Tetrapyrrole Biosynthesis in Plant and Yeast Organelles

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Since chloroplasts were found to possess their own species of ribosomes and characteristic DNA the possibility that these organelles, like mitochondria, possess a limited degree of autonomy has been discussed (Granick & Gibor, 1967). In addition to a variety of enzymes associated with photosynthesis, chloroplasts have a capacity for DNA synthesis, DNA-dependent RNA synthesis, protein synthesis and fatty acid synthesis. It was therefore decided to determine whether chloroplasts, which contain both chlorophylls and haems, possess the enzymes of tetrapyrrole biosynthesis. Both chloroplasts (Jones, 1967; Porra & Lascelles, 1968) and proplastids (Porra & Lascelles, 1968) contain ferrochelatase, which catalyses the incorporation of ferrous ions into protoporphyrin IX to form protohaem. Further, ALA† is converted into porphobilinogen by plant chloroplasts but into protochlorophyll via protoporphyrin IX in plant proplastids (Granick, 1961). Thus the chloroplast in its immature proplastid form seems competent to synthesize both haem and chlorophylls from ALA.

It was now decided to test whether the plant proplastids also possess the ability to form ALA or rely on the mitochondria for supplies of this substrate. ALA synthetase has been demonstrated in cell-free extracts of facultative photosynthetic bacteria and of liver cells of porphyric animals; in the latter case the enzyme is located in the mitochondrion. With a sensitive radiochemical assay of ALA synthetase (Irving & Elliott, 1969) no activity could be found in either proplastids or chloroplasts; in addition, the enzyme could not be found in plant mitochondrial fractions. However, in a distribution study in which this new radiochemical assay was employed ALA synthetase was detected in cell-free extracts of the yeast, *Torulopsis utilis*, grown under nitrogen; unlike strictly anaerobically grown cells, which contain no haem (Barrett, 1962), these cells contained a little (0.045 nmol/mg of protein). No activity could be detected in aerobically grown cells even though they contained as much as 1.45 nmol of haem/mg of protein.

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† Abbreviation: ALA, δ -aminolaevulate.

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Studies on the Phospholipids of Yeast Mitochondria

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Yeast mitochondria were isolated from cells grown in an aerated medium (N-mitochondria) and from partially repressed cells grown in semi-anaerobic conditions and in the presence of a high concentration of glucose (10%, w/v) (R-mitochondria).

The total phospholipid content of N-mitochondria was 0.23 μ mol of lipid P/mg of protein, and the cytochrome *c* oxidase activity was 3.0 μ mol of cytochrome *c* oxidized/min per mg of protein. The R-mitochondria contained only 0.09 μ mol of lipid P/mg of protein and showed a cytochrome *c* oxidase activity of 0.7 μ mol of cytochrome *c* oxidized/min per mg of protein.

The distribution of the main phospholipids given as percentages of total phospholipids in N-mitochondria was: phosphatidylcholine, 42%; phosphatidylinositol, 9%; phosphatidylethanolamine, 35%; cardiolipin, 12%. This was practically unchanged in R-mitochondria.

Phospholipase activity was high in both N- and R-mitochondria with respect to either endogenous or exogenous phospholipid (egg phosphatidylethanolamine). At acidic pH (below pH 5.5) the rate of disappearance of phospholipid molecules was very high with a maximum at pH 4.5, and no accumulation of lyso derivatives was detected, indicating, probably, the presence of a very active lysophospholipase. Phosphatidylcholine is the most rapidly degraded, phosphatidylethanolamine and cardiolipin are also hydrolysed, and phosphatidylinositol remains unchanged. Above pH 6.0 hydrolysis of egg phosphatidylethanolamine led to the accumulation of lysophosphatidylethanolamine. Whereas between pH 7 and 9 endogenous phosphatidylcholine and phosphatidylethanolamine were hydrolysed, the cardiolipin and phosphatidylinositol content of mitochondria remained unchanged. The phospholipids of N-mitochondria being characterized by a

very high content of unsaturated fatty acids, the release of unsaturated versus saturated fatty acids does not reflect exactly a phospholipase A₂ activity; this is also so with R-mitochondria, even though the percentage of saturated fatty acids is greater than in N-mitochondria.

The reacylating activity of yeast mitochondria in

the presence of ATP and CoA was estimated by the incorporation of [¹⁴C]oleate. Below pH 5.0, in the presence of ATP and CoA, the endogenous phospholipid content remains stable but there is no appreciable labelling. Above pH 5.5 and up to pH 9.0 there is a rapid incorporation of radioactivity into phosphatidylcholine and phosphatidylthanolamine.