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EFFECT OF SOME ENVIRONMENTAL POLLUTANTS ON THE SUPEROXIDE DISMUTASE ACTIVITY IN *LEMNA*

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Exposure of *Lemma* sp. to SO_2 resulted in an increased activity of superoxide dismutase. About 3 to 4 fold increase in the activity was observed within 30 minutes after the plants were fumigated with 10 ml/l of SO_2. Paraquat, a well known superoxide generator, doubled the enzyme activity after 1 hour of treatment with 0.1 mM paraquat. Superoxide dismutase activity was also enhanced by cadmium treatment but the response was not immediate. Optimum increase in the activity of enzyme was observed after 4 days of treatment with 40 mg/l of cadmium in the medium. Treatment with H_2O_2 very clearly inhibited the activity of superoxide dismutase in *Lemna*.

KEY WORDS: Cadmium, H_2O_2, paraquat, SO_2, superoxide dismutase.

INTRODUCTION

Plants suffer oxidative damage from active oxygen types in many unfavourable environmental conditions, like water deficit,[1] aging,[2] treatment with various herbicides and heavy metals,[3-5] and air pollutants,[6,7] high O_2 [8] or low CO_2.[9] Superoxide radicals can directly interact with membrane lipids, or be dismutated by superoxide dismutase. The peroxide ion can be catalytically converted in the presence of divalent iron or copper to highly reactive hydroxyl radicals by Fenton reaction,[10] or be enzymatically removed by ascorbate peroxidase and catalase. Superoxide dismutase, ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase comprise the pathways for the detoxification of superoxides and peroxides as described by Halliwell and Asada.[11,12]

Lemna sp. was used as the experimental plant material. *Lemna* was found to be very sensitive to the heavy metals and can be used as an indicator to the environmental pollution.[13] Nevertheless, owing to its minute size and easy manipulation under aseptic conditions *Lemna* provides an excellent material for studying the biochemical changes involved in the plant response to oxidative stress. In this work we studied the effect of the air pollutant SO_2, the water pollutant cadmium, the herbicides paraquat and H_2O_2 on the level of superoxide dismutase activity.

MATERIAL AND METHODS

All the experiments were performed with 2 weeks old stock culture of *Lemna* sp.

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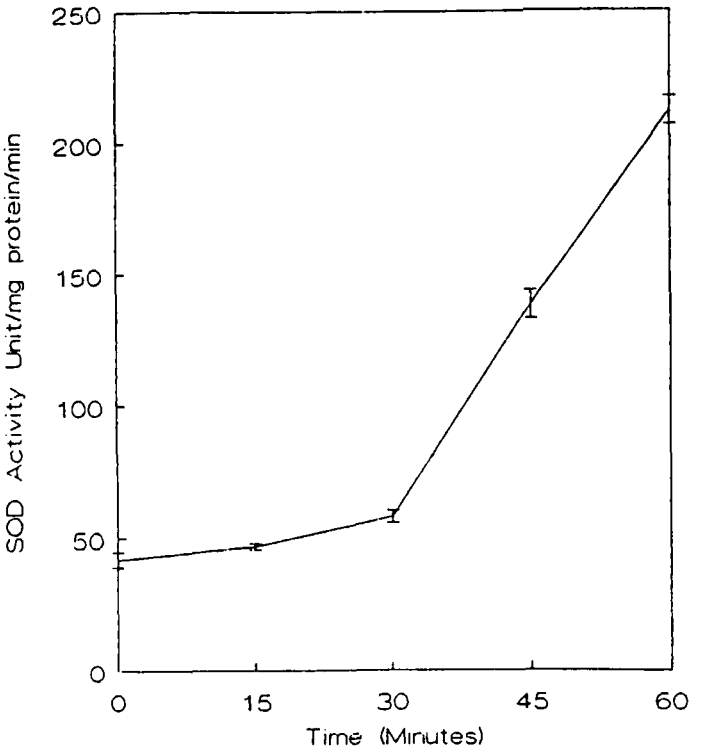

FIGURE 1. Effect of SO_2 fumigation on the superoxide dismutase activity of *Lemna* sp. Plants were fumigated with different concentrations of SO_2 and analysed after 24 hours of treatment.

collected from the botanical garden, University of Tel-Aviv which was maintained on inorganic Bonner-Devirian medium (BD-medium),[14] supplemented with a 1% sucrose under continuous light of 100 microeinsteins $m^{-2} sec^{-1}$.

