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Plant holo-(acyl carrier protein) synthase

Salah A. ELHUSSEIN,* Jan A. MIERNYK† and John B. OHLROGGE‡

Seed Biosynthesis Research Unit, Northern Regional Research Center, United States Department of Agriculture, Agricultural Research Service, 1815 North University Street, Peoria, IL 61604, U.S.A.

1. An improved method was developed for the assay of plant holo-(acyl carrier protein) synthase activity, using *Escherichia coli* acyl-(acyl carrier protein) synthetase as a coupling enzyme. 2. Holo-(acyl carrier protein) synthase was partially purified from spinach (*Spinacia oleracea*) leaves by a combination of $(NH_4)_2SO_4$ fractionation and anion-exchange and gel-permeation chromatography. 3. The partially purified enzyme had a pH optimum of 8.2 and K_m values of 2 μM, 72 μM and 3 mM for apo-(acyl carrier protein), CoA and Mg^{2+} respectively. Synthase activity was inhibited *in vitro* by the reaction product 3′,5′-ADP. 4. Results from the fractionation of spinach leaf and developing castor-oil-seed (*Ricinus communis*) endosperm cells were consistent with a cytosolic localization of holo-(acyl carrier protein) synthase activity in plant cells.

INTRODUCTION

In plants, acyl carrier protein (ACP) is a nuclear-encoded plastid protein that participates in fatty acid biosynthesis, desaturation and acyltransferase reactions (Ohlrogge, 1987). The phosphopantetheine prosthetic group of ACP is attached to a serine residue near the middle of the polypeptide chain. This same prosthetic group structure is found in CoA, where it serves the similar function of carrying acyl groups activated as thioesters. During the biosynthesis of ACP in *Escherichia coli*, the enzyme holo-ACP synthase (EC 2.7.8.7) catalyses the transfer of 5′-phosphopantetheine from CoA to apo-ACP (Alberts & Vagelos, 1966; Elovson & Vagelos, 1968; Polacco & Cronan, 1981).

The mechanism of holo-ACP synthesis has not previously been examined in plants. Because of the general similarities between plant and bacterial fatty acid synthesis, it might be expected that ACP assembly would also be similar. However, it has recently been recognized that plant ACPs are synthesized as larger precursor proteins, with a 5–6 kDa transit peptide. Therefore, as with most plastid proteins, ACP is synthesized in the cytoplasm and post-translationally imported into plastids, where proteolytic processing to the mature peptide occurs (reviewed by Ohlrogge, 1987). These observations raise the question of where the prosthetic group is attached: in the cytoplasm or within the plastids? Herein we describe the subcellular localization of holo-ACP synthase in higher plants and some properties of the enzyme partially purified from spinach (*Spinacia oleracea*) leaves.

MATERIALS AND METHODS

Plant material

Spinach (*Spinacia oleracea* L., cv. 424) and castor-oil-seed (*Ricinus communis* L., cv. Baker 296) seeds were germinated and the plants were glasshouse-grown at 26 °C with a 12 h day/12 h night photoperiod. Young enlarging spinach leaves from plants less than 4 weeks old were selected. Developing castor-oil seeds were selected approx. 25 days after anthesis, at the beginning of the exponential phase of storage-lipid accumulation (Simcox *et al.*, 1979).

Reagents

Holo-ACP was isolated from *E. coli* (frozen cell paste of strain B purchased from Grain Processing, Muscatine, IA, U.S.A.) as described by Rock & Cronan (1980). Apo-ACP was prepared by removal of the 5′-phosphopantetheine prosthetic group with HF by the method of Prescott *et al.* (1969). Briefly, 1 mg of holo-ACP was incubated with 2.5 ml of 50 % (v/v) HF at 4 °C for 72 h, then shell-frozen and freeze-dried. The residue was dissolved in a small volume of 100 mM-Tris/HCl, pH 8.0, and then dialysed overnight against 10 mM-Tris buffer, pH 8.0. The conversion of holo-ACP into apo-ACP was monitored by the periodic removal of samples for analysis by ACP assay. There was a greater-than-95 % conversion in 48 h without significant degradation of the protein as detected by SDS/PAGE (results not shown).

Sephadex G-25, Sephacryl S-200 and a Mono-Q HR 5/5 anion-exchange column were from Pharmacia. DE-53 DEAE-cellulose and DE-81 DEAE-paper were from Whatman.

