Characterization of fatty acid desaturase activity in rat lung microsomes

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Abstract Preparations of rat lung microsomes containing 0.030–0.050 nmole of cytochromes P-450 and b_5 per mg microsomal protein have been observed to contain significant levels of fatty acid desaturase activity. Both stearoyl CoA and palmitoyl CoA are desaturated to their monounsaturated analogues, oleic acid and palmitoleic acid, respectively. Activity (per mg microsomal protein) of the lung preparations varied according to the diet of the animals prior to killing in the order: fat free diet > normal rat chow > starvation. All preparations exhibited approximately 50% inhibition when incubated in the presence of 0.10 mM CN^-. Maximal activity was obtained with 0.50 mM NADH, less activity with equal amounts of NADPH, and there was no synergistic interaction of NADH and NADPH together. The rate of desaturation was linear with protein concentrations between 0.15–1.5 mg microsomal protein/incubation at incubation times up to 8 min. A pH optimum range of 7.0–7.4 was observed. For all variables of fatty acid desaturase activity which were examined, the rate of desaturation of stearoyl CoA was approximately twice that for palmitoyl CoA. These results indicate that the same fatty acid desaturation system which is functional in the liver is also present in significant amounts in mammalian lungs.

Supplementary key words fatty acid desaturation • cytochrome b_5 • lung mixed-function oxidase

Microsomal oxidative Δ9-desaturation of fatty acids is accomplished by a mixed-function oxidase which catalyzes the conversion of saturated fatty acyl CoA's to the corresponding monoenoic acid ester; the conversion most often studied is the metabolism of stearoyl CoA ($C_{18:0}$). The electrons required for this desaturase are transferred from NADH or NADPH through cytochrome b_5 to a cyanide-sensitive factor (1–4). This factor is thought to be the rate-limiting component for desaturation. Cytochrome P-450 does not appear to be involved in this mixed-function oxidase reaction (1).

Fatty acid desaturase activity has been observed in yeast (5), in the intact rat (6), the isolated, perfused lung (7), and in microsomal preparations from various mammalian tissues including liver (1, 3, 8), adipose tissue (5, 9, 10), and lactating mammary glands (11–13). Desaturase activity was not found in microsomes prepared from rat kidneys or testes, even though significant amounts of cytochrome b_5 were isolated from these tissues (9). Significant desaturase activity was also reported in microsomes prepared from intestinal mucosa (14); however, this has not been confirmed (9).

This study reports the characterization of the fatty acid desaturase activity present in microsomes prepared from lungs of rats which have received several dietary regimes. These lung microsomal preparations have been found to accomplish the conversion of both stearoyl CoA ($C_{18:0}$) to oleoyl CoA ($C_{18:1}$) and palmitoyl CoA ($C_{16:0}$) to palmitoleoyl CoA ($C_{16:1}$).

MATERIALS AND METHODS

Male, Charles River C-D rats (180–220 g) (Charles River Breeding Laboratories, Wilmington, Mass.) were given ad libitum access to a fat-free diet ("Fat Free" Test Diet, Nutritional Biochemicals, Cleveland, Ohio) and tap water for four days prior to being killed, unless otherwise stated. "Starved" rats received the fat-free diet on days one and two and then no food for days three and four. "Normal diet" animals received the standard diet of the rat colony (Purina Rat Chow, Ralston Purina, St. Louis, Mo.) until being killed. All animals were killed by cervical fracture between 9:00 and 10:00 A.M., and the lungs were removed and immediately minced with scissors. The minced lungs were washed three times with cold Tris–KCl buffer (0.02 M Tris–0.15 M KCl, pH 7.4) and filtered through cheese cloth. This washing procedure removed much of the contaminating hemoglobin as evidenced by the slight pink coloration of the resultant microsomes as compared to the bright red color of microsomes prepared from unwashed lungs. The washed mince was homogenized in a motor driven Teflon-glass homogenizer with 0.10–0.15 mm clearance in three volumes of the cold Tris–KCl buffer. The mincing procedure also greatly facilitated this homogenization. The homogenate was centrifuged at 9,000 *g* for 15 min and the resulting supernatant solution was then centrifuged at 165,000 *g* for 38 min. The resultant pellet was resuspended in a Tris–HCl buffer, pH 7.25, at a final concentration of 10–15 mg microsomal protein per ml. Microsomal protein was determined by the method of Sutherland et al. (15). Concentrations of cytochromes b_5 and P-450 were determined on an Aminco DW2 spectrophotometer (American Instruments, Silver Spring, Md.) utilizing

