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Sphingobium sp. SYK-6 LigG involved in lignin degradation is structurally and biochemically related to the glutathione transferase omega class

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ABSTRACT

SpLigG is one of the three glutathione transferases (GSTs) involved in the process of lignin breakdown in the soil bacterium *Sphingobium* sp. SYK-6. Sequence comparisons showed that SpLigG and several proteobacteria homologues form an independent cluster within cysteine-containing GSTs. The relationship between SpLigG and other GSTs was investigated. The X-ray structure and biochemical properties of SpLigG indicate that this enzyme belongs to the omega class of glutathione transferases. However, the hydrophilic substrate binding site of SpLigG, together with its known ability to stereoselectively deglutathionylate the physiological substrate α-glutathionyl-β-hydroxypropiovanillone, argues for broadening the definition of the omega class.

Structured summary of protein interactions:
SpLigG and **SpLigG** bind by X-ray crystallography (View interaction).

1. Introduction

Glutathione transferases (GSTs) constitute a multigenic family with fundamental roles in cellular detoxification of a wide range of exogenous and endogenous compounds. Most GSTs characterized until now catalyze the conjugation of the tripeptide glutathione (GSH) to compounds containing an electrophilic center, generally increasing the solubility and diminishing the toxicity of the resulting compounds. The highly reactive thiolate anion of GSH is usually stabilized by a serinyl or tyrosinyl residue conserved in the GSH binding site (G-site) of GSTs [1]. In contrast, several classes of GSTs contain at this place a catalytic cysteine conferring them thiol transferase activity [1]. Whereas a number of cysteine-containing GST classes span several kingdoms and phyla (for example omega (O) in mammals, fungi and insects [2]; S-glutathionyl hydroquinone reductase (GHR) in fungi and prokaryotes), others are limited to a specific kingdom or phylum (lambda (L) and DHAR in plants; beta (B) and LigG in bacteria). Although the principle in vivo activities of cysteine-containing GSTs remain unclear, recent advances indicate that they are able to bind a large range of small molecules and could thus be involved in various metabolic pathways. Supporting this view, human omega class GSTs could participate in arsenic biotransformation [3], while bacterial and fungal GHRs could be involved in the catabolism of chlorinated quinones such as pentachlorophenol, a fungicide used for wood preservation [4]. In addition *Arabidopsis thaliana* lambda GSTs were recently shown to bind oxidized anthocyanidins and tocopherols, suggesting putative roles in response to oxidative stress [5].

In *Sphingobium* sp. strain SYK-6 (an aerobic Gram-negative soil bacillus), *ligE*, *ligF* and *ligG* are the three GST encoding genes found in an operon involved in the process of lignin breakdown. SpLigE and SpLigF catalyze the transfer of GSH to an aryl ether substrate resulting in a β-thioether intermediate and SpLigG catalyzes elimination of GSH from the conjugate product [6,7]. Sequence

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comparison shows that SpLigG possesses several proteobacteria homologues with which it forms an independent cluster among cysteine-containing GSTs (Fig. 1).

In this study we present the three-dimensional structure of SpLigG, and the comparison of its biochemical properties with omega and xi (GHR) GSTs from *Phanerochaete chrysosporium* characterized recently by our group [8]. The results show a clear relationship between SpLigG and omega GSTs.

2. Materials and methods

2.1. Cloning, site-directed mutagenesis, production and purification of recombinant proteins

The cloning of the *Sphingobium* sp. SYK-6 LigG and LigF into pET-21 (+) has been described previously [9]. The LigG_C15S mutant of the active site cysteine has been obtained by PCR-based site-directed mutagenesis using two complementary mutagenic primers (5′TCACATTCCCGGC**A**GCCCCTTCTCCGAGCG3′ and 5′CGCT CGGAGAAGGGGC**T**GCCGGGAATGTGA3′). *Escherichia coli* BL21(DE3) culture conditions and protein purification procedures were similar to the ones previously described [8]. For the production of the selenomethionine tagged SpLigG, recombinant plasmid carrying the gene of interest was electroporated into methionine auxotrophic strain of *E. coli* BL21 (DE3) and further production and purification steps were performed as described by Koh et al. [10]. After purification to homogeneity, electrospray mass analysis of SpLigG (30 830 Da) showed that the N-terminal methionine residue had been cleaved, that a GSH adduct was present and that all eight methioninyl residues had been substituted.