Treatment With Pollutants

Plants were exposed to SO_2 in 300 ml volume chamber containing 1.5 g of *Lemna* in 100 ml of BD-medium. For the experiments with cadmium, paraquat and H_2O_2, 1.5 g of plant material was treated with these chemicals in 100 ml of BD-medium in Erlenmeyer flasks. Subsequently, after the treatment, plants were taken out, washed successively with distilled water, and homogenized in 0.1 M ice cold phosphate buffer (pH 7.5) and then filtered through the cheese cloth. The homogenate was centrifuged at 32,000 g for 30 minutes and the supernatants were used for the biochemical determinations.

Enzyme Assay

Superoxide dismutase was assayed spectrophotometrically as described by McCord and Fridovich.[15] The inhibition of xanthine oxidase dependent reduction of 10 μM ferricytochrome *C* was monitored at 549 mm in 50 mM K-phosphate buffer (pH 7.8), 100 μM EDTA and 50 μM xanthine. The enzyme unit was defined as the amount of

the enzyme required to inhibit the reduction of cytochrome *c* by 50%. Protein content was measured according to Bradford.[16]

RESULTS AND DISCUSSION

It is widely accepted that gaseous SO_2 turns to SO_3^{2-} and HSO_3^- within leaf tissues.[17] Only a minor fraction of these ion species are incorporated into sulfur metabolites in plants. Most of the SO_3^{2-} and $HSO3^-$ is photo-oxidized to the less toxic SO_4^{2-} in chloroplast, and this photo-oxidation is accompanied by propagation of superoxide radicals.[18] The superoxide radicals is dismutated to H_2O_2 and O_2 either spontaniously or by superoxide dismutase. *Lemna* plants were exposed to increasing amount of SO_2 in air (2.5 ml/l to 10 ml/l), for 24 hours under continuous illumination at room temperature and analysed for the effect of SO_2 treatment on superoxide dismutase activity. Figure 1 shows the induction of superoxide dismutase activity as a response to SO_2 stress. These results suggest that superoxide dismutase is playing an important role in the removal of superoxide radicals, which were produced through sulfite mediated chain reactions, similar to the system in chloroplast.[11,12] Sulphur dioxide treatment caused pigment bleaching and reduced protein content as well (result not shown). The time course analysis of the effect of SO_2 on superoxide dismutase induction demonstrated fast increase in the level of enzyme activity, as shown in

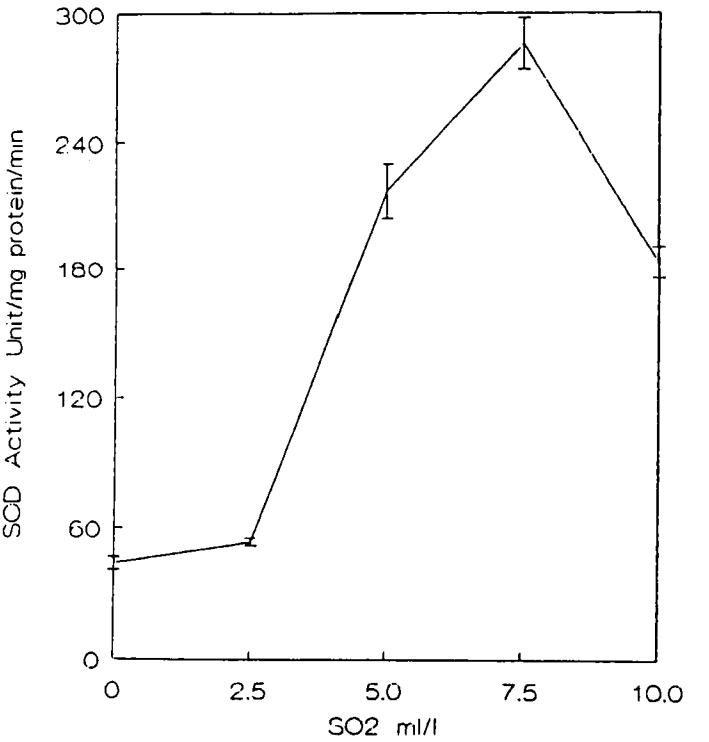

FIGURE 2. Time course of the response to SO_2 of the superoxide dismutase activity of *Lemna* sp. Plants were treated with 10 ml/l of SO_2 for different differnt time periods.

