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Study of the Δ12-Desaturase System of *Lipomyces starkeyi*

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ABSTRACT: The specific activity of the microsomal Δ12-desaturase system, which transforms oleic acid into linoleic acid, was about 16 pmol/min/mg protein. However, most of the total activity was nonsedimentable even after a 200000 × *g* centrifugation for 100 min. The study of various physicochemical parameters showed that this enzymatic complex, functioning optimally between pH 7 and 8, had low thermal stability. Ca^{2+}, which may cause an aggregation of the microsomes, and Hg^{2+} completely inhibited the activity, whereas Mg^{2+}, Mn^{2+}, and Zn^{2+} were activators. The Δ12-desaturase system was relatively specific toward oleic acid, though isomers of this fatty acid also had an action, either as substrates or as competitive inhibitors, on the activity of the system. The study of the effect of the exogenous oleoyl-CoA and elaidoyl-CoA on the specific activity of the Δ12-desaturase system showed a preference toward oleoyl-CoA.

Lipids 31, 253–259 (1996).

We have previously shown (1) that the lipids from yeast species belonging to the related genera *Lipomyces* and *Waltomyces* contain notable amounts of essential long-chain fatty acids (linoleic and α-linolenic acids), which are precursors of very long-chain polyunsaturated fatty acids such as dihomo-γ-linolenic and arachidonic acids found as traces. In this paper, we have also shown that oleic acid was the most abundant fatty acid in the lipids (40% of total fatty acids) of *Lipomyces* and *Waltomyces* and that its desaturation produced a relatively important amount of linoleic acid (19% of total fatty acids).

Although desaturases are key enzymes in the regulation of unsaturated fatty acid biosynthesis, studies dealing with the desaturation enzymatic complexes of microorganisms are not numerous, and most of the literature concerns the desaturases contained in rat liver microsomes. Among the microbial desaturase systems studied are those of *Bacillus* species (2), *Mortierella alpina* (3,4), *Mucor* sp. INMI (5), *Saccharomyces cerevisiae* (6), *Yarrowia lipolytica* (7,8), and *Cryptococcus curvatus* (9).

In the present work, we have studied the influence of various physical and chemical parameters on the specific activity of the microsomal Δ12-desaturase system of *Lipomyces starkeyi* CBS 1807 responsible for the formation of linoleic acid from oleic acid. The activity of the Δ6 desaturase system, which may desaturate oleic acid into $C_{18:2n\text{-}9}$, has not been studied here because this latter fatty acid was not detected in the fatty acid composition of the strain *L. starkeyi* CBS 1807 (1) nor in that of the microsomes prepared from the cells of this yeast.

MATERIALS AND METHODS

Microorganisms. Lipomyces starkeyi CBS 1807 was obtained from the Centraal Bureau voor Schimmelcultures (Delft, The Netherlands).

Biochemicals. [1-^{14}C] Oleic acid (58 mCi/mmol) was obtained from Amersham (Amersham France, Les Ulis, France). It was diluted with cold oleic acid to a 10 mCi/mmol specific activity. Coenzyme A and ATP were purchased from Sigma (Sigma Chimie, Saint-Quentin Fallavier, France), and NADH was provided by Boehringer (Boehringer Mannheim, Meylan, France).

Culture medium and growth conditions. The strain was grown in medium G as described by Lomascolo *et al.* (1). Cultivation was conducted at 28°C for 15 h. At the end of the exponential growth phase, cells were harvested by centrifugation (7000 × *g* for 10 min) at 4°C and washed twice with a cold pH 7.5 buffered solution (buffer A) containing 0.05 M potassium phosphate, 0.65 M sorbitol, 1 mM EDTA, 1 mM DTT, and 1 mM $MgCl_2$.

Preparation of microsomes. The washed cells were suspended in 25 mL of buffer A and ground for 3 min in a CO_2 cooled Braun MSK bead grinder (Braun, Melsungen, Germany). The homogenate was then centrifuged at 25000 × *g* for 40 min, and the resulting supernatant fluid was centrifuged at 105000 × *g* for 100 min. The pellet, consisting in the microsomal fraction, was finally suspended in 2 mL of buffer A. This corresponded to 4.0 to 6.5 mg of protein per mL determined according to the procedure of Lowry *et al.* (10). Microsomes were used immediately for assays of desaturase activity. Aliquots of microsomes were kept at −20°C for 24 h before the determination of NADH:cytochrome *c* reductase (EC 1.6.2.1), succinate:cytochrome *c* reductase (EC 1.3.99.1), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activities.

