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Glutathione In Cyanobacteria

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Introduction

Glutathione, the tripeptide gamma glutamyl cysteinyl glycine (γ-glu-cys-gly), a nearly universal constituent of eukaryotic cells (Fahey, et al., 1984), has been found in many eubacteria (Fahey et al., 1978) including cyanobacteria (Fahey, personal communication). A related compound, γ-glu-cys, has recently been found in halobacteria (Newton and Javor, 1985). Numerous speculations on the function of glutathione have been put forth but they are based on scanty evidence. Glutathione may protect cells from peroxides (Mills, 1957). However, trends in glutathione production are not predictable; other functions for glutathione may exist for which the cell regulates its production.

The microelectrode and geochemical studies show that dramatic environmental gradients in microbial mat communities occur in the first 2 mm of the surface. Within this region steep gradients of oxygen, light, and hydrogen sulfide occur. The adaptations of several organisms to sulfide conditions by means of dissimilatory sulfur pathways have recently been described (Cohen, et al., 1975; Garlick, et al., 1977) which suggest an influence on local environments and the sulfur cycle.

Organisms adapt physiologically to their environment according to need, such as chromatic adaptation of phycobiliproteins in cyanobacteria. Similarly, changes in microbial mat environments of light, oxygen, and hydrogen sulfide may alter the need for glutathione. Light is responsible for both the photolytic damage to cells and oxygenic photosynthesis in cyanobacteria. The presence of O_2 and photolytically produced free radicals may increase the need for glutathione because of increased peroxide production. Conversely, the presence of H_2S, which combines with O_2 to form thiosulfate, may reduce the need for glutathione where the two gases coexist. We sought to determine the effects of light and O_2 on glutathione production. A preliminary study on the effects of the glutathione synthetase inhibitor, buthionine sulfoximine (S-n-butyl homocysteine sulfoximine or BSO for short), was also initiated.

Materials and Methods

Total glutathione, both oxidized and reduced forms of glutathione, and homoglutathione (Fahey and Newton, 1983) were assayed by the enzymatic method of Tietze (1969) modified by Fahey, et al., (1975) and expressed as nanograms of oxidized glutathione per mg residual dry weight.

Inhibition of glutathione synthesis by the -glutamylcysteine synthetase inhibitor BSO (S-n-butyl homocysteine sulfoximine) of molecular weight 232 (Griffith and Meister, 1979) was tested on *Anacystis nidulans* by its addition to the culture media in varying concentrations ranging from 10 uM to 5 mM. Cells were harvested by centrifugation and assayed for glutathione.

An axenic culture of *Anacystis nidulans* was obtained from Yehuda Cohen and grown on media BG-11 (Rippka, et al., 1979).

Aphanothece halophytica-dominated waters collected from the 150 per mil pond (Pond 4) were maintained at 25°C in closed vessels. A light period of 12 hours was followed by a dark period of 12 hours (40 to 0 $uE\ m^{-2}\ s^{-1}$ from incandescent 40 watt bulbs), very low light.

Growth curves of *Anacystis nidulans* and total glutathione were determined in a series of 9 2-liter Erlenmyer flasks fitted with cotton plugs and containing 750 ml BG-11 culture media and 75 ml. inoculum of stationary phase cells. Cell density was measured by absorbance at 550 nm. Cultures were sequentially harvested by centrifugation and total glutathione measured.

Light and dark effects on glutathione levels in *Anacystis nidulans* under normal oxygen tension were determined after placing 200 ml of log phase cells with a sterile supplement of 5 mM $NaHCO_3$ into 500 ml Erlenmyer flasks in light (20 and 0 $uE\ m^{-2}\ sec^{-1}$ Sylvania F40-GRO fluorescent lighting for 24 hours) and dark condtions. Aeration was provided at a rate of 0.35 liters per minute.

Light and dark effects on glutathione levels in *Anacystis nidulans* under reduced oxygen tension were determined after placing 200 ml of log phase cells with a sterile supplement of 5 mM $NaHCO_3$ in 500 ml Erlenmyer flasks in light (20 and 0 $uE\ m^{-2}\ sec^{-1}$ from Sylvania F40-GRO fluorescent light) and dark conditions for 24 hours. Oxygen tension was lowered by passing cotton filtered N_2 through the media at a rate of 0.35 liters per minute.

Results and Discussion

Results of light and dark studies under normal and reduced oxygen tensions were compared to determine the effect of reduction in oxygen tension on glutathione levels.

The growth rate of *Anacystis nidulans* and concurrent production of glutathione is presented in Figure IV-18. The generation time of *Anacystis nidulans* was approximately 12 hours. Throughout growth, glutathione levels remained between

