Fat Metabolism in Higher Plants

XLVIII. PROPERTIES OF OLEYL COENZYME A DESATURASE OF CARTHAMUS TINCTORIUS

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SUMMARY

A membrane-bound oleyl coenzyme A desaturase from maturing Carthamus tinctorius seeds specifically catalyzed the conversion of oleyl-CoA to linoleyl-CoA. Any modification of the substrate, that is, a trans double bond, a shift in the position of the cis double bond, chain length, and the substitution of acyl carrier protein for CoA as the thioester moiety resulted in complete loss of activity. The system was strongly inhibited by a number of -SH reagents and metal chelators; carbon monoxide at various concentrations was ineffective; cyanide partially inhibited. Although DPNH and molecular oxygen were components of the system, DPNH could be replaced by photochemically reduced ferredoxin. This result would suggest that DPNH did not interact directly with molecular oxygen and the desaturase but served only as a source of electrons. Attempts at solubilization were unsuccessful.

Linoleic acid (cis-9, cis-12-octadecadienoic acid) constitutes more than 75% of the total fatty acids of oil rich seeds of Carthamus tinctorius L (safflower) (1). Oleyl-CoA desaturase, the enzyme responsible for linoleate formation, manifests maximum activity in safflower seeds 14 to 18 days after flowering. Preliminary work of McMahon and Stumpf (2) described a cell-free system from maturing safflower seeds that catalyzed the conversion of oleyl-CoA to linoleyl-CoA. Any modification of the substrate, that is, a trans double bond, a shift in the position of the cis double bond, chain length, and the substitution of acyl carrier protein for CoA as the thioester moiety resulted in complete loss of activity. The system was strongly inhibited by a number of -SH reagents and metal chelators; carbon monoxide at various concentrations was ineffective; cyanide partially inhibited. Although DPNH and molecular oxygen were components of the system, DPNH could be replaced by photochemically reduced ferredoxin. This result would suggest that DPNH did not interact directly with molecular oxygen and the desaturase but served only as a source of electrons. Attempts at solubilization were unsuccessful.

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EXPERIMENTAL PROCEDURE

Materials—Palmitic, palmitoleic, stearic, oleic, elaidic, cis-vaccenic, linoleic, and linolenic acids were obtained from Sigma; methyl esters of fatty acids were the products of Applied Science Laboratories, State College, Pennsylvania. [1-14C]Palmitic acid (35 μCi per pmole), [1-14C]palmitoleic acid (272 μCi per pmole), [1-14C]stearic acid (35.5 μCi per pmole), [1-14C]oleic acid (53.5 μCi per pmole), [1-14C]elaidic acid (62 μCi per pmole), and [1-14C]-linoleic acid (57 μCi per pmole) were obtained from Amersham-Searle, Arlington Heights, Illinois. cis-[14C]Vaccenic acid (labeled on odd carbons; approximately 60 μCi per pmole) was synthesized by an Escherichia coli fatty acid synthetase system (6) with [1,3-3H]malonyl-CoA (7.5 μCi per pmole) prepared by the method of Trans and Brady (7) as modified by Kusaka and Goldman (8). Radioactive acyl thiosteres of CoA (henceforth termed as thioester substrates including [1-14C]palmitoyl-CoA, [3-14C]palmitoleoyl-CoA, [1-14C]stearoyl-CoA, [1-14C]oleoyl-CoA, [1-14C]elaidoyl-CoA, cis-[odd-14C]vaccenyl-CoA and [1-14C]linoleyl-CoA, specific activity 5 μCi per pmole) were prepared by the enzymatic procedure of Galliard and Stumpf (9). Purity and characterization of these products were established by criteria described earlier (3). [1-14C]Stearyl-acyl carrier protein (ACP) and [1-14C]oleyl-ACP (both of specific activity 5 μCi per pmole) were prepared essentially by the method of Birge, Silbert, and Vagelos (10) with the following modifications: Dithiothreitol was used to reduce ACP, 1.0 at Tris-HCl buffer, pH 8.0, was used during acetylation, and a 20-fold molar excess of acetate anhydride was used. These acyl-ACPs had more than 40% thioester as determined by Barron and Mooney analysis (11), the remainder being nonthioester linkage of the acyl group to the protein. All calculations were corrected accordingly. All other chemicals were from commercial sources. ACP used for synthesizing acyl-ACPs was purified from E. coli B by the method of Sauer et al. (12).

