Effect of L-methionine sulfoximine on the enzymes of nitrogen metabolism in barley leaves

P A KUMAR and Y P ABROL
Nuclear Research Laboratory and Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012, India
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Abstract. L-methionine sulfoximine, a potent inhibitor of glutamine synthetase decreased nitrate reductase activity by 50% at the end of 12 h of treatment while nitrite reductase was insignificantly affected. By 3 h the inhibition of glutamine synthetase activity was complete. Elevated levels of ammonia induced by L-methionine sulfoximine did not influence glutamate dehydrogenase. It is inferred that ammonia accumulation does not affect photosynthetic electron transport which supplies reducing power to nitrite reductase. The failure of glutamate dehydrogenase activity to be induced by high ammonium levels shows that it is not involved in the process of ammonia assimilation in the leaves.

Keywords. Ammonia; methionine sulfoximine; nitrate reductase; nitrite reductase; glutamate dehydrogenase; barley.

1. Introduction

Glutamine synthetase (GS, EC 6.3.1.2) is the principal enzyme involved in the assimilation of ammonia arising from various sources in the plant system (Kumar and Abrol 1990). Inhibition of GS activity by L-methionine sulfoximine (MSO) in photorespiring tissues leads to increased ammonium levels (Martin et al 1983; Kumar et al 1984). Accumulation of toxic levels of NH$_4^+$ may result in uncoupling of photophosphorylation and consequently inhibition of CO$_2$ fixation (Platt and Anthon 1981; Achhireddy et al 1983). This view was later contradicted by Ikeda et al (1984) and Walker et al (1984) who showed that MSO-induced NH$_4^+$ accumulation did not affect CO$_2$ fixation.

Nitrate reductase (NR) and nitrite reductase (NiR) depend on the products of photosynthesis and photosynthetic electron transport, respectively, for the supply of reducing power (Beevers and Hageman 1983). There are no reports in the literature regarding the effects of MSO-induced ammonia accumulation on NR and NiR. Similarly, it is not known whether high amounts of ammonia accumulating as a consequence of MSO treatment, induce glutamate dehydrogenase (GDH) activity. The objective of the present study was to examine the effect of MSO on the activities of the enzymes of nitrogen assimilation in barley leaves.

2. Materials and methods

Barley (Hordeum vulgare L. cv DL-157) seedlings were grown in cement pots (38 x 72 cm) filled with sandy loam soil. The plants were given Hoagland's solution (10 mM KNO$_3$) at weekly interval. Third and fourth leaves of 20–25-day old seedlings were selected for the study. The leaves were cut under water and fed with various treatment solutions via the transpirational stream at a light intensity of
800 μE m⁻² s⁻¹. The pre-treatment of the leaves with either NO₃⁻(10 mM) or glycine (10 mM) was done for 3 h. The concentration of MSO throughout the experimentation was 2.5 mM. Ammonia in the leaf extracts was determined according to Kumar et al (1984).

NR activity was assayed according to Klepper et al (1971). NiR activity was determined following an in vivo procedure developed in our laboratory. Fine slices of leaves (<1 mm) were suspended in a medium containing 300 μmol potassium phosphate (pH 6.9), 3 μmol methyl viologen and 0.5 μmol sodium nitrite. Reaction was initiated by the addition of 20 μmol of sodium dithionite prepared in 0.05 mM phosphate buffer. After incubation for 15 min at 33°C, the reaction was stopped by vigorous agitation of the reaction mixture. Nitrite disappeared was estimated by comparing with a zero time control in which reaction was stopped immediately after the addition of sodium dithionite. GS activity in the leaves was estimated following the procedure of Mohanty and Fletcher (1980). NADH-GDH activity was assayed in the crude mitochondrial fractions, as described by Mohanty and Fletcher (1980).