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Fatty acid extraction and analysis. Fatty acid methyl esters were obtained as follows. The assay reaction mixture (1.1 mL), added with 0.7 mL of an ethanolic solution of 4 M KOH, was saponified at 80°C for 1 h in sealed ampules. For the analysis of the microsomal fatty acid composition, 1.1 mL of a suspension of microsomes was used instead of the reaction medium. After removal of unsaponified substances with hexane, the mixture was acidified with 0.8 mL of 4 M HCl, and free fatty acids were extracted three times with 2.5 mL of hexane. After evaporation of the solvent using a flow of N_2, the residue was methylated in the presence of 0.75 mL of 12.5% boron trifluoride in methanol for 2 min at 90°C in sealed ampules. After cooling in ice, 0.75 mL of distilled water was added to decompose the excess of BF_3, and the fatty acid methyl esters were extracted three times with 1.5 mL of hexane. The solvent was then evaporated, and the samples were kept in 0.1 mL of hexane at –20°C under N_2. Fatty acid methyl esters were analyzed by gas chromatography as described by Lomascolo *et al.* (1).

Assays of NADH:cytochrome c *reductase, succinate:cytochrome* c *reductase, and glucose-6-phosphate dehydrogenase activities.* All assays were carried out at 30°C in a Uvikon 930 UV/Visible spectrophotometer (Kontron Instruments, Zürich, Switzerland). (i) NADH:cytochrome *c* reductase: the reaction mixture contained in a final volume of 1.5 mL: 50 mM Tris HCl buffer pH 7.2, 0.06 mM NADH, 0.06 mM ferricytochrome *c*, 3 mM NaN_3, and 0.05 mL of a suspension of microsomes. The reaction was started by the addition of microsomes, and the reduction of cytochrome *c* was followed by recording the absorbance increase at 550 nm. The millimolar extinction coefficient used for cytochrome *c* was 29 $L.mmol^{-1}.cm^{-1}$. (ii) Succinate:cytochrome *c* reductase: the reaction mixture for this enzyme assay was the same as above, except that NADH was replaced with disodium succinate. (iii) Glucose-6-phosphate dehydrogenase: the reaction mixture contained in a final volume of 1.5 mL: 100 mM Tris HCl buffer pH 7.5, 1 mM glucose-6-phosphate, 0.15 mM NADP, 20 mM $MgCl_2$, and 0.1 mL of microsomes. The reaction was started by the addition of microsomes, and the reduction of NADP was followed by recording the absorbance increase at 340 nm. The millimolar extinction coefficient used for NADP was 6.22 $L.mmol^{-1}.cm^{-1}$.

Assay of Δ12-desaturase activity. The reaction mixture (final volume, 1.1 mL) contained: 0.1 M potassium phosphate buffer, pH 7.2, 0.65 M sorbitol, 1 mM DTT, 5.5 mM $MgCl_2$, 6.5 mM ATP, 0.5 mM CoA, 0.7 mM NADH, 18–20 μM [1-^{14}C] oleic acid (in 5 μL of ethanol, i.e., 0.2 μCi), and 0.15 mL of microsomes (0.6–1 mg protein).

The reaction was started by the addition of [1-^{14}C] oleic acid and was performed for 10 min at 40°C (except for the study of the effects of temperature on activity) with constant shaking in air. The reaction was stopped by adding 0.7 mL of 4 M KOH in ethanol. For the study of the influence of cations, the assay reaction medium (potassium phosphate buffer pH 7.2, sorbitol, DTT, $MgCl_2$, ATP, CoA and NADH, microsomes and cation) was preincubated for 10 min before adding [1-^{14}C]. The preincubation time was 2 min for the study of the influence of chemical amino reactive reagents. After saponification and methylation of fatty acids as described above, fatty acid methyl esters were separated by argentation thin-layer chromatography using toluene/acetonitrile (97:3, vol/vol) as mobile phase. Plates of Silica gel 60G (Merck, Darmstadt, Germany) were impregnated with 10% silver nitrate according to Wilson and Sargent (11). Commercial standards of $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ were cochromatographed on each plate. Following their visualization using 2′-7′-dichlorofluoroscein, the spots corresponding to saturated, monounsaturated, and biunsaturated fractions were separately scraped off the plates. The silica gel containing the fractions was suspended in Packard Instagel Plus scintillation liquid (Packard, Rungis, France) added with 77% (by vol) of distilled water in vials protected from light in order to prevent the formation of silver oxide. Radioactivity was counted after one hour in a 1600 TR Liquid Scintillation Analyzer (Packard Instruments S.A., Rungis, France). From the amount of radioactivity found in the diene fraction vs. the radioactivity recovered from the monoene fraction, the enzyme activity could be determined and expressed as picomoles of oleic acid converted to linoleic acid per min and per mg of microsomal proteins.

