5-Aminolaevulinate Synthetase of *Micrococcus denitrificans*

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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.

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Enzymic Patterns in Plant Tissue Culture

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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

differentiation, a change in enzymes can give better information about a causal connexion, as is the case in an intact plant.

In our experiments, and also in the literature, there are examples of many enzymes where there are characteristic changes in the isoenzyme patterns. For this purpose we have been working with plant tissue cultures of various origins (*Anagallis arvensis*, *Brassica oleracea*, *Daucus carota*, *Nicotiana tabacum* and *Sinapis alba*) to see whether the desired changes in the isoenzymes exist, and if from these results corresponding conclusions can be drawn. Moreover, it will be considered whether this change in enzymic activity depends on the new synthesis of one or many enzymes, or whether there is an activation of already existing enzymes.

Hormonal Control of Cell Division and Differentiation *in vitro*

By I. K. VASIL (*Department of Botany, University of Florida, Gainesville, Fla., U.S.A.*)

Excised 1 cm terminal root segments from germinating seeds of tomato (*Lycopersicum esculentum*) and whole mature seeds of sweet pea (*Lathyrus odoratus*) were cultured *in vitro* on modified White's and/or Murashige & Skoog's nutrient media to study the morphogenetic and fine structural effects of various plant growth substances.

Segments of tomato roots were cultured in modified White's medium in suspension cultures on a shaker. Roots grown in a medium without any auxins show normal growth and double their length in about 10 days; lateral roots appear in normal sequence. The histology of these roots compares closely with that of control roots taken from young seedlings grown on moist filter paper for 7–10 days. In media containing indol-3-ylacetic acid, extension growth of the roots is terminated, the cytohistological zonation of the root tip is lost, and a very large number of loci of intense meristematic activity appear all along the root segment in the region of the pericycle. This activity gives rise to lateral root primordia, which push their way into the cortical region of the parent root but do not emerge out of the root. As a result, the entire root takes on a nodular appearance. Kinetin induces the accumulation of numerous starch grains, which are characteristically grouped around the nucleus in most cells of the root. The typical organization of the root tip is lost, there is no extension growth, and the vascular elements differentiate and extend right up to the root-cap region. Gibberellic acid causes appreciable extension growth, enlargement of cortical and pith cells, and the formation of numerous normal lateral roots. Histochemistry and fine structure of such cultured roots has been studied.

Various phenoxyacetic acids significantly alter the course of morphogenetic development of germinating sweet-pea seeds in Murashige & Skoog's nutrient medium. Extensive cell proliferation takes place from the pericycle region of the hypocotyl, giving rise to an undifferentiated mass of callus tissue. Cells undergoing repeated mitoses are small and show large nuclei with swollen and vacuolated nucleoli, and a non-vacuolated cytoplasm that is rich in RNA. The nuclei of the callus cells are lobed and show numerous enlarged and fragmenting nucleoli with a large population of ribonucleoprotein particles around them. Actinomycin D inhibits cell proliferation caused by phenoxyacetic acids. The swelling and fragmentation of the nucleoli and the formation of numerous ribonucleoprotein particles at the nucleolar sites are interpreted as the response of cells to auxin treatment leading to the formation of increased amounts of rRNA, ribosomes and proteins. These observations confirm the results obtained in histochemical and biochemical studies on auxin action in plant tissues.

Distribution of Photosystems 1 and 2 in the Chloroplast Lamellar Structure

By P. V. SANE (*Shraddhanand peth, Post-Ajani Nagpur, India*)

Spinach chloroplasts were fragmented by using a French pressure cell. The fragments were separated by fractional centrifugation to obtain 1000g (1K), 10000g (10K), 40000g (40K) and 160000g (160K) fractions. The 160K fraction, on the basis of its chlorophyll *a/b* ratio, photochemical activities, cytochrome *P-700* content and Mn content, appeared to contain only photosystem 1, whereas the 10K fraction seemed to be enriched in photosystem 2. The ultrastructural studies showed that the 160K fraction originated from stroma lamellae and possibly end-grana membranes, whereas the 10K fraction originated from the grana stacks. On the basis of these studies a model for the distribution of function among the lamellar structure of a higher-plant chloroplast will be discussed.