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# A combined biochemical and computational studies of the rho-class glutathione s-transferase sll1545 of *Synechocystis* PCC 6803

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ABSTRACT

Peroxides are one of the most important radicals that cause oxidative stress. Certain Glutathione S-transferases (GSTs) have been reported to show peroxidase activity. We report a novel peroxidase activity of *Synechocystis* GST- sll1545. The recombinant protein was purified to homogeneity and characterized. Low *Km* (0.109 μM) and high *V*max (0.663 μmol min$^{-1}$) values suggest a high preference of sll1545 for cumenehydroperoxide. Disc inhibition assay confirmed the ability of the enzyme to protect cells against peroxide-induced damage. sll1545 has very low sequence and structural similarity with theta and alpha class GSTs that exhibit glutathione-dependent peroxidase activity. Recent data from our laboratory shows that sll1545 is also strongly active against dichloroacetate (DCA), which is a characteristic of zeta class GST. Interestingly, sll1545 shows less than 20% sequence identity with zeta class GST. Molecular dynamic simulation results show that sll1545 was much more structurally different from alpha/theta classes. Our results suggest that sll1545 shows structural variation from zeta, theta/alpha classes of GSTs but have related enzymatic activity. Phylogenetic analysis reveal that sll1545 is evolutionally very distinct from the known GSTs. Overall, the data suggest that *Synechocystis* sll1545 does not belong to any known GST class and represent a novel GST class, which we have named rho.

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## 1. Introduction

Peroxides and hydroxyl radicals are highly reactive and biologically toxic chemical molecules. Even though peroxides are not free radicals, they participate in redox reactions. A comparison of peroxides with dioxygen suggests that peroxides are a more reactive oxygen species [1]. While colliding with organic molecules, peroxides may extract an electron from it; this electron renders the propagation of a chain reaction, leading to the oxidation of bio-molecules such as membrane lipids, proteins, and DNA [2]. Unlike superoxides, hydrogen peroxide ($H_2O_2$) and cumenehydroperoxide (CuOOH) are highly diffusible through membranes and aqueous compartments and can directly inactivate sensitive enzymes at a low concentration [3]. Similar to superoxides, peroxides are stable and, therefore, are less toxic than other reactive oxygen species. However, they provide the environment for the generation of highly reactive hydroxyl free radicals inside the cells [4]. These highly reactive hydrogen free radicals in oxygen-derived species are known to be involved in cell injury or death.

Cells catalyze the detoxification process of peroxide radicals using various enzymes including peroxidase, catalase, and glutathione peroxidases. Some reports have suggested that glutathione s-transferases (GSTs) also show peroxidase activity in the presence of reduced glutathione (GSH) [5–9]. GSTs are enzymes that play an important role in the phase II of cellular detoxification. They catalyze the conjugation of the thiol group of GSH to electrophilic compounds resulting in soluble products that can be removed from the cytosol by ATP dependent GS–X pumps. Moreover, a growing number of non–detoxification functions have now been attributed to GSTs [10,11].

Three–dimensional structures of several GSTs have been solved, including complexes with substrate- GSH, substrate analogues, and products. Structurally, most GSTs exist in dimeric state though monomeric [12,13] and a tetrameric GST [14,15] has been reported. Despite the low level of sequence identity across the classes, all

*Abbreviations:* GST, glutathione S-transferase; GSH, reduced glutathione; CuOOH, cumene hydrogen peroxide; Non-Se-GPX, non-selenium dependent glutathione peroxidase; RMSD, root mean square deviation; RMSF, root mean square fluctuation; ED, essential dynamics; PCA, principal component analysis.

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GSTs follow a similar canonical fold, with each subunit consisting of two distinct domains. The N–terminal domain adopts a typical thioredoxin fold, consisting of central β–sheets flanking with α–helices. This domain is highly conserved and provides most of the residues for GSH binding site. It is connected to the C–terminal domain by a short linker. The C–terminal domain contributes most of the residues that interact with the hydrophobic substrate. Differences in the C–terminal domain are thought to be responsible for differences in substrate specificity between the classes [10,16]. Generally a conserved active site tyrosine, serine or cysteine residue is reported to be involved in the catalytic reaction[17]. The active site is located along the interface between the two domains with each domain contributing essential residues for functional activity. With respect to sequence similarity, GSTs that share greater than 30% identity are assigned to the same class while those with less than 30% similarity are assigned to separate classes. On the basis of sequence similarity, immunological cross reactivity and substrate specificity, the cytosolic GSTs have been grouped into at least many classes. The cytosolic GSTs have been divided into the following classes: mu, alpha, pi, theta, sigma, zeta, and omega. Organism-specific classes of cytosolic GSTs include nu (nematode), lambda, phi and tau (plants), beta (prokaryotes), delta, epsilon, iota and chi (bacteria, insects) [18–20]. It is thought that the multiple GST classes arose by a process of gene amplification followed by divergence, perhaps involving a mechanism similar to DNA shuffling, resulting in novel catalytic activities.

