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Stearyl-CoA Desaturase of Bovine Mammary Microsomes

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Stearyl-CoA desaturase from the microsomal fraction of lactating bovine mammary tissue had a specific activity of 0.4 nmoles oleate formed min^{-1} mg^{-1} protein. NADH was required for desaturase activity. However, oxidized NAD^+ and $NADP^+$ supported measurable desaturase activity. K_m values for stearyl-CoA and NADH were 25.0 μM and 3.0 μM, respectively. Desaturase was depressed by increasing concentrations of other acyl-CoA esters, i.e., palmityl-CoA and oleyl-CoA (>10 μM). Sn-1,2 diglycerides (1–2.0 mM) depressed desaturase slightly in the order 0–20%, as did L-α-glycerolphosphate (0.2–3.6 mM). 1-Acyl-*sn*-glycerol-3-phosphorylcholine (>0.1 mM) depressed desaturase activity markedly. Sonication of the microsomal preparation stimulated desaturase activity. The addition of ethanol depressed desaturation, and EDTA inhibited desaturation. Palmityl CoA was equally desaturated by the microsomes. The acyl-CoA desaturase was very stable when stored at −30° C as a freeze-dried microsomal preparation, i.e., activity was retained after 12-month storage.

Labeled stearate and oleate were isolated as esters (triglycerides and phospholipids) and as free fatty acids, indicating the presence of acyl transferases and acyl-CoA hydrolase in mammary microsomes.

Acyl-CoA desaturase(s) occur widely in animal, avian, plant, and microbial tissues (1–17). Some of the properties and components of these enzymes have been studied and generally they are classified as mixed-function oxidases (1, 12, 16). Recently we suggested that the stearyl desaturase of lactating bovine mammary tissue may facilitate milk triglyceride synthesis (6). The present studies examine the basic properties and some factors influencing the activity of bovine mammary stearyl-CoA desaturase.

EXPERIMENTAL

Preparation of the microsomal enzyme. Mammary tissue was excised from lactating cows immediately after slaughter. Upon removal of connective tissue, the mammary tissue was minced in a meat grinder. The crude homogenate was homogenized in a Waring Blendor at top speed for 25 sec, after a 1:1 dilution, using a phosphate-bicarbonate buffer, pH 8.0 (70 mM $KHCO_3$, 85 mM K_2HPO_4, and 9 mM KH_2PO_4). This was rehomogenized in a small mill (Polyscience Corp., 11) for 15 sec. All manipulations were carried out at 4°C.

The homogenate was centrifuged in a Sorvall refrigerated centrifuge using a G.S.A. rotor (r = 5.75 in.) at 6000g for 10 min to remove cellular debris. The supernatant fraction was further centrifuged at 19,600g for 15 min to remove mitochondria. After each centrifugation, the supernatant fraction was strained through two layers of cheesecloth to remove fat. The resulting supernatant fluid was centrifuged in a Beckman Model L2-65 ultracentrifuge with type 21 rotor at 44,000g for 75 min. The final microsomal pellets were pooled and quickly frozen in a dry ice-acetone bath and then freeze dried.

Assay conditions. A typical incubation medium contained, 20 nmoles [1-^{14}C]stearyl CoA (120,000 cpm), 256 nmoles NADH,[1] 0.4–0.5 mg microsomal

[1] *Abbreviations.* NAD, NADH, NADP, NADPH oxidized and reduced nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, respectively. C16:0-CoA, C18:0-CoA, C18:1-CoA—palmityl-, stearyl- and oleyl-coenzyme A, respectively; NL = neutral lipids; PL = phospholipids, TG = triglycerides; FFA =

protein, and potassium phosphate buffer (66 mM), pH 7.4, was used to make final volume to 1 ml. All reagents were made up in this phosphate buffer unless otherwise stated. The assay tubes were preincubated for 5 min at 30°C prior to the addition of enzyme solution or substrate to initiate the reaction. Incubation tubes were loosely capped during incubation at 30°C.