Unless otherwise noted, biochemicals were provided by Sigma or P-L Biochemicals. Purified Triton X-100 was obtained from Pierce Chemicals (Rockford, IL, U.S.A.). [^{3}H]Palmitate (23 Ci/mmol) was from New England Nuclear. Inorganic chemicals and solvents were of reagent grade or better.

Preparation of holo-ACP synthase

Typically, 3–5 g of plant tissue were ground with a mortar and pestle in 2 vol. of homogenization buffer

Abbreviations used: ACP, acyl carrier protein; PAGE, polyacrylamide-gel electrophoresis; DTT, DL-dithiothreitol; RuBisCo, ribulose 1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39); cv., cultivar.

* Permanent address: Agricultural Research Corporation, P.O. Box 126, Wad Medani, Sudan.

† To whom correspondence and reprint requests should be sent.

‡ Present address: Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312, U.S.A.

(100 mM-Tris/HCl, pH 8.0, containing 5 mM-$MgCl_2$, 1 mM-EDTA, and 1 mM-DTT). The homogenate was passed through four layers of cheesecloth, then centrifuged at 27000 *g* for 20 min in an SS-34 rotor in a Sorvall RC-5B refrigerated centrifuge. Supernatant proteins were precipitated by addition of solid $(NH_4)_2SO_4$ to 60% saturation. Precipitated proteins were then dissolved in a small volume of homogenization buffer. Before ion-exchange chromatography or kinetic analyses, samples were desalted with a column (1 cm × 7 cm) of Sephadex G-25 equilibrated in homogenization buffer.

Enzyme assays

Holo-ACP synthase was assayed by a two-stage discontinuous reaction system. In the first, apo-ACP plus CoA were converted into holo-ACP plus 3′,5′-ADP. The holo-ACP was then converted into [^{3}H]palmitoyl-ACP by *E. coli* acyl-ACP synthetase in the second stage. The reaction mixture for holo-ACP synthase contained, unless otherwise noted, the following components at the indicated final concentrations: holo-ACP synthase in 100 mM-Tris/HCl, pH 8.0 (~ 200 μg of protein), apo-ACP (6 μM), CoA (500 μM), $MgCl_2$ (10 mM) and DTT (2 mM), in a final volume of 60 μl. After incubation at 37 °C for 50 min, the reaction was stopped by heating at 70 °C for 10 min, followed by a brief centrifugation to remove denatured protein. A 15 μl sample of the first reaction supernatant was then added to the second reaction system. In addition to the reaction-1 supernatant, reaction system 2 contained *E. coli* acyl-ACP synthase, [^{3}H]palmitate, ATP and other cofactors, in a final volume of 50 μl, as described by Kuo & Ohlrogge (1984). After incubation at 37 °C for 60 min, 25 μl samples were spotted on to Whatman DE-81 paper, partially dried, and then washed four times for 3 min in a 4:1 (v/v) mixture of propan-2-ol and phosphate-buffered saline (200 mM-sodium phosphate/200 mM-NaCl, pH 7.1). After drying, the filters were placed into scintillation vials with 1 ml of 200 mM-NaOH and heated at 65 °C for 15 min. The radioactivity released from the filters was quantified by liquid-scintillation spectrometry, and the results were converted into mol of holo-ACP formed by means of a standard curve prepared by allowing *E. coli* ACP, measured gravimetrically, to react with acyl-ACP synthetase as described for the holo-ACP synthase reation.

Heat treatment after completion of the first reaction was necessary to decrease background radioactivity, which otherwise interfered with measurement of holo-ACP (Elhussein *et al.*, 1987). In all instances, control reactions were used to assess holo-ACP contamination of apo-ACP and/or the apo-ACP synthase preparations. Appropriate corrections were made for any interference (due to manipulations of the holo-ACP synthase reaction) with the second-stage acyl-ACP synthetase coupling reaction.

Marker-enzyme activities were determined by established methods (Kruger *et al.*, 1963; Macdonald & Preiss, 1986; Miernyk, 1985). The quantification of RuBisCO protein was accomplished by rocket immunoelectrophoresis, using antibodies to fescue-grass (*Festuca arundinacea* Schreb) RuBisCO generously supplied by Dr. D. D. Randall, Department of Biochemistry, University of Missouri, Columbia, MO, U.S.A.