For each activity value given in this paper, at least two microsomal preparations obtained from different cultures were used, with at least three independent measurements made on each preparation. The activity value given was the mean of these six or more determinations.

The diene fraction obtained by thin-layer chromatography after 0 and 10 min of reaction at 40°C (standard conditions) has been identified as linoleic acid ($C_{18:2n\text{-}6}$) by gas chromatography by comparison of the retention time of the resulting peak with that of a commercial sample of pure linoleic acid.

RESULTS AND DISCUSSION

Localization of Δ12-desaturase system activity. Little activity (<2%) was associated with the mitochondrial fraction (25000 × *g* pellet). Table 1 shows that only a part of the activity of the Δ12-desaturase system was contained in the microsomal 105000 × *g* pellet. Total Δ12-desaturase system activity was 30 times higher in the 25000 × *g* supernatant (which contained microsomes plus cytosol) and 16 times higher in the 105000 × *g* supernatant than in the 105000 × *g* pellet. The specific activity measured on microsomes obtained by a 200000 × *g* centrifugation was the same as that found in microsomes contained in the 105000 × *g* pellet (16 pmol/min/mg protein).

The measurement of NADH:cytochrome *c* reductase, succinate:cytochrome *c* reductase, and glucose-6-phosphate dehydrogenase activities verified the nature of the pellet obtained after centrifugation at 105000 × *g*. A rotenone-insensitive NADH:cytochrome *c* reductase is also a characteristic enzyme from the endoplasmic reticulum in rat liver (12), and of the microsomes of mammalian and plant cells in which

TABLE 1
Distribution of Δ12-Desaturase System Activity

		Formation of linoleic acid	
Cell fraction	Protein content (mg)	Total activity ($nmol/min^{-1}$)	Specific activity[a] (pmol/min/mg protein)
Supernatant at 25000 × *g*	40	4.8 (100%)	131.1 ± 11
Microsomes, pellet at 105000 × *g*	9	0.2 (4%)	20.8 ± 1
Supernatant at 105000 × *g*	37	2.9 (60%)	74.1 ± 14

[a]Values of specific activity are given as mean ± SEM (n = 3).

it is linked to cytochrome b_5 and is involved in the fatty acid desaturation (13). Desaturases in the microsomal electron transport system occur together with NADH:cytochrome b_5 reductase and cytochrome b_5. Our results show that the activity of NADH:cytochrome *c* reductase measured in 105000 × *g* pellet extracts which had been frozen at −20°C was the same with or without rotenone (7.5 μL of a 1 $mg.mL^{-1}$ ethanolic solution): 0.021 μmol cytochrome *c* reduced/min/mg protein ±0.004 (n = 9). On the other hand, the succinate:cytochrome *c* reductase, characteristic of mitochondrial activities (14), did not have any activity in the microsomal pellet, whereas the specific activity of this enzyme was 9 nmol cytochrome *c* reduced/min/mg protein in the 25000 × *g* pellet (i.e., in the mitochondria). Finally, glucose-6-phosphate dehydrogenase, characteristic of the microsomes plus cytosol fraction (15) was present in the microsomal pellet and the supernatant of the 105000 × *g* centrifugation (0.1 ± 0.02, n = 4 and 0.37 ± 0.13, n = 4 μmol NADP/min/mg protein, respectively). The 105000 × *g* pellet was thus effectively composed of microsomes and free of mitochondria. Unless otherwise specified, this pellet was used in all the subsequent studies.

Fatty acid composition of microsomes. The long-chain fatty acid composition of microsomes is given in Table 2: oleic acid, linoleic, and palmitic acids represented more than 80% of total fatty acids. This composition was similar to that of the whole cells of *L. starkeyi* (1).

