SOME PROPERTIES OF A FATTY ACID ω-HYDROXYLATION SYSTEM SOLUBILIZED FROM LIVER MICROSOMES

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1. Introduction

Ichikawa and Yamano [1, 2] reported that the purified cytochrome *P*-450 from rabbit liver microsomes was readily reduced by NADPH, ferredoxin-NADP reductase and ferredoxin (spinach electron transport system). However, the activities of aniline hydroxylation and aminopyrine demethylation could not be restored by the reduction of the cytochrome *P*-450 with such spinach electron transport system. We have recently found that the ω-hydroxylation system of medium-chain fatty acids in pig kidney microsomes was resolved into two protein fractions, one of which was replaced by the spinach electron transport system [3]. In the present work, this finding was extended to the fatty acid ω-hydroxylation system in rat liver microsomes. In addition, it was found that the treatment of the enzyme preparations with ether resulted in a great decrease of the activity, which could be markedly restored by Triton X-100.

2. Methods

Rat livers were homogenized with 4 parts of 1.15% KCl in a glass homogenizer for 1.5 min. After the homogenates were centrifuged at 10,000 *g* for 20 min, the microsomes were obtained by centrifuging the supernatant at 105,000 *g* for 60 min, washed once, and suspended in 1.15% KCl to a protein concentration of approximately 25 mg per ml. The microsomes were then solubilized by use of Triton X-100, and separated into two protein fractions in essentially the same manner as that described in the preceding paper [3] with the pig kidney microsomes. The two protein fractions thus obtained from the liver microsomes were designated as Fraction I and II in this paper. The former fraction contained cytochrome *P*-450, while the latter fraction had NADPH-cytochrome *c* reductase activity. Ferredoxin and ferredoxin-NADP reductase were prepared from spinach leaves by the methods of Tagawa and Arnon [4], and Shin et al. [5], respectively. Ether-extracted enzyme preparations were prepared by mixing Fraction I or II with excess ether for 1 min, followed by centrifugation. Successive extractions were carried out in the same way with fresh ether. Egg yolk lecithin was purified by thin-layer chromatography, and used in some experiments. The activities of laurate ω-hydroxylation and aminopyrine demethylation were determined by the methods of Kusunose et al. [6], and Nash [7], respectively.

3. Results and discussion

3.1. *Reconstitution of the fatty acid ω-hydroxylation system by Fraction I, and Fraction II or the spinach electron transport system*

Although the liver microsomes were active in laurate ω-hydroxylation [8], neither Fraction I or Fraction II alone had significant activity. As table 1 shows, however, the recombination of these two fractions restored the laurate ω-hydroxylation activity.

Table 1
Laurate ω-hydroxylation by solubilized preparations from rat liver microsomes.

System	Products (nmoles)
Fraction I	0.00
Fraction II	0.06
Fraction I + Fraction II	1.56
Fraction I + ferredoxin	0.00
Fraction I + ferredoxin-NADP reductase	0.00
Fraction I + ferredoxin + ferredoxin-NADP reductase	0.29
Ferredoxin + ferredoxin-NADP reductase	0.00

Reaction mixture containing 50 μmoles of tris-HCl buffer (pH 8.1), 0.2 μmole of NADPH, 2.4 nmoles of laurate-1-^{14}C (7.2×10^4 cpm), and enzyme preparations, in a total volume of 0.5 ml, was incubated at 37° for 20 min. Fraction I, 0.26 mg protein; Fraction II, 0.33 mg; ferredoxin, 48 μg; ferredoxin-NADP reductase, 5.4 μg. No stimulation by ferredoxin and ferredoxin-NADP reductase was observed with Fraction II alone in the experiments of tables 1 and 2.

Table 2
Oxidative demethylation of aminopyrine by solubilized preparations from rat liver microsomes.

System	Products (nmoles)
Fraction I	4.18
Fraction II	7.23
Fraction I + Fraction II	36.90
Fraction I + ferredoxin	0.00
Fraction I + ferredoxin-NADP reductase	0.00
Fraction I + ferredoxin + ferredoxin-NADP reductase	19.80
Ferredoxin + ferredoxin-NADP reductase	0.00

Reaction mixture containing 50 μmoles of tris-HCl buffer (pH 8.1), 0.5 μmole of NADPH, 2.5 μmoles of aminopyrine, and enzyme preparations, in a final volume of 0.5 ml, was incubated at 37° for 20 min. Fraction I, 0.67 mg of protein; Fraction II, 0.94 mg; ferredoxin, 48 μg; ferredoxin-NADP reductase, 5.4 μg. This experiment was carried out simultaneously with that of table 1.