Chromatographic methods

The product of the coupled holo-ACP synthase/acyl-ACP synthetase reactions was analysed by h.p.l.c., a Waters Associates model 510 system and a Mono-Q anion-exchange column being used. Mobile phases were 20 mM-piperazine buffer, pH 6.0, containing 20% (v/v) propan-2-ol (A), and the same solution containing 1 M-NaCl (B). The column was run at 5 ml/min with a 10–70% (v/v) gradient of B in A over 15 min.

Desalted holo-ACP synthase preparations were chromatographed on a 50-ml-bed-volume column of DEAE-cellulose, previously equilibrated with 40 mM-Tris/HCl, pH 7.8, containing 1 mM-DTT and 5 mM-$MgCl_2$. Elution was with a 0–400 mM-gradient of NaCl in equilibration buffer. In some instances a 0.9 cm × 6 cm column of DEAE-cellulose, equilibrated and eluted under identical conditions, was used for the rapid separation of adsorbed and non-adsorbed fractions of holo-ACP synthase.

Gel-permeation chromatography was performed on a column (1.5 cm × 30 cm) of Sephacryl S-200. The mobile phase was typically 100 mM-Tris/HCl, pH 8.0, containing 5 mM-$MgCl_2$, 1 mM-EDTA and 1 mM-DTT. In some experiments the typical mobile phase was augmented with 0.03% (v/v) Triton X-100 or 100 mM-NaCl.

Subcellular localization

The glasshouse-grown spinach plants were kept in the dark at 25 °C for 24 h before analysis in order to reduce the leaf starch content. Developing castor-oil seeds were selected, and the seed coats and embryos were dissected from the endosperm. The plant tissues were coarsely chopped with scissors into 4 vol. of ice-cold homogenization medium consisting of 100 mM-imidazole buffer, pH 6.9, 500 mM-sucrose, 0.05% (w/v) fatty-acid-free bovine serum albumin, 2 mM-$MgCl_2$, 1 mM-EDTA and 2 mM-benzamidine, then they were homogenized with a mortar and pestle. The homogenate was passed through eight layers of cheesecloth, one layer of Miracloth and one layer of 20 μm-pore-size Nitex mesh, each previously moistened with homogenization medium. All subsequent steps were conducted at 4 °C. Organelle-enriched fractions were prepared by rate-zonal sedimentation (Miernyk, 1985). The organelle-enriched fractions were either used immediately or frozen and stored at −20 °C. Fractions were suspended in a small volume of 100 mM-Tris/HCl, pH 8.0, containing 5 mM-$MgCl_2$, 1 mM-EDTA and 1 mM-DTT, then disrupted by two 15 s bursts with a Brinkman Polytron homogenizer at the no. 6 setting. Before analysis, membranous material was removed by centrifugation as described under 'Preparation of holo-ACP synthase' above.

Other analytical methods

Radioactivity was quantified with a Beckman LS-9800 liquid-scintillation spectrometer and Biocount scintillation fluid. Analysis of enzyme kinetic data was by the method of Garland & Dennis (1977), with iterative curve-fitting by non-linear regression. Protein analysis was by the dye-binding method of Read & Northcote (1981).

RESULTS

In a preliminary report (Elhussein *et al.*, 1987) we described methods and conditions for assay of holo-ACP synthase activity using a two-stage discontinuous assay. As presented below, in the first stage of the assay, holo-ACP is formed from apo-ACP and CoA by holo-ACP synthase. In the second stage, holo-ACP is quantitatively acylated with [^{3}H]palmitate by *E. coli* acyl-ACP synthetase. It was necessary to heat-treat the reaction mixture after the first stage. This procedure eliminated a very high ATP-dependent background activity which we attribute to palmitoylation of CoA by acyl-CoA ligase during the second reaction stage. *E. coli* apo-ACP was used as the substrate for the assay because of the limited availability of purified plant ACP.

First stage:

$$\text{apo-ACP} + \text{CoA} \rightarrow \text{holo-ACP} + 3',5'\text{-ADP}$$

Second stage:

$$\text{holo-ACP} + [^3\text{H}]\text{palmitic acid} \xrightarrow[\text{ATP}]{} [^3\text{H}]\text{palmitoyl-ACP}$$

By using this very sensitive assay system, the activity of holo-ACP synthase was demonstrated in all higher-plant tissues tested, namely spinach and pea (*Pisum sativum*) leaves, and seeds of safflower (*Carthamus tinctorius*) and the castor-oil plant. Spinach leaves, however, yielded the most consistently active enzyme preparations, with activities per g fresh weight approx. 10-fold higher than those of the other tissues tested.