Δ12-Desaturase system specific activity in standard conditions. We have verified that the specific activity (16 pmol/min/mg protein ± 4, n = 10) was proportional to the reaction time when measured after up to 10 min in the standard reaction conditions described in the Materials and Methods section. This specific activity was the same when the microsomal protein amount in the reaction mixture was doubled.

The specific activity was in the same range as that found in *M. alpina* under similar conditions (3). On the other hand, it was threefold lower than that of *Mucor* sp. INMI (5) and sixfold lower than that of *Y. lipolytica* (7). Culture conditions, particularly aeration and temperature, may influence the production or the activity of the Δ12-desaturase system. The different procedures used for the obtention of microsomes may also result in differences of activity. Notably, as most of the Δ12-desaturase activity was found as a nonsedimentable form in the 105000 × *g* and 200000 × *g* supernatants, i.e., not in the standard microsomal fraction, the procedures for disruption may influence the activity found in the microsomes. A high proportion of Δ12-desaturase activity was also observed in 160000 × *g* and 200000 × *g* supernatants of microsomal preparations from *Y. lipolytica* (7).

Effects of temperature and pH on the specific activity of the Δ12-desaturase system: (i) Influence of temperature: the reaction was performed in the same conditions as described for the standard reaction but for different temperatures. The results given in Figure 1 show that the optimal temperature was 40°C for 10 min of reaction. The study of the thermal denaturation of the Δ12-desaturase system was realized by incubating the microsomal extracts for 15 and 30 min at various temperatures (30 to 45°C) before adding labelled oleic acid. After this treatment, the enzyme activity was determined in the standard conditions (Fig. 2A). Above 45°C, the Δ12-desaturase system did not show activity after 10 min. This sensitivity to temperatures higher than 40°C is likely to be due to the low apparent activation energy of the thermal denaturation of the enzyme complex (31 $kJ.mol^{-1}$ ± 19, n = 3), determined using an Arrhenius plot (Fig. 2B). (ii) Influence of pH: the reaction was realized as described for the standard reaction but for pH values varying from 5 to 9. The buffers used were citric acid/disodium phosphate 0.1 M (pH 5), potassium phosphate 0.1 M (pH 6–8), and Tris HCl 0.1 M (pH 9). The enzymatic system functioned optimally when microsomes were placed in a reaction medium with a pH between 7 and 8 (Fig. 3), corresponding to the usual intracellular pH range. It is to be noted that the pH at the active sites of the Δ12-desaturase system in the

TABLE 2
Long-Chain Fatty Acid Composition of Microsomes of *Lipomyces starkeyi* CBS 1807[a]

Fatty acid	Percentage of total fatty acids[b]
$C_{14:0}$	1.3 ± 0.21
$C_{15:0}$	0.2 ± 0.02
$C_{16:0}$	27.0 ± 2.5
$C_{16:1}$ (*c*9)	2.3 ± 0.35
$C_{17:0}$	0.2 ± 0.03
$C_{17:1}$ (*c*10)	0.2 ± 0.03
$C_{18:0}$	5.0 ± 0.39
$C_{18:1}$ (*c*9)	35.0 ± 0.74
$C_{18:2}$ (*c*9, *c*12)	22.1 ± 0.34
$\alpha C_{18:3}$ (*c*9, *c*12, *c*15)	1.4 ± 0.25

[a]Centraal Bureau voor Schimmelcultures (Delft, The Netherlands).
[b]Values are given as mean ± SEM (n = 3).

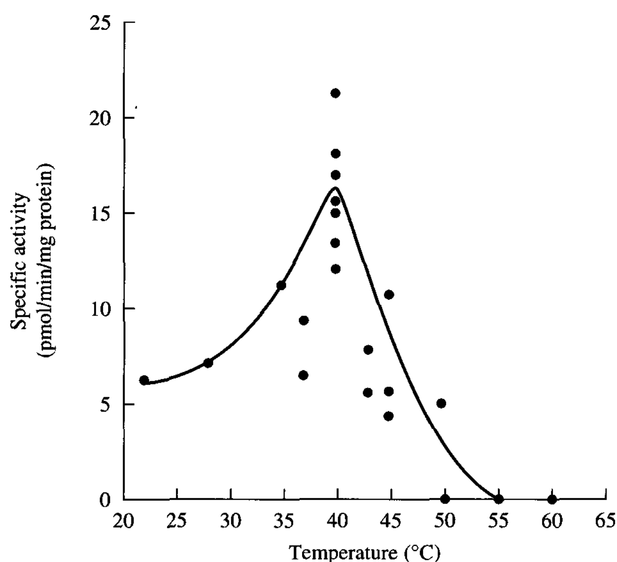

FIG. 1. Influence of temperature on the specific activity of the Δ12-desaturase system.

