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PARTICIPATION OF THE MICROSOMAL ELECTRON TRANSPORT SYSTEM INVOLVING CYTOCHROME P-450 IN ω-OXIDATION OF FATTY ACIDS

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SUMMARY

The participation of the microsomal electron transport system involving cytochrome P-450 in ω-oxidation of fatty acids by a rat liver preparation was examined since ω-oxidation involves microsomal reactions requiring both NADPH and molecular oxygen.

ω-Oxidation of fatty acids was inhibited by CO and by the antibody against NADPH–cytochrome *c* reductase. The addition to the reaction mixture of drugs which interact with cytochrome P-450 inhibited ω-oxidation. It is concluded that the microsomal electron transport system involving cytochrome P-450 functions in ω-oxidation of fatty acids.

INTRODUCTION

It has been shown that starvation, feeding a high fat diet and alloxan diabetes cause an increase in the cytochrome P-450 content of liver microsomes[1,2]. In animals under these conditions fatty acids are used as the main energy source. Therefore, it is likely that cytochrome P-450 plays a role in metabolism of fatty acids. Cytochrome P-450 is reduced by NADPH through NADPH–cytochrome *c* reductase (EC 1.6.2.3) and reacts with molecular oxygen[3–7]. The reduced form of the haemoprotein is capable of binding CO (refs. 8–10). In metabolism of fatty acids, ω-oxidation involves microsomal reactions requiring both NADPH and molecular oxygen[11,12]. Recently, ω-oxidation of physiological fatty acids such as stearic acid and lauric acid by a liver preparation has been reported[13]. Lauric acid is oxidized at the ω-carbon by either a liver or a kidney preparation[14], but stearic acid is oxidized by liver but not by kidney[15]. So, the participation of the microsomal electron transport system involving cytochrome P-450 in ω-oxidation was examined. In a preliminary paper[16] we reported that ω-oxidation of stearic acid is inhibited by CO. LU AND COON have reported about the role of cytochrome P-450 in ω-oxidation of lauric acid without details[17]. This paper reports in detail that ω-oxidation depends upon NADPH oxidation catalyzed by an electron transport system involving cytochrome P-450, and that there are differences between the mechanisms for ω-oxidation of stearic acid and lauric acid.

MATERIALS AND METHODS

Radioactive fatty acids, [1-^{14}C]stearic acid and [1-^{14}C]lauric acid, were purchased from the Radiochemical Centre and purified by chromatography on silicic acid before use. Analytical grade 100-mesh silicic acid (Mallinckrodt) was used. Rabbit antibody against NADPH–cytochrome *c* reductase, which had been highly purified from rat liver microsomes was kindly provided by Dr. T. OMURA of the Institute for Protein Research, Osaka University.

Male rats of the Sprague–Dawley strain were used in these experiments. The livers were homogenized in 2.5 vol. of the medium described by CLAYTON AND BLOCH[18], and the homogenates were centrifuged at 10000 × *g* for 20 min. The resulting supernatant was used for studies on ω-oxidation in the postmitochondrial fraction. The microsomes were prepared as follows; livers were homogenized in 5 vol. of 1.15 % KCl solution, and the homogenates were centrifuged at 10000 × *g* for 20 min. The resulting supernatant was centrifuged at 105000 × *g* for 1 h. The precipitate was resuspended in KCl solution and recentrifuged.

ω-Oxidation of fatty acids was assayed essentially according to the method of PREISS AND BLOCH[13]. The standard incubation mixture for the postmitochondrial supernatant contained 0.1 M potassium phosphate buffer (pH 7.4) and 0.5 mM $NADP^+$, 1 mM NAD^+, 0.5 mM $MnCl_2$, 5 mM isocitrate, 0.1 mM radioactive substrate (0.1 μC); stearic acid or lauric acid, and 0.1 ml of the postmitochondrial supernatant in a total volume of 1 ml. The incubation mixture for microsomes contained 0.1 M potassium phosphate buffer (pH 7.4) and 0.5 mM $NADP^+$, 2.5 mM $MgCl_2$, 5 mM glucose 6-phosphate, an excess of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 0.1 mM radioactive substrate (0.1 μC) and microsomes (0.3 mg protein) in a total volume of 1 ml. Incubations were carried out at 37° for 30 min with shaking. Metabolites were analyzed by the method of PREISS AND BLOCH[13]. Radioactivity was measured with a Packard, Tri-Carb, liquid scintillation spectrometer. Protein was determined by the method of LOWRY *et al.*[19].

RESULTS

Nucleotide requirements

As shown in Table I, ω-oxidation of stearic acid by the postmitochondrial supernatant decreased when NAD^+ or $NADP^+$ was omitted. The omission of NAD^+ did not affect ω-oxidation by microsomes, but the omission of $NADP^+$ reduced it remarkably.