extinction coefficients of 110 mM^{-1} cm^{-1} and 91 mM^{-1} respectively (16).

Desaturation of [1-^{14}C]stearoyl CoA or [1-^{14}C]palmitoyl CoA was determined as previously described (8). The standard incubation mixture contained 1.0–1.5 mg microsomal protein, 1.0 mM NADH (Sigma Company, St. Louis, Mo.), and 70 μM [1-^{14}C]stearoyl CoA or 70 μM [1-^{14}C]palmitoyl CoA in a final incubation volume of 0.50 ml. Incubations were performed for 6 min at 37°C in a shaking water bath. With the exception of temperature, variations in each of these concentrations were made dependent upon the individual experimental design. [1-^{14}C]stearoyl CoA and [1-^{14}C]palmitoyl CoA were obtained from New England Nuclear, Boston, Mass., with specific activities of 51.8 mCi/mmole (Lot #678-279) and 57.8 mCi/mmole (Lot #748-221), respectively. Two-tenths mg of [1-^{14}C]stearoyl CoA (0.010 mCi) or 0.17 mg of [1-^{14}C]palmitoyl CoA (0.010 mCi) was added to 10 mg of the corresponding cold fatty acyl CoA (Sigma Company) in Tris-HCl, pH 7.25, for use in the incubation mixtures. All incubations were performed in duplicate or triplicate. The reaction was stopped by the addition of 1.0 ml of 10% KOH in methanol, followed by saponification at 80°C for 30 min. The mixture was then acidified with 2.0 ml of 4 N HCl; the fatty acids were extracted with 30 ml of petroleum ether or hexane, and converted to methyl esters with 4 ml of 14% boron trifluoride in methanol (17). The methyl esters were extracted into petroleum ether or hexane and separated by thin-layer chromatography on silica gel GF plates (Analtech Inc., Newark, Del.) containing 10% $AgNO_3$ by developing in ether–hexane 1:9 (v/v). It should be noted that separation of the monounsaturated from the saturated esters was markedly facilitated by prewashing the plates with ethyl acetate. The spots were identified under ultraviolet light by comparison with authentic standards after spraying the plates with 0.50% rhodamine B (in methanol). The spots were scraped off and counted in a toluene scintillator (4 g PPO and 0.25 g POPOP per 1 of toluene) in a Beckman LC-100 scintillation counter (Beckman Instruments, Fullerton, Cal.). Desaturation activity was determined by dividing the radioactivity found in the monounsaturated ester by the sum of the radioactivities in both the saturated and unsaturated esters; this ratio was then converted to nmoles of product formed/mg of microsomal protein/minute. Approximately 80% of the initial radioactivity was routinely recovered utilizing this procedure. Incubation blanks were obtained by addition of the microsomes after addition of the KOH solution. These blanks routinely gave an apparent metabolism of 0.01–0.02 nmoles product formed/mg of protein/minute. Values that were less than twice this background were considered not detectable.

