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# Superoxide dismutase level in response to paraquat and high temperature in the cyanobacterium *Gloeocapsa* sp.

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## Abstract

Paraquat (methylviologen) at concentrations above 0.05 mM inhibited the growth of photoautotrophic cyanobacterium *Gloeocapsa* sp. in axenic cultures. The growth rate was not affected by concentrations of 0.01 mM or less. This concentration resulted after a lag period in a moderate increase in superoxide dismutase level. After removal of paraquat, the cyanobacterium continued to generate higher levels of superoxide dismutase. There was a lag period of one hour before resumption of normal enzyme activity. Addition of puromycin at concentration of 0.5 mg $cm^{-3}$ had no effect on cell survival, but greatly enhanced the sensitivity of the culture to the toxicity of paraquat. The data showed an increase of SOD activity by temperatures above the normal growth temperature level. However, this increase was suppressed by chloramphenicol which revealed that the induction of superoxide dismutase by high temperatures was associated with *de novo* protein synthesis.

## Introduction

Molecular oxygen itself has low toxicity, but products of oxygen partial reduction can lead to free radical mediated reactions in the cell with toxic consequences (Rabinowitch and Fridovich 1983, Scandalios 1990, Mishra *et al.* 1993). Aerobic organisms use several protective enzymes to remove these toxic oxygen species. The enzyme superoxide dismutase catalytically scavenge oxygen radical in the cell and provide a major defense against oxygen toxicity (Hassan and Fridovich 1977). It has been found in all oxygen metabolizing organisms including plants and algae (Asada *et al.* 1975, Miller and Macdowell 1975, Henry and Hall 1977, Johnke *et al.* 1991, Daza *et al.* 1993)

Paraquat (methylviologen) increases intracellular production of oxygen as a necessary part of its cytotoxic effect. The reduced form transfers an electron to

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*Abbreviation:* SOD - superoxide dismutase.

molecular oxygen producing the superoxide radical. It has been shown to increase the rate of superoxide synthesis in animals, plants and in *E. coli* (Hassan and Fridovich 1978, Krall *et al.* 1988, Shaaltiel *et al.* 1988). Paraquat is now produced in multiton quantities and widely used as a herbicide. Its mechanism of action in prokaryotic cyanobacterial strains have widely varying degrees of sensitivity towards oxidative stress (Abeliovich *et al.* 1974, Asada *et al.* 1975, Okada *et al.* 1979). In addition, it appears possible that heat stress might alter the superoxide dismutase (SOD) level. The induction of SOD biosynthesis by the temperature change was observed in mammalian cells (Loven *et al.* 1985, Sheil *et al.* 1986) and in *E. coli* (Privalle and Fridovich 1987, Schöner and Krause 1990, Daza *et al.* 1993, Mishra *et al.* 1993).

In the present study, the possible changes in SOD activity in the cyanobacterium *Gloeocapsa sp.* in response to paraquat and heat stress were investigated.

## Materials and methods

Unless otherwise stated, the exponentially growing cultures were used for this study. The photoautotrophic cyanobacterium *Gloeocapsa sp.* was grown in the liquid medium BG-11 at 25 °C (Stainer *et al.* 1971). Growth of the cultures was monitored spectrophotometrically at 800 nm. Chlorophyll *a* content was measured in 90 % acetone extracts at 663 nm (Kallas and Castenholz 1982). The extinction coefficient for Chl *a* of 28.04 mg $mg^{-1}$ $cm^{-1}$ was used. The cultures were heat treated in circulating water bath thermostated to the elevated temperatures (28, 31, 34, 36, 39, 42 and 45 °C).

A sterile stock solution of paraquat (1,1'-dimethyl-4, 4'-bi-pyridinium, *Sigma*), was added aseptically to the growing cultures to reach the different concentrations. In the recovery studies, the paraquat treated cells (0.01 mM) were collected by centrifugation, washed twice with BG-11 medium and resuspended in the same medium at normal growth conditions.

