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 *Rhodopseudomonas 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 6.25μM to less than 0.25μM. 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 (6.13μM), 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, chloramphenical-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.

We are grateful to the Science Research Council for financial support and to Dr P. B. Garland for the use of his split-beam spectrophotometer. *T. utilis* mitochondria were supplied by Mr R. A. Clegg, Mr B. A. Haddock, Dr P. A. Light and Mr C. I. Ragan.

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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

activity with increasing pH, presumably due to a shift in the equilibrium of the tautomeric system resulting in the increased formation of the sodium salt of β-hydroxyacrolein. Evidence supporting the coupling of phosphorylation to the oxidative metabolism of malondialdehyde has been obtained from experiments using radioactive (^{32}P)phosphate.

A sex difference, in which the rate of aldehyde-stimulated oxygen uptake by mitochondria isolated from female rat livers was much greater, confirms previously observed effects with hepatic aldehyde oxidase preparations (Deitrich, 1966).

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Malate Oxidation by Tomato Fruit Mitochondria

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Intact particles from the outer walls of tomato fruit have been shown to retain the property of respiratory control during the oxidation of tricarboxylic acid-cycle intermediates. In these circumstances the oxidation of these substrates is stimulated by ADP (State 3 according to Chance & Williams, 1955) and then decreases as ADP becomes limiting (State 4). A gradual improvement in both respiratory control and ADP/O uptake ratios has been demonstrated up to incipient ripeness; thereafter the ratios decrease with advancing senescence of the fruit (Hobson, 1970).

Green tomatoes at any stage during development yield particles that oxidize malate rapidly after the addition of a small quantity of ADP, but subsequent additions of the nucleotide are progressively less effective. Exogenous supplies of thiamin pyrophosphate increase the rate of malate oxidation on ADP addition, and constant respiratory control ratios are produced as successive amounts of phosphate acceptor are made available.

Mitochondria from tomatoes during ripening become progressively less dependent on exogenous thiamin pyrophosphate for the production of constant respiratory control ratios after a series of ADP additions, but added cofactor continues to stimulate the oxidation of malate. In addition, the State 4 rate immediately after the display of respiratory control slowly increases with time to a constant value. It has been found that in the presence of added CoA this initial inhibition in State 4 disappears and the rate remains linear.

The progressive decrease in the stimulation of malate by ADP on mitochondria from green fruit is probably due to oxaloacetate accumulation, which is counteracted by the presence of thiamin pyrophosphate. The higher rate of malate oxidation by particles from ripening tomatoes leads to greater oxaloacetate production, the further metabolism of which is dependent on the availability of thiamin pyrophosphate. It appears likely that oxaloacetate concentrations reach such a value at the end of the State 3 oxidation period that the initial part of the subsequent State 4 is inhibited. Since CoA prevents this, it would appear that this cofactor is present in limiting quantities when malate oxidation is rapid. Thus not only the concentrations of the various cofactors but possible changes in susceptibility of the oxidative system to both oxaloacetate and thiamin pyrophosphate (Hulme & Rhodes, 1968) may have important bearings on the onset of the climacteric respiration rise and the ripening processes.

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Phosphorylation Associated with Cyanide-Insensitive Respiration in Plant Mitochondria

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Isolated plant mitochondria possess a variable proportion of their respiration that is not inhibited by cyanide or antimycin A (Bonner, 1961). It has been suggested that the cyanide-insensitive respiration utilizes an alternative oxidase system that bypasses phosphorylation sites II and III (Storey & Bahr, 1969). Reversal of electron transport but not phosphorylation has been observed in plant mitochondria with succinate as substrate in the presence