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Desaturation of Myristoyl-CoA to Myristoleoyl-CoA by Hen Liver Microsomal Δ^9-Desaturase

AZIZ C. AWAD, HAN-SEUNG SHIN, DALE R. ROMSOS, AND J. IAN GRAY*

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824-1224

The desaturation of myristoyl-CoA to myristoleoyl-CoA was measured in microsomal preparations of hen liver. The desaturation was maximal at pH 7.4. The enzymatic activity was linear with time up to 10 min and proportional to microsomal protein concentrations. The initial velocity was linear with substrate concentrations between 13 and up to 200 μM. A decrease in desaturation activity was observed at substrate concentrations greater than 266 μM. There was an absolute requirement for reduced pyridine nucleotide (NADH), while a maximum activity was observed at a myristoyl-CoA:NADH mole ratio of 1. Competitive inhibition studies of myristoyl-CoA desaturation suggest that the inhibitors, stearyl and oleyl-CoA, were more effective than palmitoyl-CoA. Free CoA did not inhibit the Δ^9-desaturase system. The desaturation of myristoyl-CoA was stimulated by bovine serum albumin and reduced by cytoplasmic proteins. The effect of cytoplasmic proteins on the enzymatic reaction was completely abolished by trypsin digestion and boiling for 30 min. On the basis of these data, it was concluded that 9,10-desaturation of acyl-CoA derivatives containing 14−18 carbon fatty acyl chains is catalyzed by the same enzyme.

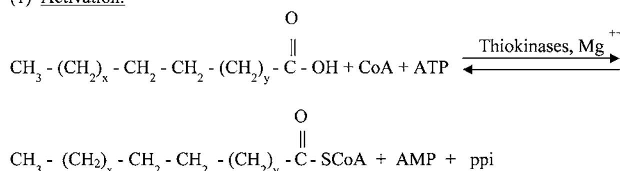
KEYWORDS: Myristoleoyl-CoA; desaturation; enzymatic reaction; competitive inhibition

INTRODUCTION

Enzymatic desaturation of saturated fatty acids to monounsaturated fatty acids is known to occur in various aerobic organisms including yeast and several animal species (*1*). Studies with liver homogenates have established that the desaturation of fatty acids involves two enzymatic reactions, viz., the activation of the fatty acid to the acyl-CoA derivative by thiokinases in the presence of CoA, adenosine triphosphate (ATP), and Mg^{2+}, followed by the desaturation of the derivative by the acyl-CoA desaturase system (**Figure 1**).

Holloway (*2*) reported that mammalian Δ^9 fatty acyl-CoA desaturases are bound to the endoplasmic reticulum and have an obligatory requirement for reduced nicotenamide adenine dinucleotide (NADH), molecular oxygen, cytochrome b_5, and NADH cytochrome b_5 reductase (EC 1.6.2.2). These components have been characterized by Spartz and Strittmatter (*3*) and Strittmatter et al. (*4*). The purified cytochrome b_5 reductase (EC 1.6.2.2) and cytochrome b_5 consist of a hydrolytic catalytic segment exposed to the aqueous environment and a hydrophobic portion that is involved in binding to the microsomal membrane. The terminal stearoyl CoA desaturase is a more nonpolar polypeptide of 53 000 Da and contains 62% hydrophobic amino acid residues (*4*). It is completely submerged in the hydrocarbon region of the membrane with the catalytic domain exposed to the aqueous environment to permit reduction of its nonheme iron by cytochrome b_5 and interaction with the hydrophilic portion of substrate molecules (*5*).

(1) Activation:



(2) Desaturation:

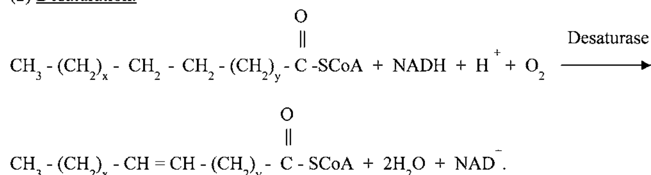


Figure 1. Enzymatic desaturation of saturated fatty acids to form monounsaturated fatty acids (*19*).

Using purified stearoyl-CoA desaturase, Enoch et al. (*5*) reported that 9,10-desaturation of fatty acyl-CoA derivatives with chain lengths of 12−19 carbon atoms is catalyzed by the same enzyme, with stearoyl-CoA being the best substrate. These studies, coupled with those of Raju and Reiser (*6*) who demonstrated the lack of desaturation of lipid-bound stearate, established fatty acyl-CoA as the true substrate for stearoyl CoA desaturase. Similar conclusions have been established for the Δ^6 and Δ^5 fatty acyl CoA desaturases (*7*).

* To whom correspondence should be addressed. Tel: 517-355-0123. Fax: 517-353-5406. E-mail: gray@msu.edu.

