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Communication

Selenium as Inducer of Glutathione Peroxidase in Low-CO_2-Grown *Chlamydomonas reinhardtii*[1]

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ABSTRACT

Culture of the green alga *Chlamydomonas reinhardtii* in the medium containing sodium selenite caused the activity of ascorbate peroxidase to disappear and the appearance of glutathione peroxidase. The induced maximum activity of glutathione peroxidase reached 350 micromole (milligram chlorophyll hour)$^{-1}$ under assay conditions used. The enzymic properties of the selenite-induced glutathione peroxidase closely resembled those of animal glutathione peroxidase that contains selenium.

Living matter contains catalase, AsA-POD,[2] and GSH-POD, enzymes which all decompose one of the toxic form of oxygen, H_2O_2 (6). Catalase is found only in peroxisomes, has a low affinity for H_2O_2, and cannot decompose the organic hydroperoxides. Thus, AsA- and GSH-PODs detoxify such active oxygens in energy-generating organelles (6, 17).

In photosynthetic organisms, light energy is directed to O_2 reduction when the amount of CO_2 available for photosynthesis is limiting (4, 6). Oxygen is also reduced during the induction period that takes place when dark-adapted photosynthetic organisms are transferred to lighted conditions (7, 11). In addition, green algae, grown in low concentrations of CO_2 (as in the air) constantly form H_2O_2 in pseudocyclic electron transport to produce ATP. The ATP is used to concentrate CO_2 or HCO_3^- in the cells or chloroplasts from the surrounding medium (15, 19). The rate of formation of H_2O_2 is 100 to 150 μmol (mg Chl h)$^{-1}$ (15, 19). It is not known what enzyme detoxifies H_2O_2 which is synthesized at such high rates. We report here induction of GSH-POD in *Chlamydomonas reinhardtii* by a small amount of sodium selenite in the culture medium.

MATERIALS AND METHODS

Chlamydomonas reinhardtii Dangeard was grown aseptically in Allen's medium (1) with or without sodium selenite (3 mg L^{-1}). The culture was bubbled with sterile air or air containing 5% CO_2 and was illuminated 55 to 80 μE m^{-2} s^{-1} or at 240 μE m^{-2} s^{-1}.

The algal cells were collected by centrifugation at 3,000*g* for 5 min and suspended in 100 mM Tris-HCl buffer (pH 8.3) containing 300 mM sucrose. The cells were disintegrated by twice passing them through a cooled French pressure cell at 400 kg cm^{-2}, and then the cell homogenate was centrifuged at 10,000*g* for 20 min. The obtained supernatant was used as the enzyme solution.

The activity of GSH-POD was assayed spectrophotometrically (18). The assay mixture consisted of 100 mM Tris-HCl (pH 8.3), 1.0 m*M* glutathione, 0.1 m*M* H_2O_2, 0.2 mM NADPH, 2 units of glutathione reductase, 0.1 mM NaN_3 and enzyme solution. The activity of AsA-POD was determined as described previously (12).

Chl (19) and protein (3) were determined by the methods described in cited references.