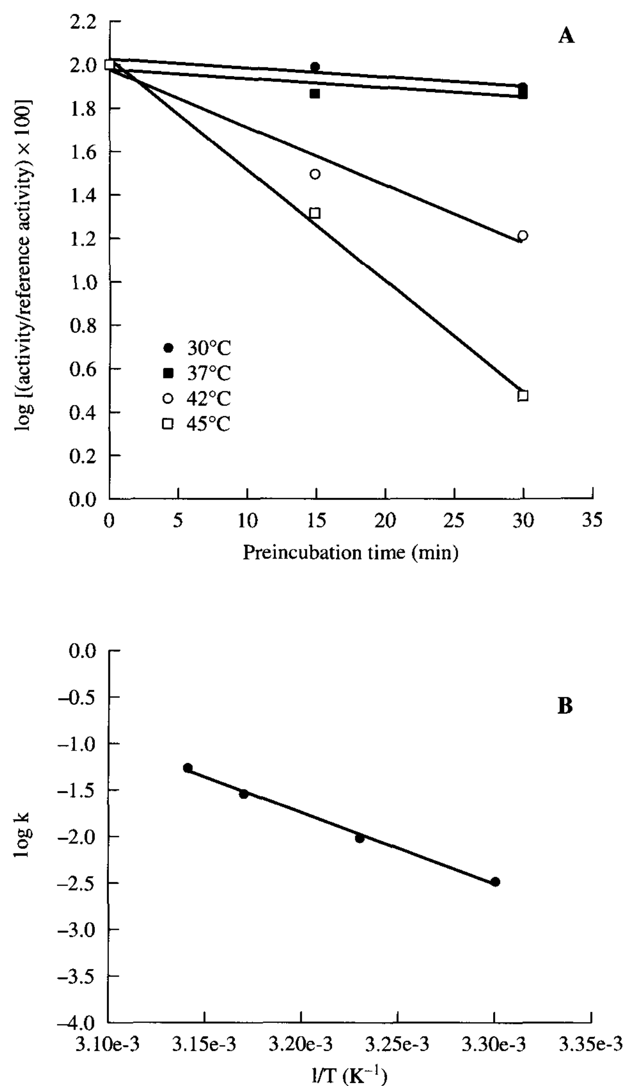

FIG. 2. (A) Thermal denaturation. Residual specific activity of the Δ12-desaturase system after 15 and 30 min of preincubation at different temperatures (30, 37, 42, and 45°C). The reference activity was measured in the standard reaction conditions (10 min at 40°C). (B) Thermal denaturation of the Δ12-desaturase system (Arrhenius plot).

microsomes may be different from the pH of the reaction medium.

Apparent K_m. By varying the concentration of labelled oleic acid in the reaction mixture, an apparent K_m value of 50 μM (±9, n = 3) was determined from the Lineweaver-Burk plot represented in Figure 4. It was in the same range as that found for *Y. lipolytica* (7).

Influence of effectors. Table 3 indicates the relative Δ12-desaturase specific activity (%) in the presence of different cations in the reaction mixture, compared to a reference realized in standard conditions. The activity was completely inhibited by Hg^{2+}. Among the other cations, Ca^{2+} presented the most important effect since it inhibited 80% of the activity. However, it is to be noted that this cation was employed in the protocol of Käppeli *et al.* (16) in order to precipitate the microsomal fraction, recovered by a rapid centrifugation at 15000 × *g*. It may thus mean that in the presence of Ca^{2+} in the reaction mixture, microsomes formed aggregates of lower specific activity. The fact that EDTA (Table 4), which is a metal chelating agent, inhibited 60% of activity indicated that a cationic ion is necessary to the functioning of the Δ12-desaturase system. Mg^{2+} is generally used in desaturase assays. In our case, the activity in the presence of 3 mM Mg^{2+} was 30% lower than in the standard conditions (5.5 mM). However, Zn^{2+}, and more especially Mn^{2+} had, at the concentration of 3 mM, a same or higher activating effect as 5.5 mM Mg^{2+}.