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As free fatty acyl-CoA derivatives can be highly toxic within the cell, there are particular species of cytoplasmic proteins that bind to them to protect cell membranes and membrane-bound enzymes from their cytotoxic effect. This is analogous to the presence of albumin in the blood circulatory system (*8*). The functional role of these proteins is not clearly understood. Brophy and Vance (*9*) and Jeffcoat et al. (*10*−*12*) suggested that these proteins act as fatty acid-binding proteins, which regulate the availability of fatty acids or fatty acyl-CoA for the lipid-metabolizing enzymes. The latter investigators, working with purified Δ^9-desaturase, demonstrated that bovine serum albumin (BSA) is not obligatory for the desaturation reaction per se, suggesting that its role is to protect the fatty acyl-CoA substrate from the effect of acylthiolester hydrolases. In this study, the initial objective was to develop an assay system to monitor the desaturation of myristoyl-CoA to myristoleoyl-CoA by the Δ^9-desaturase system in hen liver microsomes. This assay procedure was subsequently used to study several properties of the enzyme system including specific activity, substrate specificity, and the interaction of the substrate with BSA and cytosolic proteins (CPs).

MATERIALS AND METHODS

Materials. Myristoleoyl-, myristoyl-, palmitoyl-, stearoyl-, and oleoyl-CoA (lithium salt), tetradecanoic, *cis*-9-tetradecenoic, and pentadecanoic acids, BSA, trypsin, trypsin inhibitor, and NADH were obtained from Sigma Chemical Co. (St. Louis, MO). The standards were of greater than 98% chemical purity according to the manufacturers. Magnesium chloride and potassium hydroxide were obtained from Aldrich Chemical Co. (Milwaukee, WI). Laying hens (White Leghorn) were obtained from the Michigan State University Poultry Farm (East Lansing, MI).

Preparation of Hen Liver Microsomes. For each set of experiments, three laying White Leghorn hens were sacrificed. The whole livers were excised and cooled immediately over ice. The livers were minced and mixed together to decrease possible variations in desaturation yield from one single liver preparation to another. A 20 g sample of the ground livers was immediately homogenized in a Waring blender for 30 s with 60 mL of chilled 0.1 M potassium phosphate buffer (pH 7.4) containing 0.3 M sucrose. The resulting homogenate was centrifuged at 15 000*g* for 30 min at 2 °C. The sedimented cellular debris and mitochondria were discarded. Microsomes containing the Δ^9-desaturase system were sedimented from the postmitochondrial supernatant fraction by centrifuging at 105 000*g* for 1 h at 2 °C. The resulting microsomal pellet was either suspended in the homogenizing buffer or in the microsomal supernatant (MS) (containing the cytoplasmic proteins) to provide a final concentration of 2.66 mg microsomal protein/mL. The protein concentration was determined by the method of Lowry et al. (*13*). The remaining ground livers were stored at −20 °C for no longer than 10 h. Preliminary studies in our laboratory indicated that there was no loss in enzymatic activity over the duration of the holding period.

Enzyme Assays. Myristoyl-CoA was converted to myristoleoyl-CoA using the Δ^9-desaturase system of hen liver microsomes. BSA (60 mg) and 1000 nmol of C14:0 CoA were dissolved in 2.4 mL and 200 μL of distilled water, respectively. Samples were incubated at room temperature (25 ± 2 °C) for 5 min. NADH (6.2 mg) was dissolved in 1 mL of distilled water (NADH:C14:0 CoA mole ratio of 9), and 0.94 mg of $MgCl_2$ was dissolved in 0.1 mL of distilled water (pH was adjusted to 7.4 with 1 N HCl). Hen liver microsomes (3 mL) were added and suspended in homogenizing buffer (2.66 mg microsomal protein/mL homogenizing buffer). The total volume of the digest was adjusted to 7.5 mL by the addition of 0.8 mL of distilled water. The final microsomal protein concentration in the assay mixture was 1.06 mg/mL. The digest was incubated at 37 °C for 5 min.

Lipid Extraction and Fatty Acid Methyl Esterification. The enzymatic reaction was stopped by adding 4 mL of 10% methanolic KOH to saponify the lipids. The methanolic KOH solutions were heated at 65 °C for 30 min and then acidified with 7 N HCL. The samples were extracted three times with a mixture of hexane:2-propanol (3:2, v/v). The combined solvent extracts were evaporated under nitrogen. The internal fatty acid standard C15:0 (0.02 mg) was added to each of the samples, which were then derivatized to their fatty acid methyl esters by the boron trifluoride/methanol esterification procedure of Morrison and Smith (*14*).