The reaction was terminated by either of two methods depending on subsequent analysis. When desaturation alone was being measured 2 ml of 10% methanolic KOH was added and the mixture was saponified at 85°C for 40 min, cooled, acidified with 0.35 ml of concd. HCl, and then the free fatty acids were thrice extracted with 2 ml of petroleum ether (bp 30–55°C). The petroleum ether extracts were pooled and evaporated to dryness with nitrogen. The fatty acids were redissolved in *n*-hexane, and from aliquots the total radioactivity was determined using a liquid scintillation counter. This extraction method gave 100% recovery of the radioactivity incubated. The fatty acids were methylated using 1 ml boron trichloride in methanol (14%) and after addition of 0.5 ml saturated saline solution the methyl esters were thrice extracted with hexane. These extracts were pooled and concentrated under nitrogen for gas chromatography.

Determination of radioactivity in methyl esters. The radioactive methyl esters were analyzed by gas–liquid chromatography using a model 5000 Barber-Colman (Rockford, Ill.) instrument with a radioactive monitoring system and conditions described previously (6, 18). The radio assay system was calibrated using a standard [1-^{14}C]methyl myristate solution. The radio activity in the stearate and oleate chromatographic peaks was quantified from their respective areas (Fig. 7). Using these data, the quantity of oleic acid formed from the substrate stearyl-CoA was computed. The specific activity of the stearyl desaturase was calculated as nmoles oleate formed per minute per milligram of microsomal protein.

Distribution of radioactivity in lipid classes. When the distribution of ^{14}C label in the various lipid classes were required the assays were stopped by the addition of 4 ml of chloroform/methanol/2:1 (v/v), followed by vigorous shaking. The lipids were then extracted according to the Folch procedure (37), dried, dissolved in chloroform, and the total radioactivity was quantified. Duplicate aliquots of this lipid extract were then fractionated by thin-layer chromatography on silica gel G plates (Brinkmann, Westbury, NY) according to described procedures (18, 19). Lipids were identified by co-chromatography with pure standards. (Applied Science, State College, PA). After identification, the lipid spots were transferred to scintillation vials and the radioactivity associated with each was determined in a liquid scintillation counter (Packard Tri-Carb, Chicago, Ill). Using these data the total radioactivity in each lipid class was calculated.

Measurement of radioactivity. Radioactivity in lipid fractions was counted using a Packard Tri-Carb liquid scintillation counter in 10 ml of a cocktail containing 5 g of 2,5 diphenyloxazole and 0.3 g of 1.4 *bis*[2(4 methyl-5-phenyloxazolyl)] benzene per liter of toluene. Radioactivity in aqueous solution was counted using "Aquasol" (New England Nuclear, Boston, MA). Protein was determined by the method of Lowry *et al.* (20).

Buffers. Potassium phosphate buffer (pH 7.4, 66 mM) was used throughout the experiments unless otherwise specified. Diethanolamine–HCl buffer (0.05 M) and bicarbonate buffer (0.025 M) were used in determining pH stability of desaturase.

Chemical purity of stearyl[1-^{14}C]CoA and stearyl CoA. The chemical purity and the freeze–thaw stability of the substrate stearyl-CoA (labeled and nonlabeled) was measured to ensure that additions of substrate were quantitative. Using Whatman 3 MM chromatography paper in a solvent system *n*-butanol:acetic acid:water (5/2/3) (v/v), sodium stearate had an R_f value similar to stearyl CoA. These were separated by a solvent system of 30 ml 1 M ammonium acetate, pH 5.0, plus 70 ml of ethanol (95%). Different stearyl-CoA solutions were co-chromatographed with coenzyme-A, stearic acid, and sodium stearate. Purified stearyl-CoA was used as a standard. All samples containing stearyl-CoA gave a spot with an R_f (0.56) identical to the spot given by pure stearyl-CoA; 97–98% of the ^{14}C spotted as [1-^{14}C]stearyl-CoA alone had an R_f of 0.56. The substrate was stable for at least 1 month and not affected by repeated freezing and thawing.

The chemical purity of reduced pyridine dinucleotide. Preweighed vials of NADH (2 mg/vial) were purchased from the Sigma Chemical Co. (St. Louis, MO) and were stored at room temperature in a desiccant bag. The NADH was made up with potassium phosphate buffer (66 mM, pH 7.4) to an appropriate volume 30 min prior to experiments. Purity of NADH was based on a molar extinction coefficient of 6.2×10^3 at 340 mμ. Samples of NADH, less than 1 hr in solution ranged from 90 to 100% purity. Samples stored for up to 5 hr at 20°C showed approximately 80% purity. Samples stored overnight at 10°C showed almost complete

free fatty acids; DG = diglycerides; Lyso PC = lysophosphatidylcholine; L-α-GP = L-α-glycerolphosphate.

loss of absorbancy at 340 mμ, indicating complete oxidation of NADH.

