Enzymatic Desaturation of Stearyl Acyl Carrier Protein*

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SUMMARY

Stearyl acyl carrier protein, chemically prepared from 1-14C stearic acid and Escherichia coli acyl carrier protein, is desaturated to oleic acid by extracts of photoauxotrophic Euglena gracilis and of spinach. The soluble stearyl acyl carrier protein desaturase system from Euglena has been separated into three components, a reduced triphosphopyridine nucleotide oxidase, the desaturase, and a nonheme iron protein (ferredoxin). The enzyme requires reduced triphosphopyridine nucleotide and molecular oxygen. Desaturation is inhibited by KCN but is not affected by carbon monoxide.

Enzyme systems catalyzing the oxidative desaturation of stearyl- and palmityl-CoA to the corresponding monounsaturated fatty acids have been found in particulate fractions of yeast (1), liver (2-5), and Xanthobacterium phlei (6). According to Mudd and Stumpf (7) and James and Stumpf (8), the synthesis of oleic acid from acetate in extracts of avocado mesocarp and in lettuce chloroplasts is also an aerobic process. These plant extracts, however, do not convert free stearate or palmitate into the corresponding olefins.

Recently, Stumpf's laboratory (9-11) reported the presence of acyl carrier protein in plants and the dependence of plant fatty acid biosynthesis on acyl carrier molecules of the type originally found in Escherichia coli (12-15). Using various chemically synthesized fatty acyl derivatives of E. coli ACP,1 we have shown that crude extracts of photoauxotrophic Euglena gracilis and spinach chloroplasts elongate the ACP-thioesters effectively to long chain fatty acids. Moreover, on aerobic incubation in the presence of ferredoxin both systems produced oleic acid (16). These results prompted us to investigate the desaturation of stearyl-ACP as a possible biosynthetic route to oleate in plant systems.

As reported in preliminary communications (17, 18), chemically synthesized stearyl-ACP is desaturated effectively to oleic acid by soluble fractions of photoauxotrophic E. gracilis and by spinach chloroplasts. The soluble desaturase system from Euglena is separable into three protein components: a TPNH oxidase, a fraction with the properties of a ferredoxin, and the desaturase. All three proteins are required for the desaturation of stearyl-ACP to oleic acid.

MATERIALS AND METHODS

Stearic acid-1-14C (10 mCi per mmole), obtained from New England Nuclear, was freed of polar impurities by chromatography on silicic acid columns (12). Stearyl-ACP was synthesized by the mixed anhydride method as described previously (16). Incubation of mixed anhydride with ACP in buffered tetrahydrofuran solution for 2 to 5 min gave the best results as judged by the quantity of radioactivity released from the thioester by hydroxylamine. However, the proportion of thioester which could be enzymatically desaturated under optimal conditions varied from preparation to preparation and ranged from 10 to 40%. Stearyl-CoA was also prepared by the mixed anhydride method (19). Coenzyme A, TPN+, TPNH, and glucose 6-phosphate were purchased from Boehringer and Sohne, and yeast glucose 6-phosphate dehydrogenase was obtained from Sigma. Electrophoretically pure E. coli ACP isolated according to the method of Majerus, Alberts, and Vagelos (14) was kindly supplied by Dr. Philip Gold and Mrs. J. Gussin of this laboratory. Clostridium pasteurianum ferredoxin was purchased from Worthington. Adrenodoxin was a generous gift from Dr. Tokui Kimura, St. Paul's University, Tokyo, Japan.

Preparation of Crude Euglena Extract—About 10 ml of photoheterotrophically grown starter culture of E. gracilis Z were transferred to a mineral medium (20) and cells were grown photoauxotrophically as described earlier (16). The harvested cells were suspended in 5 volumes of 0.5 M sucrose solution containing 0.01 M phosphate buffer (pH 7.2), 0.002 M sodium acetate, 0.01 M NaCl, and 0.001 M EDTA, and were broken in either a French pressure cell at 3000 psi or by a 1-mm homogenization with glass beads (21). Intact cells and cell debris were removed by centrifugation at 110,000 x g and the resultant supernatant was used as a source of crude enzyme. Spinach chloroplasts to be used for desaturase assays were prepared in 0.5 M sucrose solution containing 0.01 M phosphate (pH 7.2) and 0.001 M sodium
The unsaturated products were isolated by way of the mercuric in dimethyl azelaate, the dicarboxylic acid derived from oleate. The radiolysis of this fraction, 87% of the radioactivity was found to consist predominantly of octadecanoic acid. On oxidative degradation of saturated fatty acids (16), also convert stearyl-ACP to oleate.