Gas Chromatographic (GC) and GC-MS Analyses. GC analyses were carried out on a Hewlett-Packard 5890 A gas chromatograph (Avondale, PA) equipped with a flame ionization detector, split mode (1/12), and a fused silica capillary column (30 m × 0.25 mm) coated with a 0.25 μm film of DB-225 (J & W Scientific, Folsom, CA). Operating conditions of the gas chromatograph were as follows: temperatures—injector, 275 °C; detector, 300 °C; column, 165 °C; hold 5 min; 0.5 °C/min to 180 °C, hold 20 min, increase 10−215 °C, hold 10 min. Fatty acid methyl esters were identified by comparing their relative GC retention times with those of known standards. Standard curves were generated from known concentrations of pure methyl ester standards of C14:0 and *cis*-9-C14:1 ranging from 7.8 to 62.5 pg and containing 0.01 pg of C15:0. The areas of the C14:0, C14:1, and C:15:0 peaks were determined using a digital integrator. The C14:0 and C14:1 peak areas were divided by the internal standard area to obtain the response ratios for both standards and samples. The standard response ratios for C14:0 and *cis*-9-C14:1 were plotted against their corresponding concentrations. Standard response ratio plots should bracket the sample response ratio. The C14:0 and *cis*-9-C14:1 concentrations in the sample extracts were quantified from the standard response ratio plots and corrected for the dilution factor (df = 2). Initial analyses indicated that there was no endogenous *cis*-9-C14:1 in the medium. Thus, the *cis*-9-C14:1 observed was derived as a result of the desaturation reaction per se.

The identity of *cis*-9-C14:1 was confirmed by comparing the mass spectra of authentic *cis*-9-C14:1 standard and that derived from the desaturation of C14:0. The GC-MS analyses were carried out on a JEOL A × 505H double focusing mass spectrometer equipped with a Hewlett-Packard 5890 J GC. The fatty acid methyl esters, dissolved in hexane, were injected using a splitless injection technique onto a 30 m × 0.25 mm i.d., 25 μm coated DB 225 capillary column interfaced directly into the ion source. The GC oven temperature was programmed from 100 to 165 °C at 5 °C per min and held for 5 min, then to 172 °C at 0.5 °C per min, and finally to 230 °C at 10 °C per min. The injector and transfer line temperatures were maintained at 230 and 230 °C, respectively. Fatty acids were analyzed in the m/z range of 45−600 for the methyl ester derivatives in the positive electron ionization mode (EI). The analyses indicated that the fatty acid resulting from the desaturation of C14:0 by the hen liver desaturase system was indeed *cis*-9-C14:1.

Trypsin Digestion. To a 6 mL aliquot of the MS containing 31.5 mg/mL CPs, 340 mg of trypsin was added, and the mixture was incubated for 1 h at 37 °C. Proteolysis was stopped with the addition of 680 mg of trypsin inhibitor.

Effects of Heat Treatment and Trypsin Digestion. The study was designed to determine the effects of heat treatment and trypsin digestion of the CPs with MS on the desaturation reaction. MS (3 mL) containing 31.5 mg CP/mL was prepared, and 1000 nmol of C14:0 CoA was dissolved in 200 μL of distilled water. Samples were incubated at room temperature (25 ± 2 °C) for 5 min. NADH (6.2 mg) was dissolved in 1 mL of distilled water (NADH:C14:0 CoA mole ratio of 9), and 0.94 mg of $MgCl_2$ was dissolved in 0.1 mL of distilled water (pH was adjusted to 7.4 with 1 N HCl). Hen liver microsomes (3 mL) were added and suspended in homogenizing buffer (2.66 mg microsomal protein/mL homogenizing buffer). The total volume of the digest was adjusted to 7.5 mL by the addition of 0.2 mL of distilled water. The final microsomal protein concentration in the incubation mixture was 1.06 mg/mL. This digest was incubated at 37 °C for 5 min. The MS was trypsin-digested in one set of experiments and boiled for 30 min in another set.

Statistical Analysis. All treatments were performed in duplicate (n = 2). Statistical analyses were based on the polynomial regression and analysis of variance (ANOVA) procedures of SYSTAT 7.0 (*15*). ANOVA was also used to analyze the data and included NADH,

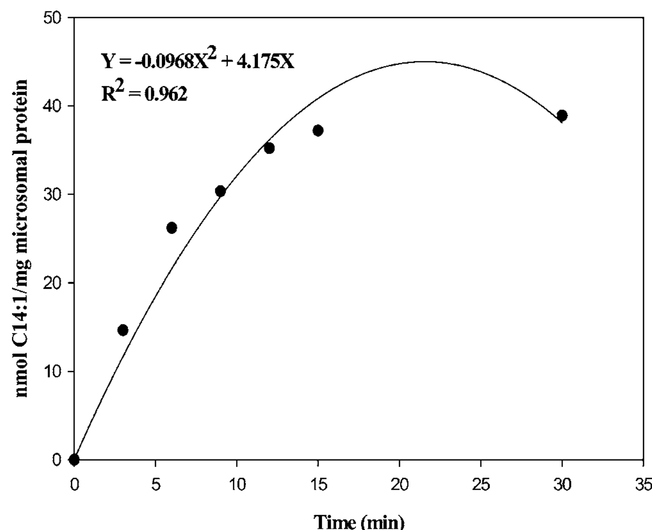

Figure 2. Desaturation of myristoyl-CoA as a function of time by the Δ^9-desaturase of hen liver microsomes. The complete system contained 8 mg of hen liver microsomal protein, 40 mM potassium phosphate (pH 7.4), 120 mM sucrose, 133 μM C14:0CoA, 60 mg of BSA, 1.16 mM NADH, and 1.31 mM $MgCl_2$, pH 7.4, in a total volume of 7.5 mL.

