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Temperature shift-induced changes in the antioxidant enzyme system of cyanobacterium *Synechocystis* PCC 6803

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Abstract

The 24 h effect of low (20 °C) and high (43 °C) temperature on the antioxidant enzyme activities and lipid peroxidation was investigated in intact cells of the cyanobacterium *Synechocystis* PCC 6803 grown at 36 °C. At low temperature treated cells, the superoxide dismutase, catalase and glutathione peroxidase activities were significantly higher and the protein content lower than in high temperature treated cells. The increase of hydroxyl free radical level and malonyldialdehyde formation, when algal cells were exposed to low temperature, were due to the stimulated production of superoxide radicals O_2^- and hydrogen peroxide (H_2O_2).

Key words: catalase, glutathione peroxidase, lipid peroxidation, protein content, superoxide dismutase

Introduction

Cyanobacterium *Synechocystis* PCC 6803 is a prokaryotic organism, that lacks differentiated organelles but retains a well developed intracytoplasmic membrane, the so-called thylakoid membrane (Stanier and Cohen-Bazire 1977). This alga is quite similar to the chloroplasts in higher plants and green algae (Allen *et al.* 1964). It seems to possess an evolution position between bacteria and plants. When the cells were exposed to low temperature near 0 °C, their viability declined (Rao *et al.* 1977), the photosynthetic oxygen evolution, the electron transport and the phosphorylation diminished (Jansz and Maclean 1973), and the absorption spectrum of carotenoids changed (Brand 1977). Glutamate and pteridines are released from the cells (Jansz and Maclean 1973); the permeability of the cells altered. In spite of correlation between temperature and oxygen concentration (Witas *et al.* 1984), the importance of oxygen radicals has only recently been pointed out in connection with oxygen toxicity and protection against deleterious oxygen effects by the peroxide

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metabolism enzymes, especially superoxide dismutase (Fridovich 1974) which seems to be present in all aerobic organisms (McCord *et al.* 1971). Superoxide radicals and hydrogen peroxide radicals have been shown to be formed by photosynthesizing organisms and it was increased by cold shock (Patterson and Myers 1973).

Therefore, our studies deal with down and upward shift of growth temperature on antioxidant enzyme activities and lipid peroxidation of *Synechocystis*.

Materials and methods

Growth and culture conditions: Cyanobacterium *Synechocystis* PCC 6803 was obtained from the Pasteur Culture Collection. The cells were grown photoautotrophically in an inorganic medium (Allen 1968) at constant temperature (36 °C) for three days. The cultures were kept under continuous irradiance of 30 μmol(photon) m^{-2} s^{-1} provided from incandescent lamps and supplied with a mixture of 97 % of sterile air and 3 % (v/v) of CO_2. In the third day of growth, the cell suspension in test tubes or flasks were transferred, immersed and swirled in orbital shaking incubator oscillating at 300 rpm and maintained at 20 °C or 43 °C for 24 h as designated period. The cells were harvested by centrifugation at 4 °C and after being washed with cold deionized water, they were stored frozen until analysis. Each sample was treated in the cold in an MSE ultrasonic desintegrator for total 5 min at an intervals of 15 - 20 s followed by an equal periods of cooling in ice. The 10 000 × *g* supernatant served as the enzyme source.

Biochemical measurements: Total superoxide dismutase (t-SOD, EC 1.15.1.1), was measured by the method based on the inhibition of adrenaline-adrenochrom oxidation. One unit of the enzyme was defined as the amount of superoxide dismutase required for 50 % inhibition of adrenaline-adrenochrom at 25 °C (Beauchamp and Fridovich 1971, Misra and Fridovich 1972, Matkovics *et al.* 1977).

Catalase (C-ase, EC 1.11.1.6) activity was estimated by the method involving measurement of H_2O_2 decomposition at 240 nm and 25 °C (Beers and Sizer 1952).

Glutathione peroxidase (GP-ase, EC 1.11.1.9) activity was measured by the chemical method. GSH and cumene hydroperoxide were used as substrates (Sedlak and Lindsey 1958, Chiu *et al.* 1976 and Matkovics *et al.* 1988). One enzyme unit was regarded the amount of enzyme which is able to transform 1 μmol of substrate in 1 min.

Hydroxyl radical ($HO^{\bullet}$) was measured by the deoxyribulose method described by *e.g.* Winterbourn (1991) and Matkovics *et al.* (1991). The malonyldialdehyde formed can be estimated by the thiobarbituric acid (TBA) method described by Placer *et al.* (1966) and modified by Matkovics *et al.* (1988). Under the mentioned conditions the D-deoxyribose decomposition is a sensitive detector of ($HO^{\bullet}$) radical.