Solubilization of diglycerides, L-α-glycerophosphate and lysophosphatidylcholine. L-α-Glycerophosphate (90 mM) was made up in potassium phosphate buffer (pH 7.4, 66 mM). 1,2 Dimyristin (13 mM), 1,2 dipalmitin (25 mM), and distearin (10 mM) were dispersed in Tween 80 and ethanol 2:1 (v/v). 1,2 Diolein (16 mM) was made up in Tween 80 and ethanol 1:1 (v/v). 1-Acyl sn-glycerol-3-phosphorylcholine (lysophosphatidylcholine, 20 mM) was dissolved in ethanol/water 1/1 (v/v). Before addition to incubation medium all the suspensions were sonicated for 5 min until the solutions became clear.

Materials. All chemicals were of reagent grade. The [1-^{14}C]stearyl CoA was purchased from New England Nuclear, Boston, MA. Chemically pure stearyl-CoA was purchased from P-L Biochemicals, Milwaukee, WI. Sodium salts of oxidized and reduced pyridine dinucleotides (NAD, NADH, NADP, NADPH) were obtained from Sigma Chemical Co., St. Louis, MO. The diglycerides and lysophosphatidylcholine (LysoPC) were procured from Nutritional Biochemicals, Cleveland, OH. All lipid materials were purified by thin-layer chromatography before use. L-α-Glycerolphosphate was obtained from Sigma Chemical Co.

RESULTS

Stearyl-CoA desaturase required oxygen, NADH, and stearyl-CoA for activity. Because of the low activity of stearyl-CoA synthetase in bovine microsomes (6) preformed stearyl-CoA was used in all experiments. Desaturation was linear with time over a range of protein concentrations (Figs. 1 and 2). The rate of desaturation of stearyl-CoA was proportional to the microsomal protein concentration up to 1.5 mg and above this concentration, i.e., 1.5–6 mg, desaturation remained constant. All subsequent incubations contained 0.5 mg microsomal protein and were maintained for 20 min at 30° C.

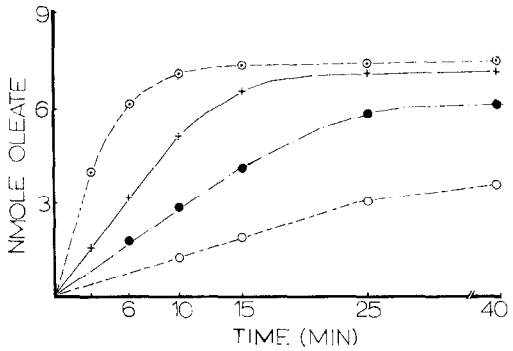

FIG. 1. The rate of formation of oleate from stearyl CoA in the presence of increasing quantities of microsomal protein from bovine mammary tissue. The assay system, contained 20 nmoles [1-^{14}C]stearyl CoA (120,000 cpm); 250 nmoles NADH, and varying amounts of protein made up to 1 ml in 66 mM potassium phosphate buffer, pH 7.4. ○-----○, 0.25; ●—●, 0.5; +—+, 0.8; ⊙——⊙, 1.6 mg protein, respectively.

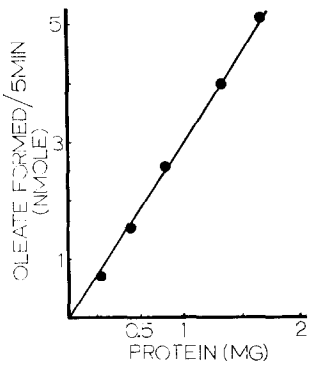

FIG. 2. The rate of formation of oleate from stearyl-CoA at low concentrations of bovine mammary microsomal protein. Assay conditions as in Fig. 1.

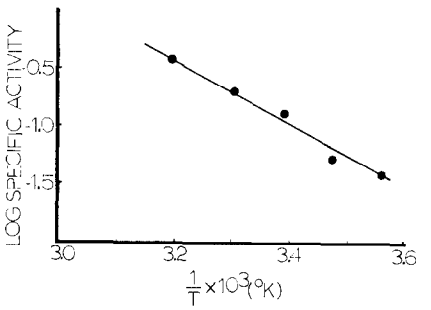

FIG. 3. Arrhenius plot showing the effect of temperature on the specific activity of stearyl-CoA desaturase from bovine mammary microsomes. Conditions as stated in Methods.