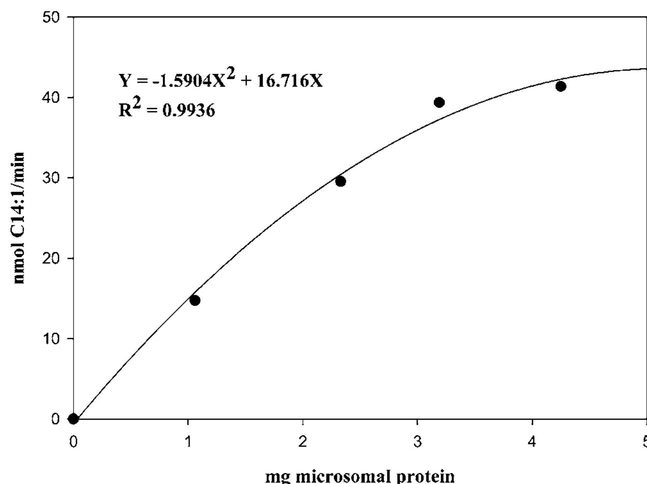

Figure 3. Desaturation of myristoyl CoA as a function of microsomal protein concentration by the Δ^9-desaturase of hen liver microsomes. The complete system contained 40 mM potassium phosphate (pH 7.4), 120 mM sucrose, 133 μM C14:0CoA, 60 mg of BSA, 1.16 mM NADH, and 1.31 mM $MgCl_2$, pH 7.4, in a total volume of 7.5 mL. Incubations were at 37 °C for 5 min.

competitive inhibitors, heat treatment, trypsin digestion, BSA, and MS as main effects and BSA × MS as interaction. The Tukey multiple comparison procedure was used for mean separation (*16*). If levels within a factor were significantly different, they were examined further using the Student's *t*-test for differences among individual means. Results were judged to be statistically significant based on the type I error rate of 5%.

RESULTS AND DISCUSSIONS

Mammalian desaturases have been known for many years to be bound to the endoplasmic reticulum and require oxygen and NADH (*17*, *18*). In mammalian tissues, the formation of monoenes from free saturated fatty acids is a two-stage reaction: activation of the saturated fatty acid to fatty acyl-CoA by acyl-CoA synthetase, followed by desaturation of the acyl-CoA derivative (*19*). In the activation reaction, the energy is generally supplied by ATP conversion to adenosine monophosphate and pyrophosphate (*20*). Previous studies of desaturation generally used free fatty acids as the substrate in the presence of CoA, Mg^{2+}, and ATP, and thus, the rates of both reactions were measured. In the present study with Δ^9-desaturase of hen liver microsomes, the desaturation was studied as a single reaction using the activated form of the substrate (myristoyl-CoA) in order to ensure that the results were not due to inhibition of the initial activation of free myristic acid to myristoyl-CoA. When the fatty acyl-CoA is used as the substrate, the activation reaction of the substrate does not take place; therefore, it is not a rate-limiting step in the desaturation reaction.

Factors that Modify the Yield of Desaturation of Myristoyl-CoA. Hen liver microsomes, like microsomes from other sources (*1*), catalyzed the oxygen-dependent and reduced pyridine nucleotide-dependent desaturation of myristoyl-CoA to myristoleoyl-CoA. To test the reliability of the assay conditions, the effects of time, microsomal protein concentration, substrate concentrations, reducing agent, pH, competitive inhibitors, BSA, and MS on enzyme activity were investigated.

Effect of Incubation Time. Under the assay conditions, the amount of myristoleate formed was directly related to the incubation times. When the desaturation reaction was stopped after 5 min, a linear relationship between desaturation and time was observed (**Figure 2**). A similar relationship was observed when the reaction was terminated at 10 min. The linear relationship between desaturation rate and incubation times (up to 10 min) is indicated by the equation $Y = 3.7643X$ ($R^2 = 0.93$). The rate of monoene production declined sharply after 30 min of incubation, which may be due to the decrease in substrate concentration as a result of its utilization in other enzymatic reactions. In the liver cell, any saturated acyl-CoA can suffer a variety of fates, such as being hydrolyzed to the unesterified fatty acid, being converted into lipid, or undergoing β-oxidation, chain elongation, or desaturation (*21*).