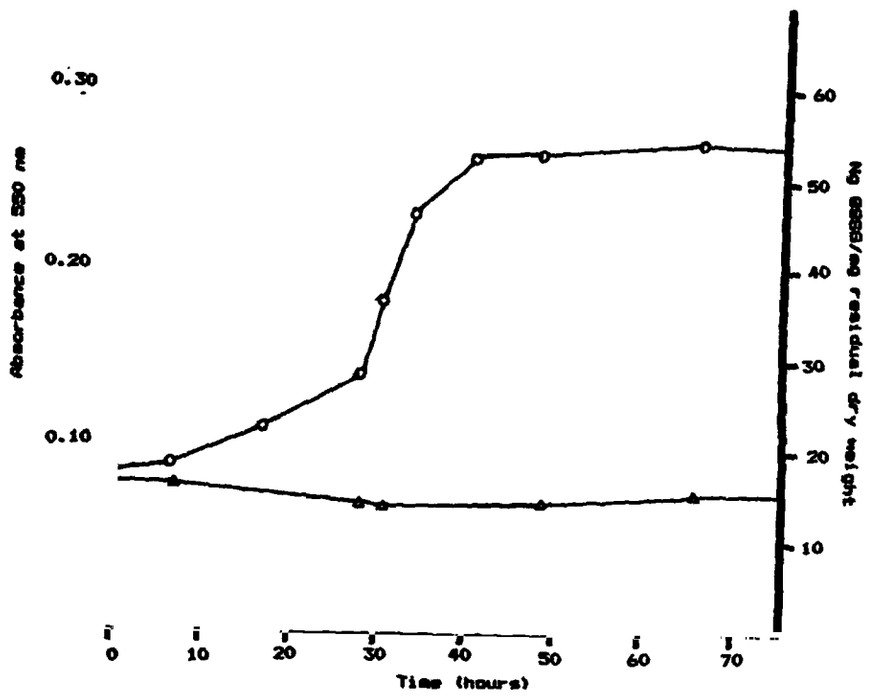

Figure IV-18. *Anacystis nidulens* growth curve comparing levels of oxidized glutathione (GSSG) per mg of residual dry weight. —▲— = glutathione levels; —○— = growth

16.0 and 13.0 ng glutathione per mg residual dry weight of cells when grown under incandescent lighting of 40 uE m^{-2} sec^{-1}, thus showing a slight trend toward reduced levels during log phase. This allowed the subsequent comparison between light and dark incubated cells which would presumably no longer exist in the same growth phase.

Results of light and dark incubation of *Aphanothece halophytica*-dominated planktonic microbial community from Pond 4 and *Anacystis nidulans* under high and low oxygen tension is presented in Table IV-5. Unexpectedly, both *Aphanothece halophytica* samples and *Anacystis nidulans* cultures show increased levels of glutathione after 24 hours of continuous dark. While *Aphanothece* samples exhibited only approximately a 10 percent increase, *Anacystis* (O_2) and *Anacystis* (N_2) cultures showed approximately a 100 percent and 50 percent increase, respectively, thus revealing a diurnal variation.

Different light sources were used for growing *Anacystis* and for the light and dark study under normal and reduced oxygen tension. The former used 40 uE m^{-2} sec^{-1} from 40 watt incandescent lamps and the latter 20 uE m^{-2} sec^{-1} from F40-GRO fluorescent lighting. Apparently, there is an increased glutathione level; the lower intensity presumably also differs qualitatively.

It appears that light-grown *Anacystis nidulans* cells have equal amounts of glutathione while dark-grown cells produce more glutathione in the presence of increased O_2. Since *Aphanothece halophytica*-dominated planktonic communities grow under reduced oxygen tensions in this high salinity pond, this may account for the relatively lower percentage increase at night.

The results of the glutathione synthesis inhibition by BSO are presented in Table IV-6. Although some concentrations of BSO apparently result in approximately one third of the control level of glutathione, the highest concentration of BSO used (5 mM) showed no inhibition. Thus BSO is not an effective inhibitor of glutathione synthesis in *Anacystis nidulans*. It is not known whether the lack of inhibition is due to lack of sensitivity by the enzyme, lack of transport into the cell, or detoxification by intracellular or extracellular means.

These preliminary studies do show an environmentally determined pattern of cellular glutathione. The effects, if any, of H_2S, organic sulfur, or other metabolites remain to be tested.

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Plate Depth (mm) (plates)	*P. jugosus* (presumptive)	Manganese oxidizers: quantity	diversity	spread away from sediment inoculum
1	–	+	+	+
2	++	+	–	–
3	+	+	–	–
4	++	+	–	–
5	++	++	+	+
6	+++	+++	+++	+++
7	+	+	+	–
8	+	+	+	–
9	+	+	+	–
10	+	+	+	–

Key to diversity

–	absent
+	present, more than or equal to 3 different colony morphotypes
++	present, more than or equal to 3-5 different colony morphotypes
+++	present, more than or equal to 6 different colony morphotypes

Table IV-5. Colonies of *Paratetramitus jugosus* and manganese-oxidizing bacteria as a function of depth.

	Light	Dark
Aphanothece (7/10/84)	4.9	5.6
Aphanothece (7/24/84)	4.4	5.4
Anacystis (O_2)	41.4	82.5
Anacystis (N_2)	43.2	57.1

Table IV-6. Effect on glutathione levels by light and dark conditions of *Aphanothece halophitica*-dominated planktonic community and on axenic cultures of *Anacystis nidulans* under high (O_2) and low nitrogen (N_2)

BSO Concentration (μM)	0	10	100	500	1000	5000
ng glutathione mg^{-1} residual dry weight	50	23.9	22.4	20.4	20.8	56.2

Table IV-7. Effect of increasing concentrations of BSO on glutathione levels in *Anacystis nidulans*

aerobic	anaerobic	sulfide concentration (mM)								
	0	0.01	0.02	0.05	0.1	0.2	0.5	1	2	4
no sulfide	+++	+++	+++	+++	++	++	++	+	+-	-
++										

+++ = very good growth; ++ = good growth; + = little growth; +- = more or less growth - = no growth.

Table IV-8. Anaerobic growth of *Oscillatoria* from Alum Rock spring site 3 in the presence of sulfide after 5 days.