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MALATE DEHYDROGENASE AND ISOCITRATE DEHYDROGENASE IN ROOT NODULES OF *TRIGONELLA*

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Key Word Index—*Trigonella foenum graecum*: Leguminosae; nitrogen fixation; root nodule; nodule cytosol; bacteroids; dehydrogenase enzymes.

Abstract—The nodule cytosolic dehydrogenase enzymes malate dehydrogenase and isocitrate dehydrogenase in root nodules of *Trigonella foenum-graecum* may provide reducing power and keto acids for the ammonia assimilatory enzymes to assimilate the ammonia exported from bacteroids.

INTRODUCTION

Nitrogenase [nitrogen (acceptor) oxidoreductase; EC 1.7 99.2] requires a reductant for the conversion of nitrogen to ammonia [1]. Free living aerobic nitrogen fixers and *Rhizobium* probably use reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the reductant for nitrogen fixation [2, 3].

Nitrogenase synthesis is repressed by ammonia [4–9]. Ammonia generated by bacteroidal nitrogenase is exported to the nodule cytosol [10–13]. We have reported that *Trigonella foenum-graecum* has an efficient ammonia assimilatory mechanism to make productive use of the ammonia provided by the microsymbiont [14]. Ammonia is fixed into amino acids in the presence of reducing power and the corresponding keto acids, which are provided by the nodule cytosolic dehydrogenase enzymes. Malate dehydrogenase (MDH: EC 1.1.1.37) is present in bacteroid extracts from nodules of *Pisum sativum* [15] and *Phaseolus vulgaris* [16]. Isocitrate dehydrogenase (IDH: EC 1.1.1.42) has been detected in extracts of bacteroids from nodules of *P. sativum* [15] and *Lupinus angustifolius* [17]. However, few reports have addressed the interrelationships between carbon metabolism and the nitrogen fixation process in rhizobia bacteroids and their plant hosts [18–20].

Our purpose was to establish the presence of MDH, IDH, glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.41) and 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.43) during the period of nitrogen fixation in *T. foenum-graecum* and to relate temporal changes in their activities to nitrogen fixation.

RESULTS AND DISCUSSION

Nitrogenase activity of the intact root nodules increased until the 5th week after planting of *T. foenum-graecum* and thereafter declined (Fig. 1). The activities of

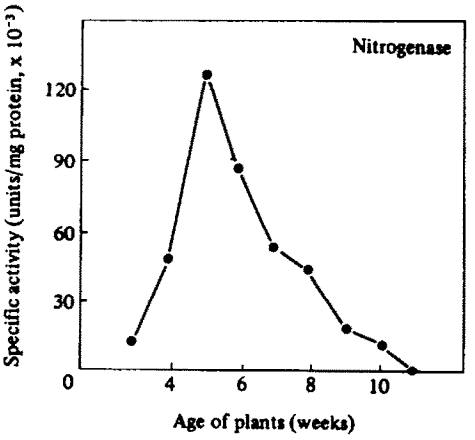

Fig. 1. Levels of nitrogenase activity during the growth cycle of *Trigonella foenum-graecum* (●).

MDH (Fig. 2A) and IDH (Fig. 2B) in the nodule cytosol and bacteroidal fractions were also found to increase as nitrogenase activity increased during the first 5 weeks of plant growth. The activities of G6PD (Fig. 2C) and 6PGD (Fig. 2D) in the nodule cytosol and bacteroids were 10% of the rate of the MDH and IDH activities. Possibly MDH and IDH may be more active than G6PD and 6PGD in providing reducing power and keto acids for nitrogen fixation in *T. foenum-graecum*.

The coincidence of high bacteroidal IDH activity at the time of high rates of nitrogen fixation may indicate that IDH provides reductant for the fixation of nitrogen in the nodules of *T. foenum-graecum*. Similarly, Kurz and La Rue [15] reported bacteroidal IDH as the probable source of reductant for nitrogen fixation in *P. sativum*. Bacteroidal MDH and IDH may also provide keto acids for ammonia assimilation.

We have shown earlier that ammonia incorporation in nodules of *T. foenum-graecum* occurs in the cytosol by an efficient glutamine synthetase–glutamate synthase (GS–GOGAT) pathway, which requires the reducing factors reduced nicotinamide adenine dinucleotide

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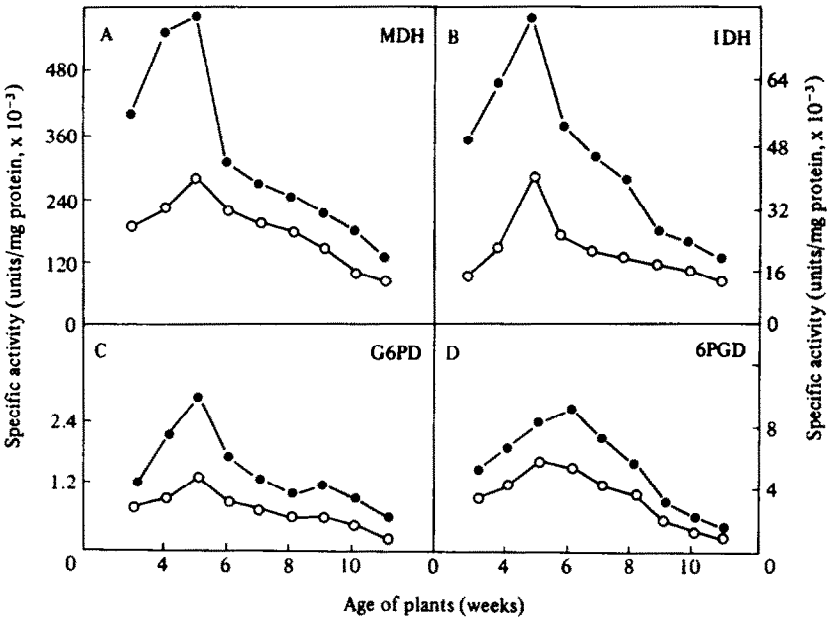

