Plant Physiol. (1974) 54, 484–486

Fat Metabolism in Higher Plants

LXII. STEARYL-ACYL CARRIER PROTEIN DESATURASE FROM SPINACH CHLOROPLASTS[1]

Received for publication February 25, 1974 and in revised form May 29, 1974

BRUCE S. JACOBSON,[2] J. G. JAWORSKI, AND P. K. STUMPF
Department of Biochemistry and Biophysics, University of California, Davis, California 95616

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, dichlorophenolindophenol, 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[3] 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 chloroplast 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 48,000*g* for 10 min. The 48,000*g* supernatant fraction was then centrifuged at 105,000*g* for 1 hr. The 105,000*g* supernatant fraction comprised the chloroplast stroma fraction. The 48,000*g* 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 48,000*g* 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 48,000*g* for 10 min, and the supernatant fraction was separated from the floating fat layer and centrifuged at 105,000*g* for 1 hr. Each of the 105,000*g* supernatant fractions were assayed for protein concentration by the Lowry method (9) and adjusted to a concentration of 2 mg of protein/ml.

^{14}C-Stearyl-ACP Preparation. ^{14}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-^{14}C-acetic acid, specific activity 5 mCi/mmole, 10 μmoles of NADP, 10 μmoles of NADH, 80 μmoles of glucose-6-P, 1 unit of glucose-6-P dehydrogenase, 40 μmoles of ATP, 4 μmoles 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 palmityl-ACP. All the acyl groups were attached as thioesters. The final specific activity was 45 mCi/mmole.

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 ^{14}C-stearyl-ACP, activity 45 mCi/mmole, 100 μmoles of Tricine buffer, pH 7.9, and a ferredoxin reducing system consisting of either 50 nmoles of dichlorophenolindophenol, 10 μmoles of sodium ascorbate, and 50 μgm of Chl of spinach lamellae, or 0.5 μmoles of $NADP^+$, 4 μmoles 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 μmoles of $^{14}C\text{-}HCO_3^-$, specific activity 6.7 μCi/mmole, 5 μmoles of $MgSO_4$, and 2 μmoles of tris-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

[1] This research was supported in part by National Institutes of Health Grant GM 19213-02.

[2] Present address: Department of Biological Sciences, Harvard University, Cambridge, Massachusetts 02138.

[3] Abbreviation: ACP: acyl carrier protein.

reaction mixture was vacuum desiccated for 30 min to remove unreacted $^{14}CO_2$, 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 *Carthamus tinctorius* (5, 16), and it completely replaced the NADPH-ferredoxin-NADP reductase system with the stromal enzyme (Fig. 1). Although dichlorophenolindophenol and ascorbic acid were routinely used as the electron donor system to photosystem I, N,N,N′,N′-tetramethyl-*p*-phenylenediamine (0.1 mM) served equally well as an electron donor. The photoreduction of ferredoxin was completely dependent on light and no desaturation occurred when the reaction was carried out in the dark.

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 105,000*g* supernatant fraction, indicating the possibility of a membrane-bound enzyme system. Employing ribulose 1,5 diP carboxylase activity 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 source, 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*

Substrate	Substrate Added	Desaturated
	nmoles	%
Palmityl-CoA	0.25	0
Palmityl-ACP	0.25	0
Stearyl-ACP	0.25	60
Stearyl-CoA	0.25	<1
Stearic acid	0.50	0
Oleyl CoA	0.50	0
Oleic acid	0.50	0

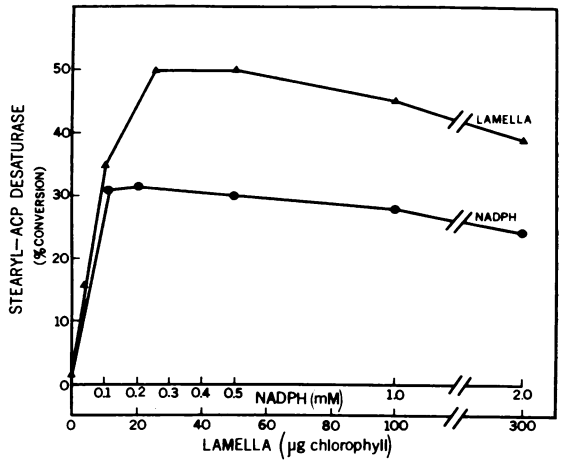

FIG. 1. Effect of increasing concentrations of NADPH and lamella on chloroplast stearyl-ACP desaturase. With lamella, ascorbate, DCPIP, and light were also present. Experimental details are described in "Materials and Methods."

