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RAT LIVER PROTOPORPHYRINOGEN IX OXIDASE: SITE OF SYNTHESIS AND FACTOR INFLUENCING ITS ACTIVITY

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Summary. Rat liver protoporphyrinogen IX oxidase is not formed in mitochondria in contrast to the claims made for the yeast enzyme (Poulson and Polglase, FEBS Lett. (1974) 40, 258). Inhibition of mitochondrial protein synthesis in regenerating rat livers by thiamphenicol led, instead, to a slight increase in protoporphyrinogen oxidase activity. Protoporphyrinogen IX oxidase was not induced in rat liver by triiodothyronine, an inducer of mitochondrial protein synthesis, or by AIA, an inducer of heme synthesis. Significant increases in activity were observed to be associated with rapidly growing cells, such as regenerating livers and rat ascites hepatoma cells.

Protoporphyrinogen IX oxidase, the pentulimate enzyme in heme synthesis, catalyses the oxidation of protophorphyrinogen IX to porphyrinogen IX. The yeast and rat liver enzymes have been partially purified, and characterized (1, 2). Both are located in the mitochondrion (1, 2). Yeast protoporphyrinogen oxidase is reportedly repressed in cells grown in the presence of glucose (3), and is inhibited in cells grown in the presence of chloramphenicol but not in cycloheximide-treated cells (3). Hence it was suggested (3) that yeast protoporphyrinogen IX oxidase is formed in mitochondria, a conclusion which infers a mitochondrial location for the gene of this enzyme. In view of the fact that the products of several mammalian mitochondrial genes remain unidentified (4, 5), including a peptide with an apparent molecular weight similar to that reported for liver protoporphyrinogen IX oxidase (2), we have investigated the effects of altered mitochondrial protein synthesis on liver protoporphyrinogen IX oxidase.

Methods

Protoporphyrinogen IX oxidase was measured in an incubation media containing 0.5% Triton X-100, 0.1 M Tris-HCl, 1 mM EDTA, 0.1 mM cysteine, pH 8.7, and 0.5 to 1 mg of mitochondrial

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protein in a total volume of 1 ml as described (2). The incubation mixture was equilibrated to 25°C in the dark in the presence of air, and the reaction was then started by addition of freshly prepared protoporphyrinogen IX to a final concentration of 30 μM. The formation of protoporphyrin IX was followed either by spectral scanning of the mixture at regular intervals as in (2), or kinetically by continously monitoring the formation of protoporphyrin IX at 542 nm minus 558 nm in an Aminco DW spectrophotometer. An extinction coefficient of 9.84 $mM^{-1} \cdot cm^{-1}$ was used for these wavelength pairs. Samples without enzyme were run simultaneously to determine the nonenzymatic rate of protoporphyrin IX formation.

Enzyme substrate was prepared as in (1). Protoporphyrin IX was prepared from its dimethyl ester (Sigma) by hydrolysis overnight in 7 N HCl (1). Protoporphyrinogen IX was prepared by oxidation of protoporphyrin IX (0.25 mM) with freshly ground 3% (W/W) amalgam (1 g/ml of solution) for 2 min under N_2 in the dark. Protoporphyrinogen IX was adjusted to pH 7.5 with 40% H_3PO_4 and kept under N_2 until used in the assay.

Male, Sprague-Dawley rats (120-150 g) were used. Partial hepatectomy was performed by excising 75% of the liver. Thiamphenicol (Sigma) (600 mg/kg body weight) was injected twice daily for a period of three days after hepatectomy to inhibit mitochondrial protein synthesis (6). Published methods were used for purification of mitochondria from normal, regenerating rat liver and rat ascites hepatoma (7).

Cytochrome oxidase activity (8) and heme aa_3 (9) were determined as published. The content of cytochrome oxidase subunits I and II in mitochondria isolated from thiamphenicol-treated and control regenerating livers was determined by immunoreplication (10). Mitochondria were separated on SDS-Page gels, blotted onto nitrocellulose sheets, and decorated with antisera against cytochrome oxidase subunits I and II and then with ^{125}I-labeled protein A. The subunits were located autoradiographically, cut from the strips and radioactivity was counted.

Results

Mitochondria from normal, resting, rat liver catalyzed the conversion of protoporphyrinogen IX to protoporphyrin IX at a rate of 11 nmoles/hr/mg protein, in good agreement with previously reported results (2). Similar values were obtained by scanning the spectra after various periods of incubation (2) or by the kinetic methods described here. The reactions were linear in both assays for a period of 1-2 hours and with increasing mitochondrial protein up to 2 mg. The non-enzymatic rate of protoporphyrin IX formation was 1-1.5 nmoles/hr.

