RESISTANCE TO HIGH OXYGEN TENSION, STREPTONIGRIN, AND ULTRAVIOLET IRRADIATION IN THE GREEN ALGA *CHLORELLA SOROKINIANA* STRAIN ORS

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INTRODUCTION

Because of the reactivity of oxygen with biologically important compounds, e.g. unsaturated lipids, SH-containing proteins and cofactors, and redox compounds (see review by Haugaard, 1968), cells in aerobic environments require some mean(s) for protection from indiscriminate oxidation. Since the superoxide free radical, O_2^-, is apparently a major product of the reaction of O_2 with reduced compounds (Misra and Fridovich, 1972), Fridovich and co-workers have proposed an O_2 protective mechanism based on the O_2^--scavenging enzyme, superoxide dismutase (SOD) (McCord and Fridovich, 1969; McCord et al., 1971; Gregory and Fridovich, 1973 *a*).

This report describes the combined occurrence of the following physiological characteristics in a mutant strain of the green alga *Chlorella sorokiniana* (Shihira and Krauss): (*a*) resistance to high partial pressures of O_2, (*b*) resistance to the O_2^--generating antibiotic, streptonigrin, and (*c*) resistance to UV irradiation. The mutant also contained a 3.5-fold higher level of SOD when grown under air than did the wild-type strain. These correlations provide substantial evidence that an O_2^- detoxification system (probably involving SOD) functions to protect the mutant strain from, not only O_2 toxicity, but also, some fraction of UV-mediated damage.

MATERIALS AND METHODS

Organisms

Wild-type *C. sorokiniana* was obtained from R. Krauss at The University of Maryland. The mutant (designated strain ORS) was originally described by Morhardt and Ward (1968) on the basis of its ability to grow autotrophically under high O_2 tension (see Fig. 1).

Culture Conditions and Growth Rates

Liquid cultures were grown at 39°C by the test-tube culture method of Myers (1950) with bilateral illumination from 20-W fluorescent lamps (three on each side, 7 cm from the growth tubes). Medium Cg10[1] (Van Baalen, 1967) was the growth medium. Cultures were agitated by continuous bubbling with 1% CO_2 in air or 1% CO_2 in oxygen.

Optical density (OD) of cultures at 600 nm was measured with a Lumetron colorimeter (Photovolt Corp., New York). A suspension of wild-type strain at OD = 1.0 contains 0.28 mg dry weight/ml.

Quantitative Cell Plating

Survival after exposure to streptonigrin or UV radiation was determined by quantitative surface plating of treated cells as described by Van Baalen and O'Donnell (1972). Plates were incubated in room air under tungsten lights at 31–35°C. Colony-forming units were counted after 5–6 days.

Streptonigrin Treatment

The mechanism of action of streptonigrin involves production of O_2^- (White et al., 1971; Gregory and Fridovich, 1973 *b*). The drug was kindly provided by the National Cancer Institute, Drug Research and Develop-

[1] 1 liter of Cg10 contains (in milligrams): Na_2-EDTA, 10; $FeCl_3 \cdot 6\ H_2O$, 3.89; trace elements (H_3BO_3, 2.86; $MnCl_2 \cdot 4\ H_2O$, 1.81; $ZnSO_4 \cdot 7\ H_2O$, 0.222; MoO_3 [85%], 0.018; $CuSO_4 \cdot 5\ H_2O$, 0.079; $CoCl_2 \cdot 6\ H_2O$, 0.010); $MgSO_4 \cdot 7\ H_2O$, 250; KNO_3, 1,000; $Ca(NO_3)_2 \cdot 4\ H_2O$, 25; KH_2PO_4, 50; and glycyl glycine, 1,000. pH brought to 8.2 with 2 N NaOH.

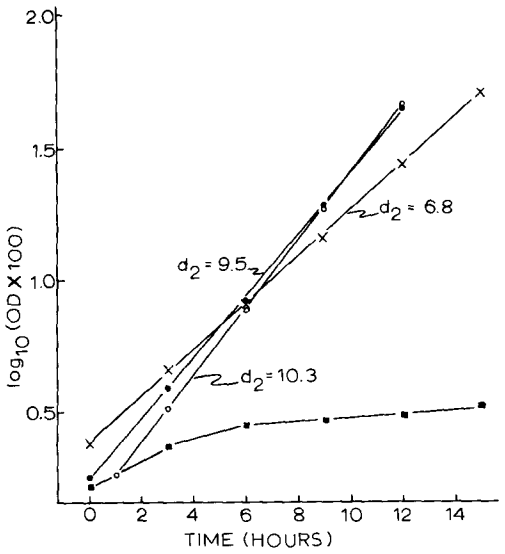

FIGURE 1 Autotrophic growth rates of wild-type and ORS strains. O——O, wild-type on 1% CO_2 in air; ●——●, ORS strain on 1% CO_2 in air; ■——■, wild-type on 1% CO_2 in O_2; ×——×, ORS strain on 1% CO_2 in O_2. All cultures were started at time 0 from inocula, grown on 1% CO_2 in air, taken in the exponential growth phase (OD of 0.6–0.7). Growth rates are in doublings per day, d_2, calculated according to Guillard (1973).

ment Branch, Division of Cancer Treatment, Bethesda, Md.