RESULTS

The dependence of the rate of fatty acid desaturation on enzyme concentration was found to be linear for both stearoyl CoA and palmitoyl CoA desaturation up to a maximum protein content of 4.0 mg of microsomal protein per ml of incubation mixture. Between 4.0 and 6.0 mg of microsomal protein/ml, the desaturation of stearoyl CoA became slightly nonlinear while the desaturation of palmitoyl CoA became markedly nonlinear. This range of protein concentrations yielding linear rates of desaturation is identical with that reported for the hepatic microsomal desaturation system (1, 18). The desaturation rate for both substrates was linear for 9–10 min under these experimental conditions. Beyond 10 min of incubation the desaturation of both substrates became markedly nonlinear. This time dependence is quite similar to the 6–7 min linearity in hepatic microsomes observed by Oshino, Imai, and Sato (1). The 9–10 min linearity in this study is quite different, however, from the 30 min linearity observed in hepatic microsomes by Paulsrud et al. (18). This difference may be explained by the fact that in the study of Paulsrud et al. (18) the fatty acyl CoA was continuously being formed by a CoA-generating system, whereas in the present study and in the study of Oshino et al. (1) the fatty acyl CoA was initially added to the incubation medium.

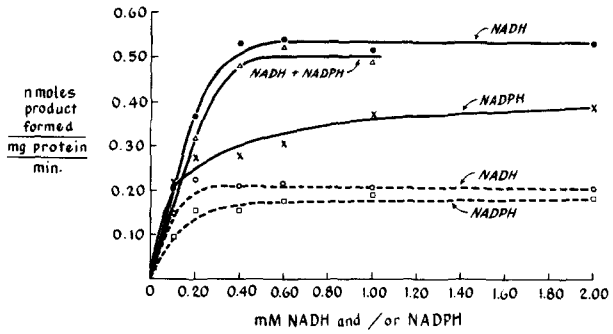

Fig. 1. Effect of pyridine nucleotide concentration on lung microsomal desaturase activity. Incubation mixture (0.50 ml), containing 70 μM [1-^{14}C]stearoyl CoA or 70 μM [1-^{14}C]palmitoyl CoA, 1.0–1.5 mg microsomal protein, and varying concentrations of NADH, NADPH, or NADH and NADPH together, incubated in duplicate at 37°C for 6 min. ●—●, △—△, and x—x represent [1-^{14}C]stearoyl CoA desaturation in presence of NADH only, NADH and NADPH together, and NADPH only, respectively. ○---○ and □---□ represent [1-^{14}C]palmitoyl CoA desaturation in presence of NADH and NADPH, respectively.

This lung mixed-function oxidase reaction appears to be only slightly dependent upon pH in the range of 6.6–7.8. An apparent pH optimum around pH 7.0–7.3 was observed.

To elucidate the nature of the energy requirements for this enzyme system, different amounts of either NADH or NADPH were added to the incubations containing 70 μM stearoyl CoA or palmitoyl CoA. **Fig. 1** shows that desaturation of stearoyl CoA was stimulated maximally in the presence of 0.40–0.60 mM NADH. Further increasing the NADH concentration above 0.60 mM did not result in any further increase in desaturase activity. In the presence of NADPH the rate of stearoyl CoA desaturation showed a slight but continuous increase throughout the range of NADPH examined, 0.10–2.0 mM. When NADH and NADPH were added together, the rate of stearoyl CoA desaturation was not greater than that resulting from an equal, total concentration of NADH alone. These results suggest that 0.40–0.60 mM NADH represents a maximally stimulating energy source and that this pulmonary mixed-function oxidase enzyme system does not exhibit a pyridine nucleotide synergism similar to

TABLE 1. Effect of dietary treatment and cyanide on lung microsomal fatty acid desaturation[a]

Substrate[b]	0.10 mM CN^-	Source of Microsomes		
		starved	*normal diet*	*fat-free diet*
Stearoyl CoA	−	0.071 ± 0.004	0.236 ± 0.008	0.447 ± 0.032
	+	0.043 ± 0.003	0.101 ± 0.003	0.176 ± 0.012
Palmitoyl CoA	−	0.019 ± 0.002	0.128 ± 0.012	0.276 ± 0.010
	+	N.D.[c]	0.057 ± 0.004	0.120 ± 0.009

[a] Incubations performed with microsomes prepared from pooled lungs from 3–5 rats. Values (mean ± S.E.M.) represent average nmoles of product (oleic or palmitoleic acid) formed per mg microsomal protein per minute on triplicate incubations containing 1.0 mM NADH and 2.0–3.0 mg lung microsomal protein per ml of incubation mixture.