Spinach ferredoxin and ferredoxin-TPN reductase were purified by the method of Boger, Black, and San Pietro (13). Ferredoxin was assayed by the method of San Pietro (14) and ferredoxin-TPN reductase activity was determined using the diaphorase assay of Jagendorff (15). Antibody to spinach ferredoxin (anti-spinach ferredoxin) was kindly provided by Dr. C. G. Kannangara of this laboratory. High purity spinach plastocyanin and partially purified Euglena cytochrome f was obtained by the method of San Pietro, Indiana University, Bloomington, Indiana. Preparation of Cell Fractions—Safflower seeds, 14 to 18 days after flowering, were obtained from University fields or from

1 By [odd-14C] is meant 14C labeled in carbons 1, 3, 5, etc. of the fatty acid hydrocarbon chain.
greenhouses. Subcellular fractions were prepared as described earlier (5) and designated as follows: 500 × g supernatant, cell-free extract; 12,000 × g pellet, mitochondrial pellet; 105,000 × g pellet, microsomal pellet; and 105,000 × g supernatant, soluble fraction. When organelle integrity was desired for subcellular localization of the desaturase, 0.1 M Tricine-HCl buffer, pH 7.2, containing 0.25 M sucrose was used. Unless otherwise stated, the microsomal fraction was employed throughout this study. As reported earlier (5), the safflower desaturase was highly unstable and fresh microsomes were employed each time.

Sucrose Gradient Centrifugation—The mitochondrial pellet was suspended in 20% (w/w) sucrose made up in 0.1 M potassium phosphate buffer, pH 7.2. Portions of this crude particulate suspension (15 to 20 mg of protein) were then layered on linear sucrose gradient prepared from 12 ml of 20% (w/w) sucrose and 12 ml of 60% (w/w) sucrose also made up in 0.1 M potassium phosphate buffer, pH 7.2, in Spinco rotor SW 25/1 cellulose nitrate tubes. Gradients were centrifuged at 25,000 rpm for 7 hours and subsequently fractionated into 1-ml fractions. Fractions were maintained at 0-5°C and assayed as quickly as possible.

Enzyme Assays—Standard oleyl-CoA desaturase assays were conducted as reported earlier (5). For marker enzyme studies, fumarase was assayed according to the method of Racker (16) and succinic dehydrogenase by the method of Pennington (17) as modified by Proteus and Clark (18). Glucose 6-phosphatase activity was determined according to the method of Huber and West (19) and the phosphate released was measured by the method of Fiske and Subbarow (20). TPNH-cytochrome c reductase was assayed according to the method of Masters, Williams, and Kamin (21) with 1.0 mM cyanide and 1.0 mM azide included to inhibit TPNH oxidase activity. Both the fumarase and TPNH-cytochrome c reductase activities in sucrose gradient were measured with the Cary model 14 dual beam recording spectrophotometer.

Desaturase Reactions Coupled to Photochemically Reduced Ferredoxin—To gain some insight into the electron transport process of the desaturase reaction, the possibility of ferredoxin, plastocyanin, and cytochrome f as possible electron donor-carriers was explored. Assays were conducted in which DPNH was omitted and replaced by photochemically reduced ferredoxin in a manner analogous to that described by Yoch and Arnon (22) for the nitrogen fixation system of photosynthetic bacteria. Each reaction vessel contained, in addition to [1-14C]oleyl-CoA and the buffer, spinach ferredoxin, 50 μg; washed spinach chloroplast fragments (equivalent to 500 μg of chlorophyll); sodium ascorbate, 10 μmoles; 2,6-dichlorophenoldinophenol, 0.05 μmoles. Incubations were done for 30 min at 30°C in light from an incandescent source (intensity approximately 50 × 10^3 lux). Added in combination with ferredoxin were 200 μg of either spinach plastocyanin or Euglena cytochrome 552, analogous to higher plant cytochrome f. Experiments were also carried out in which anti-spinach ferredoxin was added to the standard desaturase assay. Chloroplasts were prepared by the method of Arnon, Allen and Whatley (23) and chlorophyll was determined by Arnon's method (24).

