Note

Effect of Intracellular Glutathione on Heat-induced Cell Death in the Cyanobacterium, Synechocystis PCC 6803

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A correlation was found between the rate of cell death induced by heat and the GSH content of Synechocystis PCC 6803: cells accumulating GSH above the control level were more tolerant to heat than the control cells, and those containing a lower concentration of GSH were more sensitive. Lethal heating caused a decrease of GSH content, and a rapid intracellular oxidation in cells containing the decreased amount of GSH.

Key words: cyanobacteria; glutathione; lethal heat shock; oxidative stress

GSH has been reported to protect cells from oxidative damage induced by H₂O₂ and ionizing radiation. GSH reacts with the superoxide anion radical, and plays a sacrificial defense role against oxidative stress in bacterial cells, because GSH-deficient Escherichia coli cells are approximately twice as sensitive to killing by both H₂O₂ and chlorine compounds. Moreover, Davidson et al. have concluded that oxidative stress was involved in the heat-induced cell death of Saccharomyces cerevisiae, because they found that mutants without the antioxidant genes encoding catalase, SOD and cytochrome c peroxidase were more sensitive to the lethal effect of heat than wild-type cells.

The antioxidative enzymes depending on GSH such as glutathione peroxidase, glutathione S-transferase and glutathione thiol-transferase operate in eukaryotes. The GSH-dependent enzyme other than glutathione reductase is absent from the cyanobacterial cells, Anabaena sp. strain 7119 and Spirulina maxima. The gene encoding glutathione reductase in Anabaena PCC 7129 has been cloned. One group of cyanobacteria (six among ten species tested), including Synechocystis PCC 6803, has distinct but low Asc peroxidase activity acting for H₂O₂ scavenging, but the other group lacks this activity. In the Asc-GSH cycle, which involves successive oxidation and reduction of Asc, GSH and NADPH, the stroma of spinach chloroplasts, in which the Asc-GSH cycle functions to decompose H₂O₂, may contain both Asc and GSH at milli-molar concentrations. Cyanobacterial cells contain a high concentration of intracellular GSH (2-5 mM), mainly in the reduced form; the GSH content in these prokaryotes is 20-250 times higher than that of Asc (20-100 μM). However, the detection of Asc by HPLC, or by a colorimetric method which is able to measure micro-molar Asc was unsuccessful with an extract from Synechocystis PCC 6803 cells, and a homologous or similar sequence to the genes encoding the enzymes related to Asc biosynthesis has not yet been detected in the published Cyanobase (T. Ishikawa, personal communication). This background information led us to examine the antioxidative role of GSH itself. We describe here that cyanobacterial cells which accumulated GSH above the control level became more tolerant against heat-induced death than the control cells, and that those containing a lower concentration of GSH were more sensitive.

To evaluate the antioxidative role of GSH, GSH-deficient E. coli, or S. cerevisiae, treatment of a porcine kidney epithelial cell line with alkylating agents, incubation of yeast with BSO, which is the inhibitor of GSH synthetase I, and the administration of BSO to whole animals have been employed. On the other hand, the incubation of S. cerevisiae, or of the filamentous cyanobacterial cells, Phormidium lapidum, with precursor amino acids can increase the intracellular GSH level. Over-expression of GSH reductase and GSH synthetase has been shown to increase GSH in leaves of poplar trees.

We have previously reported that Synechocystis PCC 6803 cells accumulated GSH (5.9-7.0 mM) by incubating with 10 mM Glu, 2 mM Cys and 10 mM Gly for 3 h while continuously stirring in white light, whereas cells incubated without amino acids showed little change of GSH content (2.6-3.5 mM). On the other hand, the incubation of cells with 2 mM 2-ME severely decreased the cellular GSH contents (1.6-2.3 mM). The procedures to modulate the GSH level caused only slight changes in the activities of such antioxidative enzymes as SOD, catalase and GSH reductase (data not shown). We used three kinds of cells containing different levels of GSH to

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Abbreviations: Asc, ascorbic acid; BSO, buthionine sulfoximine; GSH, glutathione; Glu-Cys, γ-glutamylcysteine; 2-ME, 2-mercaptoethanol; SOD, superoxide dismutase
evaluate the role of intracellular GSH in heat-induced cell death.

Control cells showed 7.5% survival after 5 min of a lethal heat treatment at 50°C, while cells containing lower amount of cellular GSH after treatment with 2-ME survived by only 0.2% (Fig. 1). In contrast, the survival of cells which had an enhanced GSH content by preincubation with three amino acids was 74%. Extracellular GSH added to the medium at 7 mm, a similar concentration to that observed in the GSH-accumulated cells, did not protect the death of the control cells. These results indicate that the concentration of intracellular GSH was related to the heat-induced cell death of Synechocystis PCC 6803.