The crude enzyme system did not desaturate stearyl-CoA significantly. The results of these experiments are summarized in Table I.

**TABLE I**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Incubation time</th>
<th>Saturated fatty acids</th>
<th>Unsaturated fatty acids</th>
<th>Distribution of radioactivity in unsaturated fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearyl-ACP</td>
<td>0 min</td>
<td>15,000</td>
<td>62</td>
<td>≤C18</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>17,200</td>
<td>1,700</td>
<td>11</td>
</tr>
<tr>
<td>Stearyl-CoA</td>
<td>0 min</td>
<td>20,000</td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>18,300</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

**Fractionation of crude extract**

Conditions for incubation were the same as described in Table I, except that 0.2 μmole of substrate was used.

**RESULTS**

Desaturation of Stearyl-ACP by Crude Extracts

Crude extracts of photosynthetic Euglena, which had previously been shown to elongate ACP derivatives of intermediate chain length fatty acid to long chain saturated and unsaturated fatty acids (16), also convert stearyl-ACP to oleate. The unsaturated products were isolated by way of the mercuric acetate adducts and shown by gas-liquid chromatography to consist predominantly of octadecenoic acid. On oxidative degradation of this fraction, 87% of the radioactivity was found in dimethyl azelaate, the dicarboxylic acid derived from oleate. The crude enzyme system did not desaturate stearyl-CoA significantly. The results of these experiments are summarized in Table II.

**Preliminary Purification of Desaturase System**

After high speed centrifugation (100,000 × g for 90 min) of crude extract, the desaturase activity remained in the soluble fraction (Table II). Enzyme precipitated with ammonium sulfate (70% saturation) was passed through a Sephadex G-25 column. After these treatments the recovery of activity was about 130%, indicating removal of inhibitory substances. Inhibition was also noted when boiled soluble fraction was added to the Sephadex-treated enzyme preparation.

**Cofactor Requirements**

Like the acyl-CoA desaturases from yeast (1) and mammalian liver (2), partially purified stearyl-ACP-desaturating enzyme...
TABLE III
Requirements for desaturation of stearyl-ACP

<table>
<thead>
<tr>
<th>Experiment, enzymes, and additions</th>
<th>Desaturation (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0 to 75% (NH₄)₂SO₄ fraction, 0.9 mg of protein, TPNH</td>
<td>7.5</td>
</tr>
<tr>
<td>TPNH, boiled enzyme</td>
<td>0.2</td>
</tr>
<tr>
<td>DPNH</td>
<td>2.6</td>
</tr>
<tr>
<td>TPN⁺</td>
<td>0.3</td>
</tr>
<tr>
<td>TPNH, anaerobic*</td>
<td>0.2</td>
</tr>
<tr>
<td>TPNH, air²</td>
<td>5.9</td>
</tr>
<tr>
<td>20 to 45% (NH₄)₂SO₄, 0.45 mg of protein (A), TPNH</td>
<td>1.1</td>
</tr>
<tr>
<td>45 to 75% (NH₄)₂SO₄, 0.26 mg of protein (B), TPNH</td>
<td>1.3</td>
</tr>
<tr>
<td>A + B, 0.71 mg of protein, TPNH</td>
<td>9.9</td>
</tr>
<tr>
<td>2. A + B + TPNH</td>
<td>7.0</td>
</tr>
<tr>
<td>A + B + TPNH + ACP (1.36 μmoles)</td>
<td>5.9</td>
</tr>
<tr>
<td>A + B + TPNH + ACP (6.80 μmoles)</td>
<td>4.3</td>
</tr>
</tbody>
</table>

* Incubations were carried out in Thunberg tubes. Air was replaced by flushing three times with helium followed by evacuation.

² This experiment served as a control. After flushing with helium and evacuation, air was admitted and the tube was incubated for 10 min.

Enzymic Desaturation of Stearyl-ACP

The composition of the reaction mixture and the experimental conditions were the same as described in Table I, except that 0.05 μmole of substrate and 0.2 μmole of pyridine nucleotide instead of TPNH-generating system were used. The incubation time was 10 min.

