Fat Metabolism in Higher Plants

LXII. STEARYL-ACYL CARRIER PROTEIN DESATURASE FROM SPINACH CHLOROPLASTS

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ABSTRACT

Stearyl-acyl carrier protein desaturase (EC 1.14.99.6), present in the stroma fraction of spinach (Spinacia oleracea) chloroplasts, rapidly desaturated enzymatically prepared stearyl-acyl carrier protein to oleic acid. No other substrates were desaturated. In addition to stearyl-acyl carrier protein, reduced ferredoxin was an essential component of the system. The electron donor systems were either ascorbate, dichlorofenolindophenol, photosystem I and light, or NADPH and ferredoxin-NADP reductase. The desaturase was more active in extracts prepared from chloroplasts obtained from immature spinach leaves than from mature leaves. Stearyl-acyl carrier protein desaturase also occurs in soluble extracts of avocado (Persea americana Mill) mesocarp and of developing safflower (Carthamus tinctorius) seeds.

Soluble stearyl-ACP desaturases (EC 1.14.99.6) have been isolated from Euglena gracilis (12–14), spinach chloroplasts (13, 14) and developing safflower seeds (5). In addition, an extract capable of desaturating stearic acid has been prepared from soybean cotyledons (4). Efforts in this laboratory to demonstrate a stearyl-ACP desaturase in spinach chloroplasts had been generally unsuccessful until recently. After an enzymatic method was developed for the synthesis of stearyl-ACP (6) which consistently yielded an active substrate, stearyl-ACP desaturase activity was readily detected. This communication will, therefore, report on the details of the substrate specificity, electron donors, and distribution of the desaturase.

MATERIALS AND METHODS

Enzyme Preparations. Fresh spinach (Spinacia oleracea) was purchased from a local market. Chloroplasts from mature and immature leaves were prepared as described earlier (7). The chloroplasts were broken by a French pressure cell at 15,000 p.s.i. and the suspension of disrupted chloroplasts was centrifuged at 48,000g for 10 min. The 48,000g supernatant fraction was then centrifuged at 105,000g for 1 hr. The 105,000g supernatant fraction comprised the chloroplast stroma fraction. The 48,000g pellet, containing the chloroplast lamellae, was resuspended in 30 ml of the original medium employed in the isolation of the chloroplasts and was homogenized with a Potter-Elvehjem homogenizer. The suspension was then recentrifuged at 48,000g for 10 min. The lamellae were resuspended in the isolating medium at a concentration of 2 mg of Chl/ml.

Preparation of safflower (Carthamus tinctorius) enzyme extract was as described previously (5). Avocado (Persea americana Mill) mesocarp was ground in five volumes (w/v) of 0.1 m potassium phosphate, pH 7.4, and 0.1 mM mercaptoethanol with a Sorvall Omnimix homogenizer. The homogenate was centrifuged at 48,000g for 10 min, and the supernatant fraction was separated from the floating fat layer and centrifuged at 105,000g for 1 hr. Each of the 105,000g supernatant fractions was assayed for protein concentration by the Lowry method (9) and adjusted to a concentration of 2 mg of protein/ml.

"C-Stearyl-ACP Preparation. "C-Stearyl-ACP was enzymatically prepared as previously described (6). The stearyl-ACP was generated by incubating anaerobically for 30 min at 22 C the following reaction mixture: 10 µCi of 2-4C-acetic acid, specific activity 5 mCi/m mole, 10 µmole of NADP, 10 µmole of NADH, 80 µmole of glucose-6-P, 1 unit of glucose-6-P dehydrogenase, 40 µmole of ATP, 4 µmole of reduced coenzyme A, 1 mg of crude E. coli ACP, and 60 mg of protein from immature chloroplast stroma in 20 ml of the isolation media (7). The reaction was stopped by adding an equal volume of 10% trichloroacetic acid and the precipitated stearyl-ACP was purified as previously described (6). The final substrate contained 85 to 90% stearyl-ACP with the remainder as palmitoyl-ACP. All the acyl groups were attached as thioesters. The final specific activity was 45 mCi/m mole.

Enzyme Assays. Stearyl-ACP desaturase was assayed by incubating 1 mg of protein of enzyme extract with 75 µg of purified spinach ferredoxin (15), 0.25 nmoles of "C-stearyl-ACP, activity 45 mCi/m mole, 100 nmoles of Tricine buffer, pH 7.9, and a ferredoxin reducing system consisting of either 50 nmoles of dichlorofenolindophenol, 10 µmole of sodium ascorbate, and 50 µg of Chl of spinach lamellae, or 0.5 µmole of NADP, 4 µmole of glucose-6-P, and 0.15 units of glucose-6-P dehydrogenase. The final reaction volume was 1 ml and the incubation was carried out at 15 C for 10 min with 1000 ft-c of white light. The reaction was stopped and analyzed as previously described (5).

Ribulose-1,5 diP carboxylase was assayed by incubating 20 µg of protein of enzyme extract, 1 µmole of ribulose-1,5 diP, 5 µmole of "C-HCO3", specific activity 6.7  µCi/m mole, 5 µmole of MgSO4, and 2 µmole of Tricine buffer, pH 8.3, in a total volume of 0.2 ml for 20 min at 22 C. The reaction was stopped by addition of 0.2 ml of glacial acetic acid, the
reaction mixture was vacuum desiccated for 30 min to remove unreacted \(^{14}C\)O\(_2\), and remaining \(^{14}C\) was counted in 10 ml of Bray's solution (2) by a Beckman liquid scintillation spectrometer.