The level of oxidation developed during lethal heating at 50°C was measured by using 2′,7′-dichlorofluorescin diacetate as a fluorescent probe for intracellular H$_2$O$_2$\(^{(8)}\) as in the case of the lethal heat treatment to S. cerevisiae\(^{(9)}\) (Fig. 2). A progress increase in fluorescence intensity was observed during the incubation at 50°C, whereas there was no such increase when control cells were incubated at 25°C, indicating that intracellular oxidation had been caused by the heating. During the course of the lethal heat treatment, the fluorescence intensity increased rapidly in those cells containing a lower concentration of cellular GSH, whereas a lower fluorescence intensity was observed in the cells with a higher GSH level. These results indicate that the accumulation of H$_2$O$_2$ proceeded according to the intracellular GSH concentration. However, after the death of almost all the 2-ME-treated cells caused by the heat treatment within 5 min, the accumulation of H$_2$O$_2$ still continued with further incubation time, but at a lower rate than that observed in the early stage of heating.

The contents of such thiol compounds as GSH, GSSG, Glu-Cys and Cys in cells preincubated under various conditions were determined by HPLC\(^{(10)}\) before and after the heat treatment (Table 1). Although the method could determine authentic Cys, Glu-Cys, Asp, Glu, cystine, GSH and GSSG were detected in all of the cell extracts, but Cys and Glu-Cys were not detected in cells preincubated without Cys. When cells were preincubated with Cys (2 mm), but without Glu and Gly, free Cys and Glu-Cys were clearly detected, although the con-
Table 1. Contents of GSH and GSSG in *Synechocystis* PCC 6803 Cell before and after the Lethal Heat Treatment

<table>
<thead>
<tr>
<th>Cells*</th>
<th>Before treatment</th>
<th>After lethal heat treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH (mM)†</td>
<td>GSSG (mM)†</td>
</tr>
<tr>
<td>Preincubated in K-phosphate</td>
<td>3.05±0.41</td>
<td>0.23±0.06</td>
</tr>
<tr>
<td>+ Glu + Cys + Gly</td>
<td>6.42±0.55</td>
<td>0.71±0.21</td>
</tr>
<tr>
<td>+ 2-Mercaptoethanol</td>
<td>1.88±0.29</td>
<td>0.06±0.05</td>
</tr>
</tbody>
</table>

* Harvested cells were preincubated to modulate GSH content in 50 mM K-phosphate at pH 7.5, or in the presence of either Glu (10 mM), Cys (2 mM) and 10 mM Gly, or 2-ME (2 mM) for 3 hr with continuous stirring under white light (40 μmol m⁻² sec⁻¹).  
Cells were heated at 50°C for 10 min.  
Cells (1.8-2.3×10⁶) were resuspended in 700 μl of 10% PCA containing bathophenanthroline disulfonic acid. The converted thiol compounds in the resulting PCA extracts (0.5 ml) were analyzed by HPLC as described. The intracellular concentration was calculated by using the reported average cellular volume of *Synechocystis* PCC 6803 of 1.2 (±0.2)×10⁻¹¹ ml.  
† not detected.

Contents were lower than that of GSH (GSH, 5.5 mM; GSSG, 0.33 mM; Glu-Cys, 0.89 mM; Cys, 0.3 mM). Before the lethal heat-treatment, the GSH content was 87-92% of the total amount of glutathione compounds (GSH+GSSG), similar to that in other biological systems. These results obtained by the HPLC method confirmed that preincubation with a precursor amino acid or with 2-ME increased or decreased intracellular GSH, respectively, with almost all glutathione being present in the reduced form.

After the lethal heat treatment (50°C for 10 min), the cellular GSH levels decreased dramatically to less than 30% of that detected before the treatment. However, the amount of GSSG did not increase significantly, which may suggest the release of GSSG from the damaged cells. However, those cells accumulated more GSH than the control cells still maintained a relatively high level after the treatment. These results indicate that heat-induced lethal stress produced the moiety which is reactive with GSH, and resulted in the accumulation of H₂O₂ as detected by the fluorescent increase of the probe for intracellular H₂O₂, as shown in Fig. 2. GSH can react with superoxide, singlet oxygen and the hydroxyl radical.

Cyanobacteria contain carotenoids and tocopherols in the thylakoid membranes for singlet oxygen scavenging, and two types of SOD, namely FeSOD in the cytosol and MnSOD in the thylakoids, for superoxide scavenging. For H₂O₂ scavenging, catalase activity has been found to be associated with all cyanobacterial species. Mutsuda et al. have recently isolated catalase-peroxidase that is the sole hydrogen peroxide-scavenging system in *Synechococcus* PCC 7942, characterized its properties, analyzed the nucleotide sequence, and expressed its gene in *E. coli*. Catalase-peroxidase from *Anacystis nidulans* has also been purified and characterized.

The results shown here enable us to conclude that GSH itself played an antioxidative role in the cyanobacterium *Synechocystis* PCC 6803. Oxidative stress causes the depletion of cellular GSH and other thiols, and then lipid peroxidation before cell death in porcine kidney cells, in addition to the change in the protein thiol-disulfide redox status and the activation of heat shock factor after the depletion of GSH. Thus, while cyanobacterial SOD and catalase activities would scavenge active species of oxygen until they were inactivated by heat, GSH also plays an antioxidative role, and also reduces the formation of protein disulfide by oxidative stress. To support these functions of GSH, cyanobacterial cells may contain a higher concentration of GSH, and accumulate GSH by the addition of precursor amino acids, although they have no GSH-dependent antioxidative enzymes other than glutathione reductase.

References