TABLE 3
Influence of Different Cations at 3 mM on the Activity of the Δ12-Desaturase System

Cations (chloride salts)	Relative Δ12-desaturase system activity[a] (%)
Mn^{2+}	123 ± 6
Zn^{2+}	106 ± 3
Hg^{2+}	0
Mg^{2+}	71 ± 2
Co^{2+}	59 ± 2
Cu^{2+}	97 ± 3
Ca^{2+}	19 ± 2

[a]This percentage was the ratio between the activity obtained in presence of each cation and the activity measured in the standard conditions (16 pmol/min/mg protein). Values are given as mean ± SEM (n = 3). Each cation was incubated 10 min with microsomes before adding oleic acid.

Table 4 gives the relative activity (%) of the Δ12-desaturase complex in the presence of effectors reacting with peculiar amino acid residues and of EDTA. An inhibitory effect was observed in the presence of *N*-ethylmaleimide (100%), iodine (90%), Woodward reagent K (i.e., *N*-ethyl-5-phenyl-

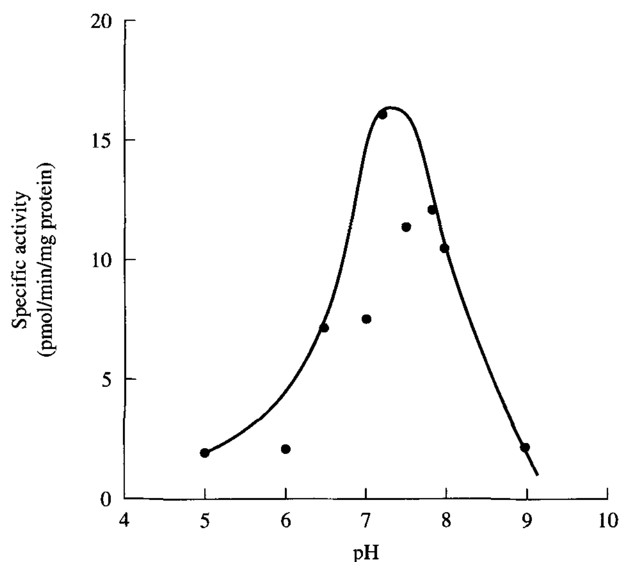

FIG. 3. Influence of pH on the specific activity of the Δ12-desaturase system.

isoxazolium 3′-sulfonate, 90%), *N*-bromosuccinimide, and iodoacetic acid (50–60%). Cystein (characterized by *N*-ethylmaleimide and iodoacetic acid), tyrosine (iodine and *N*-bromosuccinimide), aspartic and glutamic acids (the Woodward reagent K), and histidine (iodoacetic acid and *N*-bromosuccinimide) thus seem to be necessary to the reaction. It is to be noted that the inhibitory effect of iodine may also be due to a reaction with the double bonds of the unsaturated fatty acids involved in the reaction.

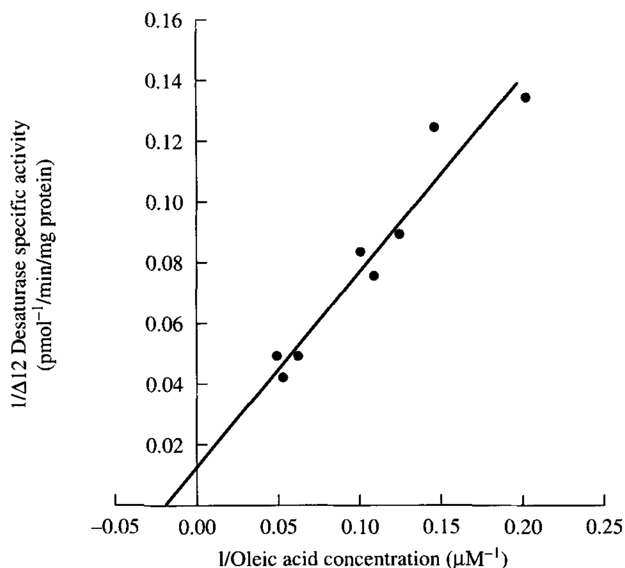

FIG. 4. Δ12-Desaturase system specific activity in standard reaction as a function of oleic acid concentration: double-reciprocal (Lineweaver-Burk) plot.