Protein determinations were done by the Lowry procedure (25) and total iron was determined by atomic absorption spectroscopy. A number of treatments were tested for their ability to solubilize the desaturase preparatory to its purification. Such treatments included extensive washing of the microsomes, sonication, freeze-thaw and sonication, high salt and EDTA, detergents Triton X-100, Brij-35, Lubrol WX, sodium cholate and sodium deoxycholate, and phospholipases A, C, and D. Solubilization attempts were also done on an acetone powder of the microsomes. However, attempts to solubilize the desaturase were unsuccessful.

RESULTS

A careful kinetic analysis of the oleyl-CoA desaturase system presented a number of difficulties. In the first place, preliminary experiments established that the oleyl-CoA desaturase from safflower is a particulate enzyme. Secondly, there was a high reduced pyridine nucleotide oxidase activity in safflower particulate preparations. Thirdly, oleyl-CoA was likely to be in a micellar form, since the critical micelle concentration of long chain acyl-CoAs was about 4 μM (26). Fourthly, kinetic studies of the oleyl-CoA desaturase reaction were further complicated by the presence of an acyltransferase in safflower microsomes (5). The optimal conditions for the standard desaturase assay were therefore chosen to obtain a high amount of linoleate for

![Fig. 1. Time course of oleyl-CoA desaturase reaction. The assay system was 5 μmoles of [1-14C]oleyl-CoA; 1 μM of DPNH; 100 μM potassium phosphate buffer, pH 7.2; 1 mg of microsomal protein in a total volume of 1 ml. Incubation was 30°C with air as the gas phase.](http://www.jbc.org/)

![Fig. 2. Linoleate formation in the oleyl-CoA desaturase reaction as a function of safflower microsomal protein. Incubation time was 30 min as in the standard assay (see legend of Fig. 1).](http://www.jbc.org/)
Fig. 1 shows the time course of the desaturase reaction and Fig. 2 the effect of increasing amounts of microsomal protein on the extent of desaturation. On the basis of these results, an arbitrary 30 min time of incubation and 1 mg of microsomal protein were chosen for the standard and all other assays performed with the oleyl-CoA desaturase.

**Localization**—Data presented in Table I show that the microsomal pellet had the highest desaturase activity among the subcellular fractions analyzed. Although there was no activity in the supernatant fraction, the mitochondrial pellet showed considerable desaturase activity. When these subcellular fractions were analyzed for mitochondrial and microsomal marker enzymes (Table II), it was apparent that the microsomal pellet did not have a heavy mitochondrial contamination. On the other hand, the mitochondrial pellet had approximately 25% of the TPNH-cytochrome c reductase activity of the microsomal pellet. Since glucose 6-phosphatase, which is often used as a microsomal marker for microsomal fractions derived from animal tissues, had the least activity in the microsomal pellet, this enzyme appeared to be a poor marker enzyme for plant microsomal fractions.

**Table I**

*Oleyl-CoA desaturase activity in various subcellular fractions*

Experimental conditions and methods were as described in the text and in legend of Fig. 1.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>[1-14C]Linoleate</th>
<th>Conversion from [1-14C]oleyl-CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free extract</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>12,000 g pellet</td>
<td>2.2</td>
<td>44</td>
</tr>
<tr>
<td>12,000 g supernatant</td>
<td>1.5</td>
<td>30</td>
</tr>
<tr>
<td>Microsomal pellet</td>
<td>2.6</td>
<td>52</td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

The mitochondrial pellet was further examined by a 20% to 60% linear sucrose gradient (Fig. 3). In this gradient, the mitochondria banded quite sharply at a density of 1.17 as indicated by the marker enzymes, fumarase, and succinic dehydrogenase. However, neither TPNH-cytochrome c reductase nor glucose 6-phosphatase could be detected in any fraction. It is possible that these enzyme activities which were fairly low in the pellet itself (7.2 x 10^{-2} units per mg for TPNH-cytochrome c reductase and 2 μg inorganic phosphate released per mg of protein for glucose 6-phosphatase), had been diluted to a level too low for detection. The activity of oleyl-CoA desaturase did not give a well-defined band, but was distributed over a wide range in the gradient with a maximum around density of 1.14.