Effect of Microsomal Protein Concentration. The rate of desaturation of myristoyl-CoA was shown to be directly proportional to the amount of the microsomal protein over the range of 1.06 to 3.19 mg per mL of incubation mixture (**Figure 3**). The linear relationship between the desaturation rate and the microsomal protein concentration (up to 3.19 mg per mL incubation mixture) is indicated by the equation $Y = 12.554X$ ($R^2 = 0.99$). The enzyme activity was slowed at protein concentrations greater than 4.25 mg per mL incubation mixture. A relatively high microsomal protein concentration of 3.19 mg per mL incubation mixture was purposely employed in all of the assays, unless otherwise indicated, to ensure that there was sufficient production of myristoleate over the incubation time of the assay in order to be quantifiable by the GC procedure. At constant levels of substrate, monoene formation was slowed at high protein concentrations and slowed by prolonged incubation. These latter two findings were also observed in rat brain desaturation systems (*22*) and could be due, in part, to the addition or accumulation of desaturation product.

Effect of Substrate Concentration. The relationship of initial velocity to substrate concentration for the hen microsomal Δ^9-desaturase reaction is shown in **Figure 4**. The plot was linear between 13 and 133 μM. Inhibitory effects of substrate concentration occurred at concentrations in excess of 300 μM. The assays of initial rates could not be measured accurately at substrate concentrations lower than 13 μM, because it was difficult to have sufficient product to be accurately determined by GC. Because of the inhibition at high substrate concentrations and variation at the very low concentrations (i.e., less than 13 μM), it was difficult to determine K_m for the desaturation reactions. Unless otherwise indicated, most assays were carried

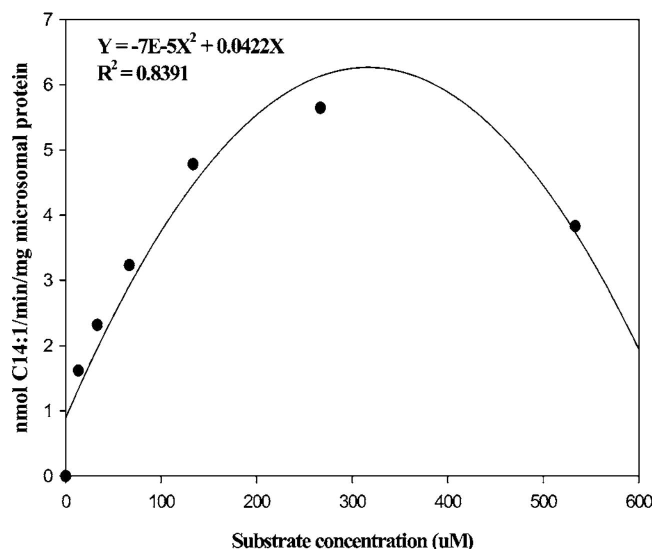

Figure 4. Relationship of initial velocity to substrate concentration for the hen microsomal Δ^9-desaturase-catalyzed reaction. The complete system contained 8 mg of hen liver microsomal protein, 40 mM potassium phosphate (pH 7.4), 120 mM sucrose, 60 mg of BSA, 1.16 mM NADH, and 1.31 mM $MgCl_2$, pH 7.4, in a total volume of 7.5 mL. Incubations were at 37 °C for 5 min.

out at a substrate concentration of 133 μM, allowing recovery of product significantly above background values (if any) and avoiding the possibility of substrate inhibition. Furthermore, the use of this constant substrate concentration decreased the likelihood of nonfirst-order reaction conditions of reduced substrate availability, by incorporation into lipids by acyltransferase. These data, together with those in **Figures 2** and **3**, clearly show that the assay described here gives a true measure of the initial velocity under pseudo first-order reaction conditions.

Effect of Reducing Agent. The reducing equivalent in the desaturation reaction is used to reduce the Δ^9-desaturase system through an electron transport chain (*18*). The microsomal nonphosphorylating electron transport chain involved in the fatty desaturation includes a flavoprotein, the desaturase enzyme attributable to a hemo-protein and cytochrome b_5 (*18*). The reducing equivalent required for the desaturation site is supplied by the NADH via cytochrome b_5. The rate of cytochrome b_5 reduction by NADH in rat liver microsomes is in the order of 1000 nmol per min per mg protein. In contrast, the activity to desaturate stearyl CoA is, at most, less than 10 nmol of oleate formed per min per mg protein in even highly "induced microsomes" (*18*). Thus, the capacity of the microsomal system to supply reducing equivalents to cytochrome b_5 is greater than that required for the desaturation of fatty acyl CoAs.