TABLE 4
Influence of Different Chemical Amino Reactive Reagents and of EDTA on the Activity of the Δ12-Desaturase System

Reagent (final concentration 3 mM)	Relative Δ12-desaturase system activity[a] (%)
2,4-Pentanedione	111 ± 3
N-bromosuccinimide	57 ± 5
Woodward reagent K[b]	13 ± 2
2,5-Dimethoxytetrahydrofurane	97 ± 4
Phenylmethylsulfonyl fluoride	86 ± 4
Iodoacetic acid	55 ± 4
N-ethylmaleimide	0
Trifluoroacetic anhydride	62 ± 4
2-Mercaptoethanol	75 ± 5
Iodine	10 ± 2
4-Dimethylaminobenzaldehyde	96 ± 4
EDTA	42 ± 1

[a]This percentage was the ratio between the activity obtained in presence of each reagent and the activity measured in the standard conditions (16 pmol/min/mg protein). Values are given as mean ± SEM (n = 3).
[b]Woodward reagent K: *N*-ethyl-5-phenylisoxazolium 3′-sulfonate. Each effector was incubated 2 min with microsomes before adding [^{14}C] oleic acid.

Effect of isomers of oleic acid and of oleoyl-CoA and elaidoyl-CoA. In our conditions of determination of the activity of the Δ12-desaturase system, at least two activities are involved: in a first step, acyl CoA synthetase catalyzes the formation of oleoyl-CoA from oleic acid and CoA, then oleoyl-CoA is desaturated into linoleoyl-CoA (we have verified that no desaturation occurred when CoA was absent in the reactant medium). In an attempt to evaluate the substrate specificity of the Δ12-desaturase system, the desaturation of oleic acid into linoleic acid was measured in the presence of equimolar quantities (0.02 mM) of labelled oleic acid and cold isomers of this fatty acid (Table 5). The Δ12-desaturase activity was about 25% inhibited in the presence of petroselinic, petroselaidic, and *cis*-vaccenic acids. Elaidic acid had a slightly higher inhibitory effect, whereas *trans*-vaccenic acid inhibited the reaction by only 10%. If the system had the same affinity toward these isomers as toward oleic acid, the activity would be 50% inhibited. There is thus at least one enzyme in the desaturase complex which presents a higher affinity toward oleic acid than its isomers. This enzyme may be the acyl-CoA synthetase, the Δ12 desaturase, or an intermediary enzyme, such as an oleoyl-CoA acyl transferase responsible for the formation of 1-acyl 2-oleoyl-*sn*-3-phospholipids as suggested by Ferrante *et al.* (7). It is to be noted that mammalian liver and *Bacillus* acyl-CoA synthetases are known to be very nonspecific (17).

In a second set of experiments, we have studied the influence of the addition of cold oleoyl-CoA or elaidoyl-CoA to the reaction mixture containing microsomes (105000 × *g* pellet) or the 105000 × *g* supernatant. Inhibition of the microsomal desaturase complex by 0.02 mM oleoyl-CoA and elaidoyl-CoA, which were supposed to compete with the [^{14}C] oleoyl-CoA produced by the acyl-CoA synthetase, was only 12 and 3%, respectively (Table 5). This was lower than expected, as cold oleoyl-CoA should have reduced the mea-

TABLE 5
Influence of the Addition of Different Isomers of Oleic Acid and of Oleoyl-CoA and Elaidoyl-CoA on the Activity of the Δ12-Desaturase System in the Presence of 0.02 mM [^{14}C] Oleic Acid

Fatty acids and acyl-CoA	Final concentration (mM)	Percentage of residual Δ12-desaturase system activity[a]
Cold oleic acid (*cis*-9)[b]	0.02	51 ± 4
Elaidic acid (*trans*-9)[b]	0.02	70 ± 4
Petroselenic acid (*cis*-6)[b]	0.02	78 ± 6
Petroselaidic acid (*trans*-6)[b]	0.02	76 ± 5
cis-Vaccenic acid (*cis*-11)[b]	0.02	77 ± 2
trans-Vaccenic acid (*trans*-11)[b]	0.02	88 ± 3
Oleoyl-CoA[b]	0.02	88 ± 1
Elaidoyl-CoA[b]	0.02	97 ± 1
Oleoyl-CoA[b]	0.20	0
Elaidoyl-CoA[b]	0.20	0
Oleoyl-CoA[c]	0.02	45 ± 4
Elaidoyl-CoA[c]	0.02	51 ± 2

[a]This percentage was the ratio between the activity obtained in presence of each isomer of oleic acid or of each molecule of acyl-CoA and the activity measured in the standard conditions. Values are given as mean ± SEM (n = 3).
[b]Reactions were realized with microsomes (105000 × *g* pellet).
[c]Reactions were realized with the 105000 × *g* supernatant.