The rate of desaturation of stearyl CoA increased with temperature from 8 to 40°C (Fig. 3). From an Arrhenius plot an energy of activation of 12.4 kcal/mole was calculated.

The optimum pH for stearyl desaturase was 6.8. However, the enzyme was active over a wide pH range, i.e., 5.5–9.0 (Fig. 4).

Because of the amphiphathic nature of the substrate and competition by other enzymes

(acyl-CoA hydrolase, acyl-CoA transferases) for this substrate it is difficult to determine a valid K_m for stearyl-CoA desaturase. Using substrate concentrations ranging from 3.0 to 55 μM, K_m values obtained from Lineweaver-Burk plots ranged from 15.7 to 25 μM (Fig. 5). Apparent substrate inhibition occurred at concentrations of stearyl CoA greater than 50 μM. This phenomenon has been observed previously and is discussed below. The apparent K_m (25 μM) was determined from a linear regression of the average specific activity values which did not include the values at which substrate inhibition was observed.

Both NADH and NADPH supported desaturation of stearyl-CoA, with NADH being the preferred cofactor. The apparent K_m for NADH was 3.0 μM (Fig. 6). A nonlinear relationship between the amount of oleate formed and added cofactor was consistently observed at low concentrations of NADH, i.e., the ratio of oleate formed to added NADH was greater than one (Table I) indicating possible endogenous regeneration of NADH in the microsomes. This was corroborated by the experimental data showing that oxidized cofactors, NAD or NADP, were capable of supporting desaturation (Table I). In the absence of added cofactors the acyl-CoA desaturase was inactive (Fig. 7).

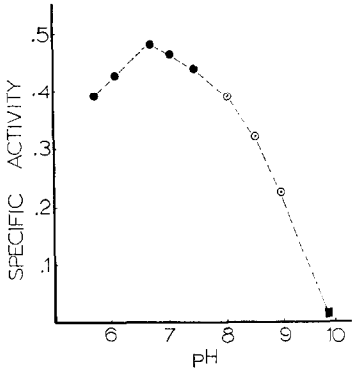

FIG. 4. The effect of pH on the specific activity of bovine mammary stearyl desaturase activity. Conditions of assay same as in Methods. Potassium phosphate buffer (●), 66 mM was used between pH 5.5–7.5; diethanolamine-HCl buffer (⊙) pH 8–9; and potassium bicarbonate buffer (■), at pH 10.

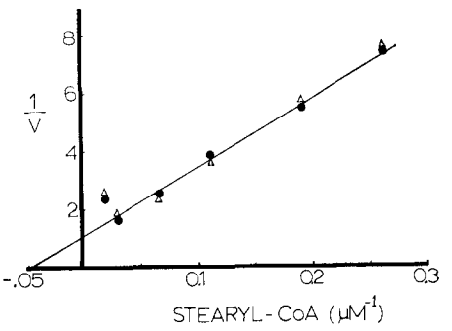

FIG. 5. A Lineweaver-Burke plot of the desaturation of staryl-CoA by microsomes from lactating bovine mammary tissue at varying stearyl-CoA concentrations. Assay system which was incubated for 20 min at 30°C contained 250 μM NADH, 0.5 mg microsomal protein, and varying concentrations of [1-^{14}C]stearyl CoA.

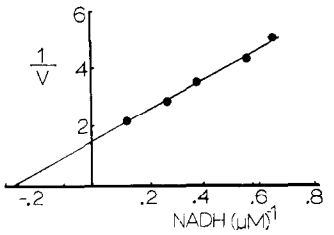

FIG. 6. Lineweaver-Burk plot for NADH, using stearyl CoA desaturase from bovine mammary microsomes. Incubations contained 0.5 mg protein, 20 μM [1-^{14}C]stearyl CoA, varying concentrations of NADH, and were kept at 30°C for 20 min.

