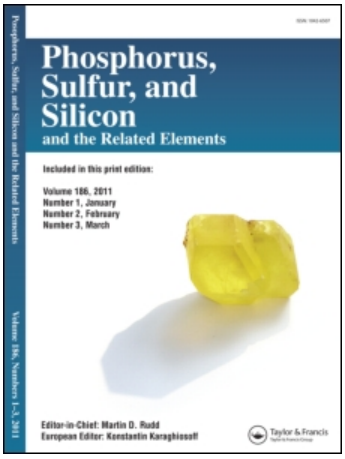

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INDUCTION OF GLUTATHIONE PEROXIDASE BY SELENITE AND ITS PHYSIOLOGICAL FUNCTION IN *Chlamydomonas reinhardtii*

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Abstract Feeding of selenite to *Chlamydomonas* grown under illumination and ordinary air (0.03% CO_2 concentration) caused the activity of glutathione peroxidase (GHSP) to increase and reach a peak after 24 hr. The inhibitive effect of cycloheximide and immunochemical titration showed that the increase in GSHP activity results from an increase in the amount of protein. Transfer of *Chlamydomonas* cells either from low CO_2 to high CO_2 (5%) or from the light to the dark together with the addition of selenite stopped the increase of the enzyme activity, indicating that low CO_2 concentration in the atomosphere and high light intensity were also required for the induction of the GSHP with high activity.

INTRODUCTION

Glutathione peroxidase (GSHP) is widespread in many tissues and organs of mammalians and functions in the protection of the cell from oxidative damage by scavenging of hydrogen peroxide (H_2O_2) and lipid peroxides[1]. GSHP is a selenium-containing enzyme and the active site consists of selenocysteine encoded by TGA, normally nonsense codon[2].

We have previously found that culture of the unicellular green alga *Chlamydomonas reinhardtii* in a medium containing sodium selenite caused the activity of

ascorbate peroxidase (AsAP) to disappear and GSHP to appear[3]. The enzymic, physicochemical and immunological properties of GSHP closely resembled those from mammalian sources[4].

In this study we report on the induction of GSHP by the addition of sodium selenite to Chlamydomonas cells grown in a medium without selenium and the effect of external environments such as CO_2 concentration and light intensity on the induction of GSHP. We also discuss the physiological function of the enzyme in Chlamydomonas reinhardtii.

MATERIALS AND METHODS

Chlamydomonas reinhardtii Dangeard was grown aseptically in Allen's medium without sodium selenite under continuous illumination (10,000 lux) at 27°C and bubbling with ordinary air (0.03% CO_2) or air containing 5% CO_2. The preparation of crude enzyme and the assay of GSHP and AsAP were done by the methods described previously[4]. Immunochemical titration was performed by the method of Cannons and Merrets[5].

RESULTS AND DISCUSSION

When cultured under illumination in the medium that contains no selenium, Chlamydomonas cells contained AsAP activity. No GSHP activity was detected at all[3]. Transfer of the cells to a medium containing 3 mg of sodium selenite per liter caused the activity of GSHP to appear and reach a peak at the growth stage (5 days). In order to elucidate in detail the appearance of this enzyme, selenite was fed to the cells grown for 5 days in the medium without selenite. As shown in Fig. 1, GSHP activity increased to reach a peak after 24 hr and then decreased gradually, while AsAP activity was

perfectly lost after 6 hr. The increase of GSHP activity by the addition of selenite was inhibited by cycloheximide but not by chloramphenicol and streptomycin. The immunochemical titration using the antibody raised against bovine erythrocyte GSHP was done on the crude extracts prepared from the cells adapted for 5 hr and 18 hr to sodium selenite. The amount of GSHP activity at 5 hr and 18 hr inhibited by a fixed amount of the antibody was identical for both enzyme preparations (Fig. 2). These results indicate that the increase of the enzyme activity is due to de novo synthesis of the enzyme protein and not to an activation of the pre-existing protein.

When the concentration of CO_2 was increased from the air level (0.03%) to 5% CO_2 in air at the time of addition of sodium selenite, the increase of GSHP activity was perfectly suppressed. When the cells were again transferred to the low CO_2 concentration, the activity increased to the similar level to that observed in the cells continuously grown at air level of CO_2 (Fig. 3).

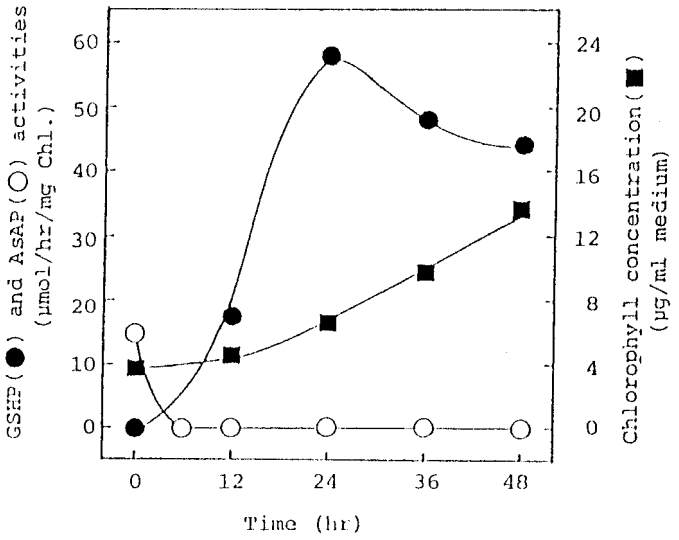

FIGURE 1. Effect of the addition of sodium selenite on GSHP and AsAP activities.

