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Factors modulating superoxide dismutase activity in needles of spruce trees (*Picea abies* L.)

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Introduction

Superoxide dismutases (SOD) are considered to be a major enzymic defense against oxygen toxicity in cells (Fridovich, 1986). These enzymes contain either Cu/Zn, Mn or Fe in their catalytic center (Fridovich, 1986). Most abundant in plants are Cu/Zn–SODs which are characterized by a broad pH-optimum between pH 7 and pH 10 and an inhibition by cyanide and H_2O_2 (Fridovich, 1986). SODs detoxify superoxide radicals originating from physiological functions, such as photosynthesis under excess light energy (Robinson, 1988), as well as different environmental stress factors, such as herbicides and air pollutants (O_3, SO_2, NO_2) (Rabinowitch and Fridovich, 1983; Fridovich, 1986). Since it is generally assumed that air pollution is one of the major reasons for forest decline in Central Europe, we compared SOD activity in needles of healthy and injured spruce trees growing in the field.

Materials and Methods

Experiments were performed with needles from Norway spruce trees (*Picea abies* L.) growing in the field. Extracts of spruce needles were prepared as described elsewhere (Polle *et al.*, 1989). After dialysis, the activity of superoxide dismutase was determined according to the method of Misra and Fridovich (1972). This assay is based on the autooxidation of epinephrine to adrenochrome at pH 10.2. $O_2^{\cdot-}$ serves as the chain-propagating species in this reaction. SOD competes for $O_2^{\cdot-}$, thus inhibiting adrenochrome formation. By definition, 1 unit of SOD is the amount of extract that inhibits the maximal rate of adrenochrome formation by 50%.

Results

To determine SOD activity, we adapted extraction and assaying procedures (after Misra and Fridovich, 1972) to extracts from spruce needles (Polle *et al.*, 1989). Fig. 1 shows a typical calibration curve for spruce SOD. Increasing amounts of spruce extract exhibited increasing inhibition of adrenochrome formation with a saturation level of 80%. In comparison with spruce extract, a commercially available SOD preparation from horseradish reached a saturation level of 90% in this assay system (not shown). The failure to obtain complete inhibition was attributed

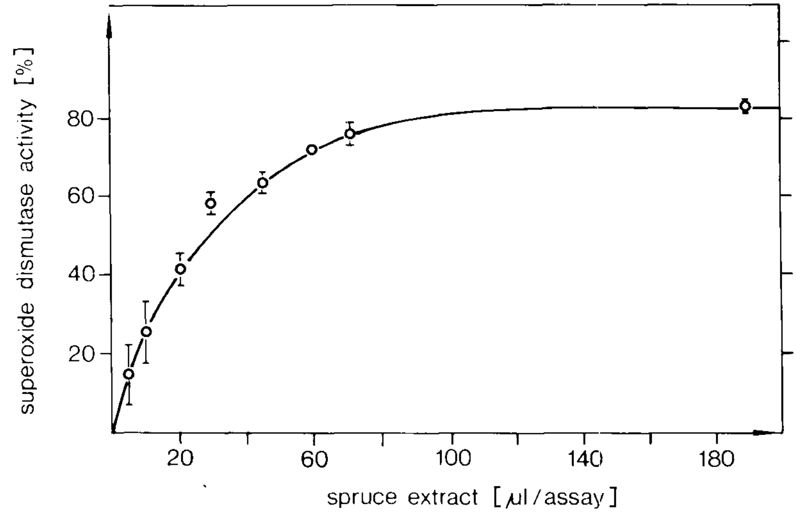

Fig. 1. SOD activity in extracts of spruce needles. One unit of SOD corresponded to 27 µl of extract in a total assay volume of 1 ml. The extract contained a protein concentration of 0.6 mg/ml, n = 5, bars represent standard error.

to alternative oxidative pathways (Misra and Fridovich, 1972; Fridovich, 1986). possibly caused by interactions with other components present in crude dialyzed spruce extracts.