The specific activity of microsomal stearyl desaturase preparations from five different animals ranged from 0.36 to 1.3 nmoles oleate produced per minute per milligram protein. In the present study microsomes from the same cow were used in all experiments. Bovine mammary stearyl desaturase activity was very stable when the microsomal preparations were stored at −30°C as a freeze-dried powder. Thus, the specific activity of the desaturase averaged 0.48, 0.45, and 0.44 nmoles, oleate formed per milligram protein per minute after 0-, 4- and 8-month storage. Preliminary analyses using the methods of Gaylor *et al.* (16) revealed that the cytochromes P_{450} (P_{420}) and b_5, associated with the enzyme preparation, remained rather stable during the same storage period.

TABLE I

COMPARATIVE FORMATION OF OLEATE FROM STEARYL-CoA BY MICROSOMES FROM LACTATING BOVINE MAMMARY GLAND AT VARYING CONCENTRATIONS OF NADH AND THE EFFECT OF NAD^+ AND $NADP^+$ ON DESATURATION.[a]

	Concentration of cofactor (μM)		nmoles oleate formed/20 min/0.5 mg protein	nmole oleate formed/nmole of added NADH
	Oxidized	Reduced NADH		
Expt. A	—	3.41	4.09	1.20
	—	3.41	3.60	1.05
Expt. B	—	0.51	1.21	2.36
	—	1.54	1.78	1.15
	—	2.56	2.64	1.03
	—	3.84	3.28	0.85
Expt. C	15.9 NAD^+	—	0.63	—
	16.0 $NADP^+$	—	1.52	—

[a] The assay medium containing 20 μM [1-^{14}C]-stearyl CoA and 0.5 mg protein was incubated at 30°C for 20 min. Assays lacking added NADH failed to desaturate stearyl-CoA (see Fig. 7).

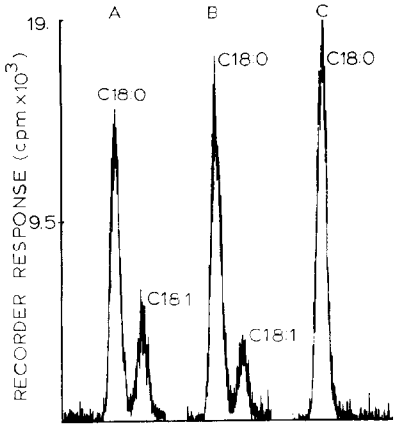

FIG. 7. Radio-gas chromatogram showing the effects of limiting quantities of NADH on stearyl CoA desaturase. The standard assay system was used except that A, B, C contained 5.0, 2.0, and 0 μM NADH, respectively.

The inclusion of EDTA (1 mM) in the microsomal preparation consistently inhibited desaturase activity by >95% under otherwise optimum conditions.

Negligible stearyl-CoA desaturase activity was detected in crude microsomal preparations from liver and adipose of lactating animals which possessed mammary desaturase activity. Corresponding tissues from lactating rat showed a reverse pattern of activity (Table II).

TABLE II

RELATIVE ACTIVITIES OF STEARYL DESATURASE FROM MAMMARY, ADIPOSE, AND LIVER TISSUES OF BOVINE AND RAT[a]

Tissue	Physiological state of animal	Specific activity (nmoles oleate formed/min/mg protein)
Bovine mammary tissue	Lactating	0.3–0.7
Bovine mammary tissue	Nonlactating	Trace
Bovine adipose tissue	Lactating	Not detectable
Bovine liver tissue	Lactating	Not detectable
Rat liver	Lactating	1
Rat adipose tissue	Lactating	1
Rat mammary tissue	Lactating	Not detectable

[a] Each assay containing 0.5 mg microsomal protein, 20 μM [1-^{14}C]stearyl CoA, 250 μM NADH was incubated at 30°C for 20 min.

The microsomes desaturated palmityl CoA and had a specific activity of 0.45 n-moles palmitoleate formed per minute per milligram of microsomal protein under conditions which were optimal for stearyl-CoA desaturase activity.

Because oleyl-CoA is the product of the desaturase reaction and palmityl-CoA is apparently a substrate for the same enzyme, the effects of varying concentrations of oleyl- and palmityl-CoA on stearyl desaturase was assayed. Both of these acyl thioesters depressed desaturase and the palmityl-CoA had a more marked effect (Table III).