Spinach holo-ACP synthase was quite heat-labile (Fig. 1), and was unstable during chromatographic procedures intended for its purification. Its instability was especially encountered in chromatofocusing and anion-exchange chromatography. Various potential stabilizing agents were tested for their effectiveness by incubation with crude spinach extracts for up to 48 h. Of these, 1 mM-EDTA and 5 mM-$MgCl_2$ slightly stabilized the activity of the crude enzyme extract and proved to be, together with DTT (1–2 mM), essential during anion-exchange chromatography and gel filtration.

By using 4-ml-bed-volume columns of DEAE-cellulose, it was possible to separate the activity from spinach leaves into two fractions: one that was not adsorbed and was eluted in the void volume of the column, and another that was adsorbed, then eluted with salt. A 50-ml-bed-volume DE-53 column eluted with a 0–0.4 M-NaCl gradient resolved the activity into three fractions, two of which were eluted within the salt gradient. The ratio of adsorbed to unadsorbed enzyme activity was, however, lower with the larger column (Fig. 2) compared with that obtained with more rapid batch elution from smaller columns (results not shown). This could be due to the instability of the enzyme and the longer time needed for chromatographic development in the former case (24 h). However, even with the small columns the ratios varied. Where the adsorbed and non-adsorbed fractions were pooled separately, precipitated by $(NH_4)_2SO_4$, redissolved and dialysed, the ratio of adsorbed to non-adsorbed enzyme activity was about 3:1. On the other hand, where fractions were assayed directly, i.e. in the presence of NaCl in the salt-eluted fractions, the ratio was 1:1. The effect of NaCl upon activity of the two fractions was not studied; however, earlier work with crude extracts had shown that up to 0.4 M-NaCl had no effect on either holo-ACP synthase or the second reaction stage of the assay. When re-chromatographed on the same column, the unadsorbed fraction remained unadsorbed, indicating that the lack of binding was not due to column overloading.

Gel permeation of preparations from spinach using Sephacryl S-200 resolved one major peak of activity (Fig. 3). Elution with buffer containing Triton X-100/0.1 M-NaCl (to minimize possible protein aggregation) gave similar results. Holo-ACP synthase activity was eluted after ovalbumin and before cytochrome *c*, indicating that the plant enzyme has a relatively low M_r. The chromatographic methods employed allowed an approx. 40-fold purification of spinach leaf holo-ACP synthase to a specific activity of 850 nmol·min^{-1}·mg of protein^{-1}. Measurement of holo-ACP synthase activity was completely dependent upon CoA and apo-ACP. Reaction was linear with respect to time and amount of added protein. Formation of a single radioactive product, palmitoyl-ACP, was verified by h.p.l.c. analysis (Elhussein *et al.*, 1987). Activity of spinach leaf holo-

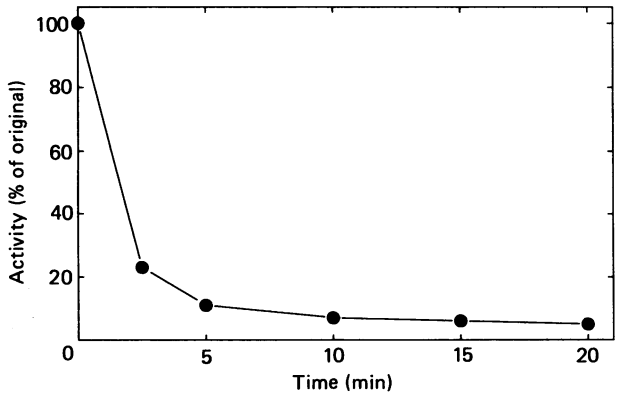

Fig. 1. Stability of spinach leaf holo-ACP synthase at 52 °C

The original activity was 96 pmol/min.