Furthermore, the spinach electron transport system could partially replace Fraction II. Fig. 1 illustrates that the activity was proportional to the amounts of ferredoxin in this reconstituted system. No significant activity was observed with Fraction II and the spinach electron transport system, similar to the fatty acid ω-hydroxylation system in pig kidney microsomes [3].

Furthermore, it was found that the oxidative demethylation of aminopyrine also required both Fraction I and II for the maximal activity, and that the latter fraction was partially replaced by the spinach electron transport system (table 2).

3.2. *Restoration of the activity of the ether-extracted enzyme preparations by Triton X-100*

Lu et al. [9–11] reported that a heat-stable lipid fraction was necessary for the reconstitution of the hydroxylation of fatty acids, drugs and alkanes by the solubilized enzyme system from rabbit and rat liver microsomes. Therefore, we have also examined the possible role of lipids in our system. For this purpose, both Fraction I and II were extracted 10 times with ether. As table 3 (expt. 1) shows, this treatment resulted in a great decrease of the laurate ω-hydroxylation activity, and the addition of the ether extracts from either Fraction I or II partially restored the activity. In order to determine the nature of the restoring factor, the ether extracts were separated by thin-layer chromatography into several fractions,

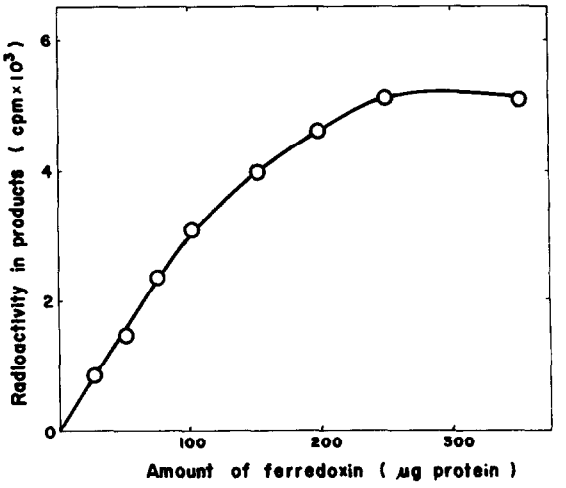

Fig. 1. The effect of various concentrations of ferredoxin on laurate ω-hydroxylation. Reaction conditions were the same as described in table 1. Fraction I, 0.32 mg of protein; ferredoxin-NADP reductase, 5.4 μg.

Table 3
Restoration of the activity of laurate ω-hydroxylation by the ether-extracted enzyme preparations

Experiment	System	Products (nmoles)
1	Fraction I + Fraction II	0.90
	Ether-extracted Fraction I	
	+ ether extracted Fraction II	0.05
	+ ether extracts from Fraction I (0.2 mg)	0.25
	+ ether extracts from Fraction II (0.2 mg)	0.23
2	+ Triton X-100 (0.2)	0.45
	Ether-extracted Fraction I	
	+ ether-extracted Fraction II	0.15
	+ active fraction (0.2 mg)	0.78
	+ Triton X-100 (0.2 mg)	0.69

Experiment 1: Fraction I, 123 μg; Fraction II, 166 μg; ether-extracted Fraction I, 123 μg; ether-extracted Fraction II, 166 μg
Experiment 2: ether-extracted Fraction I, 246 μg; ether-extracted Fraction II, 322 μg. Active fraction was separated from the ether extracts of Fraction I by use of thin-layer chromatography. Other conditions were the same as in table 1.

which were scraped off and tested for the activity, separately. The R_f of the active fraction did not coincide with that of phospholipids or free fatty acids, but coincided with that of Triton X-100. The spots of the active fraction were not stained with Dittmer's reagent [12], but stained with anthrone reagent. Moreover, Triton X-100 added showed the same effect as did the active fraction (table 3, expt. 2). Egg yolk lecithin had no effect, when added instead of Triton X-100. It suggests that the restoration of the activity by the ether extracts was due to the Triton X-100, which had been used for the preparation of Fraction I and II [3]. Although these findings do not necessarily rule out the participation of lipids in the ω-hydroxylation activity, further extensive studies remain to be done. Recently, several papers appeared showing that detergents such as Triton X-100 or deoxycholate stimulate enzyme activities involved in lipid metabolism [13–15].

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