Effect of CO on ω-oxidation

In the reduced form cytochrome P-450 has been shown to combine with CO (refs. 8–10), so the effect of CO on ω-oxidation of fatty acids by the postmitochondrial fraction was tested. As shown in Table II, conversion of stearic acid to polar metabolites by the liver preparation was reduced when the reaction was carried out under a gas phase containing CO, and the conversion of lauric acid by either a liver or a kidney preparation was also inhibited by CO. In all cases the activity was inhibited 95 % under N_2–O_2–CO (40:20:40, by vol.) as compared with the activity measured under N_2–O_2 (80:20, v/v).

TABLE I

NUCLEOTIDE REQUIREMENT FOR ω-OXIDATION OF STEARIC ACID

System	*Liver postmitochondrial fraction (0.2 ml)* % *conversion*	*Liver microsomes. Specific activity (mμmoles/min per mg protein)*
Complete	20.8	1.02
NAD^+ omitted	14.3	0.93
$NADP^+$ omitted	11.5	0.04
NAD^+ and $NADP^+$ omitted	5.6	0.02

TABLE II

EFFECT OF CO ON ω-OXIDATION OF FATTY ACIDS BY POSTMITOCHONDRIAL FRACTIONS

Gas phase (by vol.)	*Stearic acid* *Liver*		*Lauric acid*			
			Liver		*Kidney**	
	Conversion (%)	*Inhibition* (%)	*Conversion* (%)	*Inhibition* (%)	*Conversion* (%)	*Inhibition* (%)
N_2–O_2 (80:20)	10.22		22.99		9.16	
N_2–O_2–CO (40:20:40)	0.48	95	1.34	94	0.46	95

* 0.4 ml of enzyme preparation.

TABLE III

CO INHIBITION OF ω-OXIDATION OF STEARIC ACID BY LIVER POSTMITOCHONDRIAL FRACTION AT DIFFERENT CO/O_2-RATIOS

Gas phase: CO–O_2–N_2 *(by vol.)*	CO/O_2 *ratio*	*Conversion* (%)	n*	$1-n$	K**
0:20:80		9.68			
5:20:75	0.25	2.78	0.29	0.71	0.10
10:20:70	0.5	1.69	0.17	0.83	0.10
20:20:60	1.0	1.09	0.11	0.89	0.12
40:20:40	2.0	0.48	0.05	0.95	0.11

* n = (rate with CO)/(rate without CO).
** $K = (n/1-n) \times (CO/O_2$ ratio).

Table III shows the effects of various CO/O_2 mixtures on the rate of ω-oxidation. Increasing the CO/O_2 ratio in the gas mixture increased the inhibition and the partition constant[20] was close to unity. The absolute value of the constant was 0.1, which appears to be lower than that for other reactions involving cytochrome P-450 (ref. 21).

Inhibition of ω-oxidation by rabbit antibody against NADPH–cytochrome c reductase of rat liver microsomes

NADPH–cytochrome *c* reductase is a member of the microsomal electron transport system involving cytochrome P-450. Antibody against this enzyme inhibits

the enzymic activity of microsomes. As shown in Fig. 1, the antibody also inhibited the ω-oxidation of stearic acid by microsomes.

Effect of phenobarbital treatment on ω-oxidation

Liver microsomes prepared from rats which had been treated with phenobarbital *in vivo*, have increased amounts of components of the electron transport system involving cytochrome P-450 (refs 4, 22). But phenobarbital treatment decreased the ω-oxidation by microsomes (Table IV).

Effect of methylcholanthrene treatment on ω-oxidation

It has also been reported that the amount of cytochrome P-450 in liver micro-

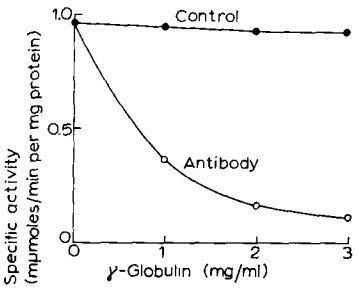

Fig. 1. Effect of antibody against NADPH–cytochrome *c* reductase on ω-oxidation of stearic acid by microsomes.

TABLE IV

EFFECT OF PHENOBARBITAL TREATMENT ON ω-OXIDATION OF STEARIC ACID BY LIVER MICROSOMES

Rats weighing 100–120 g were divided into two groups. One group served as the control and the other group was fed with a diet containing 0.1% phenobarbital for a week. Results were expressed as mean ± S.E. for the five animals in each group.