Identification of Ferredoxin as Active Component in Fraction B

Enzyme Fraction B was chromatographed on a DEAE-cellulose column and assayed for desaturating activity by incubation with Fraction A. The activity was eluted with 0.3 to 0.38 M LiCl in a reddish brown fraction after the bulk of the protein and a pink protein (cytochrome?) had emerged (Fig. 1). The spectral properties of this fraction are those of a nonheme iron protein or ferredoxin. Since molecules of this type are known to participate in steroid hydroxylations (32, 33), the involvement of a ferredoxin in stearyl-ACP desaturation was explored. Eluate Fractions 500 to 700 (Fig. 1) were therefore examined for photosynthetic pyridine nucleotide reductase activity by incubation with spinach chloroplasts (22). The photosynthetic pyridine nucleotide reductase activity coincided closely with the material absorbing at 330 μm and with Fraction B activity for desaturation.

TPNH Oxidase and Desaturase as Components for Stearyl-ACP Desaturation

TPNH oxidase is known to catalyze electron transfer from TPNH to ferredoxin in plants (23). The presence of a ferredoxin in the stearyl-ACP desaturation system of Euglena therefore implicated a TPNH oxidase as a further component necessary for oleate formation. TPNH oxidation was observed when either FMN, FAD, or ferredoxin was added as an electron ac-
Supernatant of extracts from auxotrophic Euglena was chromatographed on a DEAE-cellulose column. TPNH oxidase activity was found in certain early effluent fractions. These TPNH oxidase-positive fractions had no desaturase activity when tested alone or in combination with ferredoxin fractions. Two fractions could therefore be used as components for assay ing stearyl-ACP desaturase activity in column eluates. In this manner desaturase activity was localized in fractions eluted by 0.1 M to 0.15 M LiCl from DEAE-cellulose (Fig. 2). The evidence obtained at this point indicated the need of three enzyme components for the desaturation of stearyl ACP (18). The further purification of these components is described below.

**TPNH Oxidase—**TPNH oxidase-positive fractions from a chromatogram of crude Euglena extracts on a DEAE-cellulose column were combined and the fraction precipitating between 45 and 65% ammonium sulfate was collected. The protein was dissolved and desalted on a Sephadex G-25 column. A mixture of TPNH oxidase (45 to 65% (NH$_4$)$_2$SO$_4$) fraction after the first DEAE-cellulose chromatography (430 mg of protein) and desaturase (0 to 60% (NH$_4$)$_2$SO$_4$) precipitate after DEAE-cellulose chromatography (230 mg of protein) in 15 ml was placed on a Sephadex G-25 column (2.6 X 76.7 cm) and eluted with sucrose-ascorbate solution. The flow rate was about 7 ml per hour. Desaturase (O—O) activity was measured as described in Fig. 2 and TPNH oxidase (●—●) activities were measured under standard assay conditions. Absorbance at 455 nm (—-) was a measure of flavoprotein. The void volume of the column was 144 ml.

**Desaturase**—Desaturase activity was localized in fractions eluted by 0.1 M to 0.15 M LiCl from DEAE-cellulose (Fig. 3). The TPNH oxidase thus obtained had a spectrum typical for a flavoprotein with absorption maxima between 380 and 400 and 440 and 460 nm. One milligram of the enzyme oxidized 20 mmoles of TPNH per min in the presence of Euglena ferredoxin, spinach ferredoxin (43 μg), Clostridium ferredoxin (5 μg), or FMN (1.8 mmoles). The enzyme also showed TPNH diaphorase activity (34) and ferredoxin-TPNH reductase activity (25). It did not oxidize DPNH in the presence of FMN.