Since stearyl-CoA is a substrate for both desaturase and acyl transferase(s) *in vivo* (42), and because of the possible requirement for endogenous oleic acid for the terminal (sn3-position) acylation of forming triglycerides (6) the effects of several potential acyl acceptors on stearyl-CoA desaturase were examined. 1,2 Diglycerides depressed desaturase activity only slightly but to an increasing extent as their respective concen-

TABLE III

THE EFFECT OF VARYING AMOUNTS OF OLEYL-CoA AND PALMITYL-CoA ON THE SPECIFIC ACTIVITY OF STEARYL DESATURASE FROM BOVINE MAMMARY MICROSOMES[a]

	Concentration of added Acyl-CoA (μM)	Stearyl desaturase (sp act)	Inhibition (%)
Control	0	0.35	0.0
Oleyl-CoA	18.0	0.30	14.2
	44.0	0.22	37.0
	88.0	0.21	40.0
Palmityl-CoA	18.0	0.26	25.5
	44.0	0.17	51.0
	88.0	0.11	67.0

[a] Both oleyl-CoA and palmityl-CoA were made up in potassium phosphate buffer (pH 7.4, 66 mM) and varying amounts were added to each assay tube containing 20 μM [1-^{14}C]stearyl-CoA, 250 μM NADH, and 0.5 mg protein. Assay conditions were as described in Methods.

trations were increased, e.g., 2.0 mM diolein depressed desaturase by 20%.

L-α-Glycerolphosphate at concentrations ranging from 0.2 to 3.6 mM caused a gradual depression of desaturase activity. Lysophosphatidylcholine (LysoPC) in amounts above 0.1 mM markedly inhibited desaturase activity (Fig. 8).

In view of the effect of lysoPC, a lytic agent, which probably disrupted the membrane-bound desaturase, we examined the effects of other factors which alter membranes, i.e., sonication and ethanol, on the desaturase activity. Sonication of the incubation mixture enchanced desaturase activity by 13–40% (Table IV). Sonication did not affect the K_m for stearyl-CoA nor NADH but it did increase the V by 30–40%. The inclusion of ethanol at 10% by volume consistently depressed stearyl desaturase activity.

Analyses of the distribution of labeled stearic and oleic acid in the various lipid classes consistently showed that most of the radioactivity was associated with the triglycerides (45–60%) phospholipids (20–35%), and free fatty acids 15–25%. The inclusion of diglycerides or ethanol in the assay mixture enchanced labeling of the triglycerides whereas αGP or sonication increased the relative amount of radioactivity in the phospholipids.

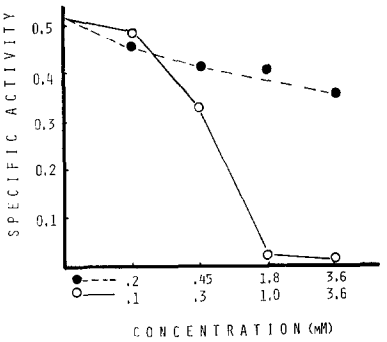

FIG. 8. The effects of L-α-glycerolphosphate (●) and 1 acyl-*sn*-glycero-3-phosphorylcholine (○) on the specific activity of stearyl CoA desaturase from bovine mammary microsomes.

TABLE IV

THE EFFECT OF SONICATION AND ETHANOL ON THE ACTIVITY OF STEARYL-CoA DESATURASE FROM BOVINE MAMMARY MICROSOMES[a]

Expt. no.	Specific activity (nmoles oleate produced min/mg protein)			Change in spec act (%)
	Control	Sonicated	10% ethanol	
1	0.50	0.700	—	+40.0
2	0.42	0.475	—	+13.0
3	0.34	—	0.141	−58.6

[a] The specific activity is the average of duplicate assays. The ethanol was added to the complete assay tubes and vigorously shaken before incubation at 30°C. The microsomes in phosphate buffer 0.5 mg/ml were sonicated at 5°C for 5 min using an ultrasonic vibrator (Model 8845, Cole-Palmer, Chicago) at top frequency. The assay, which was initiated by addition of NADH, was as described in Methods.

DISCUSSION

The K_m for stearyl-CoA obtained in the present study was in agreement with those (16, 19, and 20 μM) obtained for stearyl desaturase of rat liver and other tissues (2, 15, 22). Allen *et al.* (11) reported K_m values from 0.95 to 7.3 mM for desaturase of hen liver. Conceivably these high K_m values could be explained by shortage of substrate, i.e., stearic acid was used as substrate precursor and stearyl-CoA synthetase activity may have been low in their preparation.