RESULTS AND DISCUSSION

GSH-POD, which decomposes H_2O_2 and organic hydroperoxides in mammals and birds, is a selenium-containing protein (13, 17). We have now studied the possibility that selenium added to the growth medium of a green alga would induce an animal-type GSH-POD. *C. reinhardtii* was first cultured in the light (55–80 μE m^{-2} s^{-1}) in the culture medium that contains no selenium compounds. The cells contained AsA-POD corresponding to the activity of 15 to 20 μmol (mg Chl h)$^{-1}$, but no GSH-POD activity. The cells was transferred to a medium containing 3 mg of sodium selenite per liter and illuminated at 240 μE m^{-2} s^{-1}. The culture was bubbled with sterile air. The change in the activity of GSH-POD under these conditions is shown in Figure 1, together with the Chl concentration in the culture medium. The enzyme activity increased to 350 μmol (mg Chl h)$^{-1}$ (equivalent to 4.4 μmol [mg protein min]$^{-1}$) at the growth stage corresponding to 3 to 4 μg of Chl ml^{-1}. Thereafter, the activity decreased. When the culture was illuminated at 55 μE m^{-2} s^{-1}, the activity of GSH-POD was only one-fifth that at 240 μE m^{-2} s^{-1}. This suggests that the decrease in the activity after d 4 (Fig. 1) was due to a decrease in the light intensity at the cell surface in the culture which was dense by that time. Sodium selenite was effective only if added to the culture medium, as is true for the induction of formate dehydrogenase in *Escherichia coli* (10) and glycine reductase in *Clostridium sticklandii* (16), both enzymes containing selenium (14). GSH-POD in *C. reinhardtii* was induced only when the culture was bubbled with air (340 ppm CO_2). Cells grown in a 5% CO_2 atmosphere, in which the organism does not develop the mechanism for concentrating CO_2 (2, 19), contained an activity of 36 μmol (mg Chl h)$^{-1}$ at the growth stage corresponding to 5 μg of Chl ml^{-1}. GSH-POD was totally absent in cells grown in the absence of sodium selenite.

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[2] Abbreviations: AsA-POD, ascorbic acid peroxidase; GSH-POD, glutathione peroxidase.

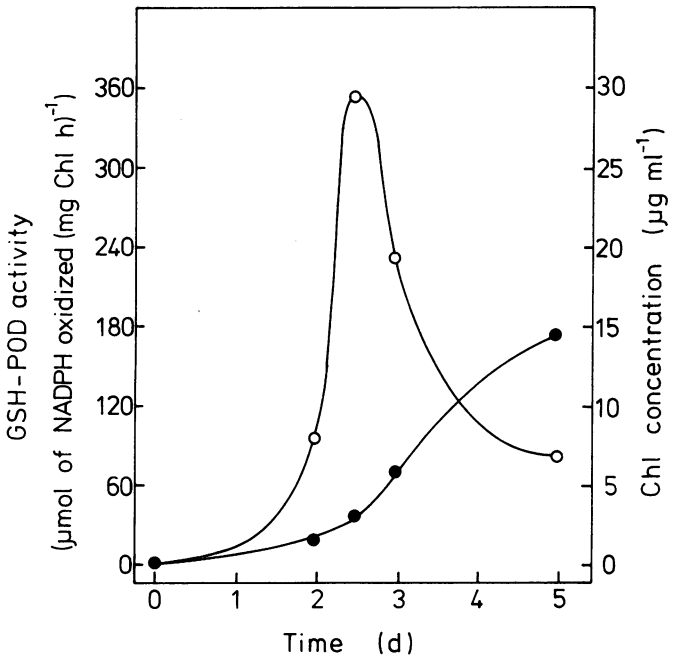

FIG. 1. Changes of the activity of GSH-POD (○) during the growth of *C. reinhartdii* in the presence of 3 mg of sodium selenite per liter; (●) concentration of Chl in the culture medium. The initial Chl concentration was 0.3 μg ml^{-1}.

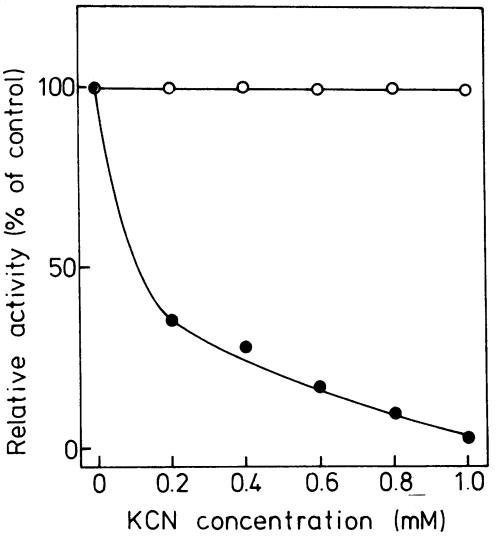

FIG. 2. Changes in the activities of GSH- (○) and AsA- (●) PODs with various concentrations of potassium cyanide. The activities of GSH- and AsA-PODs were 350 and 19 μmol (mg Chl h)$^{-1}$, respectively, in the absence of KCN.