The effect of NADH concentrations on the desaturation of myristoyl-CoA is shown in **Figure 5**. A wide range of NADH: myristoyl-CoA mole ratios was assessed under the experimental conditions described in **Figure 1**. In the absence of NADH, no product formation was observed suggesting the absence of endogenous reducing equivalents. At NADH:myristoyl-CoA mole ratios ranging from 1 to 9, the desaturation was maximal. A decrease in desaturation was observed at a NADH:myristoyl-CoA mole ratio of 12. A NADH:myristoyl-CoA mole ratio of 9 was employed for all of the assays, unless otherwise indicated, to ensure that the electron transport system was not rate-limiting in the desaturation reaction.

Effect of pH. The velocity of enzyme-catalyzed reactions depends on the pH. The importance of pH in the desaturation

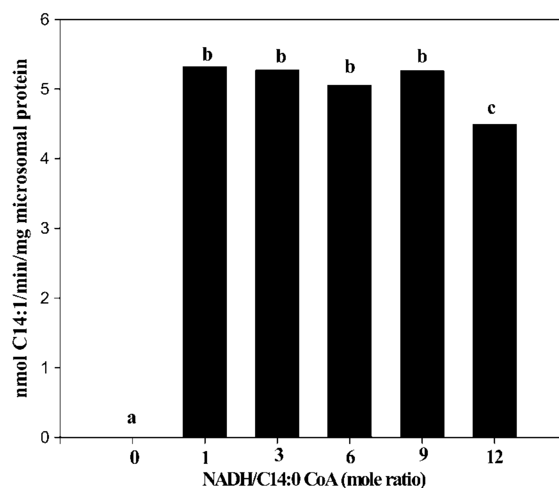

Figure 5. Effect of increasing concentrations of reduced pyridine nucleotide (NADH) on the desaturation of myristoyl-CoA by the Δ^9-desaturase system in hen liver microsomes. The complete system contained 8 mg of hen liver microsomal protein, 133 μM C14:0 CoA, 40 mM potassium phosphate (pH 7.4), 120 mM sucrose, 60 mg of BSA, and 1.31 mM $MgCl_2$, pH 7.4, in a total volume of 7.5 mL. Incubations were at 37 °C for 5 min. a–c: means with no common superscript are significantly different ($p < 0.05$).

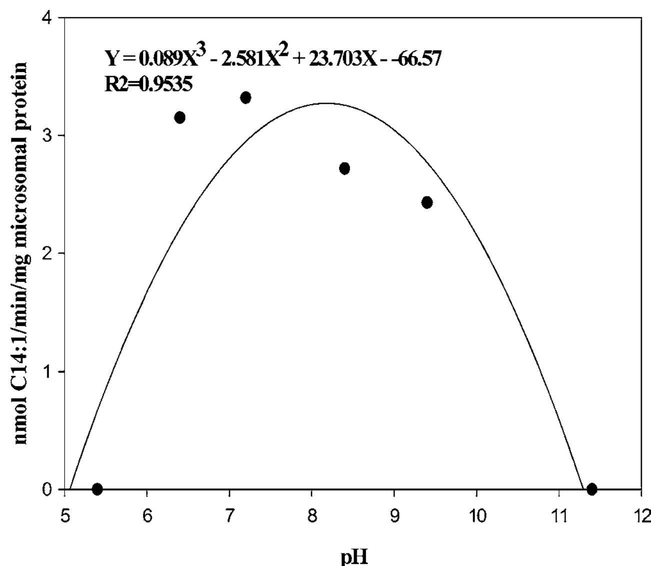

Figure 6. Effect of pH on the desaturation of myristoyl-CoA by the Δ^9-desaturase of hen liver microsomes. The complete system contained 8 mg of hen liver microsomal protein, 133 μM C14:0 CoA, 40 mM potassium phosphate (pH 7.4), 120 mM sucrose, 60 mg of BSA, 1.16 mM NADH, and 1.31 mM $MgCl_2$ in a total volume of 7.5 mL. Incubations were at 37 °C for 5 min.

reaction is due to its ability to change (i) the ionization state of the Δ^9-desaturase system, (ii) the membrane structure where the Δ^9-desatuarese system is embedded, (iii) the ionization state of the substrate, which in turn affects the reactivity of the reaction, and (iv) the solubility of the substrate solubilizing protein (*21*). The Δ^9-desaturase system gave a parabola-shaped curve of velocity vs pH (**Figure 6**), which is typical of most enzymes. The optimal pH for the formation of monoenes from myristoyl-CoA in hen liver microsomes was 7.4. At extreme pH values, the desaturation reaction was completely inhibited.

