Free Rad. Res. Comms., Vols. 12-13, pp. 845-850
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OXIDATIVE STRESS RESPONSES IN THE UNICELLULAR CYANOBACTERIUM *SYNECHOCOCCUS* PCC 7942

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Oxidative stress responses were tested in the unicellular cyanobacterium *synechococcus* PCC 7942 (R-2). Cells were exposed to hydrogen peroxide, cumene hydroperoxide and high light intensities. The extent and time course of oxidative stress were related to the activities of ascorbate peroxidase and catalase. Ascorbate peroxidase was found to be the major enzyme involved in the removal of hydrogen peroxide under the tested oxidative stresse. Catalase activity was inhibited in cells, treated with high H_2O_2 concentrations, and was not induced under photooxidative stress. Catalase was specifically induced in cells treated with cumene hydroperoxide.

Superoxide dismutase activity increased under conditions generating superoxide, such as high light intensities. The induction of the antioxidative enzymes was light dependent and was inhibited by chloramphenicol.

KEY WORDS: Ascorbate peroxidase, catalase, superoxide dismutase, hydrogen peroxide, cumene hydroperoxide.

INTRODUCTION

Higher plant chloroplasts and cyanobacteria were found to generate H_2O_2 during photooxidation[1] and photorespiration.[2] The toxicity of H_2O_2 to cyanobacteria involves the production of hydroxyl radicals [3] and the direct inhibition of Calvin cycle enzymes.[4] The hydroxyl radicals were found to interact with cell components, causing lipid peroxidation and DNA strand brakes. [5,6] Removal of H_2O_2 is therefore essential in order to prevent damage under normal growth and stress conditions.

In chloroplasts, the ascorbate peroxidase pathway is a major route for H_2O_2 removal.[7] Cyanobacteria also posses ascorbate peroxidase and its ascorbate regenerating enzymes: dehydroascorbate reductase and glutathione reductase.[8] Cyanobacteria in contrast to chloroplasts were found to contain only low intracellular concentrations of ascorbate, and to exhibit catalase activity which was not found in chloroplasts. The importance of cyanobacterial catalase is questionable since in *Nostoc muscorum* catalase activity was reduced in diluted culture [8] but was increased in *Anabaena cylindrica* under high oxigen pressure. [9] The present investigation demonstrates that cyanobacterial ascorbate peroxidase, rather then catalase, is the major H_2O_2 detoxyifing enzyme under stress conditions.

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MATERIALS AND METHODS

Cell growth

Synechococcus R2 (PCC 7942) was cultivated in BG-11 mineral medium with 1 g/l $NaNO_3$. Cells were grown at 35°C in 250 ml erlenmeyer flasks, on a shaker and illuminated with fluorescent cool white light (I = 50 uE/m^2/sec). For large scale growth experiments a New Brunswick 10 liter culture fermentor was used.

Oxidative Stress Experiments

Cells were treated with the oxidative agents in 10 ml cultures. The cells were then harvested, washed in 0.1 M K_2HPO_4 pH 7.5 plus 5 mM EDTA and sonicated at 4°C. Cell extracts were tested for enzymatic activity.

Enzymatic Assays

Ascorbate peroxidase activity was measured by the decrease in ascorbate content at λ = 290 nm due to H_2O_2 addition to the assay mixture. [10] SOD activity was estimated by the decrease in the rate of horse heart cytochrome C reduction at λ = 549 nm, upon addition of cell extract to the assay mixture.[9] Catalase activity was determined from the rate of oxygen evolution, upon addition of H_2O_2 to cell extracts.[8]

Analytical Methods

Chlorophyll a was determined from 80% (v/v) acetone extracts.[16] Protein content was determined according to Bradford.[17]

RESULTS AND DISCUSSION

The Pattern of Antioxidative Enzymes During Cell Growth

During growth in batch culture, cyanobacterial cells are subjected to different light intensities per cell, due to increacing culture density. In diluted cultures the light intensity per cell is high and it decreases during cell growth.

Figure 1 demonstrates the antioxidative enzymatic activities in *Synechococcus* R-2 during growth in batch culture fermentor. Immediatly after inoculation ascorbate peroxidase and SOD were induced due to the exposure of cells to high light. Four hours after inoculation and during exponential growth phase, ascorbate peroxidase and SOD activities decreased due to increase in cell density (Figure 1). The positive corelation between ascorbate peroxidase activity and light intensity per cell was also confirmed using cultures at identical cell density subjected to increasing light intensities (Figure 2). Hence induction of ascorbate peroxidase does not relate to the age of the culture, and is therefore assumed to be a response to photoproduction of superoxide and H_2O_2. Catalase activity was not affected during exponential growht of cell culture, but increased towards late stationary growth phase (data not shown).