The culture was treated with paraquat, divided into two portions and puromycin (0.5 mg $cm^{-3}$) was added to one of these subcultures. Growth was followed in both cultures by either monitoring the absorbance at 800 nm or by determination of the number of colonies. Samples (0.1 $cm^{-3}$) were removed at appropriate time intervals, diluted aseptically with sterile BG-11 medium and plated on agar solidified BG-11 medium. The plates were incubated under light for 2 weeks and the percent survival was calculated by counting the number of colonies as compared with the control cultures.

**SOD assay:** Cyanobacterial cells were collected by centrifugation and suspended in 50 mM Tris-HCl (pH 7.5). The cell suspension was sonicated for 10 min at 15 s intervals followed by 15 s cooling periods on ice. The suspension was centrifuged for 20 min at 12 000 × *g* to remove the cell debris and the intact cells. The supernatant was immediately assayed for SOD activity following the procedure described by Marklund and Marklund (1974) by inhibition of autoxidation of pyrogallol in 50 mM Tris-HCl buffer at pH 8. 2. One unit of the enzyme activity is defined as the amount

of the SOD which inhibits the pyrogallol autoxidation by 50 % and the specific acitivity was calculated as the enzyme activity units per mg of protein. Protein was determined according to Lowry *et al.* (1951).

## Results and discussion

The cell population of cultures of *Gloeocapsa* sp. increased logarithmically after 30 h of inoculation. Addition of paraquat at concentrations of 10 mM or higher caused immediate toxicity to the axenic cultures. The cyanobacterium was strikingly

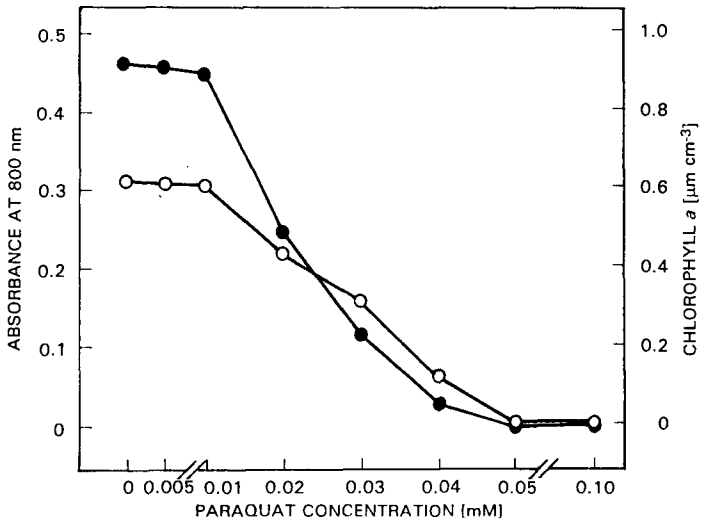


Fig. 1. Growth of *Gloeocapsa sp.* in response to treatment with various concentrations of paraquat. Growth of the culture was monitored by measuring the absorbance at 800 nm (*open circles*) and chlorophyll *a* content (*closed circles*).

sensitive to paraquat in the BG-11 nutrient medium as indicated by photobleaching and the immediate drop in the growth rate. Therefore, lower concentrations of paraquat were applied in the present investigation. The growth rates (Fig. 1) as monitored by measuring absorbance at 800 nm and chlorophyll *a* content were not affected by concentration of paraquat 0.01 mM or less. However, doubling the concentration reduced the growth rate by 30 % and at 0.03 and 0.04 mM by approximately 50 and 80 %, respectively, as estimated by measuring the absorbance. The chlorophyll *a* content was reduced in a more or less similar pattern (Fig. 1). Increasing paraquat concentration to 0.05 and 0.1 mM completely inhibited the growth of the cyanobacterium, the green colour of the cultures changed into blue-greenish before turning into white turbid colour.