Cyanobacteria have a high concentration of GSH, suggesting the presence of GSH utilizing enzymes. However, there is a lack of detailed studies on cyanobacterial GSTs. Wiktelius et al. [20] and our study [21] first demonstrated the presence of GSTs in cyanobacteria that utilize isothiocyanates and belong to chi class. In our recent study, we showed that *Synechocystis* PCC 6803 sll1545 can also dehalogenate DCA and classified it to be of a novel class, which we named rho class [22]. In the current study, by using *in vitro* and *in silico* experiments, we report the novel features of the rho class GST- sll1545 of *Synechocystis* PCC 6803. We showed the novel GSH-dependent peroxidase activity of a GST of sll1545 that has not been reported in any cyanobacteria. All the GSTs with peroxidase activity have been reported to be of theta or alpha classes [6,8,9] of GSTs. Our computational results suggest significant structural and sequence difference between sll1545 and theta/alpha class GSTs, and hence, sll1545 cannot be classified under these classes. We hereby confirm sll1545 to be of a novel rho class of GSTs.

## 2. Material and methods

### 2.1. Materials

The molecular biology kits and Ni-NTA agarose were purchased from Qiagen, CA, USA. The dNTPs and enzymes were purchased from New England Biolabs, MA, USA. All other reagents and chemicals were of the highest purity available and were purchased either from Sigma-Aldrich Chemical Company, St. Louis, MO, USA or Sisco Research Laboratories, Mumbai, India. Bacterial culture media was purchased from Himedia Laboratories, Mumbai, India.

### 2.2. Production of recombinant protein

The recombinant sll1545 protein was expressed in *Escherichia coli* (*E. coli*) M15 cells and purified using the Ni–NTA agarose matrix, as described in a previous study [22]. The purified protein was dialyzed against 50 mM phosphate buffer (pH 8.0), containing 150 mM NaCl. The Bradford method was used to determine the protein concentration using bovine serum albumin (BSA) as a standard [23].

### 2.3. Determination of the peroxidase activity of sll1545 with CuOOH

The activity of recombinant GST (sll1545) protein against CuOOH was determined using the glutathione reductase (GR) coupled assay [24]. The sll1545 protein (8 nM) was added to 100 mM phosphate buffer, pH 8.0, 1 mM EDTA, 1 U BSA, 1 mM GSH, and 1 U/mL of GR, and then incubated for 30 min at room temperature. The reaction was initiated by adding CuOOH (0.5–10 mM) followed by 0.2 mM NADPH. The consumption of NADPH was determined spectrophotometrically on the basis of extinction coefficient ($\varepsilon_{340}$ nm = 6.22 $mM^{-1}$ $cm^{-1}$) at 340 nm. This determines the conversion of CuOOH into $H_2O$ that is proportional to the activity of GST. The kinetic parameters for GSH were determined using the GSH range of 0.5–5 mM. The experiments were conducted thrice, and the background data were subtracted for all experimental values. Kinetic constants were obtained using the GraphPad Prism software (CA, USA).

### 2.4. Antioxidant assay

The antioxidant assay was performed using the agar disc diffusion method. The nutrient agar plates were prepared along with ampicillin (100 μg/mL), kanamycin (50 μg/mL), and 1 mM IPTG. The *E. coli* M15 cell suspension containing the recombinant GST gene was spread on the plates and incubated at 37 °C for 1 h. Sterilized filter paper discs (Whatman) with a diameter of 7 mm were impregnated with 100 μL of CuOOH of different concentrations viz. 10, 20, 30, 40, and 50 mM. The impregnated paper discs were subsequently placed on inoculated agar plates and incubated overnight at 37 °C. The occurrence of microbial growth was determined by measuring the diameter of the zone of inhibition. For each concentration of CuOOH, the *E. coli* M15 cells that lacked the recombinant GST gene were used as controls. The scavenging activity of recombinant GST protein was determined by measuring the inhibition zones and comparing the data with the controls. The experiment was performed in triplicates, and the mean values were considered for this study.

### 2.5. Phylogenetic analysis

The sequence of sll1545 was submitted into BLASTp to search the homologs. 500 homologs were retrieved using PSI-BLAST, out of which 66 sequences were selected that represents GSTs of different classes ranging from fungi, algae, plants, parasites and human. All the sequences were aligned with the ClustalW algorithm [25] and a phylogentic tree was constructed using BLOSUM62 matrix in the Jalview package [26].

### 2.6. Ab initio *modeling and alignment*

The monomeric model of sll1545 was generated by *I-Tasser* (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) using the *ab initio* method [27]. The MatchMakermodule of UCSF Chimera 1.10.2 [28] was used for structural alignment of the 3D structure of human alpha and theta class GSTs (PDB ID: 1GSF and 2C3N, respectively) with modeled 3D structure of sll1545. It superimposes the structures pairwise by aligning their sequences and then fitting the α-carbons of residues in the same columns of the sequence alignment. For sequence similarity analyses, the sequences representing GSTs from alpha (P08263) and theta class (P30711) and sll1545 were aligned using the ESPript 3.0 software [29] that utilizes the ClustalW algorithm [25].

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