Effect of Competitive Inhibitors. Paulsrud et al. (*23*) reported that desaturase activity in rat liver microsomes increases with increasing fatty acid carbon numbers from 10 to 18. Brett et al. (*1*), who examined four other desaturation systems, viz.,

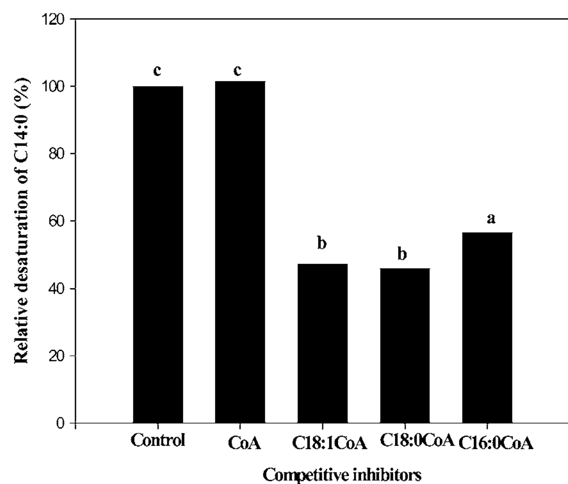

Figure 7. Effect of palmitoyl-, stearoyl-, and oleoyl-CoA and free CoA on the activity of the Δ^9-desaturase of hen liver microsomes (normalized to the control = 100%) using myristoyl-CoA as a substrate. The concentration of the myristoyl-CoA in the incubation medium (133.3 μM) was equal to the concentration of the competitive inhibitor. The total number of acyl-CoA derivatives (substrate and competitive inhibitor) in the incubation medium should not exceed 2000 nmoles (266.66 μM) since over this concentration the substrate itself was detrimental to the enzyme activity. a–c: means with no common superscript are significantly different ($p < 0.05$).

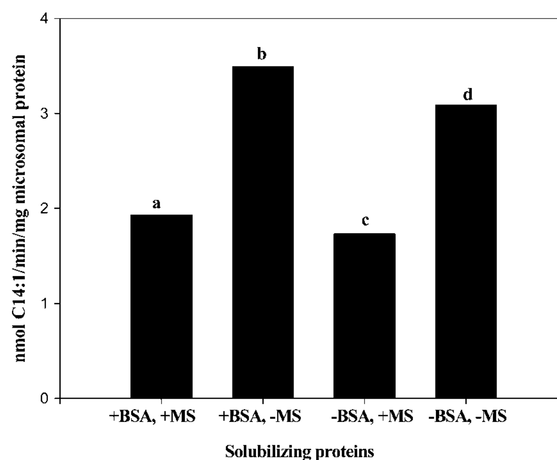

Figure 8. Effect of BSA and MS on the desaturation of myristoyl-CoA. The complete system contained 8 mg of hen liver microsomal protein suspended in the homogenizing buffer, 40 mM potassium phosphate (pH 7.4), 120 mM sucrose, 133 μM C14:0 CoA, 60 mg of BSA, 1.16 mM NADH, and 1.31 mM $MgCl_2$, pH 7.4, in a total volume of 7.5 mL. Incubations were at 37 °C for 5 min. When MS was added, the 8 mg of microsomal protein was suspended in MS instead of the homogenizing buffer. +BSA indicates the presence of BSA; –BSA indicates the absence of BSA; +MS indicates the presence of MS; and –MS indicates the absence of MS. a–d: means with no common superscript are significantly different ($p < 0.05$).

microsomal preparations of goat mammary gland, hen liver, and whole cells of *Torulopsis bombicola* and *Chlorella vulgaris*, concluded that a widely distributed single enzyme, whose activity is maximal with stearic acid and high with palmitic acid also, inserts the double bond between C_9 and C_{10}. Working with purified stearyl-CoA desaturase, Enoch et al. (*5*) reported that 9,10-desaturation of fatty acyl-CoA derivatives of varying chain lengths (12–19 carbon atoms) by rat liver microsomes is catalyzed by this enzyme, with stearyl-CoA being the best substrate. This is consistent with the observation that oleate is the major monounsaturated fatty acid of rat liver lipids. Similar observations are presented in **Figure 7** when several acyl-CoA derivatives were used as inhibitors. Stearoyl-, oleoyl-, and palmitoyl-CoA derivatives were effective competitive inhibitors for the Δ^9-desaturase of hen liver microsomes. The desaturase enzyme was not inhibited by free CoA. Again, this is consistent with the results reported by Enoch et al. (*5*). The inhibition is competitive with stearoyl-, oleoyl-, and palmitoyl-CoA. Stearoyl- and oleoyl-CoA were more effective in inhibiting the desaturase activity than palmitoyl-CoA. In the presence of stearoyl- and oleoyl-CoA, the desaturation rate decreased by 52.3 and 54.1%, respectively. In the presence of palmitoyl-CoA, the decrease in the desaturation rate was 43.4% (**Figure 7**).