The paraquat toxicity has been demonstrated *E. coli* (Hassan and Fridovich 1977). However, the cyanobacterium *Gloeocapsa sp.* exhibited more remarkable sensitivity to paraquat as compared to *E. coli.* Paraquat is known to increase intracellular

production of $O_2^-$ (Asada *et al.* 1975, Shaaltiel *et al.* 1988) and some acclimation mechanism to its action is expected to occur. The SOD is a possible enzyme induced in response to oxidative stress imposed by dioxygen or by redox active compounds such as paraquat (Privalle and Fridovich 1987). The measured specific activity of SOD (Fig. 2) indicated that addition of 0.01 mM paraquat to the culture resulted after a lag period of 2 h in a moderate increase in SOD activity (42 %) as compared to the normal level. After 4 h there was an observed increase in the rate of biosynthesis of this enzyme. This lag period might be due to the time needed to establish the effective intracellular level of paraquat and for the paraquat in turn to subvert the normal electron flow in the cell. The generation of active species such as $O_2^-$, $H_2O_2$ and OH in the PS II complex during photoinhibition has been implicated by the increase in the activities of enzymes involved in the detoxification of active oxygen species such as SOD (Schöner and Krause 1990, Jahnke *et al.* 1991, Mishra *et al.* 1993). Hassan and Fridovich (1977) and Stowers and Elkan (1981) induced a similar induction pattern by paraquat, although at a much higher level, in SOD activity in the prokaryotic system of *E. coli.* This induction represents an important adaptation in which it overcomes the toxicity of paraquat by scavenging $O_2^-$ through the stimulation of SOD biosynthesis.

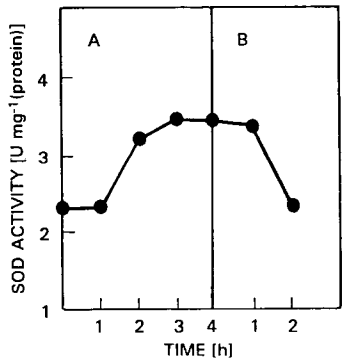


Fig. 2. Superoxide dismutase specific activity in *Gloeocapsa sp.* cultures, (*A*) treated with 0.01 mM paraquat for 4 h, (*B*) after recovery from paraquat treatment.

In an attempt to investigate the deinduction pattern, the increase in SOD level following addition of paraquat (0.01 mM) was followed by abrupt removal of paraquat. The cells were collected by centrifugation and washed three times with sterile BG-11 medium and resuspended in the same medium at normal growth temperature. The results (Fig. 2) indicated that the increased SOD activity, exerted by paraquat stress, continued for approximately one hour following its removal, then declined to normal level. The temporary high rate of SOD synthesis following removal of paraquat could be attributed to the completion process of the partially synthesized molecules and to the persistence of SOD messenger RNA. Recently, Williamson and Scandalios (1992, 1993) described the accumulation of both superoxide dismutases and catalases transcripts in plant tissue as the cumulative effect of a novel fungal toxin.

In order to assess the importance of protein synthesis as defense mechanism against paraquat toxicity, puromycin was added to prevent the changes in the intracellular level of SOD. The cultures were treated separately with paraquat (0.01 and 0.03 mM) in the presence and absence of 0.5 mg $cm^{-3}$ puromycin. The cell survival was monitored by colony counting. The data (Fig. 3) indicated that addition of puromycin had no effect on the colony- forming ability of the cultures. The cyanobacterium exhibited a marked higher sensitivity to paraquat in the presence of puromycin than in its absence. This was more obvious under treatment with 0.03 mM paraquat, as percent of cell survival decreased by about 80 %. It is most likely that puromycin as a protein synthesis inhibitor prevents the change in the intracellular level of SOD as an important defense mechanism against paraquat toxicity. Hassan and Fridovich (1978) have obtained similar results in *E. coli*.