In the presence of cyanide (20 µM NaCN), the inhibition of adrenochrome formation was completely blocked (not shown). This observation indicates that predominately Cu/Zn-containing SOD-species contributed to the activity determined with the epinephrine assay.

It has been reported for other plants that the activity of SOD is dependent upon the developmental stage of the tissue analyzed (Rabinowitch and Fridovich, 1983). However, data on developmentally determined changes in SOD activity in needles of conifers have not been published. Therefore, the activity of SOD was analyzed in 4 subsequent generations of needles of healthy trees and compared with the activity in needles of injured trees with 50% loss of needles.

In needles from healthy trees, SOD activity was highest in the youngest needles and then declined by about 25% in 4 yr old needles. In needles from injured trees, an enhanced level of SOD activity was maintained through the 4 needle generations studied.

Discussion

Enhanced activity of superoxide dismutase in younger leaves has previously been reported in several plant species (Rabinowitch and Fridovich, 1983) and was accompanied by an enhanced tolerance against SO_2 (Tanaka and Sugahara, 1980). Furthermore, higher SOD activities were found in conifer needles after ozone fumigation (Castillo *et al.*, 1987) or if the trees were growing in SO_2-polluted regions (Huttunen and Heiska, 1988). We observed in healthy needles of spruce trees growing in the field that SOD activity

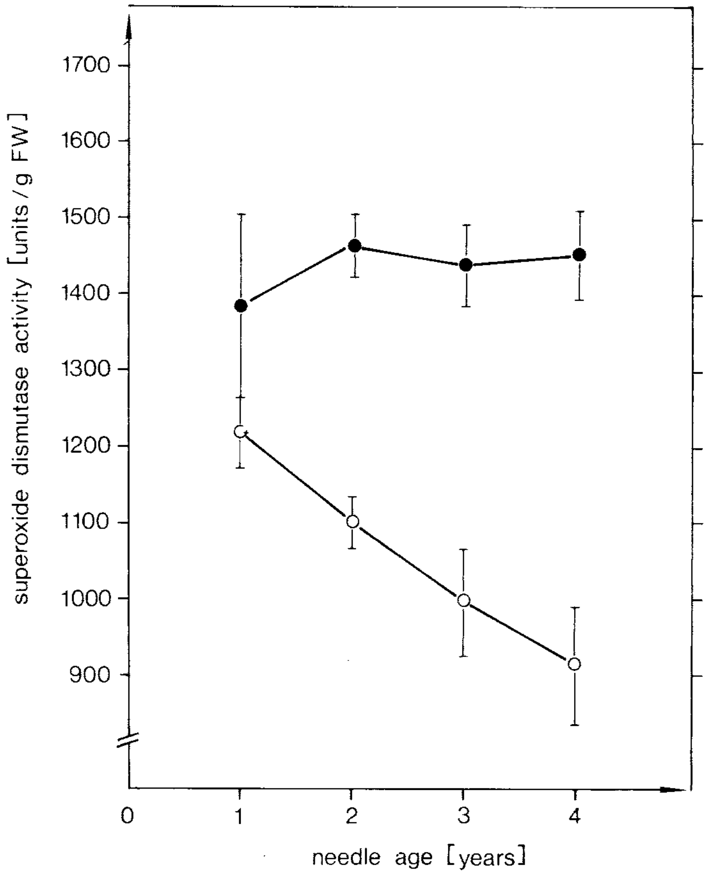

Fig. 2. SOD activity as a function of needle age and habitus of the tree. Needles of the same age from 4 trees were mixed and used for the determination of SOD activity in needles of 'healthy' (○) and 'injured' (●) trees. $n = 4$, bars represent standard error.

showed a maximum in the youngest needles and then declined. In young needles of severely injured trees, the SOD activity was slightly enhanced as compared to SOD activity in needles from healthy trees. This high level of SOD activity was maintained in the 4 needle generations analyzed. This result suggests, that among other factors, SOD activity in young needles is determined by intrinsic developmental factors, while in older needles, external environmental triggering mechanisms, such as, perhaps, air pollution, play a major role in the regulation of SOD activity.

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