The reaction medium for the assay of GSH-POD contained 0.1 mM NaN_3. Further addition of KCN up to 1 mM did not inhibit the activity of GSH-POD, but AsA-POD from *C. reinhardtii* grown without selenium at 55 μE m^{-2} s^{-1} was inhibited completely by this concentration of KCN (Fig. 2), as is *Euglena* AsA-POD that contains heme as the prosthetic group (12). The selenite-induced GSH-POD had an optimum pH 8.0 to 8.3 for activity; the activity was almost negligible at pH 7.0 and 8.9 (data not shown). The enzyme was specific for glutathione; neither guaiacol or pyrogallol could be used instead of glutathione. The

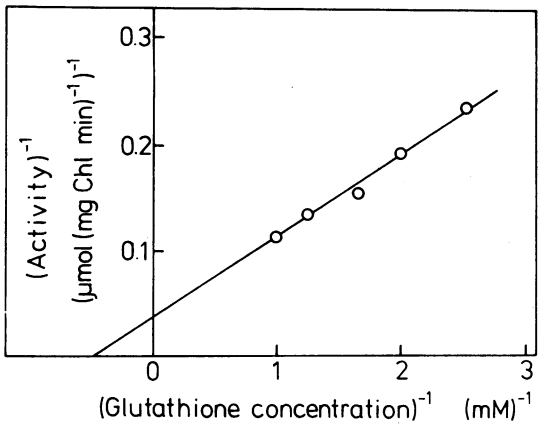

FIG. 3. Double-reciprocal plots of the activity of selenium-induced GSH-POD at different concentrations of glutathione. The concentration of H_2O_2 in the assay mixture was 0.1 mM.

apparent K_m value for glutathione was 2.2 mM in the presence of 0.1 mM H_2O_2 (Fig. 3). The K_m value for H_2O_2 was 0.15 mM in the presence of 1 m*M* glutathione (data not shown). The V_{max} of the activity was 1500 μmol (mg Chl h)$^{-1}$ in the presence of 0.1 mM H_2O_2 (Fig. 3). Cumene hydroperoxide and *tert*-butyl hydroperoxide could substitute for H_2O_2 in the enzymatic reaction. The ratio of the reactivities of H_2O_2, cumene hydroperoxide and *tert*-butyl hydroperoxide was 1:1.1:0.5. These enzymic properties of the GSH-POD induced by selenium in *C. reinhardtii* were similar to those of the GSH-POD that contains selenium (17).

The results shown here contradict a widespread belief about the distribution of peroxidase. The AsA-POD in *C. reinhardtii* grown under conventional culture conditions was replaced by an animal-type GSH-POD when the organism was grown in the presence of selenium. High light intensity and low CO_2 concentrations in the atmosphere during the culture were also needed for the induction of the GSH-POD with high activity. In the presence of glutathione at the concentration of 4 mM or more, which has been found in photosynthetic organisms (6, 9), the GSH-POD activity exhibits rates of over 700 μmol (mg Chl h)$^{-1}$. These considerations lead us to conclude that the selenium-induced GSH-POD can decompose H_2O_2 formed in large amounts when *C. reinhardtii* is concentrating CO_2. H_2O_2 formed in the cells without the GSH-POD must be decomposed by catalase, since addition of H_2O_2 to these cells caused evolution of O_2, which was inhibited by 0.1 mM sodium azide (data not shown). Selenium should be added to the culture medium for *C. reinhardtii* to study its natural way of life. Natural fresh water contains 0.2 to 2.0 μg of selenium per liter (5, 8), so it is likely that *C. reinhardtii* is originally an organism that requires selenium.

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