Properties of Ferrochelatase in Micro-organisms Adapting to Changes in Growth Conditions

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The inner membrane of rat liver mitochondria contains the enzyme ferrochelatase, which catalyses the incorporation of metal ions into porphyrins to form metalloporphyrins. This enzyme may also be readily assayed in yeast mitochondria and in extracts from the photosynthetic bacterium *Rhodospseudomonas spheroides*, two organisms in which the haemoprotein content can be varied by changes in growth conditions. We have found that in mitochondria prepared from iron-limited *Torulopsis utilis* (Light, Ragan, Clegg & Garland, 1968) the K_m of ferrochelatase for metal substrates is drastically lowered, e.g. for Co^{2+} from $6.25\mu\text{M}$ to less than $0.25\mu\text{M}$. The true value for the K_m in iron-limited cells is so low that it cannot be determined accurately by our spectrophotometric assay (Jones & Jones, 1969).

A tenfold increase in the specific activity of ferrochelatase is found in iron-limited yeast mitochondria. There was no change in the K_m of ferrochelatase for the porphyrin substrate. This suggests that the enzyme has two independent binding sites; the properties of ferrochelatase from *R. spheroides* support this view. The K_m for Co^{2+} is the same as for the yeast enzyme ($6.13\mu\text{M}$), but the porphyrin K_m is much higher than in yeast. Kinetic studies with the *R. spheroides* enzyme at various substrate concentrations indicate that the binding sites for metal and porphyrin do not interact.

Changes in the cytochromes of yeast (*Saccharomyces cerevisiae*) are found in petite mutants, chloramphenicol-treated or anaerobically grown cultures, and there is evidence that in some petite mutants, a block in the synthesis of the haem prosthetic groups is responsible for cytochrome deficiencies (Tuppy & Birkmayer, 1969). However, we have found no differences in the properties of ferrochelatase in particles from such yeast. No reactivity with porphyrin cytochrome *c* was ever detected.

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Mitochondrial Metabolism of Aldehydes

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Packer & Greville (1969) showed that glutaraldehyde, a protein-cross-linking agent (Quiocho & Richards, 1964) that traps the configurational states of mitochondria (Deamer, Utsumi & Packer, 1967) at high concentrations (2.5%), stimulates oxygen uptake by mitochondria at low concentrations (e.g. 2mM) and appears to act as a substrate. Previously it had been reported that malondialdehyde, another member of the homologous series, was metabolized rapidly *in vivo* (Placer, Veselkova & Rath, 1965), that it stimulated oxygen uptake by a rat liver homogenate (Holtkamp & Hill, 1951) and that it was readily metabolized by rat liver mitochondria in air if ATP, Mg^{2+} and inorganic phosphate were present (Recknagel & Ghoshal, 1965). Similarly the monoaldehyde formaldehyde has been shown to be metabolized to formate by rat liver mitochondria (Mackenzie, 1955; Frisell & Sorrell, 1967).

Results of our experiments, in which aldehyde metabolism was measured polarographically, confirm the observations that glutaraldehyde, malondialdehyde, acetaldehyde and formaldehyde are metabolized by mitochondria, and we report the metabolism of butyraldehyde and glyoxal, suggesting the presence of an aldehyde oxidase of low specificity in mitochondria. Investigation of cofactor requirements for the mitochondrial metabolism of malondialdehyde show that both Mg^{2+} and Mn^{2+} are stimulatory but that ATP and inorganic phosphate are neither essential nor stimulatory. No enhancement of activity was observed with other ions tested, e.g. Na^+ , K^+ , Ca^{2+} and SO_4^{2-} , suggesting that the effect of Mg^{2+} and Mn^{2+} in stimulating oxygen uptake by mitochondria in the presence of malondialdehyde is a specific effect on the metabolism of malondialdehyde rather than the effect of a general increased energy-dependent ion uptake.

Isolated hepatic aldehyde oxidase is inhibited internally by several common respiratory inhibitors (Handler, Rajagopalan & Aleman, 1964). Addition of inhibitors such as rotenone, Amytal, antimycin A and cyanide to mitochondria completely blocked the oxygen uptake established in the presence of aldehydes; the sites of action of these inhibitors are not yet clear.

The effect of pH over the range 6–9 on the rates of oxygen uptake by mitochondria in the presence of glutaraldehyde was only slight, but in the presence of malondialdehyde there was a large decrease in