The validity of K_m values determined using stearyl-CoA as substrate depends on the assay conditions used because there are many other reactions that can use stearyl-CoA. Most microsomal preparations contain active acyl-CoA hydrolases (6, 8, 14, 22, 26, 38). These preparations also contain active acyl transferases which acylate available fatty acids into glycerolipids (6, 14, 22, 26, 38). These enzymes compete with desaturase for available substrate and have the effect of increasing the apparent K_m of the desaturase enzyme for substrate acyl-CoA. Both acyl-CoA transferase(s) and hydrolase are present in bovine mammary microsomes (6, 21) and conceivably inflate the apparent K_m.

The presence of endogenous unsaturated fatty acids which depress stearyl desaturase activity (3, 26, 27) may also increase apparent K_m. However, these acids are not significant in bovine microsomes (39). Furthermore, since the activity of desaturase is affected by the nutritional state of an animal (23, 24), it may explain some discrepancies in observed K_m values because conditions which give initial velocities with an enzyme of low specific activity may not give initial velocities with an enzyme of high specific activity.

Stearyl-CoA exhibits detergent properties above its critical micellar concentration (CMC). Zahler and Cleland (28) showed that palmityl-CoA exhibited detergent properties above its CMC, (4–8 μM) and that the CMC was markedly influenced by protein concentration. Pande and Mead (4) showed that the concentration of acyl-CoA esters required for half-maximum velocity is related to the amount of enzyme (protein) incubated. This may account in part for the variability of the K_m values for stearate desaturation observed by Allen *et al.* (11). Baker and Lynen (14) showed that microsomal protein had a great affinity for stearyl-CoA and bound it very tightly. These factors indicate that the total amount of stearyl-CoA incubated may not be available to the enzyme because of the binding to the microsomal protein and because of competing enzymes. Hence, K_m values can be very variable.

The apparent substrate inhibition of bovine mammary stearyl desaturase at high substrate (55 μM) in this study was also observed with rat liver microsomes (64 μM) under similar assay conditions (4). Because of all these factors the K_m determined in this study is an apparent K_m.

The decrease in specific activity of the desaturase at increasing concentrations of microsomal protein above 1.5 mg (Fig. 1) has been observed with desaturases from other sources, but at different protein levels (15, 21). The nonlinearity observed may be ascribed to the increased activity of competing enzymes, i.e., acyl CoA hydrolases and transferases, or possibly product inhibition.

Bovine mammary stearyl desaturase activity increased with temperature from 8 to 50°C, having an activation energy (Ea) of 12.4 kcal/mole. Paulsrud *et al.* (3) using rat liver microsomes reported an Ea of 21.8 kcal/mole. This high value is explained by the inclusion of acylthiokinase in their overall reaction since stearic acid was the substrate used. An energy of activation of 12.4 kcal/mole for stearyl desaturase is very similar to that of other enzymes with $Q_{10} = 2$, (12 kcal/mole). In contrast, James *et al.* (29) observed an increase in plant stearyl desaturase activity with decreasing temperature which they related to oxygen concentration.

The apparent pH optimum of the desaturase ranged from pH 6.5 to pH 7.5. Oshino *et al.* (2) showed that buffer composition affected the specific activity of desaturase, i.e., Tris–HCl buffer (0.1 M) gave greater activity than potassium phosphate buffer (0.1 M), but both gave an optimum at pH 7.4. The negligible activity of bovine stearyl desaturase at pH 10.0 in bicarbonate buffer (0.025 M) is in contrast to the stearyl-CoA saturase from hen liver which retained 70% of its activity at pH 10.0 (25).

The K_m (3.0 μM) for NADH differs greatly from the K_m values (16 and 12 μM) obtained by Oshino (2) and Seifried (15), respectively. The bovine mammary desaturase enzyme has an order of magnitude greater affinity for NADH than for stearyl-CoA. The nonlinearity between the quantity of oleate formed and available NADH at concentrations below 3.8 μM, indicated that some NADH regeneration from oxidized cofactor was occurring in the microsomes, because the microsomal preparation lacked endogenous dinucleotides, and both NAD and NADP,

when added, supported low desaturase activity. The presence of a cofactor linked dehydrogenase could explain the relatively low K_m obtained for NADH in the present study. The observation that, of the oxidized nucleotides, NADP was a better cofactor for desaturase may indicate that isocitrate dehydrogenase is the reducing enzyme involved. The knowledge that this enzyme is particularly active in bovine mammary tissue during lactation for production of reduced NADP (30) would corroborate this suggestion. This aspect is being studied further.

