FERROCHELATASE IN WILD-TYPE AND IN CYTOPLASMIC MUTANTS OF *NEUROSPORA CRASSA*

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1. Introduction

The incorporation of iron into protoporphyrin is the last step in the biosynthesis of protohaemin. Granick [1] and also Goldberg [2] demonstrated the enzymatic nature of this reaction.

Ferrochelatase [3], haemsynthetase [4] or protohaemferrolyase (EC 4.9.1.1) [5], as this enzyme is called, was found in many mammalian organisms [6–14] as well as in micro-organisms [11]. For yeast it has been shown that ferrochelatase activity is located in the mitochondria for the aerobic cells and in the "promitochondria" of anaerobic cells respectively [15]. Jones et al. [16] were able to show that this enzyme is bound to the inner mitochondrial membrane.

Two reasons prompted us to investigate ferrochelatase in cytoplasmic mutants of *Neurospora crassa*. Mitochondria of the mi-1 mutant ("poky") and mitochondria of the chloramphenicol modification (the chloramphenicol modification arises by growing wild-type hyphae in the presence of chloramphenicol) show no or only very few christae, and the inner mitochondrial membrane seems to be altered in some way or is deficient in some of the proteins building up the christae [17]. At the same time no cytochrome aa_3 and only a low content of cytochrome b and c_1 could be detected spectroscopically in the mi-1 mutant and in the chloramphenicol modification, which could be due to a deficiency in cytohaemin and to some extent in protohaemin in the mitochondria [18].

2. Materials and methods

Strains and growth conditions: the mi-1 mutant was a generous gift of M.B.Mitchell. Hyphae were grown in Vogel's minimal medium [19] and 2% glucose with shaking. The chloramphenicol modification was cultivated in the presence of 4 mg D(-) chloramphenicol per ml medium.

Isolation of mitochondria: mitochondria were isolated by differential centrifugation after disrupting the hyphae by grinding them between two rotating discs made of corundum [17]. The crude mitochondria were purified by isopycnic centrifugation in a linear sucrose gradient [20]. Protoporphyrin was isolated according to Fischer and Pützer [21].

Determination of ferrochelatase activity: ferrochelatase activity was measured by the pyridinhaemochromogen method described by Porra and Jones [11] as modified by Riethmüller and Tuppy [15], with the one exception that Tween 20 was omitted in protoporphyrin solution. Protein was determined after Lowry et al. [22].

3. Results

Purified mitochondria of wild-type *Neurospora crassa* and of the mi-1 mutant, as well as the chloramphenicol modification, contain equal amounts of ferrochelatase activity as can be seen in table 1. In the cytoplasmic-respiration-deficient "petite" mutant of yeast the specific activity was very similar

Table 1

Ferrochelatase activity of mitochondria from different sources. Ferrochelatase was determined by incubating protoporphyrin and ferrous sulfate with a 0.5ml suspension of mitochondria (approximately 5 mg/ml) for 30 min at 37°C and pH 7.8 (0.1 M tris-SO_4 buffer). See also Materials and Methods.

Organism	Fraction	Specific activity (expressed as μmoles protohaemin formed per minute and mg protein)
Neurospora	wild-type mitochondria	1.83×10^{-4}
	mi-1 mutant mitochondria	1.56×10^{-4}
	CAP*-modification mitochondria	1.95×10^{-4}
	wild-type mitochondrial supernatant	$>0.001 \times 10^{-4}$
Yeast	wild-type mitochondria	1.90×10^{-4}
	"petite" mutant mitochondria	1.87×10^{-4}
Beef heart	mitochondria	1.44×10^{-4}

* CAP = Chloramphenicol

to that of the wild-type of *Saccharomyces cerevisiae* and the specific activity of the *Neurospora* ferrochelatase was comparable with that of the yeast enzyme. The mitochondrial supernatant showed no detectable ferrochelatase activity. Ferrochelatase could be inhibited completely by 10^{-3} M EDTA.

The effect of the pH on the ferrochelatase activity is shown in fig. 1. Ferrochelatase of *Neurospora crassa* has its pH optimum at 7.95, which differs from that of the yeast enzyme but is closely similar to that of pig liver [11]. The ferrochelatase of the cytoplasmic mutants (mi-1 and chloramphenicol modification) shows the same pH-activity curve as the wild-type enzyme. No increase of the specific activity of the *Neurospora* enzyme in the region of pH 9.0 could be detected as described for the yeast and the pig-liver enzymes.

The suggestion of Porra and Jones [11] that there are possibly two ferrochelatase isoenzymes in pig liver with different but to some extent overlapping pH optima cannot be so for the *Neurospora crassa*

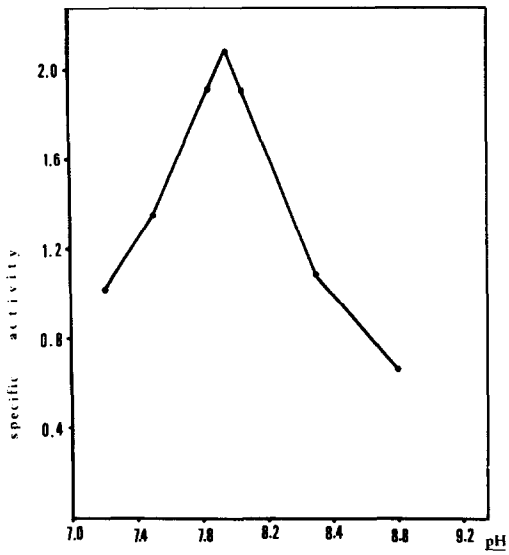

Fig. 1. Effect of pH on the specific activity of ferrochelatase. Ferrochelatase was measured by the pyridin-haemochromogen method using 0.1 M tris-SO_4 buffer of varying pH. Specific activity is defined as μmoles of protohaemin formed per minute and mg protein. See also Materials and Methods.

organism. From the data presented we conclude that in the chloramphenicol modification and in the mi-1 mutant the part of the inner mitochondrial membrane where ferrochelatase is located is unaltered in relation to this enzyme, and that ferrochelatase does not contribute to the formation of the christae. Furthermore it seems likely that ferrochelatase activity is without influence on the deficiency of membrane-bound cytochromes. The investigations will be extended to other enzymes and components involved in the biosynthesis of functional active cytochrome aa_3, b and c_1.

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