**Ferredoxin**—A 10$^6$ x g supernatant fraction obtained from 300 g of cells and containing 8.3 g of protein in 830 ml was placed on a DEAE-cellulose column (4 x 33 cm) which had been equilibrated with 0.01 M phosphate buffer (pH 7.2) in 0.1 M sucrose (buffered sucrose solution). Protein was eluted with 2 liters of a linear gradient of LiCl (0 to 0.6 M) in buffered sucrose solution. The pooled ferredoxin fractions (500 ml) were diluted with the same volume of buffered sucrose solution. Ferredoxin was then adsorbed on another DEAE-cellulose column (30-ml bed volume) and eluted with 400 ml of a linear gradient of LiCl (0.2 to 0.6 M) in the same buffered sucrose solution. The main fractions containing 35.4 mg of ferredoxin in 100 ml were combined with another batch of 41.2 mg of ferredoxin partially purified in the same way from 310 g of cells. This combined ferredoxin preparation (A$_{280}$/A$_{270}$ = 0.14) was absorbed on a DEAE-cellulose column (10-ml bed volume) and eluted with 0.6 M LiCl in buffered sucrose solution. To 8 ml of the concentrated ferredoxin solution (0.74 mg of ferredoxin, A$_{280}$/A$_{270}$ = 0.22) ammonium sulfate was added to 60% saturation and the precipitate was removed by centrifugation. The ammonium sulfate concentration was raised to 80% saturation and then 0.2 ml of 5% acetic acid solution was added carefully to the cloudy solution. The precipitated ferredoxin was collected by centrifugation and dissolved in a minimum volume of 0.02 M phosphate buffer, pH 7.0, and the solution was desalted on a Sephadex G-25 column. The final preparation contained 41.4 mg of ferredoxin. It showed an absorption spectrum (Fig. 4) similar to those reported for the ferredoxins from spinach.
Enzymatic Desaturation of Stearyl-ACP

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300 400 500 600
WAVELENGTH (mp)

FIG. 4. Absorption spectrum of Euglena ferredoxin. The solution contained 0.57 mg of protein in 1 ml of Tris-HCl buffer, pH 7.4.

(35), parsley (25), and alfalfa (36) with peaks at 276, 320, 422, and 462 mp, and a ratio of 0.38 for the absorbances at 420 and 276 mp, respectively. The visible absorption disappeared when ferredoxin was chemically reduced by dithionite. The purest preparation of Euglena ferredoxin contained 83.4 mpm atoms of iron and 88.5 mpmoles of labile sulfur per mg of protein.2 Rapid reduction of Euglena ferredoxin by TPNH and TPNH oxidase occurred on anaerobic incubation in a Thunberg tube, although the extent of reduction was small, indicating an equilibrium in favor of TPN reduction. The reduced ferredoxin was reoxidized when air was admitted.

Desaturase—Fractions eluted from DEAE-cellulose containing desaturase activity were pooled. Protein was concentrated by precipitation with (NH₄)₂SO₄ (0 to 60 %) and then placed on a column of Sephadex G-200 (2.6 x 76.6 cm) which had been equilibrated with sucrose-ascorbate solution. Desaturase activity emerged from the column as a single peak with an elution volume 1.78 times the void volume (V/V₀ = 1.78). On the same column the TPNH oxidase had V/V₀ = 2.15 (Fig. 3). The desaturase fractions showed no significant absorption in the visible range.

Further purification of the enzyme was attempted as follows. Following DEAE-cellulose chromatography, ammonium sulfate precipitation and desalting, 276 mg of desaturase in 50 ml were placed on a second DEAE-cellulose column (33 ml bed volume). Protein was eluted with 600 ml of a linear gradient of LiCl (0 to 0.4 M) in sucrose-ascorbate solution. The specific activity of the desaturase was not increased more than 2-fold by this step presumably because of the unstability of the enzyme. The purified desaturase decays with a half-life of less than 10 hours at 0°. In frozen crude extracts desaturase activity is stable for periods of at least 1 month.

Properties of Desaturase System

Ferredoxin Dependence—Fig. 5 shows the dependence of stearyl-ACP desaturation on Euglena ferredoxin. Adrenodoxin, a nonheme iron protein required for the hydroxylation of steroids in mammalian adrenal mitochondria and known to have a much more positive oxidation-reduction potential than plant ferredoxins (32), cannot replace Euglena ferredoxin for stearyl-ACP desaturation. In the presence of Euglena ferredoxin, the addition of adrenodoxin did not affect desaturation.

Adrenodoxin specificity—In the Euglena stearyl-ACP-desaturating system, Euglena ferredoxin is nearly 10 times as active as spinach ferredoxin. Surprisingly, stearyl-ACP desaturation by spinach chloroplasts is also more effectively supported by Euglena ferredoxin than by spinach ferredoxin (Fig. 6). For comparing the nonheme iron proteins from the two different sources, it was assumed that the absorbance at 463 mp is a true measure of ferredoxin concentration. On this basis, equal concentrations of Euglena and spinach ferredoxin showed the same activity as electron acceptors in TPNH oxidation by Euglena TPNH oxidase and as electron donors in TPN reduction by illuminated spinach chloroplasts.

