INVOLVEMENT OF p-OH-BENZOYL-COENZYME A IN THE BIOSYNTHESIS OF UBIQUINONE-9 IN THE RAT[1]

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SUMMARY

Evidence is presented for the involvement of the coenzyme A derivative of p-OH-benzoate in ubiquinone-9 biosynthesis in the rat. Radioactivity from p-OH-benzoyl-^{3}H coenzyme A was incorporated by rat liver mitochondria into 2-nonaprenyl-6-methoxyphenol (2-NPMP), a known intermediate in the biosynthesis of ubiquinone-9 in the rat. The reaction demonstrated typical saturation kinetics with an apparent K_m of 5×10^{-5} M. Free coenzyme A stimulated the incorporation of p-OH-benzoate into 2-NPMP in mitochondria whereas other common thiols were inhibitory. Glycine appeared to divert the p-OH-benzoyl CoA toward p-OH-hippurate instead of 2-NPMP.

INTRODUCTION

It has been shown that the aromatic ring system of ubiquinone-9 in the rat is derived from phenylalanine and tyrosine (1, 2). It was also shown that benzoate and p-OH-benzoate were the respective intermediates involved in the conversion (3). With the discovery of 2-nonaprenyl-6-methoxyphenol (2-NPMP) as an intermediate for the biosynthesis of ubiquinone-9 in rat (4),

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it became possible to test the direct conversion of p-OH-benzoate and derivatives to this lipid by mitochondria (5). It is likely that the conversion of p-OH-benzoate to ubiquinone in the rat follows the general outlines of the scheme presented by Friis, et al. (6) on the basis of the isolation of a number of polyprenyl phenols and quinones from Rhodospirillum rubrum. What is not clear, however, are the mechanisms involved in this metabolic sequence.

The purpose of this communication is to report a novel role of coenzyme A as p-OH-benzoyl-coenzyme A in the biosynthesis of 2-NPMP in the rat in vitro.

MATERIALS AND METHODS

Generally tritiated p-OH-benzoate (HBA-^{3}H) was prepared by catalytic exchange method. The compound was purified by thin layer chromatography on silica gel G using benzene:dioxane: acetic acid (105:25:4) as solvent. The purified material had specific activity of 800 mC/mM and was used without dilution. p-OH-benzoyl-coenzyme A was synthesized by condensing HBA-^{3}H with ethylchloroformate. The mixed anhydride was characterized by infra red spectrometry and the yield was determined by the method of Lipmann and Tuttle (7). The mixed anhydride was dissolved in tetrahydrofurane and was added to aqueous solution of coenzyme A. The pH of the reaction was kept at 8.0 during the reaction period. Paper chromatography (8) of the product showed one spot (R_f 0.70) characteristic of p-OH-benzoyl-coenzyme A and gave a positive nitroprusside spray reaction after alkaline hydrolysis.

For the localization of 2-NPMP in cell particulates, liver slices were incubated with HBA-^{3}H in Krebs-Ringers phosphate buffer pH 7.4 for one hour. Subcellular particles

were prepared from these slices in fortified 0.25 sucrose by the method of Bucher and McGarrahan (9). 2-NPMP was isolated from tissues and particulates, by desiccation with anhydrous sodium sulfate and extraction with ethyl ether. The lipid extract then was carried through a series of column and thin layer chromatographies (5). Synthetic 2-NPMP was used as carrier when necessary.

For studies of the biosynthesis of 2-NPMP from HBA, mitochondria were incubated one hour with HBA-^{3}H or its coenzyme A derivative in a 0.1M phosphate buffer pH 7.4 for 1 hour. The medium also contained 40 mM nicotinamide, 3.0 mM ATP, 0.50 mM DPNH, 0.20 mM TPN, 5.0 mM glutamate, 4.0 mM succinate, 1.0 mM methionine, and 0.2 mM S-adenosylmethionine.

TABLE I

DISTRIBUTION OF 2-NONAPRENYL-6-METHOXYPHENOL IN CELL PARTICULATES OF RAT LIVER AFTER ADMINISTRATION OF p-OH-BENZOATE-G-^{3}H

Particulate	2-NPMP		Gibb's Reaction
	Nanomoles/ mg Protein	Per Cent Total	
Mitochondria	0.055	84	Positive
Microsomes	0.003	3	Negative
Cytosol	0.004	13	Negative

Each value represent the average of 10 incubations of slices from 2 g liver in 10 ml phosphate buffer pH 7.4 and 1.25 x 10^{-6} Molar p-OH-benzoate-G-^{3}H (specific activity 800 mC/mM). The mixture was incubated 1 hour at 37°. Particulates were prepared from pooled liver slices after incubation. Total 2-NPMP content of rat liver = 2.5 nanomoles/g fresh.