**Table II**

*Activities of marker enzymes in various subcellular fractions*

All data are based on 1 mg of protein. Oleyl-CoA desaturase activity is defined as nanomoles of linoleate produced during the standard assay; a unit of fumarase refers to an O.D. 240 nm increase of 0.001 per min during the assay (16); a unit of succinic dehydrogenase refers to nanomoles of succinate oxidized during the incubation (17, 18); a unit of TPNH-cytochrome c reductase is the amount of enzyme that causes an increase of 1.0 in O.D.550 during the assay (21); glucose 6-phosphatase activity refers to the micrograms of inorganic phosphate produced during the reaction (19).

![Fig. 3. Distribution of fumarase, succinic dehydrogenase, and oleyl-CoA desaturase after sucrose density gradient centrifugation of the 12,000 g pellet. Activities of TPNH-cytochrome c reductase and glucose 6-phosphatase were undetectable throughout the gradient and are not shown on the diagram.](http://www.jbc.org/)

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It appeared that the oleyl-CoA desaturase activity found in the mitochondrial pellet was localized either in an organelle that did not reach sedimentation equilibrium under these experimental conditions or was associated with a dispersed population of membrane fragments which sedimented out in the usual 105,000 \( \times g \) pellet. Since the desaturase appeared to be enriched in the fraction normally associated with microsomal fragments we have assigned the enzyme to this fraction with the realization that the term referred to a 105,000 \( \times g \) pellet.

**Cofactor Requirements**—Like other microsomal desaturase enzymes, safflower oleyl-CoA desaturase catalyzed an oxygen and reduced pyridine nucleotide dependent desaturation of oleyl-CoA to linoleate (Table III). The results depicted in Fig. 4 show that at all levels of reduced pyridine nucleotide, DPNH was found to be a better electron donor than TPNH. This is similar to the results obtained with microsomal stearyl-CoA desaturase from animal tissues (27-29). From the data obtained from Fig. 4, the apparent \( K_m \) of the desaturase for TPNH was 2.5 mM and for DPNH 0.18 mM.

**Inhibitor Studies**—In Table IV, data are given for the effect of a number of inhibitors (30). Relatively little inhibition was obtained with azide even after incubation of the enzyme with the inhibitor for 1 hour. An azide inhibitory effect has been observed for stearyl-CoA desaturase from rat liver microsomes.

**Table IV**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inhibition of linoleic acid formation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanide and azide</td>
<td></td>
</tr>
<tr>
<td>0.1 mM CN(^-)</td>
<td>19</td>
</tr>
<tr>
<td>1.0 mM CN(^-)</td>
<td>58</td>
</tr>
<tr>
<td>Preliminary incubation with 1.0 mM CN</td>
<td>94</td>
</tr>
<tr>
<td>0.1 mM Azide</td>
<td>19</td>
</tr>
<tr>
<td>1.0 mM Azide</td>
<td>10</td>
</tr>
<tr>
<td>Metal chelating agents</td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>8</td>
</tr>
<tr>
<td>( \nu )-Phenanthroline</td>
<td>32</td>
</tr>
<tr>
<td>( \alpha,\alpha' )-Dipyridyl</td>
<td>35</td>
</tr>
<tr>
<td>( p )-OH-quinoline</td>
<td>100</td>
</tr>
<tr>
<td>Sodium diethyldithiocarbamate</td>
<td>100</td>
</tr>
<tr>
<td>Thiol and serine group inhibitors</td>
<td></td>
</tr>
<tr>
<td>1.0 mM Iodoacetamide</td>
<td>20</td>
</tr>
<tr>
<td>0.5 mM ( p )-Chloromercuribenzoate</td>
<td>100</td>
</tr>
<tr>
<td>1.0 mM ( N )-Ethylmaleimide</td>
<td>100</td>
</tr>
<tr>
<td>0.5 mM 5,5'-Dithiobis-2-nitrobenzoate</td>
<td>100</td>
</tr>
<tr>
<td>1.0 mM Diisopropylfluorophosphate</td>
<td>0</td>
</tr>
</tbody>
</table>

![Fig. 4](http://www.jbc.org/)  
Effect of increasing concentrations of reduced pyridine nucleotides on the oleyl-CoA desaturase activity of microsomes. The desaturase activity was measured according to the standard desaturase assay except that DPNH (\( \bullet \)) and TPNH (\( \circ \)) were added at the indicated levels.
When the enzyme was previously incubated with 1 mM cyanide concentration and 58% inhibition at 1 mM concentration. It remains to be determined how much of this iron is part of the desaturase.