Cells grown on 1% CO_2 in air were harvested at an OD of 0.7, washed in fresh Cg10, and then suspended at an OD of 0.22 in Cg10 containing streptonigrin at 66 μg/ml. During exposure to streptonigrin, cells were illuminated as in growth experiments under 1% CO_2 in air at 39°C. At various times, aliquots were taken, diluted in fresh medium, and plated.

Exposure to UV Radiation

Cells were harvested at an OD of 0.6–0.7, washed twice with Cg10 lacking nitrate, and resuspended in the same modified medium at an OD of 0.22. 6 ml of this cell suspension was irradiated at a distance of 48 cm from an unfiltered GE G15T8 lamp (dose rate = 1,141 ergs mm^{-2} min^{-1}) at room temperature as described by Van Baalen (1968). Preliminary studies showed that *Chlorella* does not photoreactivate under tungsten light filtered through an orange sheet of plastic (Rohm and Haas Plexiglas, transparent sheet no. 2422, 0.125 inch thick, Rohm and Haas Co., Philadelphia, Pa.) which has no transmission below 530 nm. All UV irradiation and subsequent plating were done under a dim safelight of the orange plastic.

Determination of SOD Activity

Cells were harvested at an OD of 0.6–0.7 and washed in phosphate buffer. Cell-free extracts were made by ultrasonic treatment with a Branson sonifier (LS 100, Branson Instruments Co., Stamford, Conn.) at 4°C in 0.05 M (Na + K) phosphate buffer pH 7.8 containing 10^{-4} M EDTA. The extract was centrifuged at 30,000 *g* for 15 min and SOD was assayed in the supernate by the method of McCord and Fridovich (1969). The enzyme was also checked once by the method of Beauchamp and Fridovich (1971). An enzyme unit was defined the same as published. Protein was determined by the Lowry method (Lowry et al., 1951).

RESULTS AND DISCUSSION

The ORS and wild-type strains are accurately compared on a per cell basis rather than a per milligram protein basis, because ORS strain cells are larger than wild-type cells and contain about twice the protein per cell. (Both strains, however, have the same DNA content, 0.1 pg/cell [Pulich, unpublished]). ORS strain cells grown under air contain between three and four times as much SOD as the wild type similarly grown (Table I). When grown under high O_2, ORS strain shows a slight decrease in SOD from its air-grown value. Although this level (4.3 U/10^8 cells) is still considerably more than that (2.8 U/10^8 cells) for the wild-type exposed to high O_2, the apparent decline in SOD is inexplicable. Presently, strain ORS is best characterized as an enlarged cell mutant, with more SOD than the wild type, particularly when grown under air.

SOD in both *Streptococcus faecalis* and *Escherichia coli* B can be induced over five times the level in anaerobically grown cells by growing the organisms under 100% O_2 (Gregory and Fridovich,

TABLE I

SOD Activity in Cell-Free Extracts of Wild-Type and ORS Strains Cultured under 1% CO_2 in Air or 1% CO_2 in Oxygen

Strain	Units/10^8 cells	Units/mg Soluble protein
Wild type, air grown	1.4	24
ORS, air grown	4.8	47
ORS, high O_2 grown	4.3	26
Wild type, exposed to high O_2 for 48 h	2.8	32

Details in text. Values are average of three measurements.

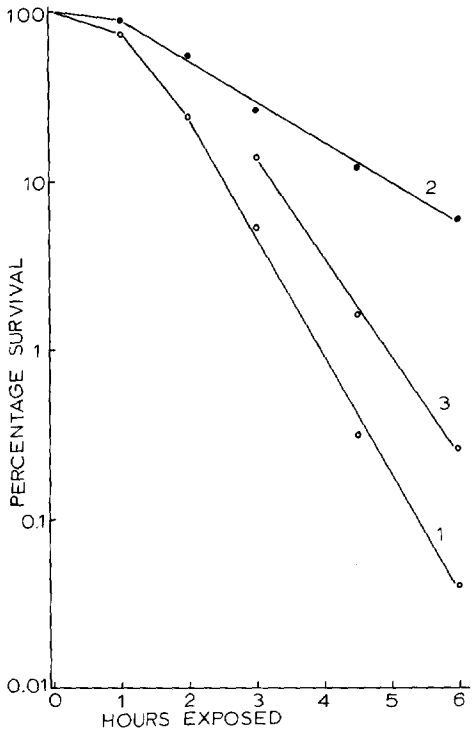

FIGURE 2 Survival curves for air-grown cells of wild-type and ORS strains, treated with streptonigrin under autotrophic growth conditions. Survival was determined by plating treated cells on solid medium. *Curve 1*, wild-type treated under 1% CO_2 in air; *Curve 2*, ORS strain treated under 1% CO_2 in air; *Curve 3*, wild-type treated under 1% CO_2 in N_2.