The fact that inhibition of enzymatic desaturation has been shown to be dependent on the chain length of the acyl-CoA derivatives indicates that the number of methylene groups of the fatty acid may regulate the desaturase activity via London–van der Waals forces that bind the fatty acid to the enzyme. As the hydrocarbon tail of the fatty acid is essentially hydrophobic, London–van der Waals dispersion forces would be able to provoke binding with the enzyme. Because the dispersion forces vary as $1/D^7$, D being the distance between the interacting molecules, the substrate and the lipoproteic enzyme must be close enough to make the binding effective. These forces increase with the number of carbon units of the molecule and in consequence may be quite large. Salem (*24*) calculated the effect of each $-CH_2-$ group at an interaction distance of 5 Å to provoke a London–van der Waals dispersion (W_{disp}) energy of $W_{disp}/N = 0.4$ kcal/mol, where N is the number of identical units. This strengthening of the London–van der Waals forces with the number of $-CH_2-$ may explain the high affinity of the enzyme toward stearoyl- and oleoyl-CoA.

Effect of BSA and CPs. BSA has been shown to bind fatty acyl-CoA derivatives and protect them from acylthiolester hydrolases (*10*). The effect of BSA on the desaturase system was investigated, and results clearly show a stimulation of enzyme activity under the experimental conditions used (**Figure 8**). The specific enzyme activity, expressed as nmol C14:1 produced per min per mg protein, was found to be 3.49 and 1.93 in the absence and presence of the MS, respectively. When BSA was not added to the incubation medium, the specific activity dropped to 3.09 and 1.73 in the absence and presence of MS, respectively. **Figure 8** also reveals that the MS has the capacity to reduce the specific activity of the desaturase system whether BSA was present or not.

The effect of heat and trypsin digestion on the capacity of the MS to decrease the desaturation activity was investigated. The MS obtained as described earlier is heat labile. The activity of the MS, which contains the CPs, was completely lost after 30 min of boiling using the assay conditions described. Boiling induced precipitation of the proteins. The increase in the desaturase specific activity due to the heat precipitation of CPs was 1 nmol per min per mg microsomal protein as compared to the control (**Figure 9**).

The effect of trypsin digestion on the MS is shown in **Figure 9**. Desaturase specific activity was increased by 1.04 nmol per min per mg microsomal protein as compared to the control. The control experiment was performed with the native MS. The marked decrease in desaturase activity in the presence of CPs could be due to (i) the incorporation of myristoyl-CoA into complex lipids by the acyltransferase, (ii) the cleavage of myristoyl-CoA to free myristic acid by acylthiolester hydrolases, and (iii) elongation of myristoyl-CoA to palmitoyl-CoA by the fatty acid synthetases. Cook and Spence (*22*) reported that the

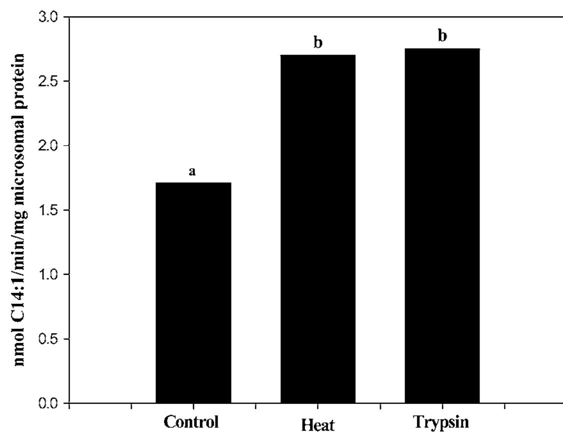

Figure 9. Effect of heat and trypsin digestion of the MS on the desaturation of myristoyl-CoA using the Δ^9-desaturase system of hen hepatic microsomes. a,b: means with no common superscript are significantly different ($p < 0.05$).

rate of incorporation of stearoyl-CoA into phosphoglycerides used as a measure of acyltransferase activity greatly exceeded its rate of desaturation. A comparison of the desaturation rate in the presence and absence of BSA (**Figure 8**) indicates that the albumin is probably protecting the acyl-CoA from the action of endogenous competing enzymes such as acylthiolester hydrolases and acyltransferase, but not from the desaturase, by binding the substrate and making it inaccessible to the competing enzymes. Albumin does not directly affect the desaturation reaction per se since it showed no effect on the desaturation rate when partially purified stearoyl-CoA desaturase was used in the absence of the competing enzymes (*10*).

In conclusion, the current research effort is directed at an understanding of the regulation of myristoyl-CoA desaturation by the Δ^9-desaturase system of hen liver microsomes. Experimental evidence has been presented to show that the assay described here gives a true measure of the initial velocity of the reaction under pseudo first-order reaction conditions. The inhibition of the desaturation of myristoyl-CoA by oleoyl-, stearoyl-, and palmitoyl-CoA suggests that all of these acyl-CoA derivatives are probably catalyzed by the same enzyme, stearoyl-CoA desaturase.

The involvement of CPs, which reduce the desaturation of myristoyl-CoA, has been established. Heat treatment and trypsin digestion were effective means to abolish the capacity of CPs to reduce the desaturation of myristoyl-CoA.

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