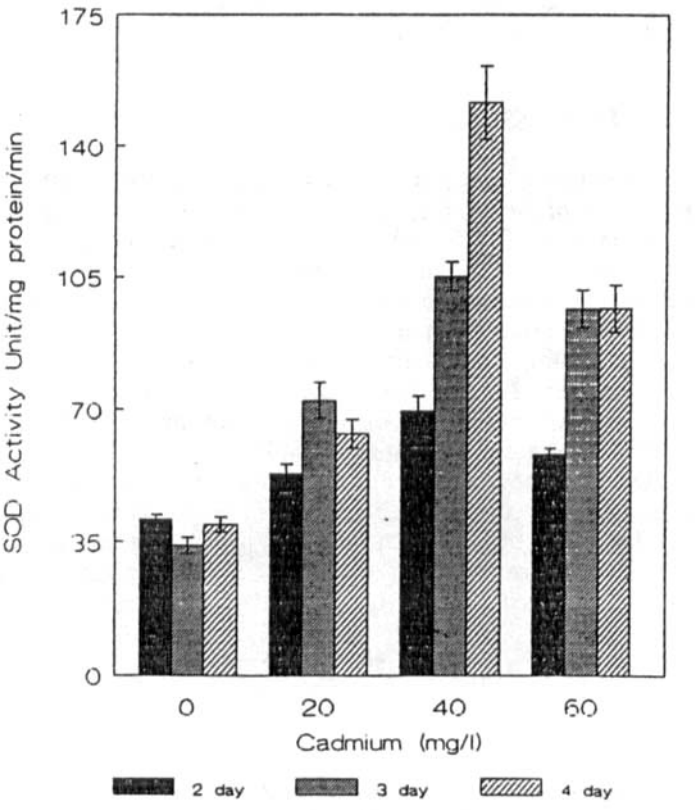

FIGURE 3. Effect of cadmium on superoxide dismutase activity in *Lemna* sp.

Figure 2. A 3 to 4 fold increase in the superoxide dismutase activity was observed within 30–45 minutes exposure of *Lemna* to 10 ml/l of SO_2. This fast response already reduced protein content of the plants. It is suggested that *Lemna* responds very quickly to the chemical chain reaction, leading to the formation of superoxide radicals. Induction of superoxide dismutase could serve as an early protection mechanism to this potent air pollutants.

Lemna plants were previously shown to tolerate up to 15 ppm cadmium during 2 weeks growth experiments.[13] After cadmium treatment, the level of superoxide dismutase was enhanced. (Figure 3). Optimum activity was observed in the presence of 40 mg/l of cadmium in the medium after 4 days treatment. At higher concentration and after a longer exposure, superoxide dismutase activity was inhibited. Cadmium was shown to induce the activity of the antioxidative enzyme peroxidase in *Lemna*.[13] The enhancement in the activity of superoxide dismutase indicates the generation of superoxide radicals in plants after cadmium treatment.

Induction in the activity of superoxide dismutase was observed when the plants were treated with a low concentration of paraquat (Figure 4). The induction of superoxide dismutase activity was fast and significant enhancement of superoxide dismutase activity was observed even within one hour of treatment. As shown, application of 0.1 mM paraquat was sufficient to induce about two fold increase in superoxide dismutase activity. *Lemna* treated with 1 mM paraquat demonstrated