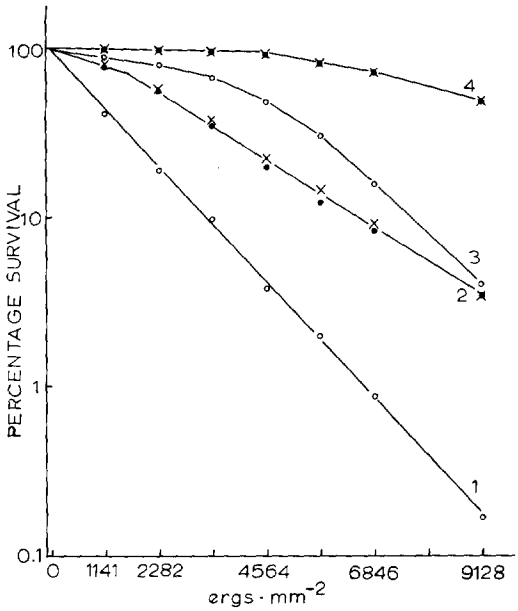

FIGURE 3 Survival curves for UV-irradiated cells of wild-type and ORS strains. *Curve 1*. Wild-type grown on 1% CO_2 in air, irradiated and plated (see text), and incubated under tungsten light filtered through orange plastic (Rohm and Haas no. 2422 Plexiglas). *Curve 2*. ORS strain grown on 1% CO_2 in air (●——●) or 1% CO_2 in O_2 (×——×); subsequent treatment same as curve 1. *Curve 3*. Wild-type treated same as curve 1, except plates incubated without orange plastic. *Curve 4*. ORS strain treated same as curve 2, except plates incubated without orange plastic. Curves 1 and 2 represent survival in the absence of photoreactivation, and curves 3 and 4 survival with photoreactivation allowed.

1973 *a,b*). Cells thus induced show increased resistance to the lethal effects of hyperbaric O_2 (20 atm), and, in the case of *E. coli* B, the antibiotic, streptonigrin. An analogous situation exists in the case of strain ORS. As illustrated in Figs. 1 and 2, air-grown strain ORS, with its higher level of SOD compared to wild type, is also much more resistant to high O_2 tension and streptonigrin than the wild type.

The inhibition by O_2 of growth of wild type is not due to a change in composition of the medium. Control tubes of Cg10 bubbled in the light with high O_2 for 24 h allow subsequent growth of wild type on 1% CO_2 in air that is identical with growth on fresh medium.

The same mechanism of streptonigrin toxicity appears operative in *Chlorella* as in *E. coli* B examined by Gregory and Fridovich (1973 *b*). This is indicated by the increased survival of wild type exposed to streptonigrin under 1% CO_2 in nitrogen. Percentage survival in this case is probably still lower than that of strain ORS because of the difficulty of maintaining O_2-free conditions when a photosynthetic cell is in the light.

Both air- and high O_2-grown strain ORS cells show identical resistance to UV, as demonstrated by the superimposable survival curves (Fig. 3). The increased resistance of strain ORS to UV is apparently not the result of a change in the system for photoreactivation: higher survival than wild-type was manifested by strain ORS under both tungsten and orange illumination, i.e. with and without photoreactivation. In fact, at any UV dose, the ratio of strain ORS percentage survival to that of wild type is greater *without* photoreactivation than it is *with* photoreactivation. These data suggest that UV resistance actually represents *protection from*, and not recovery from, UV damage. Although the sensitivity of cells to UV

can be modified by any number of factors before UV treatment (Jagger, 1967), it is possible that the O_2 protective mechanism in strain ORS also leads to a reduction in the rate of thymine-dimer formation or, more likely, prevents some type of photodynamic action, e.g., photooxidation (Kandler and Sironval, 1959), from occurring during UV treatment. The latter explanation is supported by the observations of Morhardt (1968), who found that strain ORS could tolerate a much higher intensity of visible light than the wild type before undergoing photooxidative damage.

In summary, strain ORS represents the first photosynthetic system for which the correlation between SOD and resistance to O_2 and streptonigrin toxicity has been documented. With the observation that strain ORS is also UV resistant, SOD takes on added significance as a cellular protective mechanism against lethal oxidizing conditions.

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