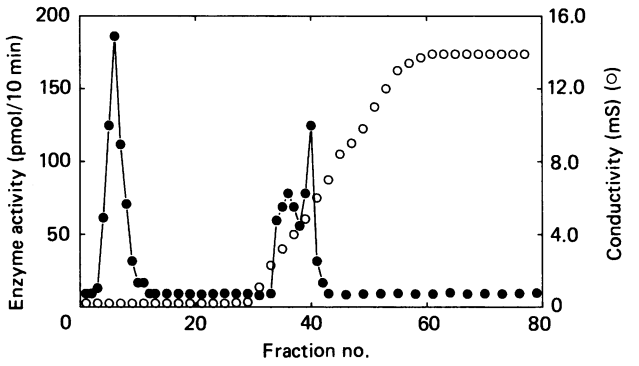

Fig. 2. Anion-exchange chromatography of spinach leaf holo-ACP synthase

A 60%-satd.-$(NH_4)_2SO_4$-insoluble fraction was dissolved, desalted on a column of Sephadex G-25, then loaded on to a 50-ml-bed-volume column of Whatman DE-53 previously equilibrated with 40 mM-Tris/HCl, pH 7.8, containing 1 mM-DTT and 5 mM-$MgCl_2$. After washing with equilibration buffer the column was eluted with a gradient of 0–0.4 M-NaCl in equilibration buffer. The original sample had an enzyme activity of 186 pmol/min. The overall recovery was 86%.

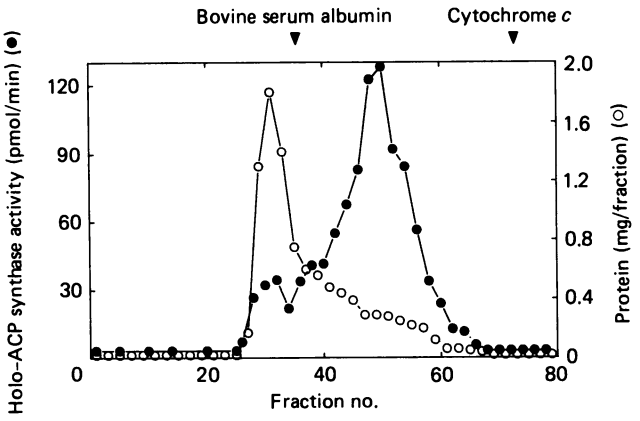

Fig. 3. Gel-permeation chromatography of spinach leaf holo-ACP synthase on a 1.5 cm × 30 cm column of Sephacryl S-200

The mobile phase was 100 mM-Tris/HCl, pH 8.0, containing 1 mM-EDTA, 5 mM-$MgCl_2$ and 1 mM-DTT. The original sample contained an original enzyme activity of 855 pmol/min. The overall recovery after chromatography was 73%.

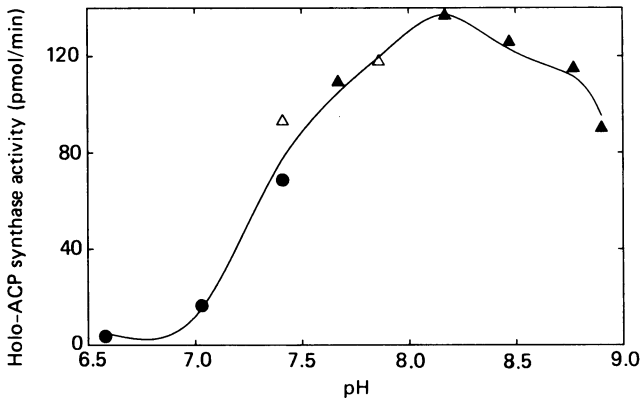

Fig. 4. Activity of partially purified spinach leaf holo-ACP synthase as a function of pH

Buffers used were: imidazole, pH 6.0–7.0 (●); Hepes, pH 7.0–7.7 (△); Tris/HCl, pH 7.7–9.0 (▲), each at 50 mM final concentration.