Lipid peroxidation (LP) was measured by the method of Placer *et al.* (1966) determining the amount of total TBA reactive substances. The calibration curve and calculation were made with malonyldialdehyde (MDA).

The quantity of protein was measured using Folin reagent by the method of Lowry *et al.* (1951).

Results and discussion

Some differences were found in the activity of the enzymes examined, and in lipid peroxidation between cold treated cells (at 20 °C) and warm treated ones (at 43 °C) for 24 h. The specific activity of SOD was increased in the cold treated cells in

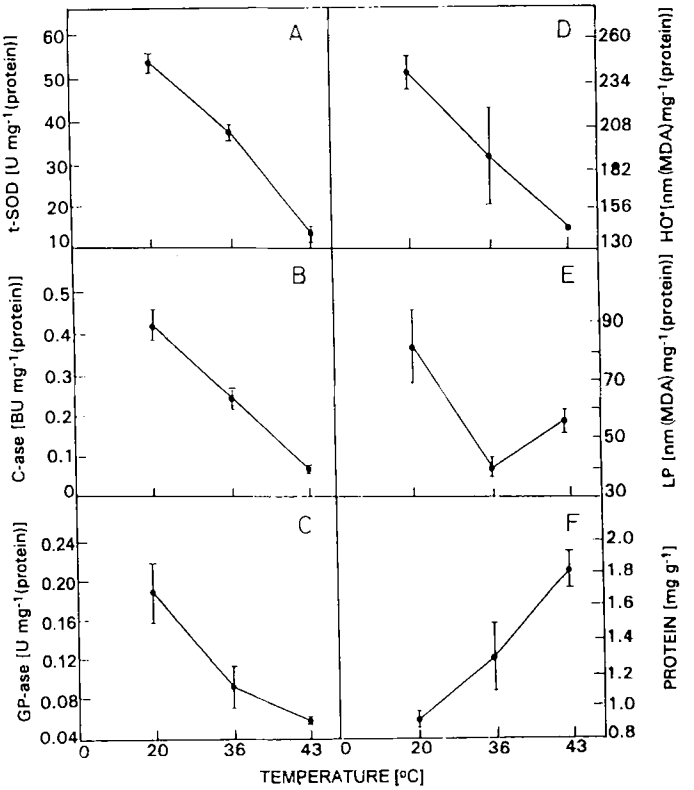

Fig. 1. Changes in the antioxidant enzyme activities: total superoxide dismutase, t-SOD (*A*); catalase, C-ase (*B*); glutathione peroxidase, GP-ase (*C*); HO• radical (*D*); lipid peroxidation, LP (*E*) and protein content (*F*) of cyanobacterium *Synechocystis* PCC 6803 in response to temperature shift under *in vivo* conditions.

comparison with the warm treated ones (Fig. 1A). The level of SOD was found to depend on the concentration of oxygen in the growth medium of the alga (Fridovich 1974). It seems that in the cold-treated cells photosynthesis became more active and oxygen evolution higher than needed. As already described by Dykens and Malcolm-Shick (1982), molecular O_2 (undergoes univalent reduction to produce superoxide anion O_2^-), which induces lipid peroxidation depolymerizes hyaluronate, and

inactivates ribonuclease (Fridovich 1978). As a result of catalytic reaction of O_2^- with SOD, high level of H_2O_2 is produced, and regarded as metabolic defect, transits the low rate of oxidative metabolism to high rate of photosynthetic metabolism (Patterson and Myers 1973), which induces catalase activity in the cold-treated cells (Fig. 1B). In contrast the warm-grown cells are associated with photooxidative death, which increases damage of photosynthetic apparatus (Abelovich *et al.* 1974, Vigh *et al.* 1990).

The glutathione peroxidase (GP-ase, EC.1.11.1.9) activity was also higher in the cold treated cells than that in the warm treated ones (Fig. 1C). It is probably due to high level of H_2O_2 and reduced level of glutathione, which act as activators for glutathione peroxidase activity. It was shown (Fig. 1D) that increased level of hydroxyl radical ($HO^•$) followed the down-shift of growth temperature, while that of malonyldialdehyde was increased on both increase and decrease in growth temperature. The reason for this probably is that the possibility of O_2 or H_2O_2 to participate in deleterious reaction with other cell components is diminished in proportion to the decrease in their concentrations (Fridovich 1978). Another explanation may reside in cell membranes containing more unsaturated fatty acids at low temperature, therefore being more susceptible to oxidative injury (Deng 1978) by superoxide radical produced from molecular oxygen.

It is presumed from our results, that treatment of cells of *Synechocystis* PCC 6803 by lower temperature induced the activity of antioxidant enzymes and malonylaldehyde formation but reduced the protein content (Fig. 1F) in comparison with the cells treated by higher temperature.

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