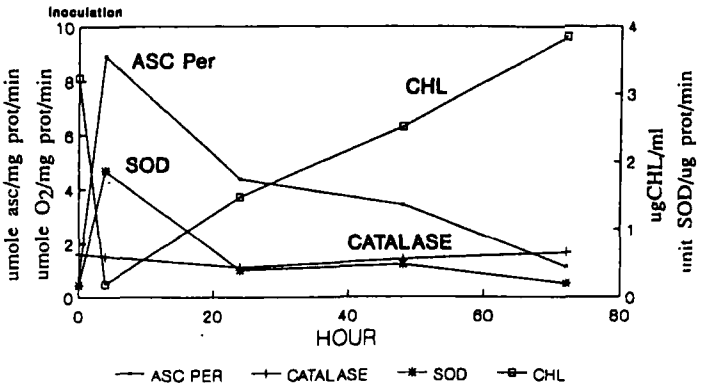

FIGURE 1 Ascorbate peroxidase, catalase and SOD activity during cell growth in culture fermentor. Enzymes were assayed as described in material and mehtods, ASC Per-ascorbate peroxidase, CHL-chlorophyll a, SOD-superoxide dismutase.

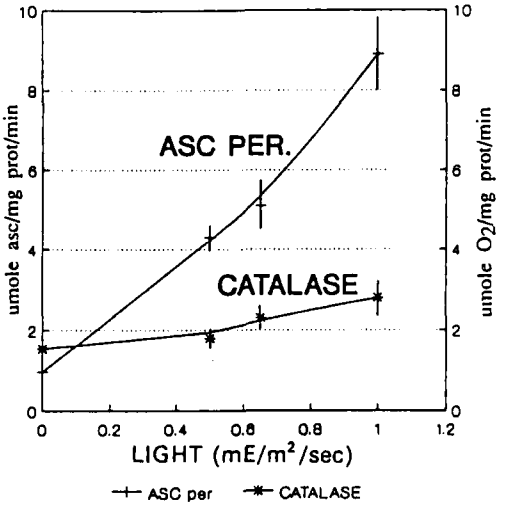

FIGURE 2 Ascorbate peroxidase and catalase activity in *Synechococcus* culture of identical density under increasing light intensities. Cultures of 0.52 OD at 720 nm were illuminated for 1 hour.

These results are in agreement with those obteined by Tel-Or *et al.*,[8] suggesting that ascorbate peroxidase is superior to catalase in the removal of H_2O_2 under photooxidative conditions. This conclusion is also supported by reports on catalase inhibition by high light.[11]

Increased activities of catalase during late stationary phase was also reported for *E. coli*[12] and is assumed to be triggered by stationary phase cell metabolisim.

Enzymatic Response to Peroxide Stress

Peroxide stress response was tested to determine whether induction of ascorbate peroxidase was driven directly by light or through the photoproduction of H_2O_2. Figure 3 shows that application of 20 mM H_2O_2 to growth medium resulted in a 4-fold increase of ascorbate peroxidase activity. The induction of ascorbate peroxidase activity was inhibited by chloramphenicol (75 ppm) suggesting the involvement of protein synthesis in the induction response. The inhibition of catalase activity observed under 20 mM H_2O_2 stress (Figure 3) is supposed to be derived from high

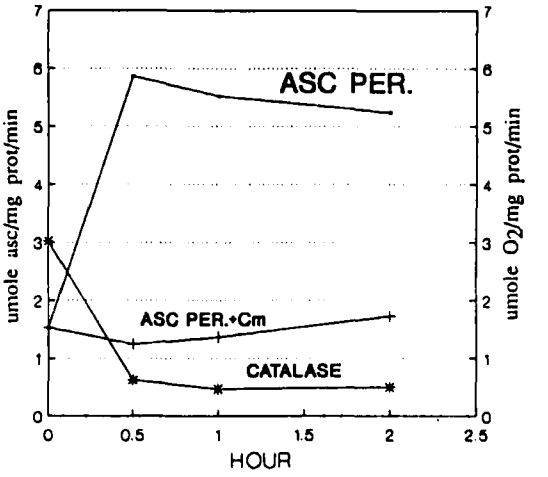

FIGURE 3 Time course of enzyme reponse during 20 mM H_2O_2 ASC PER-ascorbate peroxidase, Cm-chloramphenicol.