[b] Present at 70 μM as [1-^{14}C]acyl CoA.

[c] Activity not detectable.

that observed in the hepatic mixed-function oxidase drug metabolizing system (19, 20).

The desaturation of palmitoyl CoA was also stimulated maximally by similar concentrations of NADH (Fig. 1). NADPH again yielded slightly lower rates of desaturation over the entire range of concentrations examined. The overall rate of palmitoyl CoA desaturation was roughly one-half that observed for stearoyl CoA at all concentrations of NADH and NADPH. While the overall rates of desaturation of the two substrates were different, both substrates demonstrated similar saturation kinetics at concentrations greater than 35 μM. Thus 70 μM was selected as the routine concentration of substrate (stearoyl CoA or palmitoyl CoA) to insure enzyme-receptor saturation.

The final set of experiments in this study was designed to determine whether the desaturase system of the lung would respond to dietary manipulations similar to the hepatic desaturase system. This hepatic system is well known to be very sensitive to alterations in diet and nutritional status (8, 9, 21, 22). The results presented in **Table 1** indicate that the desaturation of both stearoyl CoA and palmitoyl CoA by rat lung microsomes proceeds at a very low rate when the microsomes are prepared from animals which have been starved for 48 hr prior to being killed. Lung microsomes prepared from animals maintained on the standard rat chow exhibited desaturase activities much greater than those found in the starved animals but less than the activities observed when the rats were given a fat-free diet for 4 days prior to killing. This response to dietary alterations is similar in nature to the response seen in the hepatic system (2, 8, 23).

The presence of a CN^--sensitive factor in the lung microsomal desaturase system is also established by the data in Table 1. Inclusion of 0.10 mM CN^- in the incubation mixtures containing microsomes from the variously treated animals resulted in a 40–60% inhibition of desaturation of both stearoyl CoA and palmitoyl CoA. The presence of 0.10 mM CN^- in the incubation containing palmitoyl CoA and "starved" microsomes resulted in a rate of conversion to palmitoleic acid which was too low to be detected in this assay.

DISCUSSION

Preparation of rat lung microsomes by the method described in this study consistently yielded concentrations of cytochromes b_5 and P-450 in the range 0.030–0.050 nmoles/mg microsomal protein. This yield is quite similar to that reported by Oppelt et al. (24). These microsomal preparations contain the enzymes required to desaturate saturated, long chain fatty acids and do so at rates dependent upon the nutritional status of the rat prior to killing. The lung mixed-function oxidase system appears to be identical with the desaturase system of the liver in this sensitivity to prior nutritional condition, in sensitivity to cyanide, and in pyridine nucleotide requirements.

Quantitatively, on a per mg microsomal protein basis, lung microsomes from starved rats exhibit desaturation rates for stearoyl CoA similar to the desaturation rate of hepatic microsomes from similarly treated animals. However, lung microsomes from rats maintained on a standard rat chow have only approximately 50% of the desaturation activity as compared to hepatic preparations from animals on the same diet. Lung preparations from rats maintained on a fat-free diet have only 10% of the desaturation activity of similarly prepared hepatic microsomes (8). This lower degree of "inducibility" of the lung system brought about by the fat-free diet is not understood and is currently under investigation.

The role of the desaturation reaction in overall pulmonary lipid metabolism is not defined. The mammalian lung is an active site for de novo synthesis of phospholipids, a fact which has gained significance since the alveoli are known to contain a surface active lining layer which is rich in phospholipids (25–27). The predominant component of this surfactant material has been established to be dipalmityl lecithin (26, 28) with lesser concentrations of several other phospholipids (26). Unsaturated fatty acids, predominantly oleic acid, are known to be important constituents of lung phospholipids and appear to be confined mainly to the β position of the phospholipid molecule (7). Whether the monounsaturation reaction described in the present study is involved in the metabolism of intracellular pulmonary lipids or in the overall metabolic formation and/or catabolism of this pulmonary surfactant remains to be established.

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