Interestingly, whereas p-chloromercuribenzoate, N-ethyl maleimide, and dithio-bis-2-nitrobenzoate (30) strongly inhibited the reaction, iodoacetamide did not give a significant inhibition (Table IV). It would appear that intact—SH groups are required for the desaturase reaction. Disopropylfluorophosphate, a reagent known to react with serine residues at the active site of a number of enzymes, failed to inhibit the system despite a preliminary incubation of the enzyme with this reagent.

The desaturase system was insensitive to a variety of mixtures and concentrations of carbon monoxide ranging from CO:O₂ (1:1:4) to CO₂:O₂ (4:1) in the gas phase of the reaction vessel. Therefore cytochrome P₉₉₉₉, a CO-binding hemeprotein which is a key component in many microsomal monooxygenase reactions (32-35), was not involved in the desaturase reaction. Carbon monoxide insensitivity has also been observed for monodesaturase enzymes from Euglena and rat liver (29, 30).

A number of inhibitors for flavin-linked dehydrogenases such as amytal (1 mM), atebrin (0.1 to 1 mM), and rotenone (0.002 to 0.1 mM) were added to the desaturation system and no significant inhibition was observed. Since these compounds are not general flavin-linked dehydrogenase inhibitors, no conclusions can be drawn pertaining to the role of flavoproteins on linking DPNH to the desaturase enzyme.

Reduction Ferredoxin-dependent Oleate Desaturation—The effect of photochemically reduced spinach ferredoxin as the electron donor in the desaturation reaction is shown in Fig. 5. Although reduced ferredoxin can clearly replace DPNH as a source of electrons, the data presented in Table V show that ferredoxin was only half as effective an electron donor as DPNH. The further addition of Euglena cytochrome 552, similar to higher plant cytochrome f, and plastocyanin did not increase the extent of ferredoxin-supported desaturation.

The important implication of the results summarized in Table V was that DPNH was not a direct obligatory reductant for the oxygen-dependent desaturation but can be replaced by another source of electrons, namely the photochemical system of spinach grana coupled to the safflower system by ferredoxin. Ferredoxin served as the link between the grana oxidation-reduction system and the safflower oxidation-reduction system. To support this conclusion, namely that ferredoxin serves as a link between the chloroplast reducing system and that of the safflower desaturase system, spinach anti-ferredoxin sera were added to the microsomal-chloroplast system. Table VI clearly shows that the antisera completely inhibits desaturation, presumably blocking the function of ferredoxin as the carrier system between chloroplasts and the desaturase. Since ferredoxin did not increase the rate of desaturation when added directly to the safflower system, it was not a limiting factor in the safflower system itself. Although ferredoxin is a highly soluble protein and can be readily leached out of particulate system, no ferredoxin requirement in the DPNH O₂ safflower microsomal system has been observed. We therefore conclude that while a tightly bound redox system is functioning in the safflower system, ferredoxin is probably not the functioning carrier.

(29). With cyanide, only 19% inhibition was observed at 0.1 mM concentration and 58% inhibition at 1 mM concentration. When the enzyme was previously incubated with 1 mM cyanide for 3 hour, there was no further increase in inhibition. Since a cyanide concentration of 1 mM can lead to nonspecific secondary reactions, the significance of partial inhibition with reference to a cyanide-sensitive heme system was not clear. While cyanide strongly inhibited the stearly-ACP desaturase from Euglena (31), the rat liver microsomal stearyl-CoA desaturase (29), the acyl-CoA desaturase from Torulopsis utilis, and a corresponding desaturase from Saccharomyces cerevisiae were cyanide resistant (31).