RESULTS AND DISCUSSION

After incubation of liver slices with HBA-^{3}H, the mitochondrial fraction has the highest percent of total 2-NPMP and the highest content per mg of protein (Table I). The results of incubation of the mitochondria with HBA-^{3}H, p-OH-benzoyl-G-^{3}H-coenzyme A (HBA-^{3}H-CoA), and HBA-^{3}H plus coenzyme A are shown in Table II. It is clear that HBA-^{3}H-CoA is the best precursor of 2-NPMP, being 6-7 fold higher

TABLE II

INCORPORATION OF HBA-^{3}H, HBA-^{3}H-CoA AND HBA-^{3}H PLUS CoA INTO 2-NONAPRENYL-6-METHOXYPHENOL

Substrate	HBA-^{3}H	HBA-^{3}H-CoA	HBA-^{3}H Plus CoA
% Incorporation	0.011	0.070	0.030

Each value represent average of 3 experiments. In each experiment, mitochondria of 2 g rat liver were suspended in 10 ml phosphate buffer pH 7.4 and incubated with 6 x 10^{-8} Molar HBA-^{3}H and HBA-^{3}H-CoA (specific activity 800 mC/mM) and 6.5 x 10^{-6} Molar CoA. The mixture was incubated for 2 hours at 37°.

than HBA-^{3}H. The addition of free coenzyme A increased the incorporation of HBA-^{3}H into 2-NPMP 3 fold. The specificity of coenzyme A and other thiol compounds is shown in Table III. The incorporation of HBA-^{3}H into 2-NPMP was greatest when coenzyme A was added. The other thiols tested did not increase the incorporation and, in fact, they had an inhibitory effect.

TABLE III

EFFECT OF COMMON THIOLS ON THE INCORPORATION OF p-OH-BENZOATE-G-^{3}H INTO 2-NONAPRENYL-6-METHOXYPHENOL

Thiol	DPM	Per Cent Incorporation
Coenzyme A-SH	11,000	0.100
Cysteine	800	0.007
Cystine	710	0.006
Glutathione-SH	375	0.003
Glutathione	610	0.005
Control	1,225	0.011

In each experiment, 2 g of liver mitochondria were incubated in 15 ml phosphate buffer pH 7.4, containing 4.1 x 10^{-7} Molar HBA-^{3}H (specific activity 800 mC/mM), and 3 x 10^{-3} Molar thiol for 2 hours at 37°.

The incorporation of tritium from HBA-^{3}H-CoA into 2-NPMP is particularly interesting in view of the suspected impermeability of acyl-coenzyme A derivatives to mitochondria. The carnitine transferring system (10) does not appear to be involved since the addition of 3 mM carnitine to the mitochondrial incubating system did not increase the incorporation of HBA-^{3}H into 2-NPMP and decreased the incorporation of label from HBA-^{3}H-CoA into 2-NPMP to 20% of control values.

Figure 1 demonstrates saturated kinetics for the biosynthesis of 2-NPMP from p-OH-benzoyl-coenzyme A in mitochondria. The apparent K_m is about 5 x 10^{-5} M with the enzyme system becoming saturated at 0.1 mM.

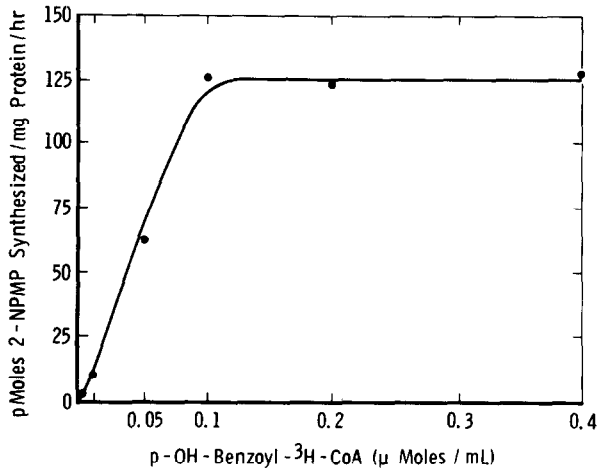

Figure 1. THE IN VITRO BIOSYNTHESIS OF 2-NPMP FROM HBA-^{3}H-CoA BY RAT LIVER MITOCHONDRIA. Each point represents the average of two experiments in which mitochondria (8 mg protein) were incubated under the usual conditions with HBA-^{3}H coenzyme A at the designated concentration for 1 hour.

Experiments with HBA-^{3}H-CoA in presence of different concentrations of glycine were of interest. Addition of 2.5 mM glycine to the mitochondrial system inhibited the incorporation of HBA-^{3}H into 2-NPMP 75% where 5.0 mM glycine completely inhibited the reaction presumably by diverting HBA-^{3}H-CoA to p-OH-hippurate (11).

From the experiments reported here, it appears that p-OH-benzoyl-coenzyme A is the reactive metabolite in the isoprenylation of p-OH-benzoyl-coenzyme A. The coenzyme A thioester of p-OH-benzoate favors electrophilic attack (12) at the ortho position to the hydroxyl since the thioester decreases electron withdrawal from the ring. This would facilitate the electrophilic substitution of the isoprenyl moiety to yield a postulated 1-hydroxy,2-polyprenyl,4-carboxythioester of benzoate.

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