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Temperature dependence of superoxide dismutase activity in plankton

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Abstract

The effect of temperature (from 1 to 37 °C) on in vitro effective superoxide dismutase (SOD) activity of several organisms was investigated and compared. Antarctic plankton, cultures of the alga *Nannochloropsis* sp., and the cyanobacterium *Synechococcus* strain WH 7803, and pure bovine erythrocyte SOD was studied. It was found that in all cases SOD activity increased with decreasing temperature within the temperature range assayed, in the Polar as well as the temperate plankton cells. This behavior of SOD is counterintuitive in terms of our experience when looking at enzyme activity or any other chemical reaction. We suggest a theoretical explanation for this apparently odd behavior. The advantage of such behavior is that the same amount of antioxidant will act better under low temperatures when reactive oxygen species (ROS) increase. Moreover, this protective process would act in vivo at a faster pace than the ex novo enzyme synthesis.

Keywords: Antarctic Ocean; Enzyme activity; Oxygen radicals; Phytoplankton; Plankton; Superoxide dismutase; Temperature effect

1. Introduction

Environmental conditions affect many aspects of cellular metabolism. In recent years, growing attention has been devoted to the damage induced by oxygen radicals to cells under stress conditions. Superoxide radicals, O_2^-, are formed as by-products of cell metabolism, especially by photosynthesis (Asada and Takahashi, 1987; Niyogi, 1999) and by respiration (Cadenas and Davies, 2000; Raha and Robinson, 2000; Sohal, 1997). These oxidant radicals can oxidize membrane lipids and cause denaturing of nucleic acids and proteins (Fridovich, 1986). Several kinds of environmental stress cause an increase in these and other oxidant radicals: high light intensity, UV radiation, nutrient deficiency, high salinity, extreme temperatures, hypoxia and various toxic compounds (for examples, see: Boyd and Burnett, 1999; Collén and Davison, 1999; Rijstenbil et al., 2000; Stoshs and Bagchi, 1995). Although these stressors operate through different mechanisms, they all induce production of reactive oxygen species (ROS). Persistent suboptimal environmental conditions constitute a stress, and cause an increase in ROS (Bohnert and Sheveleva, 1998). Chilling damage to cells is also due to active oxygen radicals (Tao et al., 1998). The superoxide anion O_2^- is not the most reactive, but is very common, and leads to

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the generation of more harmful radical species, according to the following reactions.

$$O_2^{\cdot -} + H^+ \Rightarrow HO_2^{\cdot} \quad (A)$$

$$O_2^{\cdot -} + 2H^+ \Rightarrow H_2O_2 \quad (B)$$

$$H_2O_2 + M^{2+} \Rightarrow M^{3+} + OH^- + OH^{\cdot} \quad (C)$$

All such radicals can oxidize various vital cell components:

$$R^{\bullet} + \text{substrate} \Rightarrow \text{oxidized form of substrate} \quad (D)$$

The antioxidant system of the cell acts through several compounds and processes to eliminate the excess oxidant. It includes the protective effect of some compounds (cytochrome *c*, ascorbate, β-carotene, etc.) and scavenging of the radicals by specialized enzymes. We have examined the activity of superoxide dismutase (SOD). It is the first enzyme in the scavenging process, and catalizes the disproportioning reaction that converts superoxide into peroxide, which, in turn, is converted to oxygen and water by catalase. SODs are a group of metalloenzymes, highly efficient catalysts, with rates limited almost only by diffusion, and constitute the main components of the antioxidant pathway (Foyer et al., 1994; Salin, 1991). Their metal ions are cyclically reduced and oxidized during catalysis. Although the exact details of the catalytic mechanism are still unclear, the cyclic redox reactions of the metals as shown in reactions (E) and (F) are generally accepted (Gray and Carmichael, 1992):

$$M^{n+} \cdot SOD + O_2^{\cdot -} \Leftrightarrow [M^{n+} \cdot SOD - O_2^{\cdot -}] \Leftrightarrow M^{(n-1)+} \cdot SOD + O_2 \quad (E)$$

$$M^{(n-1)+} \cdot SOD + O_2^{\cdot -} + 2H^+ \Leftrightarrow [M^{(n-1)+} \cdot SOD - O_2H_2] \Leftrightarrow M^{n+} \cdot SOD + H_2O_2 \quad (F)$$

Plankton organisms in polar areas are usually subject to low temperatures that, in spite of constituting their usual environment, are suboptimal for growth and metabolism and that generate ROS. SODs constitute important defense enzymes in the antioxidant system. We tried to determine if the activity of this enzyme complex was differentially modulated by temperature in these cold-adapted organisms versus temperate plankton organisms. We had anticipated a different biochemical behavior. We determined SOD activity of Antarctic plankton collected on a cruise to the Weddell Sea and compared it with that of temperate phytoplankton, and with pure SOD enzyme. The Weddell Sea samples consisted of mixed plankton – mostly sizes equal and below microplankton – taken with Niskin bottles, and the temperate phytoplankters were: the Haptophyte *Nannochloropsis* sp., and the Cyanophyte *Synechococcus* sp. strain WH 7803.

2. Materials and methods

2.1. Organisms and sampling

Antarctic plankton samples were taken during cruise E-DOVETAIL-97 to the Weddell Sea, during austral summer from the following stations:

Sta.	Cruise station	Latitude S	Longitude W	In situ temperature °C
1	HI-2F	58° 15′ 14″	48° 7′10.5″	0.99
2	HI-3F	58° 30′ 18.9″	48° 8′ 18.7″	0.70
3	NB-4	63° 24′ 10.1″	56° 39′ 48.3″	−1.12

Samples were taken at the depth of the chlorophyll maximum, as measured with a fluorometer attached to the Niskin bottles. The water volume taken varied between 2 and 5 l, depending on the biomass present.

Nannochloropsis sp. and *Synechoccus* WH 7803 cultures were maintained at 20 °C. Cells were collected by centrifuging 50 ml of culture (7000 ×*g*, 10 min) and resuspended in phosphate buffer 50 mM, pH = 7.5.

2.2. Analytical methods

SOD activity is usually measured as inhibition of a substrate oxidation reaction relative to controls without SOD. Such inhibition is referred here to as *effective* SOD activity. SOD activity in Antarctic plankton and temperate phytoplankton cultures was measured by following the reduction of nitroblue tetrazolium (NBT) by an aerobic mixture of NADH and phenazine methosulphate (PMS) that produces superoxide chemically at pH 8.3 (Nishikimi et al., 1972; Ewing and Janero, 1995; Rao, 1989).

Frozen filters were mechanically ground in grinding tubes with motor-driven striate teflon pestles (Thomas Scientific Co.) on ice, in 3 ml of cold phosphate buffer (pH 8.3) containing 0.1% Triton X-100. The resulting homogenate was centrifuged at 0–2 °C in a refrigerated centrifuge at 10,000 g for 15 min. The supernatant was transferred to a tube on ice and 0.2 ml aliquots were used for each assay. Cells from cultures were collected by centrifuging 50 ml of culture (7000 g, 10 min) and resuspended in phosphate buffer. The cell suspension was disrupted with an MSE model sonicator, on ice, at 20 W, four times, each time during 20 s with an interval