5-Aminolaevulinate Synthetase of *Micrococcus denitrificans*

By G. H. TAIT (*Department of Chemical Pathology, St. Mary's Hospital Medical School, London W.2, U.K.*)

5-Aminolaevulinate synthetase, the enzyme that catalyses the first step in porphyrin biosynthesis, has previously been detected in only two non-photo-synthetic bacteria, *Spirillum itersonii* (Clark-Walker *et al.*, 1967) and *Propionibacterium shermanii* (Menon & Shemin, 1967).

Micrococcus denitrificans was grown anaerobically in a defined nitrate-containing medium (Chang & Morris, 1962), and 5-aminolaevulinate synthetase activity (60 nmol of 5-aminolaevulinate/h per mg of protein) was detected in high-speed supernatants prepared from disrupted cells. Organisms grown with 1 μ M-iron instead of the normal concentration of 20 μ M had only one-sixth of the enzyme activity. Organisms grown aerobically with either nitrate or ammonia also had low 5-aminolaevulinate synthetase activities (about 12 nmol of 5-aminolaevulinate/h per mg of protein). The activities of 5-aminolaevulinate dehydratase (40 nmol of prophobilinogen/h per mg of protein) and of succinate thiokinase (7 μ mol of succinhydroxamate/h per mg of protein) were unaffected by the growth conditions.

5-Aminolaevulinate synthetase has been purified about 1000-fold by successive protamine sulphate treatment, ammonium sulphate precipitation and chromatography on Sephadex G-200, DEAE-cellulose, hydroxyapatite and Sephadex G-100, although homogeneity has not yet been achieved. This enzyme is much more stable than are the corresponding enzymes from *Rhodospseudomonas spheroides* (Burnham & Lascelles, 1963) and *S. itersonii* (Ho & Lascelles, 1971), although the enzyme from *R. spheroides* has been purified by Thompson *et al.* (1971). The properties of the most highly purified preparation are as follows: the molecular weight is about 68000, both by chromatography on Sephadex G-100 and by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate; the pH optimum is 7.0; K_m values for pyridoxal phosphate (11 μ M), succinyl-CoA (approx. 10 μ M) and glycine (12 mM) are similar to those found with the partially purified enzyme from *R. spheroides* (Tuboi *et al.*, 1970). Haemin, at a concentration of 3 μ M, inhibited the enzyme by 50%. Protoporphyrin was a less effective inhibitor.

That both the cytochrome content (Werhoeven & Takeda, 1956) and the 5-aminolaevulinate synthetase activity are higher in organisms grown anaerobically with nitrate than in cells grown aerobically is consistent with 5-aminolaevulinate synthetase being the rate-controlling enzyme for haem synthesis in *M. denitrificans*. In iron deficiency *M. denitrificans*, unlike

a number of other bacteria (Lascelles, 1964), excretes little or no porphyrin. This finding, coupled with the low 5-aminolaevulinate synthetase activity in iron-deficient cells, suggests that iron may play some role in regulating the synthesis or breakdown of the enzyme.

- Burnham, B. F. & Lascelles, J. (1963) *Biochem. J.* **87**, 462
 Chang, J. P. & Morris, J. G. (1962) *J. Gen. Microbiol.* **29**, 301
 Clark-Walker, G. D., Rittenberg, B. & Lascelles, J. (1967) *J. Bacteriol.* **94**, 1648
 Ho, Y. K. & Lascelles, J. (1971) *Arch. Biochem. Biophys.* **144**, 734
 Lascelles, J. (1964) *Tetrapyrrole Biosynthesis and its Regulation*, p. 34, W. A. Benjamin, New York and Amsterdam
 Menon, I. A. & Shemin, D. (1967) *Arch. Biochem. Biophys.* **121**, 304
 Thompson, R. H., Warnick, G. R. & Burnham, B. F. (1971) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **30**, 1283
 Tuboi, S., Kim, H. J., & Kikuchi, G. (1970) *Arch. Biochem. Biophys.* **138**, 147
 Werhoeven, W. & Takeda, Y. (1956) in *Inorganic Nitrogen Metabolism* (McElroy, W. D. & Glass, B., eds.), p. 159, John Hopkins Press, Baltimore

Enzymic Patterns in Plant Tissue Culture

By M. BOPP and Y. P. S. BAJAJ (*Botanisches Institut der Universität Heidelberg, 69 Heidelberg, Germany*)

An important aspect of the differentiation in plant cells is the change in their enzyme pattern. It is assumed that the visible morphological steps in the cells differentiation immediately follow changes in the pattern of enzymes, either in the differentiating cells themselves or in the neighbouring cells. However, up to now it has been possible in only a few cases to associate the enzyme with the visible changes in the cells or tissues. One of these enzymes is phenylalanine deaminase, which catalyses the conversion of the amino acid phenylalanine into cinnamic acid. Its activity can be regulated through changes in the external factors. This enzyme is directly associated with the flavonoid content of the cell. Frequently with a change in the activity of the enzyme there is a corresponding change in the flavonoid content. Normally for such a study a whole plant is employed, and it is found that in this only a few cells are capable of synthesizing flavonoids. However, it is not known whether only these cells have the corresponding enzyme. So, still almost nothing can be said about the actual regulatory mechanism. This general difficulty can be avoided if one starts out with a large population of possibly similar kinds of cells, e.g. callus cultures or cell suspensions. However, such cultures are mostly capable of only a limited differentiation step. There is a possibility that in such a population, where all the cells proceed with similar