sured activity by at least 50% by simple dilution of the [^{14}C] oleoyl-CoA. This indicates that the concentration of exogenous oleoyl-CoA at the active site of the desaturase complex was about ten times lower than that of the [^{14}C] oleoyl-CoA produced from the 0.02 mM [^{14}C] oleic acid. The access of exogenous acyl-CoA to the desaturase complex, probably located at the inner side of the microsomal membrane, was thus a limiting step, certainly due to the hydrophilic properties of acyl-CoA. When oleoyl-CoA and elaidoyl-CoA were added at a higher concentration (0.2 mM), a full inhibition of the measured activity was observed. In this case, the inhibition was much stronger than expected from the results obtained with 0.02 mM: if the concentration of exogenous acyl-CoA were 10-fold higher at the active site of the Δ12-desaturase, the measured activity should have been at least 50% of the reference activity. Two hypotheses could explain the 100% inhibition that was observed: (i) an inhibition of acyl-CoA synthetase by acyl-CoA, which would reduce the amount of radioactive acyl-CoA produced from [^{14}C] oleic acid; and (ii) the presence of a transport system for acyl-CoA functioning for acyl-CoA concentrations higher than 0.02 mM.

In the absence of integral microsomes, in the 105000 × *g* supernatant, the inhibition by 0.02 mM acyl-CoA was about 50%. This indicates that the access of acyl-CoA to the desaturation system is probably more direct in the supernatant fraction, which is consistent with the hypothesis that the supernatant contains finely dispersed fragments of endoplasmic reticulum (too small to form microsomes), or a solubilized form of the desaturation system: in both cases, the limiting step of the susbstrate penetration in or through the microsomal membrane is absent. This faster access to the enzymatic system also applies to oleic acid, which could partly explain the high desaturase activity of the supernatant compared to microsomes.

The influence of the concentration of exogenous oleoyl-CoA or elaidoyl-CoA on the activity of desaturation of [^{14}C] oleic acid is shown on Figure 5. The experimental data concerning oleoyl-CoA were in quite good agreement with the model according to which the exogenous oleoyl-CoA simply dilutes the [^{14}C] oleoyl-CoA produced from [^{14}C] oleic acid by the acyl-CoA synthetase. The data concerning elaidyl-CoA were also in agreement with the dilution model, but the activities were always higher than in the presence of oleoyl-

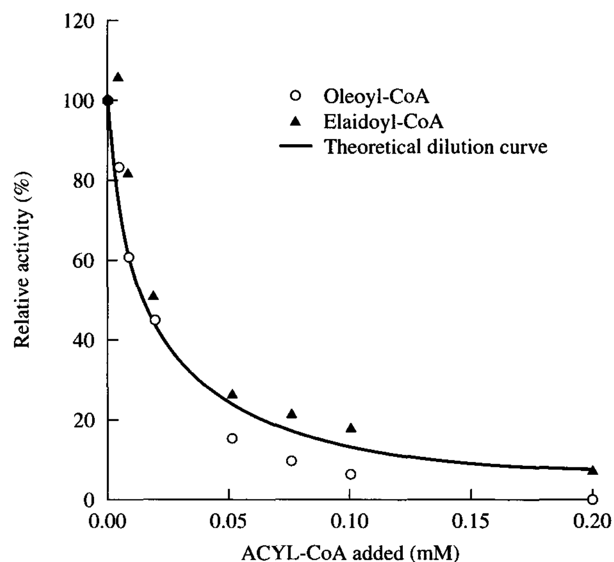

FIG. 5. Influence of the addition of cold oleoyl-CoA (○) or elaidoyl-CoA (▲) on the activity of desaturation of 0.02 mM [^{14}C] oleic acid. The dotted line represents the theoretical reduction of this activity by dilution of the intermediate [^{14}C] oleoyl-CoA with the cold oleoyl-CoA added.

CoA. This probably reflected a higher affinity of the Δ12 desaturase, or of an intermediary enzyme such as an oleoyl-CoA acyltransferase, for oleoyl-CoA than for its *trans* isomer. The study of the substrate specificity of the Δ12 desaturase system is difficult due to the involvement of numerous enzymes. However, the use of the 105000 × *g* supernatant seems to facilitate this study.

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[Received September 12, 1995; Revision accepted December 27, 1995]