Fig. 2. Levels of enzymes in the nodule cytosol (●) and bacteroids (○) of *Trigonella foenum-graecum* and various ages. A—malate dehydrogenase, B—isocitrate dehydrogenase, C—glucose-6-phospate dehydrogenase, D—6-phosphogluconate dehydrogenase.

(NADH) and NADPH and keto acids [14]. The activities of MDH and IDH concided with the peak of nitrogenase activity. Therefore, MDH and IDH in the nodule cytosol may provide reducing power and keto acids for the assimilation of ammonia exported from bacteroids. High levels of MDH and IDH in the nodule cytosol may also be of significance in maintaining a concentration gradient of ammonia, limiting its availability of the bacteroids and favouring nitrogen fixation.

EXPERIMENTAL

Growth of plants. The *T. foenum-graecum* plants were grown and maintained as described earlier [21].

Enzyme preparation. We separated the bacteroids from the nodule cytosol to extract nodule cytosol and bacteroidal enzymes as described earlier [14].

Enzyme assays. The nitrogenase activity of the intact root nodule was assayed as described in ref. [22]. The unit for nitrogenase was expressed as the amount of enzyme which produces 1.0 μl of ethylene per min at 30°. MDH, IDH, G6PD and 6PGD were assayed as described earlier [21]. MDH activity was defined as the amount of enzyme which brings about an oxidation of 1 μmol of NADH per min at 30°. The units for IDH, G6PD and 6PGD were defined as the amount of enzyme which brings about a reduction of 1.0 μmol of NADP per min at 30°. The activities of all enzymes in the manuscript are expressed in terms of specific activities (units/mg protein) and are the average values of 5 determinations. The protein was estimated by the method of ref. [23] using bovine serum albumin as standard.

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REFERENCES

1. Atkins, C. A. and Rainbird (1982) in *Advances in Agricultural Microbiology* (Subba Rao, N. S., ed.) p. 25. Oxford and IBH Publishing Company, New Delhi.
2. Rawsthorne, S., Minchin, F. R., Summerfield, R. J., Cookson, C. and Coombs, C. (1980) *Phytochemistry* **19**, 341.
3. Wong, P. P., Evans, H. J., Klucas, R. and Russell, S. (1971) *Plant Soil Special Vol.* 525.
4. Daesch, G. and Mortenson, L. E. (1972) *J. Bacteriol.* **110**, 103.
5. Drozed, J. W., Tubb, R. S. and Postgate, J. R. (1972) *J. Gen. Microbiol.* **73**, 221.
6. Tubb, R. S. and Postgate, J. R. (1973) *J. Gen. Microbiol.* **79**, 103.
7. Kurz, W. G. W., La Rue, T. A. and Chastan, K. B. (1975) *Can. J. Microbiol.* **21**, 984.
8. Klainer, D. (1975) *Arch. Microbiol.* **194**, 163.
9. Zumft, W. F. and Castillio, F. (1978) *Arch. Microbiol.* **117**, 53.
10. Bergersen, F. J. (1971) *Annu. Rev. Plant Physiol.* **22**, 121.
11. Miflin, B. J. and Lea, P. J. (1976) *Phytochemistry* **15**, 873.
12. O'Gara, F. and Shanmugam, K. J. (1976) *Biochim. Biophys. Acta* **437**, 313.
13. Givan, C. V. (1979) *Phytochemistry* **18**, 375.
14. Nautiyal, C. S. and Modi, V. V. (1982) *Phytochemistry* **21**, 505.
15. Kurz, W. G. W. and La Rue, T. A. (1977) *Can. J. Microbiol.* **23**, 1197.
16. Grimes, H. and Fottrell, P. F. (1966) *Nature* **212**, 295.
17. Robertson, J. G. and Taylor, M. P. (1973) *Planta* **112**, 1.
18. Henson, C. A. and Collins, M. (1984) *Crop Sci.* **24**, 727.
19. Karr, D. B., Waters, J. K., Suzuki, F. and Emerick, D. W. (1984) *Plant Physiol.* **75**, 1158.
20. Streeter, J. G. and Salminen, S. O. (1985) in *Nitrogen Fixation*

Research Progress (Evans, H. J., Bottomley, P. J. and Newton, W. E., eds) p. 277. Martinus Nijhoff, Dordrecht.
21. Nautiyal, C. S. and Modi, V. V. (1980) *Indian J. Exp. Biol.* **18**, 362.
22. Kurz, W. G. W., Rokosh, D. A. and La Rue, T. A. (1975) *Can. J. Microbiol.* **21**, 1009.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.