Transfer of Chlamydomonas cells from the light to the dark completely arrested the increase of the enzyme activity. Reillumination caused a renewed increase at the same rate as that in the continuously illuminated cells (Fig. 4). Illumination at 6,000 lux gave a increase of the enzyme activity with a lag phase of 6 hr but allowing to reach the same level as did that at 10,000 lux. When the cells were illuminated at 2,000 lux, the enzyme activity increased gradually with a lag phase of 12 hr to reach a level which was about 40% of the level attained by the cells illuminated at 10,000 lux. The increase of GSHP activity was inhibited 72.3% by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

These results indicate that the induction of GSHP activity by the addition of sodium selenite requires low CO_2 concentration and high light intensity in the atmosphere during the culture. It has been demonstrated that the green alga Chlamydomonas induces the CO_2-concentrating mechanism for photosynthesis when grown at low CO_2 concentration in air atmosphere[6,7]. A large amount of ATP is required for the operation of the mechanism to

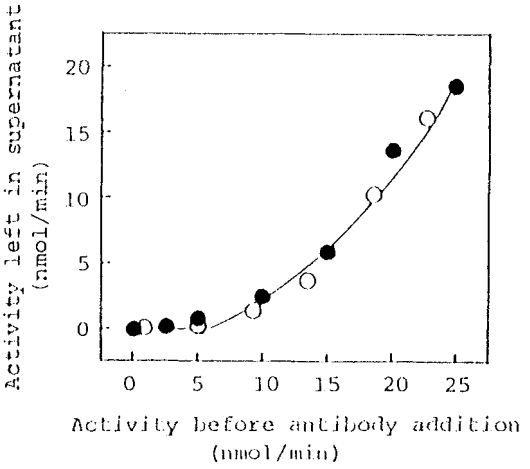

FIGURE 2. Immunochemical titration of GSHP from Chlamydomonas cells subjected to 5hr(●)and 18hr (○) of the addition of sodium selenite.

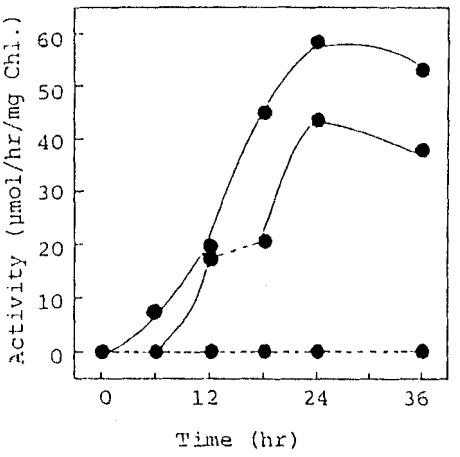

FIGURE 3. Effect of CO_2 concentration on the increase of GSHP activity. The broken line shows time under high CO_2 concentration (5% CO_2).

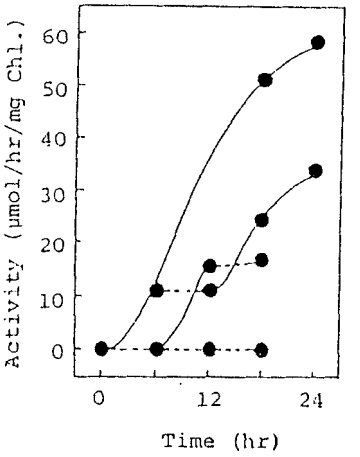

FIGURE 4. Effect of illumination on the increase of GSHP activity. The broken line shows time in the dark.

concentrate CO_2 or HCO_3^- in the cells or chloroplasts from the surrounding medium[8 9]. The energy compound is supplied from the pseudocyclic electron transport system of photosynthesis, which reduces molecular oxygen (O_2) to superoxide anion radicals (O_2^-). The generated O_2^- is immediately converted to H_2O_2 and O_2 by superoxide dismutase in chloroplasts[10]. Accordingly, it is conceivable that the transfer of Chlamydomonas cells either from low CO_2 to high CO_2 concentration or from the light to the dark concurrently with the addition of selenite arrests the operations of the CO_2-concentrating mechanism and the pseudocyclic electron transport system and, consequently, stops H_2O_2 generation in chloroplasts. The rate of H_2O_2 produced in pseudocyclic electron transport system goes up to 100 to 150 μmol/mg chlorophyll/hr[8 9]. The activity of AsAP is less than 20% that of selenite-induced GSHP and is insufficient to decompose H_2O_2 synthesized in Chlamydomonas cells grown at low CO_2 concentration. In addition, catalase located in peroxisomes has a low affinity for H_2O_2, and cannot detoxify H_2O_2 in the chloroplasts. These considerations

lead us to conclude that selenite-induced GSHP mainly works for scavenging H_2O_2 produced at high rates in the pseudocyclic electron transport to produce ATP. Inhibition of the induction of this enzyme activity by DCMU supports this view.

In relation to CO_2-concentrating mechanism, carbonic anhydrase (CA), which catalyzes the reversible hydration of CO_2, is known to play a role in concentrating CO_2 at the active site of ribulose 1,5-bisphosphate carboxylase /oxygenase and thus promoting photosynthetic CO_2 fixation[7 11]. CA is also induced by low CO_2 concentration, which induction is dependent on light intensity[12]. The simillar responce of CA activity to that of GSHP supports that GSHP induced by selenite closelycorrelates with CO_2-concentrating mechanism.

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