Table II. *Stearyl-ACP Desaturase Activity in Extracts from Various Tissue*

The reactions were carried out with a photoreducing system for 5 min at 15 C. Each reaction contained 1 mg of protein and 0.25 nmoles of substrate.

Enzyme Source	Oleic Acid Synthesized
	nmoles/5 min
Immature spinach leaves	0.11
Mature spinach leaves	0.08
Developing safflower seeds	0.15
Avocado mesocarp	0.07

Stearyl-ACP desaturase activities from various sources were also examined. Kannangara *et al.* (7) have reported higher oleic acid synthesis from ^{14}C-acetate by stroma preparations from immature leaves than from mature leaves. With ^{14}C-stearyl-ACP as substrate, these two stroma preparations were compared with desaturase activity in extracts of developing safflower seeds and avocado mesocarp (Table II). The safflower extract clearly had the highest stearyl-ACP desaturase per unit of protein. The stroma fraction isolated from immature leaves was also more active than that from the mature leaves, in agreement with findings of Kannangara *et al.* (7). The desaturase activity in the avocado mesocarp extract was considerable. In addition, extracts of cauliflower inflorets readily converted ^{14}C-stearyl-

ACP to ^{14}C-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-^{14}C-stearyl-ACP as substrate. With all preparations, ^{14}C-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.

Acknowledgments—The authors wish to thank Frank Dailey for assaying ribulose-1,5-diP carboxylase and Mrs. Barbara Clover for technical assistance.

LITERATURE CITED

1. Baker, N. and F. Lynen. 1971. Factors involved in fatty acyl-CoA desaturation by fungal microsomes. The relative roles of acyl-CoA and phospholipids as substrate. Eur. J. Biochem. 19: 200–210.
2. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in liquid scintillation counters. Anal. Biochem. 1: 279–285.
3. Holloway, P. W. 1971. A requirement for three protein components in microsomal stearyl coenzyme A desaturation. Biochemistry 10: 1556–1560.
4. Inkpen, J. A. and F. W. Quackenbush. 1969. Desaturation of palmitate and stearate by cell-free fractions from soybean cotyledons. Lipids 4: 539–543.
5. Jaworski, J. G. and P. K. Stumpf. 1974. Fat metabolism in higher plants. Properties of a soluble stearyl-ACP desaturase from maturing *Carthamus tinctorius*. Arch. Biochem. Biophys. 162: 158–165.
6. Jaworski, J. G. and P. K. Stumpf. 1974. Fat metabolism in higher plants. Enzymatic preparation of *E. coli* stearyl-ACP. Arch. Biochem. Biophys. 162: 166–173.
7. Kannangara, C. G., B. S. Jacobson, and P. K. Stumpf. 1973. Fat metabolism in higher plants. LVII. A comparison of fatty acid-synthesizing enzymes in chloroplasts from mature and immature leaves of spinach. Plant. Physiol. 52: 156–161.
8. Kawashima, N. and S. G. Wildman. 1970. Fraction I protein. Annu. Rev. Plant Physiol. 21: 325–358.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. L. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265–275.
10. Mazliak, P. and A. M. Decotte. 1973. Une nouvelle voie de biosynthese de l'acide oléique dans les vegetaux. Biochemie 55: 1481–1489.
11. Meyer, F. and K. Bloch. 1963. Effect of temperature on the enzymatic synthesis of unsaturated fatty acids in *Torulopsis utilis*. Biochim. Biophys. Acta 77: 671–673.
12. Nagai, J. and K. Bloch. 1965. Synthesis of oleic acid by *Euglena gracilis*. J. Biol. Chem. 240: PC3702–PC3703.
13. Nagai, J. and K. Bloch. 1966. Enzymatic desaturation of stearyl acyl carrier protein. J. Biol. Chem. 241: 1925–1927.
14. Nagai, J. and K. Bloch. 1968. Enzymatic desaturation of stearyl acyl carrier protein. J. Biol. Chem. 243: 4626–4633.
15. Rao, K. K., R. Cammack, D. O. Hall, and C. E. Johnson. 1971. Mossbauer effect of *Scenedesmus* and spinach ferredoxins. The mechanism of electron transfer in plant type iron-sulphur proteins. Biochem. J. 122: 257–265.
16. Vijay, I. and P. K. Stumpf. 1972. Fat metabolism in higher plants. XLVIII. Properties of oleyl coenzyme A desaturase of *Carthamus tinctorius*. J. Biol. Chem. 247: 360–366.