Rats used for liver preparation	*ω-Oxidation (mμmole/min per mg protein)*	*Cytochrome P-450 (mμmoles/mg protein)*
Control	0.91 ± 0.04	0.96 ± 0.05
Phenobarbital treated	0.70 ± 0.03	2.31 ± 0.12

TABLE V

EFFECT OF METHYLCHOLANTHRENE TREATMENT ON ω-OXIDATION OF STEARIC ACID BY LIVER MICROSOMES

Rats weighing 100–120 g were divided into two groups. The control group was injected intraperitoneally with 0.5 ml of corn oil and the other group was injected intraperitoneally with 2 mg of methylcholanthrene in 0.5 ml of corn oil 2 days before sacrifice. Results were expressed as mean ± S.E. for the five animals in each group.

Rats used for liver preparation	*ω-Oxidation (mμmoles/min per mg protein)*	*Cytochrome P-450 (mμmoles/mg protein)*
Control	0.75 ± 0.03	0.86 ± 0.04
Methylcholanthrene treated	0.59 ± 0.03	1.36 ± 0.07

somes increased when rats were treated with methylcholanthrene[23,24]. Table V shows that ω-oxidation was reduced after administration of this compound.

Effects of additions of various drugs to the reaction mixture on ω-oxidation

As shown in Table VI, aminopyrine and aniline, which are metabolized by an enzyme system involving cytochrome P-450 (refs. 22, 24–27) inhibited ω-oxidation of stearic acid. Aniline was more inhibitory than aminopyrine. These drugs also inhibited ω-oxidation of lauric acid, though to a much lesser degree.

Ethylisocyanide, which interacts with cytochrome P-450 (refs. 10, 28), inhibited ω-oxidation of stearic acid but did not inhibit that of lauric acid.

ω-Oxidations of both stearic acid and lauric acid were slightly inhibited by cyanide.

TABLE VI

EFFECTS OF ADDITIONS OF VARIOUS DRUGS TO THE REACTION MIXTURE ON ω-OXIDATION BY LIVER MICROSOMES

Drug added	*Stearic acid*		*Lauric acid*	
	Conversion (%)	*Inhibition* (%)	*Conversion* (%)	*Inhibition* (%)
None	8.11		34.3	
Aminopyrine (2 mM)	6.82	16	30.6	11
Aniline (2 mM)	2.70	67	29.0	15
Ethylisocyanide (2 mM)	2.39	71	34.2	0
KCN (2 mM)	7.06	13	28.1	18

DISCUSSION

In good accordance with the findings on ω-oxidation of sorbic acid amide by WAKABAYASHI AND SHIMAZONO[12], it was found that ω-oxidation of stearic acid requires NAD^+ and an NADPH generating system (Table I). Addition of NAD^+ is effective only in the presence of the soluble fraction. The nucleotide requirements of the reactions by the postmitochondrial fraction and by microsomes indicate the roles of NADPH for ω-hydroxylation by microsomes and of NAD^+ for the successive dehydrogenation of ω-hydroxy compounds. From analysis of the metabolites formed by the postmitochondrial fraction, PREISS AND BLOCH[13] also reported the same reaction mechanism for ω-oxidation of stearic acid.

The inhibition of ω-oxidation by carbon monoxide (Tables II and III) suggests the involvement of cytochrome P-450 in the reaction. This suggestion is strongly supported by the finding that the antibody against NADPH–cytochrome *c* reductase inhibits ω-oxidation to the same extent as it inhibits the reductase activity (Fig. 1). From these results, the electron transport system involving cytochrome P-450 seems to be the rate-limiting factor for ω-oxidation.

The absolute value of the partition constant varied between 0.1 and 0.2 in the different preparations tested. This value is lower than that for other reactions involving cytochrome P-450 reported previously[21]. Two explanations may be offered for this finding. First, there may be a specific cytochrome P-450 for ω-oxidation.

Second, there may be a difference in the mechanisms of hydroxylation by activated oxygen.

Phenobarbital treatment, which increases the content of cytochrome P-450 in liver microsomes[4,22], decreases the activity of ω-oxidation (Table IV). Therefore, the cytochrome P-450 induced by phenobarbital treatment may be indifferent from that involved in ω-oxidation. This result supports the existence of a specific cytochrome P-450 for ω-oxidation.

Methylcholanthrene treatment reduces the ω-oxidative activity of microsomes (Table V). This may be related to the suggestion that administration of methylcholanthrene causes the synthesis of a haemoprotein with spectral properties different from cytochrome P-450 (refs. 29, 30). In this case, however, methylcholanthrene treatment may change the conformation of cytochrome P-450.

ω-Oxidation of lauric acid is inhibited by CO to the same degree as ω-oxidation of stearic acid (Table II). But the effects of various drugs such as aminopyrine, aniline and ethylisocyanide on ω-oxidation of lauric acid are different from their effects on ω-oxidation of stearic acid (Table VI). These results suggest that there may be differences between the ω-oxidation of lauric acid and that of stearic acid not in the mechanism of activation of oxygen but in the mechanism of hydroxylation by activated oxygen.

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