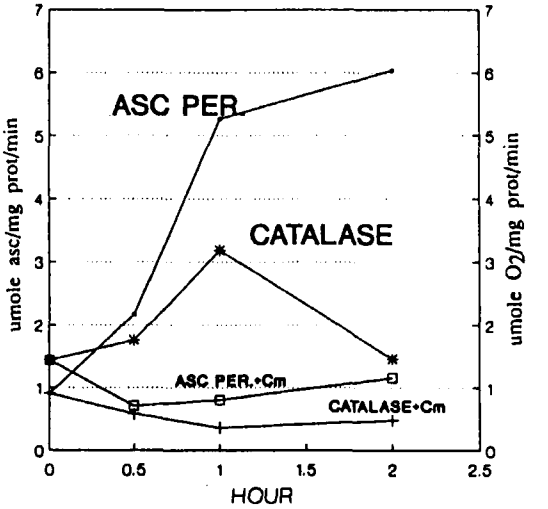

FIGURE 4 Time course of induction during 8 mM cumene hydroperoxide stress, ASC PER-ascorbate peroxidase, Cm-chloramphenicol.

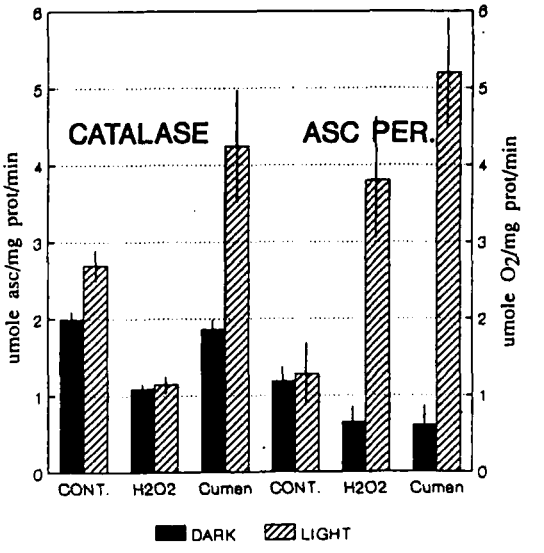

FIGURE 5 Light dependence of ascorbate peroxidase and catalase induction. Cells were treated for one hour with H_2O_2 and cumene hydroperoxide as described in Figure 2 and Figure 3. Key: Cont-control, Cumen-cumene hydroperoxide.

intracellular concentrations of monodehydroascorbate produced by the induced ascorbate peroxidase and found to be a potent inhibitor of catalase [13]. These results suggest that high intracellular concentrations of H_2O_2, under light, are required to trigger the induction of ascorbate peroxidase.

Cumene hydroperoxide, an organic peroxide, was used to simulate byproducts of lipid peroxidation and was found to be a substrate of cyanobacterial ascorbate peroxidase [8]. Peroxide stress triggered by cumene hydroperoxide, as shown in Figure 4, induced the activity of ascorbate peroxidase and catalase. Induction of these enzymes is also shown to be inhibited by chloramphenicol (Figure 4). The induction of ascorbate peroxidase under cumene hydroperoxide stress (Figure 4), was slower than the induction obtained with H_2O_2 (Figure 3). The induction of ascorbate peroxidase under simulated lipid peroxidation, via cumene hydroperoxide, demonstrates potential importance of preventing autoxidative damage caused by lipid peroxidation. Induction of catalase by cumene hydrogen peroxide was also reported in *Salmonella typhimurium*.[14] However, catalase plays a limited role in comparison to ascorbate peroxidase during peroxide stress in *Synechococcus*.

However this does not necessarily mean that catalase plays a limited role when H_2O_2 is produced endogenously.

The Light Dependence of Antioxidative Enzyme Induction

Peroxide stressed *Synechococcus* cells in the dark exhibits low activities of ascorbate peroxidase and catalase, compered to peroxide treated cells in the light. More recently it was found that ascorbate content was reduced in peroxide treated cells in the light and in the dark (data not shown). Hence it is suggested that the lower ascorbate

peroxidase activities in dark peroxide stressed cells is not due to previously proposed sensativity of the enzyme to H_2O_2 [10,15], therefore it is assumed that expression of ascorbate peroxidase is light reguleted in *Synechococcus* cells.

Acknowledgement

This research was supported by a grant from the Ministry of Technology FRG.

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Accepted by Prof. G. Czapski