10. Narayanaswamy, S., *Lloydia*, 1955, **18,** 61.
11. Narayanaswamy, S., *Phytomorphology*, 1955, **5,** 161.
12. AuLabach-Smith, C. A. and J. M. Herr (Jr.), *Am. J. Bot.*, 1984, **71,** 427.

AMMONIA ASSIMILATING ENZYMES IN *ANABAENA TORULOSA*

N. ANAND and C. CHANDRASEKARAN
Centre of Advanced Studies in Botany, University of Madras, Madras 600025, India.

ACTIVITIES of three ammonia assimilating enzymes *viz*, glutamine synthetase (GS), glutamic acid dehydrogenase (GDH) and alanine dehydrogenase (ADH) in *Anabaena torulosa* (Carm) Lagerh ex Born et Flah under N_2-fixing and nitrogen-amended (as inorganic nitrogen source) conditions are reported. Cultures of *A. torulosa* isolated from the rice fields of Kerala State, India, and made axenic by triple antibiotic treatment with chloramphenicol, streptomycin and benzyl penicillin[1] were maintained in BG_{11} medium[2] at 27 ± 1 C and 2000 lux flourescent illumination. Inorganic nitrogen source used was 20 mM KNO_3 or NH_4Cl. For each of the conditions cultures were grown in triplicates.

For extraction and assay of the enzymes the cultures were harvested by centrifugation and washed twice with 50 mM Tris/HCl buffer (pH 7.5), the filaments were disrupted under ice by using ultrasonic disintegrator for 5 min. The cell debris was removed by centrifugation at 8000 rpm for 30 min and the supernatant assayed for the activity of the enzymes. In the crude extract GS was assayed by the modified γ-glutamyl transferase assay[3]. The activities of GDH and ADH were measured (UNICAM SP 800 B spectrophotometer[4]) as decrease in extinction at 340 nm due to the oxidation of NADPH to NADP at 25°C. Protein was estimated according to Lowry *et al*[5] using bovine serum albumin as the standard.

Table 1 presents data on the specific activities of the three ammonia assimilating enzymes in *A. torulosa*.

GS, which is one of the main ammonia assimilating enzymes, showed maximal activity in nitrogen-fixing cultures and relatively low activity in nitrate and ammonium-grown cultures. This is in accordance with the report of Meeks *et al*[6], who showed that the specific activity of GS was about one half as much in ammonium grown cultures as in nitrogen-fixing cultures of *A. cylindrica*. Similarly GDH and ADH showed higher levels of activity than those reported by Meeks *et al*[6]. Haystead *et al*[7] reported trace amounts of GDH activity in *A. cylindrica*. GDH activity has been detected in some but not all non-heterocystous bluegreen algae[8,9]. Interestingly both GDH and ADH activities were more in cultures supplied with nitrogen either as nitrate or as ammonium, probably the availability of substrates has triggered the assimilatory pathways in both glutamine and alanine directions to offset the substrate inhibition of the assimilation process.

Table 1 *Specific activity (μ mol. min^{-1}, mg prot^{-1}) of ammonia assimilating enzymes in 15-day-old cultures of A. torulosa. (Values are average of three determinations).*

Enzyme	N_2 fixing	Culture condition KNO_3^- grown (20 mM)	NH_4^+ grown (20 mM)
Glutamine synthetase	0.196	0.149	0.101
Glutamic acid dehydrogenase	1.76	2.62	2.69
Alanine dehydrogenase	0.286	0.147	0.613

We thank UGC New Delhi for financial assistance.

24 October 1985; Revised 7 December 1985

1. Droop, M. R., *Brit. Phycol. Bull.*, 1967, **3,** 295.
2. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. and Stanier, R. Y., *J. Gen. Microbiol.*, 1979, **111,** 1.
3. Sampaio, M. J. A. M., Rowell, P. and Stewart, W. D. P., *J. Gen. Microbiol.*, 1979, **111,** 181.
4. Rowell, P. and Stewart, W. D. P., *Arch. Microbiol.*, 1976, **107,** 115.
5. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193,** 265.
6. Meeks, J. C., Wolk, C. P., Thomas, J., Lockav, W., Shaffer, P. W., Austin, S. M., Chein, W. and Goilomety, A., *J. Biol. Chem.*, 1977, **202,** 7894.
7. Haystead, A., Dharmawardene, M. W. N. and Stewart, W. D. P., *Plant Sci. Letts-Tokyo*, 1975, **1,** 439.
8. Pearce, T., Leach, C. K. and Carr. N. G., *J. Gen. Microbiol.*, 1969, **55,** 371.
9. Neilson, A. H. and Doudoroff, M., *Arch. Mikrobiol.*, 1973, **89,** 15.