3. Results and discussion

MSO is a potent inhibitor of GS and ammonia assimilation (Kumar et al 1983). Treatment of barley leaves with 2.5 mM MSO resulted in the accumulation of ammonia (figure 1). Pre-treatment of the leaves with nitrate enhanced the rate of ammonia accumulation. This could be due to an increased flux of nitrogen via nitrate assimilation pathway as a result of NR induction (Beevers and Hageman 1983). Similarly, pre-treatment of the leaves with glycine considerably elevated the rate of ammonia accumulation. Ammonia is released during glycine oxidation in mitochondria under photorespiratory conditions (Singh et al 1985) and the flux of ammonia through the glycolate pathway is 8-10 times more than that occurring via nitrate assimilation route (Keys et al 1978). At the end of 2 h MSO treatment the ammonium level was about 11.2 μmol/g fresh weight of the leaves (nitrate-treated). This concentration was considered to be toxic enough to affect the processes of photosynthesis in the chloroplasts (Givan 1979). In the subsequent experiment,

![Figure 1](image_url)

**Figure 1.** Effect of methionine sulfoximine on the accumulation of ammonia in barley leaves.
leaves treated with nitrate (10 mM) for 3 h were transferred to MSO and activities of the enzymes, NR, NiR, GS and NADH-GDH were assayed at different time intervals up to 24 h.

Table 1 shows the effects of MSO on the activities of the enzymes of nitrogen assimilation. GS activity is inhibited almost completely by 3 h. NR activity persisted in the leaves throughout the treatment period. However, there was a 50% decline at the end of 12 h. NiR activity was more resistant to MSO treatment. There was a decrease of only 22% at 24 h. NADH-GDH activity showed no perturbation consequent to MSO treatment. These results showed that the enzymes of nitrogen assimilation except GS, are more or less resistant to MSO treatment. NR depends on the supply of carbohydrates as the source of reducing power for its action (Beevers and Hageman 1983). Similarly, NiR derives its reducing potential from ferredoxin which is reduced during photosynthetic electron transport (Abrol et al. 1983). The insensitivity of these enzymes to MSO treatment indicates that the processes of photosynthesis in the chloroplasts are not seriously affected as a result of the accumulation of ammonia. The decline in NR activity during the later stages of MSO treatment could be attributed to the limitation of the substrate, nitrate. This was supported by the observation that in nitrogen-starved cells of Chlamydomonas reinhardii, MSO inhibited NR activity. The activity was restored by exogenous supply of nitrate (Florencio and Vega 1983). It was also observed that MSO did not affect NiR activity.

GDH is considered to play a minor role in the assimilation of ammonia in higher plants (Kumar and Abrol 1990). The possible role of GDH in an ammonia detoxification process is supported by the finding that the enzyme is induced by high levels of ammonia (Barash et al. 1973). Similarly, GDH activity increased during senescence, dark stress and proteolysis indicating its significance in situations of high ammonia levels. In the present study, we did not observe any change in GDH activity even after prolonged MSO treatment and we deduce that the enzyme is not involved in ammonia assimilation process. Cammaerts and

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<th>Enzyme</th>
<th>Time (h)</th>
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<td>NR in vivo (µmol g⁻¹ fresh wt. h⁻¹)</td>
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<td></td>
<td>2.00</td>
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<td>NiR in vivo (µmol g⁻¹ fresh wt. min⁻¹)</td>
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<td>3.24</td>
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<td>GS (µmol γ-GHA g⁻¹ fresh wt. min⁻¹)</td>
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<td></td>
<td>5.40</td>
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<td>NADH-GDH (µmol NADH mg⁻¹ protein min⁻¹)</td>
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<td></td>
<td>14.16</td>
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Values in parentheses indicate per cent change.
ND, Not detected.
Jacobs (1985) reported an increase in the level of NAD–GDH but not of NADH–GDH as a result of MSO treatment to Arabidopsis thaliana seedlings. However, NADH–GDH in the roots was induced by MSO treatment. This suggested that GDH is probably involved in ammonia detoxification in the roots.

In conclusion, it can be stated that MSO-induced ammonia accumulation does not inhibit the activities of NR and NiR. The elevated levels of ammonia do not cause any increase in the activity of GDH and thus it may not play any role in ammonia assimilation in the leaves.

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