2.2. Structure determination of SpLiG

The selenomethionylated form of SpLigG (19 mg/ml in 30 mM Tris–HCl pH 8.0, 1 mM EDTA buffer) was mixed (ratio 1:1) with DMSO containing 10 mM of glutathionylated-menadione. Droplets for the batch method under oil were prepared by mixing 1.5 µl of this solution with 1.5 µl of a solution containing 0.2 M ammonium

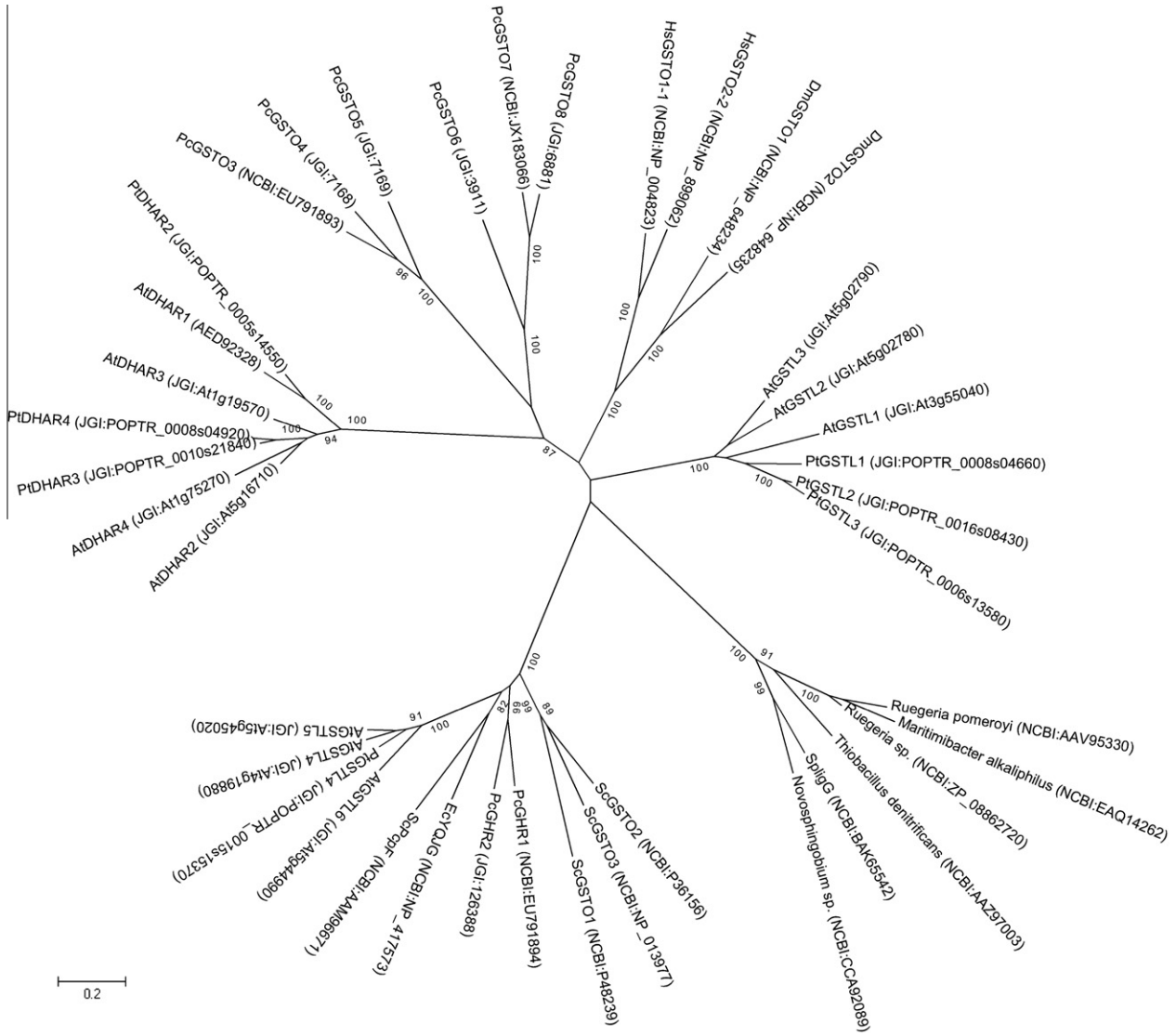

Fig. 1. Unrooted phylogenetic tree of cysteine-containing glutathione transferases from different phyla (At = *Arabidopsis thaliana*; Pt = *Populus trichocarpa*; Pc = *Phanerochaete chrysosporium*; Hs = *Homo sapiens*; Dm = *Drosophila melanogaster*; Sc = *Sphingobium chlorophenolicum*; Ec = *Escherichia coli*; Sp = *Sphingobium* sp. strain SYK-6). SpLigG homologues (bottom right) share less than 20% of sequence identity with the other represented GSTs. Sequence alignments were done by ClustalW. The tree was constructed with the neighbour-joining method in MEGA 5.0 software. The robustness of the branches was assessed by the bootstrap method with 1000 replications. Bootstrap values are placed at the nodes where the value is >70.