Clostridium ferredoxin was much less active than other nonheme iron proteins in the Euglena-desaturating system. The bacterial ferredoxin is, however, a very efficient electron acceptor for TPNH oxidation. These results are summarized in Fig. 6.

Effect of Chemical Reduction of Ferredoxin—Ferredoxin can be chemically reduced by dithionite or formamidine sulfonic acid (38). According to some reports these reductants can serve as electron donors for certain ferredoxin-requiring enzyme reactions (29). However, in the stearyl-ACP-desaturating system of Euglena, formamidine sulfonic acid could not replace TPNH (Table IV). Low concentrations of dithionite (10⁻⁵ M) par-

![Graph](http://www.jbc.org/)

FIG. 5. Effect of nonheme iron proteins on desaturation. Incubation mixtures contained 50 μmoles of triethanolamine-HCl (pH 7.2), 0.2 μmole of TPNH, 50 μg of desaturase (the most active fraction in second DEAE-cellulose chromatogram), 41 μg of purified TPNH oxidase, Euglena ferredoxin (●—●) or adrenodoxin (X—X—X), and 0.08 mpmole of stearyl-ACP. A zero time blank (1.6 μmoles) was subtracted. In R, incubation mixtures contained 20 μg of Euglena ferredoxin in addition to varying amounts of adrenodoxin.
ially replaced TPNH in the ferredoxin-dependent stearyl-ACP desaturation by spinach chloroplasts.

**Pyridine Nucleotide Specificity**—The purified Euglena enzyme system for stearyl-ACP desaturation requires TPNH but is not active with DPNH. In crude extracts either of the pyridine nucleotides serves as a reductant (Table III). In the rat liver microsome system, high concentrations of ascorbate can replace the requirement for reduced pyridine nucleotide in acyl-CoA desaturation (5). In the purified Euglena system ascorbate was inactive as a reductant even when its concentration was 50 times that of TPNH.

**Substrate Specificity**—Crude photoautotrophic Euglena extracts desaturate stearyl-ACP much more effectively than stearyl-CoA. However, in the purified reconstituted enzyme system stearyl-CoA was desaturated as rapidly as stearyl-ACP (Fig. 7). We have no explanation for this phenomenon. We have previously reported that crude extracts from photoautotrophic Euglena fail to desaturate palmitoyl- or myristyl-ACP (17) and that in spinach chloroplasts the desaturation system is specific for stearyl-ACP.

**Effect of CO and KCN**—Stearyl-ACP desaturation by Euglena enzyme was not affected by CO when samples were incubated in an atmosphere containing equal volumes of CO and air, nor was inhibition observed when a stream of CO was passed through the enzyme solution (Table IV). KCN, on the other hand, inhibits the desaturation reaction (50% at 10^{-3} M and 80% at 10^{-4} M). An inhibitory effect of KCN on acyl-CoA desaturation has also been observed in the aerobic yeast *Torulopsis utilis* and in rat liver microsomes (5), whereas the corresponding system from *Saccharomyces cerevisiae* is resistant to cyanide (1).

The CO insensitivity of the desaturase system indicates that P-450, the CO-binding hemoprotein participating in some hydroxylation reactions in liver microsomes (33), is not involved in the stearyl-ACP desaturation reaction. The same finding has been recently reported for acyl-CoA desaturation by liver microsomes (5).

**Desaturation in Heterotrophic Euglena gracilis**—As reported previously, crude extracts prepared from dark-grown Euglena desaturate acyl-CoA derivatives but not stearyl-ACP (17). Data presented in Table V show that stearyl-CoA desaturation in etiolated cells requires a component sedimentable at 1 × 10^{6} g and also a supernatant fraction. This contrast with the soluble nature of the three components that comprise the stearyl-ACP desaturase system of photoautotrophic cells. The previously described stearyl-CoA desaturases from other sources (1-6) are

