetanilide herbicides.


*Keywords:* Glutathione *S*-transferase; *Oryza sativa*; Rice; Cloning; phi Class; Herbicide activity

## 1. Introduction

Glutathione *S*-transferases (GSTs, EC 2.5.1.18) are a major family of detoxification enzyme that catalyzes the formation of conjugates between reduced glutathione (GSH)[1] and a wide variety of electrophilic substrate including many herbicides and pesticides [1,2]. Certain GSTs also catalyze peroxidase

* Corresponding author. Fax: +82 2 825 4736.
*E-mail address:* khkong@cau.ac.kr (K.-H. Kong).

[1] *Abbreviations used:* GST, glutathione *S*-transferase; GSH, glutathione; *Os*GSTF3-3, homo-dimer of phi class GST from *Oryza sativa*; IPTG, isopropyl β-D-thiogalatopyranoside; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; ETA, ethacrynic acid; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; NB, 4-nitrophenethyl bromide; CP, cumene hydroperoxide.

0048-3575/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2005.03.005

reactions or isomerization of certain steroids and are involved in hydroxyl peroxidase detoxification or tyrosine metabolism, respectively [3–5]. Other GSTs play an important role in the intracellular transport of numerous non-substrate ligands such as auxins and cytokinins [3,4,6] or anthocyanins [7,8], and thus contribute to hormone homeostasis or vacuolar anthocyanin sequestration, respectively.

Plant GSTs have been concerned in the agricultural chemistry and biochemistry because they are one of the major factors involved in the resistance of a variety of herbicides [3]. Based on sequence similarity and exon structure, the plant GSTs have been subdivided in class phi (F), ζ (Z), τ (U), θ (T), and λ (L) [3,9,10]. Individual GST isozymes can selectively detoxify specific xenobiotics with species differences in GST specificity and capacity determining herbicide selectivity [4,9,11]. In plants, studies of GSTs have been focused mainly on their ability to detoxify herbicides. Many GSTs from plants have been purified and their genes have been cloned. Among them, the enzymes from *Arabidopsis*, soybean, and maize have been extensively studied their structure, function, and physiological significance in detail [3,12]. However, the characterization and functions of GST subunits from rice, important food in Asia, are poorly understood now even if the GST purification and characterization from Dongjin rice was already reported [13].

In this study, a glutathione *S*-transferase gene homolog (GenBank Accession No. AF309384) was identified from rice (*O. sativa*) through genome sequencing. The gene was successfully cloned and expressed in *Escherichia coli*. The gene product was purified to homogeneity and was characterized in detail. To our knowledge, this is the first report on phi class glutathione *S*-transferase gene in rice.

## 2. Materials and methods

### 2.1. Materials

An RNeasy Plant Mini kit was obtained from Qiagen (Valencia, CA, USA). λZAP II and pET-26b(+) vectors used in this study were supplied from Stratagene (La Jolla, CA, USA) and Novagen (Wisconsin, USA), respectively. *E. coli* BL21(DE3) and XL1-blue were obtained from Pharmacia Biotech (Uppsala, Sweden). KOD polymerase and DNA ligation solution were from Toyobo (Osaka, Japan). Restriction enzymes were purchased from Takara Shuzo (Otsu, Shiga, Japan). Glutathione–Sepharose 4B was obtained from Pharmacia Biotech (Uppsala, Sweden). 1-Chloro-2,4-dinitrobenzene (CDNB), cumene hydroperoxide, 4-nitrophenethyl bromide, *S*-hexyl-GSH, and hematin were from Sigma Chemical (St. Louis, USA). *S*-(2,4-Dinitrophenyl) glutathione was synthesized by the method of Schramm et al. [14]. The synthesis of DNA primers was performed by COSMO genetech (Seoul, Korea). All other reagents used were of the highest grade commercially lable.

### 2.2. cDNA construction, cloning, and expression of the gene

Total RNAs were isolated from the culture cell of rice (*O. sativa* L. cv. Yamahousi) using an RNeasy Plant Mini kit (Qiagen) according to the supplier's instruction. The poly(A) mRNAs were enriched from total RNAs and used to synthesize full length first-strand cDNAs construct a unidirectional cDNA library in λ ZAP II vector as recommended by the manufacturer (Stratagene). The gene of *O. sativa* homologous to the *Triticum aestivum* gene and *Zea mays* gene for a glutathione *S*-transferase was found using a BLAST search [15]. The isolation of the complete coding sequences of the GST gene from *O. sativa* was obtained directly from the cDNA library by the polymerase chain reaction (PCR) method. Two oligonucleotide primers for PCR were designed as follows: 5′-GGAATTC CATATGGCGGCGCCTGTGACGGTGTAC-3′ (upper primer, containing an *Nde*I cutting site as underlined) and 5′-CGCGGATCCGCTATCAT GAG GGCCGCCGTGGTACA TT-3′ (lower primer, containing a *Bam*HI cutting site as underlined). The PCR product was cloned into expression vector pET-26b using *Nde*I and *Bam*HI sites. The resulted vector was designated as pET-*Os*GSTF3. The DNA sequence of *Os*GSTF3 was verified by sequencing on an Applied Biosystems