The suggestion that protoporphyrinogen IX oxidase is synthesized in mitochondria (3) was tested in partially hepatectomized rats treated with thiamphenicol (Table 1). Thiamphenicol decreased cytochrome oxidase activity and heme aa_3 by about 50% (Table 1). Cytochrome oxidase subunits I and II, which are made in mitochondria (11), are also decreased by 30-50% in thiamphenicol treated rats (Table 1), showing that mitochondrial transla-

Table 1. Effects of Thiamphenicol on Protoporphyrinogen IX Oxidase and Cytochrome Oxidase in Mitochondria from Regenerating Rat Liver

Source of mitochondria	protoporphyrinogen IX oxidase	Cytochrome oxidase		Subunit	
				I	II
	activity ($nmol \cdot mg^{-1} \cdot hour^{-1}$)	activity ($nmol \cdot min^{-1} mg^{-1}$)	heme aa_3 ($nmol \cdot mg^{-1}$)	($cpm \cdot mg^{-1}$)	
Resting rat liver	11.4 ± 0.41 (7)	2,700 (7)	0.220 (7)	-	-
Regenerating rat liver	15.2 ± 1.1 (4)	2,920 (2)	0.239 (2)	18×10^5	16×10^5
Regenerating rat liver + thiamphenicol	17.9 ± 0.6 (5)	1,350 (2)	0.104 (2)	13×10^5	8×10^5

The values are mean values based on the number of mitochondrial preparations shown in parentheses. Standard errors of the Mean are also given for protoporphyrin oxidase activities. The quantification of subunits I and II is shown for a single, typical experiment since the absolute values obtained with immunoblotting can vary greatly between experiments due to the specific activity and age of the ^{125}I protein A used.

tion is indeed, inhibited under the conditions used. In contrast, the activity of protoporphyrinogen IX oxidase increased slightly in thiamphenicol treated rats. This result shows that protoporphyrinogen IX oxidase is not synthesized on mitochondrial ribosomes, and, by inference, must be synthesized in the cytoplasm.

Triiodothyronine (T_3) (20 µg/100 g body weight for 3 days) stimulates mitochondrial protein synthesis (12), but did not significantly increase the activity of protoporphyrinogen IX oxidase (Table 2). This is in keeping with the conclusion that rat liver protoporphyrinogen IX oxidase is not synthesized on mitochondrial ribosomes. Induction of heme synthesis by allyl-iso-propylacetamide (AIA) also had no effect upon the activity of mitochondrial protoporphyrinogen IX oxidase (Table 2).

Increased protoporphyrinogen IX oxidase activity appears, however, to be associated with rapidly dividing cell types (Table 1 and 2). The activities in mitochondria from regenerating rat livers and Zajdela hepatomas ascites cells are 30-50% higher than those in mitochondria from normal, resting rat liver.

Discussion

Two types of experiments are presented which show that rat liver protoporphyrinogen IX oxidase is not translated on mitochondrial ribosomes, in contrast to that reported for the

Table 2. Influence of Altered Metabolic States on the Activity of Liver Protoporphyrinogen IX oxidase

Source of mitochondria	protoporphyrinogen IX oxidase (nmoles·mg protein^{-1}·hour)	% of control
Liver, control rates	11.0 ± 0.8 (4)	100
Liver, AIA-treated rats	11.3 ± 0.8 (6)	100
Liver, T_3-treated rats	12.2 ± 0.3 (5)	106
Regenerating rat livers	15.2 ± 1.1 (4)	133
Zajdela hepatoma cells	16.9 ± 1.8 (4)	148
nonenzymatic oxidation	1.2	

Enzyme activities are expressed as the Mean ± Standard errors based upon the numbers of mitochondrial preparations shown in parentheses.

yeast enzyme (3). Firstly, protoporphyrinogen IX oxidase is slightly stimulated in mitochondria from rats treated with thiamphenicol, whereas cytochrome oxidase activity, heme aa_3, and subunits I and II of cytochrome oxidase were decreased upon treatment. Secondly, triiodothyronine, an inducer of mitochondrial protein synthesis (12), did not induce protoporphyrinogen IX oxidase activity.

The most reasonable explanation for the differences reported here and those reported for yeast (3) is that the site of translation of protoporphyrinogen IX oxidase differs in rat liver and yeast, inferring that the genes also have different locations. An argument against a mitochondrial location of the yeast protoporphyrinogen IX oxidase gene is that the rho$^-$ strain, which lacks mitochondrial protein synthesis, produces large amounts of cytochrome c (13). However, it should be pointed out that non-enzymatic conversion of protoporphyrinogen IX to protoporphyrin IX is relatively rapid (ref. 2 and this paper), and which might be sufficient to support cytochrome c synthesis in the rho$^-$ strain. This point should be re-investigated in the future.

Rat liver protoporphyrinogen IX oxidase is not under thyroid hormone control and is not induced together with heme synthesis, as shown by its lack of response to AIA. The activity of protoporphyrinogen IX oxidase appears to be increased slightly in rapidly dividing cells, such as regenerating rat livers and rapidly growing rat ascites hepatoma cells. This increase

would have physiological relevance, however, only if protoporphyrinogen IX oxidase became rate limiting under conditions were amino levulinic acid synthetase is induced several fold. Such is the case, for example, in rapidly growing fetal and neonatal livers (14, 15, 17).

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