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**Fig. 6** Comparison of ferredoxins in desaturation, TPNH oxidation, and photoreduction of TPN. A, desaturation of stearyl-ACP by 0.2 ml of spinach chloroplast solution (30 mg of chlorophyll per ml) in the presence of Euglena TPNH oxidase (0.1 mg of protein) and ferredoxin. B, desaturation of stearyl-ACP by 0.2 ml of Euglena desaturase fraction (DEAE-cellulose chromatography, 0.24 mg of protein) and ferredoxins. C, TPNH oxidation by purified Euglena TPNH oxidase preparation. D, photosynthetic pyridine nucleotide reductase (TPNR) activity of ferredoxins in spinach chloroplasts. Spinach ferredoxin was purified by repeated DEAE-cellulose column chromatography and ammonium sulfate precipitation (37). The preparation had an A_{422}/A_{260} ratio of 0.44. •—•, Euglena ferredoxin; O—O, spinach ferredoxin; —X—X, Clostridium ferredoxin.

**Table IV**

<table>
<thead>
<tr>
<th>Enzyme protein</th>
<th>TPNH</th>
<th>Formamidine sulfonic acid</th>
<th>CO</th>
<th>Desaturation (µmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>µmol</td>
<td>µmoles</td>
<td></td>
<td>µmoles</td>
</tr>
<tr>
<td>0.2</td>
<td>0.2</td>
<td>0</td>
<td>−</td>
<td>7.3</td>
</tr>
<tr>
<td>0.4</td>
<td>0.2</td>
<td>0</td>
<td>−</td>
<td>14.4</td>
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<tr>
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<td>0</td>
<td>0.5</td>
<td>−</td>
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</tr>
<tr>
<td>0.4</td>
<td>0</td>
<td>2.5</td>
<td>−</td>
<td>1.1</td>
</tr>
<tr>
<td>0.4*</td>
<td>0.2</td>
<td>0</td>
<td>−</td>
<td>15.0</td>
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<td>0.4*</td>
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<td>0</td>
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<td>12.5</td>
</tr>
<tr>
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<td>0</td>
<td>+</td>
<td>12.1</td>
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<tr>
<td>0.4</td>
<td>0.2</td>
<td>0</td>
<td>−</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Enzyme solution was flushed with CO for 1 min.

* Dr. D. Bishop, unpublished experiment.
fatty acid biosynthesis have been isolated from plants as well as acyl-CoA derivatives (42), and in turn, in this organism palmityl-

yeast. The multienzyme complex of yeast produces long chain

In extracts of heterotrophic Euglena cultured under various conditions

<table>
<thead>
<tr>
<th>Medium</th>
<th>Culture</th>
<th>Enzyme</th>
<th>Desaturation (mhos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthetic</td>
<td>Light</td>
<td>Crude, 0.3 ml</td>
<td>0.01</td>
</tr>
<tr>
<td>Photosynthetic</td>
<td>Light</td>
<td>Crude, 0.3 ml</td>
<td>0.02</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>Light</td>
<td>Crude, 0.36 ml</td>
<td>2.85</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>Light</td>
<td>1 X 10^4 g supernatant, 0.36 ml</td>
<td>2.88</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>Dark</td>
<td>1 X 10^4 g supernatant, 0.3 ml</td>
<td>1.75</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>Dark</td>
<td>1 X 10^4 g precipitate, 0.16 ml</td>
<td>0.19</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>Dark</td>
<td>1 X 10^4 g supernatant plus precipitate, 0.46 ml</td>
<td>0.28</td>
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</table>

also particulate in nature but they do not require supernatant enzymes. It is noteworthy that stearyl-CoA desaturation occurs in extracts of heterotrophic Euglena cells whether they are grown in the light or in the dark. Cell-free extracts of Euglena grown heterotrophically in the light desaturate both stearyl-ACP and stearyl-CoA.

Stearyl-ACP is not a substrate for the particulate stearyl-CoA desaturase from T. utila.

