SO₂-INDUCED ENZYMATIC CHANGES AND ASCORBIC ACID OXIDATION IN ORYZA SATIVA

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Abstract. Field grown rice (Oryza sativa L.) plants exposed separately to 0.25 or 0.5 ppm SO₂ for 1.5 hr daily for 40 days, showed significant decrease of catalase (p < 0.001) and increase of peroxidase (p < 0.001) activities as well as decreases of protein (p < 0.001) and ascorbic acid (p < 0.001) contents associated with leaf lesions, which were proportional to SO₂-dose. Catalase and peroxidase activity levels showed an inverse relationship. It is hypothesized from the molecular structure of both enzymes and from the in vitro relationship between catalase and peroxidase activity that the tetrameric molecules of catalase in vivo might disintegrate into monomeric units with peroxidase activity, which in turn oxidise ascorbic acid and may reduce tolerance of plants to SO₂.

1. Introduction

Sulphur dioxide has a special position among air pollutants since S is one of the essential nutrients (Lougham, 1964). As a consequence, both harmful and beneficial effects are possible (Bleasdale, 1952; Reinert et al., 1969; Tingey et al., 1971; Thomas et al., 1943). However, the manner in which SO₂ affects the metabolism of the plant is not well understood, partly because of limited understanding of the normal metabolic processes of plants, the way in which they are integrated and controlled, and also because of the metabolic effects of SO₂-itself.

Photosynthetic CO₂-fixation may be inhibited in plants exposed to as low as 50 μg m⁻³ SO₂ (Black and Unsworth, 1979). It has been suggested that exposure of plants to SO₂ may lead to competitive inhibition of ribulose-1,5-diphosphate carboxylase for HCO₃⁻ (Ziegler, 1975), destruction of chlorophyll (Rao and LeBlanc, 1966; Puckett et al., 1973) or a reversible swelling of the thylakoids within the chloroplast (Wellburn et al., 1972). General increase in capacity of the key enzymes of several metabolic pathways, such as respiration, organic acid synthesis, amino acid synthesis and photorespiration are suggested to be consistent with the variation of different groups of metabolites in SO₂-exposed plants (Pierre, 1977; Pierre and Quieroz, 1981).

It has been observed that the level of ascorbic acid starts declining before the appearance of foliar injury symptoms (Keller and Schwager, 1977), and that there exists a positive correlation between decrease in ascorbic acid level and yield reduction of SO₂-exposed plants (Nandi et al., 1981). Rao (1981) also established a positive correlation between increased ascorbic acid content and tolerance of plants to SO₂. Shimagaki et al. (1980) showed that ascorbic acid, being a scavenger of superoxide radicals, may inhibit SO₂-induced chlorophyll degradation. In vitro experiments with plant extracts revealed that ascorbic acid oxidase is responsible for oxidation of ascorbic acid
(Berger, 1947), and that \( \text{SO}_2 \) may inhibit the activity of this enzyme (Ponting and Joslyn, 1948). Haisman (1974) expressed the view that oxidation of ascorbate is possibly brought about by a phenolase. Therefore, in the present paper an attempt has been made to present a possible explanation for the mechanism of ascorbic acid oxidation in \( \text{SO}_2 \)-treated plants.

2. Materials and Methods

2.1. Exposure of Plants to \( \text{SO}_2 \)

Seedlings of rice (\textit{Oryza sativa} L. cv. 'Ratna') were raised in a nursery and when 20-day old, were transplanted 10 cm apart in well manured 1.5 x 1.5 m plots. After a 20-day adaptation period, the transplants were enclosed in 1.5 x 1.5 x 1.5 m polythene chambers and were exposed to two different concentrations (0.25 and 0.5 ppm) of \( \text{SO}_2 \) for 1.5 hr daily from 40 through 79 days of their growth. During the exposure period temperature and relative humidity of the fumigation chamber were 28 ± 2 °C and 70 ± 5%, respectively.

The \( \text{SO}_2 \) gas was generated by bubbling air through 1.0% aqueous \( \text{Na}_2\text{S}_2\text{O}_3 \) solution. The gas concentration within the chamber above the crop canopy was monitored using the method of West and Gaeke (1956).

2.2. Biochemical Analysis of Leaf Samples

Fully expanded leaves that were devoid of any injury symptoms were collected from control (C), 0.25 ppm \( \text{SO}_2 \)-exposed \( (T_1) \) and 0.5 ppm \( \text{SO}_2 \)-exposed \( (T_2) \) plants at 40 (just before starting \( \text{SO}_2 \)-treatment), 50, 60, 70, 80, and 90-day ages, for determining the levels of catalase and peroxidase activities and of protein and ascorbic acid contents.

Determination of Protein: A sample of 0.5 g leaf was homogenized in 80% ethanol. The homogenate was boiled for 10 min and then centrifuged. The pellets were washed successively with 10% (w/v) cold trichloroacetic acid; ethanol-chloroform (3:1, v/v); ethanol-ether (3:1, v/v) and finally with ether. Pellets were evaporated to dryness and the protein was solubilized in 0.1 N NaOH for 15 min in a boiling water bath. After centrifugation, protein content of the supernatant was determined by following the method of Lowry et al. (1951).

Determination of ascorbic acid: The method of Keller and Schwager (1977) was used for the extraction and determination of ascorbic acid content of leaves.

Extraction and assay of enzymes: A 0.5 g leaf sample was homogenized with 10 ml 0.1 M cold phosphate buffer and 5 mM cysteine at pH 6.8, in a chilled mortar and pestle. The homogenate was centrifuged at 10,000 g for 10 min at 0° to 4 °C and the supernatant was used for enzyme assays.

A 5 ml assay mixture for catalase activity, containing 300 \( \mu \text{M} \) phosphate buffer (pH 6.8), 100 \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \) and 1 ml of diluted enzyme extract was incubated for 1 min at 25 °C. The reaction was terminated by adding 10 ml \( \text{H}_2\text{SO}_4 \) (2%) and the residual \( \text{H}_2\text{O}_2 \) was titrated against 0.02 N KMnO\textsubscript{4}. The activity was expressed as \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \) decomposed per m in per mg protein.