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Changes in the kinetic properties of *Zea mays* NADP- malic enzyme in response to sulphur dioxide exposure

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SUMMARY

The effect of sulphur dioxide exposure on the activity of NADP- malic enzyme was studied in the leaf extracts of control and exposed *Zea mays* plants. Ten - days old plants of *Zea mays* were exposed to different concentrations of sulphur dioxide (0.8 to 23 ppm) for 4 hours in a continuous flow exposure chamber under illumination (500W tungsten bulb). The visible injury symptoms in leaves produced due to the exposure were correlated with sulphur dioxide concentration. A concentration dependent decrease in the activity of the enzyme was observed in relation to sulphur dioxide exposure. The inhibition of NADP- malic enzyme by sulphur dioxide was found to be non-competitive with a K_i value of 52.6 ppm sulphur dioxide, with respect to $NADP^+$. The enzyme showed a partial competitive inhibition by sulphur dioxide with respect to malate, whereas the inhibition was competitive with a K_i value of 15 ppm, with respect to Mg^{2+}. The relatively low K_i value with respect to Mg^{2+} demonstrates a sensitive factor for sulphur dioxide damage. The K_m values were 26.3, 142 and 51 µM for $NADP^+$, malate and Mg^{2+}, respectively.

Key words : Sulphur dioxide, *Zea mays*, Inhibition, Chlorophyll, NADP- malic enzyme

Among several air pollutants, sulphur dioxide has been reported as the most widespread phytotoxic air pollutant causing extensive injury to plants (Anuradha *et al.*, 1999; Masood *et al.*, 2001; Izrael *et al.*, 2002; Agarwal and Deepak, 2003; Xiong *et al.*, 2003; Muzika *et al.*, 2004; Wang *et al.*, 2005; Amin *et al.*, 2007; Dar *et al.*, 2008). Sulphur dioxide is known to inhibit photosynthetic carbon dioxide fixation (Masood *et al.*, 1999; Gimeno and Deltoro, 2000). However, the biochemical effects of sulphur dioxide at enzymatic level are less known. Ribulose bisphosphate carboxylase which affects the first step in carbon dioxide fixation in the C_3 pathway of photosynthesis was found to be affected by sulphur dioxide (Zeigler 1972; Masood 1987; Masood *et al.*, 1999). Zeigler (1972) first reported that inhibition of photosynthetic carbon dioxide fixation by sulphur dioxide was due to competition between carbon dioxide and sulphur products for active binding sites on the enzyme. At higher concentrations, this inhibition was reported to be non-competitive (Zeigler, 1972; Mukerji and Yang, 1974). In vitro, bisulphite was found to inhibit the activities of phosphoenolpyruvate carboxylase and malate dehydrogenase, two enzymes involved in the initial steps of C_4 photosynthesis (Osmond and Avadhani, 1970). Sulphite was also found to inhibit these enzymes (Mukerji and Yang, 1974) as well as malic enzyme (Zeigler, 1974) which decarboxylates malate to release carbon dioxide for refixation by ribulose bisphosphate carboxylase.

In our earlier paper (Amin *et al.*, 2007) on the effect of sulphur dioxide on PEP-carboxylase, it was shown that sulphur dioxide causes a competitive inhibition of PEP-carboxylase with respect to HCO_3^-, but acts non – competitively with respect to PEP and Mg^{2+}. In this paper, we report the kinetics of inhibition of NADP- malic enzyme from *Zea mays* leaves by sulphur dioxide.

MATERIALS AND METHODS

Malate and dithiothreitol were purchased from Sigma Chemical Company, USA. EDTA and magnesium chloride (AR) were purchased from Romali, India. NADP was purchased from Loba- Chemie, India. Acetone, agar and HEPES were purchased from Sisco Research Laboratories Pvt. Ltd., Bombay.

The experimental plants were raised from the seeds of *Zea mays* (Hybrid - G2 variety) in pots containing Hoagland Media (EPA, 1975) with 7 - 8 seeds/pot. The pots were wrapped with the black paper and kept in the growth chamber having controlled humidity, 25^{0}C temperature and 16-18 hours photoperiod. Six pots containing 10- days old plants (3-leaf stage) were fumigated with different concentrations of sulphur dioxide for 4 hours in a continuous flow exposure chamber under illumination (500W tungsten bulb). The doses of sulphur dioxide ranged from 0.8 to 23 ppm. The plants were exposed to lowest dose first and the concentration was

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