Several workers have reported the inhibition of stearyl-CoA desaturase from various sources by fatty acids (3, 4, 21) and inhibition by stearyl- and palmityl- CoA has been also reported (4). Brenner and Peluffo (31, 32) observed no inhibition of desaturase from rat liver by oleic or palmitic acids; however, the concentration of acids was very low (0.33 μM), and initial velocities were apparently not observed. It is puzzling to to explain how these workers observe desaturase activity in the presence of 1 mM cyanide (31, 32) when it is known that desaturase contains a cyanide-sensitive factor (2, 16, 41).

A comparison of the inhibition observed in many reports (4, 21, 27) is not feasible because of varying assay conditions. Our observation that palmityl CoA caused greater inhibition of desaturase than oleyl CoA suggests that palmityl-CoA may compete with stearyl-CoA for the same site on the desaturase enzyme. This is probable since both are substrates for the same enzyme as shown herein, and by others (5). At high concentrations (88 μM) some of the inhibition by palmityl- and oleyl-CoA may be the result of their detergent action (4, 28, 33). The inhibition by oleyl-CoA suggests that it may play a regulatory role *in vivo* by depressing desaturase activity when oleyl-CoA accumulates.

The inhibitory effect of lysophosphatidylcholine on a stearyl CoA desaturase activity was also noted for rat liver desaturase (4). This effect may be caused by lytic disruption of the desaturase system which is composed of several components and required phospholipids for structural integrity and activity (8, 16, 25, 40). A similar rationale may also account for the decrease in desaturase activity caused by ethanol. Gurr and Robinson (25) showed that 1% Triton dissociated the stearyl-CoA desaturase system and decreased its activity by 50%, and other workers have employed surfactants to dissociate this enzyme complex for activity analysis (8, 16, 25, 40).

L-α-GP depressed desaturase activity slightly whereas in other experiments with mammary microsomes L-α-GP enhanced it (6, 21). However, in those studies greater amounts of microsomal protein were used and assay conditions were different. Conceivably our present data are attributable to greater activity of acyl transferases specific for L-α-GP which is consistent with the enhanced labeling of phospholipids.

The large increase in activity of stearyl desaturase upon sonication may be attributed to unfolding of the membrane system thereby increasing substrate access to and product removal from the enzyme. This is substantiated by the observation that sonicated enzyme increased the V but did not affect the K_m for NADH, indicating that sonication caused unmasking of new enzyme. From kinetic studies Vessey and Zakim (34) proposed that the increased UDP-glucuronyltransferase activity caused by sonication could be explained if the enzyme assumed different conformational states resulting in different kinetic properties.

The apparent inactivity of stearyl-CoA desaturase from the liver and perirenal adipose microsomes of lactating bovine is seemingly anomalous because monogastric animals (e.g., rat) have very active hepatic and adipose desaturase systems (24). Measurable desaturase activity in ruminant mammary tissue occurs only during lactation when it may play a vital role in providing oleic acid for milk triglyceride synthesis (6, 36). However, these observations are not conclusive as only one assay condition was used.

The ability of the microsomes to equally desaturate palmityl-CoA *in vitro* is difficult to relate to the conditions *in vivo* in which palmitic acid is plentiful yet palmitoleic acid is not produced to the same extent as oleic acid. Perhaps *in vivo* the palmityl-CoA desaturase activity has different kinetic properties, i.e., higher K_m and lower V for palmi-

tic acid than for stearic acid and conceivably palmitoleic acid may not be utilized as avidly as oleic acid *in vivo* and thereby exercise a feedback restriction of palmityl desaturase activity.

The consistent inhibitory effects of EDTA observed with bovine mammary microsomes is difficult to explain except by suggesting that it possibly chelated cations associated with the cytochromes. This apparently has not been observed by other workers (22). This effect and valid kinetic data can be obtained when desaturase is isolated in a purified state.

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