The effects of a number of metal ion chelators (30) are also given in Table IV. Since the desaturase was very sensitive to p-hydroxyquinoline and sodium diethylthiocarbamate, both causing 100% inhibition of the enzyme at 1 mM concentration, a divalent metal ion may be involved in the desaturase reaction. When analyzed for total iron, safflower microsomes were found to contain only about 2 × 10⁻¹⁷ moles of iron per mg of protein. The desaturase system was insensitive to a variety of mixtures and concentrations of carbon monoxide ranging from CO:O₂ (1:1:4) to CO₂:O₂ (4:1) in the gas phase of the reaction vessel.
Adding ferredoxin, ferredoxin-TPN reductase, Mg++, (20 mM), or sonicated microsomal lipids (0.5 mg) and TPNH. The phase or in the pellet or in a recombination of both, even after complete loss of enzyme activity towards the substrate.

The desaturase activity could not be obtained either in the soluble treated microsomes were centrifuged at 105,000 x g for 60 min, all the desaturase activity was re-

Solubilization of Desaturase—Concentrations of up to 2.5 mM KCl did not significantly inhibit the desaturase activity of the microsomes. When KCl-treated microsomes were centrifuged at 105,000 x g for 60 min, all the desaturase activity was recovered in the pellet. Extensive washing, freezing and thawing, and sonication inactivated the desaturase activity. Detergents described under “Experimental Procedures” at concentrations of up to 0.5% were inhibitory to the desaturase; when detergent-

**Table VI**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Linoleate formed (nmols)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard desaturation reaction system</td>
<td>2.0</td>
</tr>
<tr>
<td>Standard desaturation reaction system + anti-ferredoxin sera</td>
<td>2.1</td>
</tr>
<tr>
<td>Standard desaturation reaction system + rabbit serum protein</td>
<td>2.0</td>
</tr>
<tr>
<td>DPNH replacement in standard desaturation system by ferredoxin + chloroplast + DCIP + ascorbate (light)</td>
<td>1.1</td>
</tr>
<tr>
<td>DPNH replacement in standard desaturation system by ferredoxin + anti-ferredoxin sera + chloroplast + DCIP + ascorbate (light)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Substrate Specificity—The desaturase reaction showed remarkable specificity for the substrate (Table VII). Substrates tested had in common a chain length of either 16 or 18 carbon atoms, and differed only in the position, configuration, the degree of unsaturation or the nature of the thioester carrier molecule (CoA or ACP). The enzyme was capable of desaturating only oleyl-CoA; oleyl-ACP did not serve as a substrate. Therefore, the desaturase is highly specific for a C18 acyl-CoA with a cis-9,10 double bond. Any modification of this structure resulted in loss of enzyme activity towards the substrate.

**Table VII**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Unsaturated product formed (nmols)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleyl-CoA</td>
<td>2.2</td>
</tr>
<tr>
<td>Oleyl-ACP</td>
<td>0.0</td>
</tr>
<tr>
<td>cis-Vaccenyl CoA</td>
<td>0.0</td>
</tr>
<tr>
<td>Elaidyl-CoA</td>
<td>0.0</td>
</tr>
<tr>
<td>Palmitoleyl-CoA</td>
<td>0.0</td>
</tr>
<tr>
<td>Palmityl-CoA</td>
<td>0.0</td>
</tr>
<tr>
<td>Stearyl-CoA</td>
<td>0.0</td>
</tr>
<tr>
<td>Steareryl-ACP</td>
<td>0.0</td>
</tr>
<tr>
<td>Linoleyl-CoA</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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**Table VII**

**Substrate specificity of oleyl-CoA desaturase**

[14C]Acyl-thioester substrates (5 nmols) were used under the standard desaturation conditions.

Like desaturase enzymes from a number of tissues, oleyl-CoA desaturase from safflower is a particular enzyme. Sucrose gradient and marker enzyme studies would indicate that the enzyme is principally localized in a 105,000 x g pellet. A reduced pyridine nucleotide and oxygen are essential components for this enzyme. On the basis of partial fractionation and cofactor requirements, Nagai and Bloch (31) showed that for desaturation of stearyl-ACP in Euglena a rather complicated system involving a TPNH oxidase, ferredoxin, and a specific desaturase is involved. Oshino, Inami, and Sato (36) envision the electron transport in rat liver microsomal desaturation as involving a flow of electrons from either DPNH, ascorbate, or TPNH via either a DPNH-cytochrome b5 reductase or a TPNH-cytochrome b5 reductase through cytochrome b5 and then finally through a cyanide-sensitive factor to desaturase system in the presence of molecular oxygen. The precise mechanism is not known.

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