DISCUSSION

Low molecular weight proteins functioning as acyl carriers in fatty acid biosynthesis have been isolated from plants as well as from bacteria (11-15). Enzyme systems from these two sources catalyze the elongation of acetyl-ACP and also of intermediate length acyl-ACP derivatives (14-16) to long chain fatty acids which remain attached to ACP. There is now good evidence that these products can be utilized for other reactions of lipid metabolism. Thus, palmitoyl-ACP can serve as substrate for phospholipid synthesis in Clostridium butyricum (40) and in E. coli (41). Apparently the thioester linkages existing during chain elongation are preserved so that subsequent reactions, e.g. glyceride formation, can proceed without further energy input. The same coupling of chain elongation to a subsequent metabolic step seems to occur in photoautotrophic Euglena. Stearyl-ACP, the product of the ACP-dependent elongation reactions in the algal system, is also a substrate for the desaturase that forms oleate. The "ACP specificity" of the bacterial and plant enzymes may be contrasted with the "CoA specificity" of lipid metabolism in yeast. The multienzyme complex of yeast produces long chain acyl CoA derivatives (12), and in turn, in this organism palmitoyl- and stearyl-CoA are the substrates for the formation of oleic acids (1) and also for glyceride synthesis (43). In yeast, ACP derivatives do not appear to be utilized for any reactions of lipid metabolism.4

Of particular interest from the comparative point of view are the properties of etiolated Euglena cells. Unlike photoauxotrophic Euglena, the bleached phytoflagellate contains a particulate acyl-CoA desaturase and also a multienzyme fatty acid synthetase complex with properties that closely resemble the corresponding yeast enzymes.5 This unicellular organism can therefore elaborate one or the other type of enzymes of lipid metabolism depending on physiological conditions.

Because of their exceptional solubility properties, the plant stearyl-ACP desaturases are especially suitable for studying the oxidative mechanism for olefin formation in detail. Earlier work with the related but particle-bound enzymes of yeast, liver, and M. phlei had established the requirement for O2 and TPNH by these systems, but there was only suggestive evidence that additional electron carriers (flavin coenzymes, Fe) are involved. The results obtained by fractionating Euglena extracts clearly establish the plant desaturase as a multicomponent system consisting of at least two electron-transferring proteins and the desaturating enzyme proper.

Several reports on the participation of nonheme iron proteins in oxygenase reactions have appeared. Adrenodoxin supports steroid hydroxylation by adrenal mitochondria (32), rubredoxin ω-oxidation of aliphatic hydrocarbons (44, 45), and putida redoxin methylene hydroxylation (46), the two latter reactions in Pseudomonas strains. In some of these systems the ferredoxins are interchangeable but in others they are not. Adrenodoxin does not replace rubredoxin in the stearyl-ACP-desaturating system and, conversely, Euglena ferredoxin is not active in steroid hydroxylation or cytochrome reduction by adrenodoxin reductase.4 In the ω-oxidation catalyzed by Pseudomonas enzymes, spinach ferredoxin partially replaces rubredoxin (44). The nonheme iron protein specificity in oxygenase reactions appears to depend not only on their oxidation-reduction potentials but on protein structure as well. Thus, in stearyl-ACP desaturation, whether in spinach or Euglena, Euglena ferredoxin is more active than spinach ferredoxin and greatly superior to Clostridium ferredoxin, although the oxidation-reduction potentials of the bacterial and plant nonheme iron proteins are very similar.

It may be pointed out that ferredoxins from various sources, in spite of their close structural resemblances, are functionally very diverse as electron carriers (47). In anaerobic bacteria, ferredoxins are involved in nitrogen fixation and pyruvate synthesis but obviously not in the activation of oxygen. The plant ferredoxins serve both as components of the photosynthetic electron transport chain and as components of oxygenase reactions. In animal tissues the only known function of ferredoxins seems to be concerned with steroid hydroxylations.

Since all of the Euglena enzymes participating in stearyl-ACP desaturation are soluble, this system offers a much better chance than the particulate desaturase systems for detecting the hypothetical oxygen-containing intermediates. However, in none of our experiments did we observe any polar transforma
tion products of stearyl-ACP, nor was chemically synthesized 9- or 10-hydroxy stearyl-ACP dehydrated to oleic acid by crude extracts from photoautotrophic Euglena cells which actively desaturate stearyl-ACP to oleate. The only observable reaction of hydroxy-ACP is a dehydrogenation to ketosearic acid, a transformation which is unrelated to oleate synthesis (48).

4 Unpublished observations.

5 Dr. T. Kimura, personal communication.
These experiments provide the strongest evidence so far against the formation of oxygenated intermediates in fatty acid desaturation. Nevertheless, we do not regard this issue as settled. Further purification of the desaturating enzyme is needed to answer the question whether or not oxygen intervenes directly in the process by forming covalent derivatives with the fatty acid substrate.

REFERENCES

Enzymatic Desaturation of Stearyl Acyl Carrier Protein
J. Nagai and Konrad Bloch


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