RESULTS AND DISCUSSION

**Substrates.** Initial attempts by this laboratory to demonstrate stearyl-ACP desaturase activity were unsuccessful because of the unavailability of a properly prepared substrate. While \(^{14}C\)-acetate was readily incorporated into oleic acid by broken chloroplasts (7), no synthesis occurred from chemically prepared stearyl-ACP. However, enzymatically prepared stearyl-ACP was readily converted to oleic acid by a chloroplast stroma fraction. None of the other substrates tested, including stearyl-CoA, palmityl-CoA, and palmityl-ACP, were desaturated by the soluble chloroplast system (Table I). The lamellar membrane fraction had no desaturase activity with any of these substrates.

**Electron Donors.** A necessary component of the soluble stearyl-ACP desaturase system is ferredoxin (5, 14). Presumably this acts as an electron donor to the desaturase and is only active in the reduced form. NADPH is usually considered as the reductant which is coupled to the desaturase system via a ferredoxin-NADP reductase (14). Photoreduction of ferredoxin by chloroplast lamellae proved to be a very effective electron donor system with desaturases from *Cardthamus tinctorius* (5, 16), and it completely replaced the NADPHferredoxin-NADP reductase system with the stromal enzyme (Fig. 1). Although dichlorophenolindophenol and ascorbic acid were routinely used as the electron donor system to photoreduce the stromal enzyme (Fig. 1). Although dichlorophenolindophenol and ascorbic acid were used as electron donors, DCPIP and light were also present. Experimental details are described in "Materials and Methods."

**Localization and Distribution of Desaturase.** In confirmation of earlier results (12–14) we have observed that stearyl-ACP desaturase is associated with the stroma fraction of spinach chloroplasts. However, during the isolation of the chloroplast lamellae, some desaturase activity was detected in the absence of added 105,000g supernatant fraction, indicating the possibility of a membrane-bound enzyme system. Employing ribulose 1, 5 diphosphate carboxylase as a marker for chloroplast stroma contamination (8), we observed a significant amount of carboxylase activity in some lamellar fractions which could account for the presence of desaturase activity in these lamellar fractions. Thus, we have concluded that all the desaturase activity of spinach chloroplasts is associated with the soluble protein in the stroma fraction.

The research described in this communication required the use of spinach grown in both the summer and winter months. Although summer-grown spinach yielded a stearyl-ACP desaturase which was quite active when NADPH was used as the electron donor, winter-grown spinach generally gave a desaturase system which would not function with NADPH as the electron source. However, spinach desaturases prepared from summer- and winter-grown spinach were active when the photoreduced ferredoxin-lamellar system was used. The reason for the inactivity of NADPH as an electron donor in winter spinach was not resolved. Ferredoxin-NADP reductase was not a limiting factor since both types of preparations had high activity. Because of this variability in desaturase activity with NADPH as the electron donor, photoreduction of ferredoxin is preferable for routine assays of stearyl-ACP desaturase.

### Table I. Substrate Specificity of Stearyl-ACP Desaturase from Spinach Chloroplasts

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Substrate Added</th>
<th>Desaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitoyl-CoA</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>Palmitoyl-ACP</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>Stearyl-ACP</td>
<td>0.25</td>
<td>60</td>
</tr>
<tr>
<td>Stearyl-CoA</td>
<td>0.25</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>0.50</td>
<td>0</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.50</td>
<td>0</td>
</tr>
</tbody>
</table>

![Fig. 1. Effect of increasing concentrations of NADPH and lamella on chloroplast stearyl-ACP desaturase. With lamella, ascorbate, DCPIP, and light were also present. Experimental details are described in "Materials and Methods."](image)
ACP to 14C-oleic acid under our standard assay conditions. Mazliak and Decotte (10) were not able to demonstrate this reaction presumably because they used chemically acylated 1-14C-stearyl-ACP as substrate. With all preparations, 14C-stearyl-CoA was inactive as a substrate. Thus the mechanism of oleic acid biosynthesis in a nonphotosynthetic tissue, namely the developing safflower seed, in chloroplasts, and in mesocarp tissue of the avocado, appeared to be identical in that the systems from these different types of plant tissue: (a) were soluble proteins, (b) required an electron donating system which coupled to ferredoxin, and (c) required stearyl-ACP as substrate. Reduced ferredoxin, in turn, transferred the electron to the desaturase for the activation of molecular oxygen and the subsequent removal of two hydrogens from stearyl-ACP for the formation of a cis 9,10 double bond.

In comparing the stearyl desaturases of animal and plant systems, all animal systems required stearyl-CoA as substrate and the desaturase is associated with the endoplasmic reticulum (3). A similar situation exists in yeast (11) and fungi (1). However, in higher plants, all stearyl desaturases that have been examined require specifically stearyl-ACP and the desaturases is freely soluble. The implications of these differences merit further attention.

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LITERATURE CITED