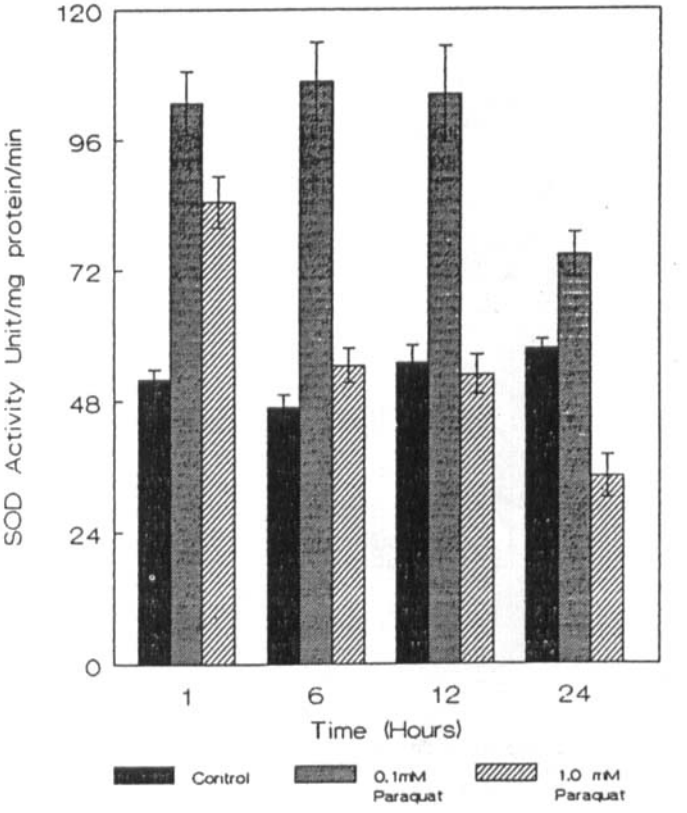

FIGURE 4. Effect of paraquat on superoxide dismutase activity of *Lemna* sp.

induced superoxide dismutase activity after one hour, but the plants showed damage due to the toxic effect of paraquat between 6 to 24 hours, and failed to show further induction of superoxide dismutase activity. Hence, *Lemna* responds similarly to chloroplast, where paraquat radicals are rapidly autoxidized to produce superoxides, leading to increased superoxide dismutase activity.[19]

Lemna plants treated with 10 mM H_2O_2 showed reduction of superoxide dismutase activity (Figure 5). Even after 6 hours of treatment, about 50 percent inhibition in the activity of superoxide dismutase was observed. In red spruce inhibition in the enzyme activity, was reported by Tandy *et al.*,[20] when treated with H_2O_2. They indicated the inhibition of many isozymes of superoxide dismutase after H_2O_2 treatment. Asada *et al.*[21] described the sensitivity of the Fe-enzyme to hydrogen peroxide. They also reported that when purified CuZn-superoxide dismutase enzyme from spinach leaves was incubated with 0.5 mM H_2O_2, almost all the activity was lost within 90 minutes. We found that *Lemna* contains CuZn-superoxide dismutase (unpublished result). Therefore, inhibition of superoxide dismutase activity after H_2O_2 treatment may be due to inactivation of enzyme. The inhibition of superoxide dismutase activity by H_2O_2 may suggest that the induction of superoxide dismutase responds to superoxide radicals rather than to peroxides. Also, it may indicate that pollution by SO_2 and cadmium, which induce the superoxide dismutase activity, produce superoxide as already shown for paraquat.

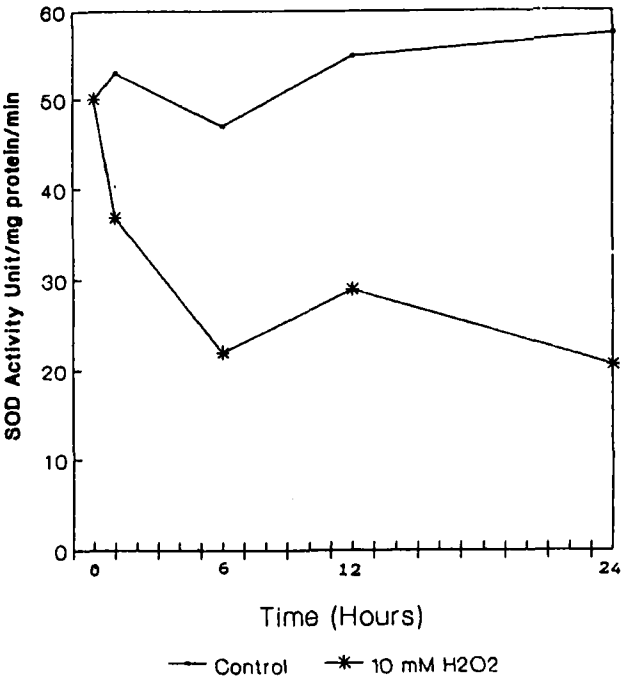

FIGURE 5. Effect of H_2O_2 on superoxide dismutase activity of *Lemna* sp.. Plants were exposed to 10 mM H_2O_2 for different time periods.

Acknowledgement

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