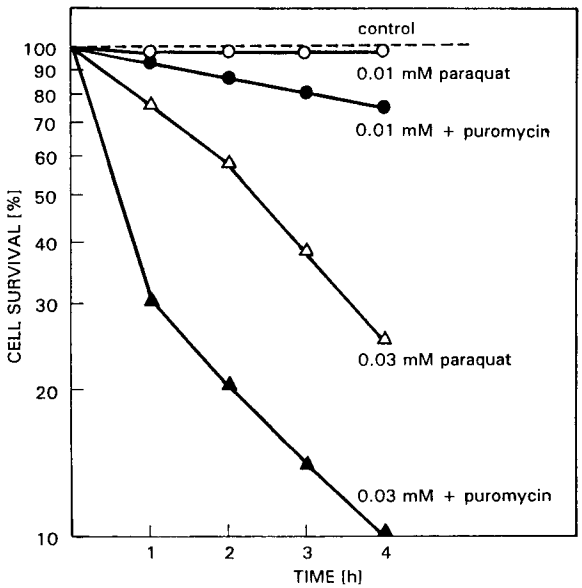


Fig. 3. Lethality of paraquat in the absence and presence of puromycin 0.5 mg $cm^{-3}$. Logarithmically growing cultures were treated with paraquat 0.01 mM and 0.03 mM with or without puromycin.

Exponentially growing cultures were exposed to temperatures higher than normal growth temperatures (Fig. 4). The SOD activity significantly increased as compared to the control cultures grown at 25 °C. The maximum activity was obtained at 42 °C and above this temperature a decrease in specific activity was detected. The response of *Gloeocapsa sp.* to elevated temperatures seems to share many properties in common with those of prokaryotic and eukaryotic systems (Daniels *et al.* 1984, Borbély *et al.* 1985). It is suggested that the high temperature stress stimulate the biosynthesis of SOD as a result of the induced production of superoxide radicals during heating. However, different organisms respond to elevated temperatures differently. Bucker and Martin (1981) has reported a linear decrease in SOD activity in *Staphylococcus aureus* at 52 °C and under oxidative stress. Meanwhile, high

temperature induced SOD activity in mammalian cells (Loven *et al.* 1985, Sheil *et al.* 1986) and in *E. coli* (Privalle and Fridovich 1987). Induction of SOD biosynthesis can take place under conditions where cellular production of superoxide radicals are increased, particularly under stress situations due to low and high temperatures (Rabinowitch and Fridovich 1993). Moreover, thermal stress causes the release of membrane bound SOD from the thylakoids thus allowing the reduced form of electron donors with specific properties to reduce $O_2^-$ radicals (Boucher and Carpentier 1993).

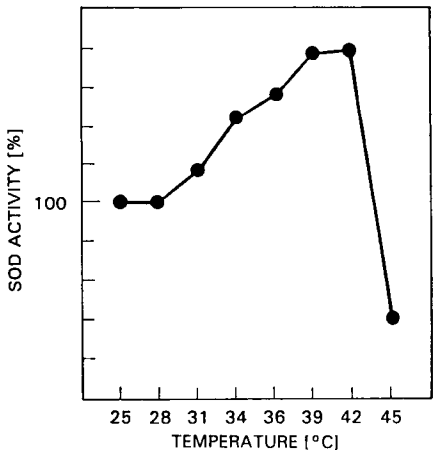


Fig. 4. Effect of temperature increase on specific activity of superoxide dismutase [% enzyme activity], after 4 h treatment.

This activity increase is a time-dependent process and at 42 °C the maximum SOD activity was achieved after 4 h. Addition of chloramphenicol at concentration of 100 μg $cm^{-3}$ to the culture medium at 42 °C, under normal conditions, for 4 h, caused a reduction in SOD activity, even lower than the normal level. This suggested that the induction process of the enzyme activity is due to protein synthesis *de novo* rather than enzyme activation. Similarly, chloramphenicol was found to inhibit protein synthesis and to prevent the appearance of SOD activity in *Anacystis nidulans* (Abeliovich *et al.* 1974).

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