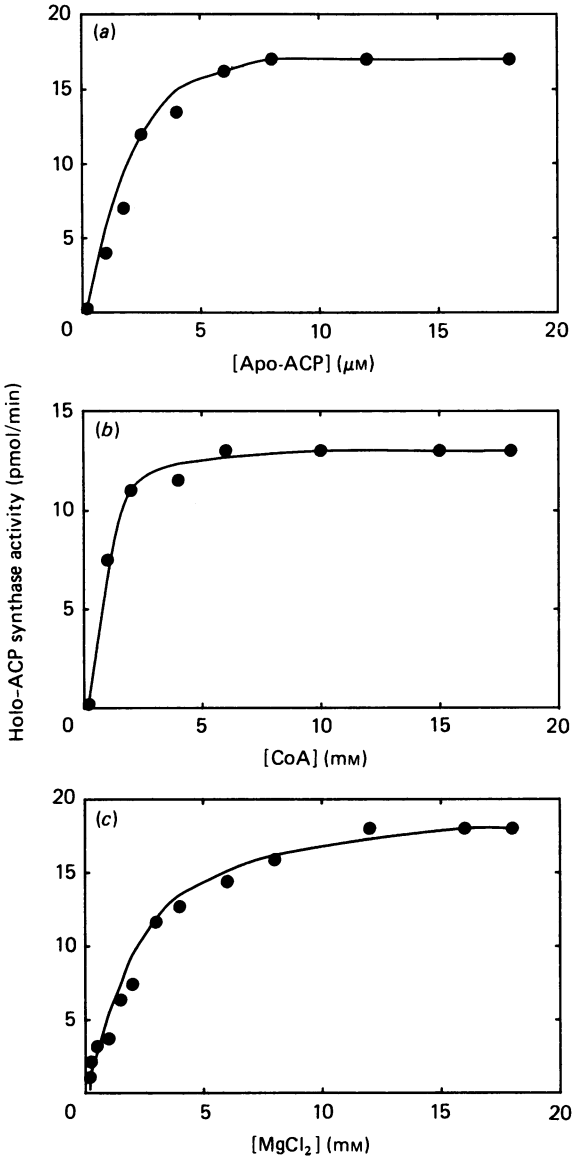

Fig. 5. Initial-rate studies of partially purified spinach leaf holo-ACP synthase as a function of (*a*) apo-ACP concentration, (*b*) CoA concentration and (*c*) $MgCl_2$ concentration

ACP synthase was low at acidic pH values and maximal at 8.0 (Fig. 4). Initial-rate studies of the spinach enzyme as a function of apo-ACP, CoA or $MgCl_2$ resulted in rectangular hyperbolae in all cases (Fig. 5). K_m values were 1.82 ± 0.28 μM, 71.59 ± 9.90 μM and 2.80 ± 0.45 mM for apo-ACP, CoA and $MgCl_2$ respectively. A product of the reaction, 3′,5′-ADP, inhibited activity of the enzyme *in vitro* (Fig. 6). Inhibition by ADP was linearly competitive with respect to CoA, with a K_i value of 0.52 ± 0.12 mM. Dephospho-CoA, the immediate precursor of CoA *in vivo*, was tested at concentrations up to 1 mM as a substrate for holo-ACP synthase, but was inactive.

Subcellular fractions were isolated from young expanding spinach leaves and developing castor-oil-seed endosperm by rate-zonal sedimentation (Table 1). On the basis of marker-enzyme distribution, the P_5 fraction (pellet after centrifugation at 5000 *g*) was enriched with plastids, the P_{27} fraction with mitochondria and the P_{100} fraction with vesicles of the endomembrane system. The membrane-limited organelles having been removed, the final S_{100} fraction (supernatant after centrifugation at 100000 *g*) is the cytosol. Although there were low levels of activity associated with the plastid and mitochondrial fractions of both tissues, the distribution of holo-ACP synthase activity most closely paralleled that of pyrophosphate:fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.90), an enzyme which in plants is confined exclusively to the cytoplasm (Kruger *et al.*, 1963; Macdonald & Preiss, 1986). Identical results were observed when alcohol dehydrogenase was used as the cytoplasmic marker for *Ricinus* endosperm (results not shown). In all instances, adequate recoveries of the marker enzymes and of holo-ACP synthase activity were

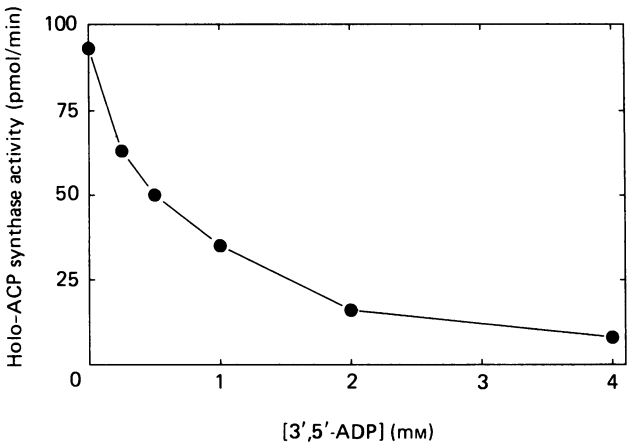

Fig. 6. Product inhibition of partially purified spinach leaf holo-ACP synthase by 3',5'-ADP

obtained. It should be noted that similar low levels of holo-ACP synthase activity were associated with P_5 and P_{27} fractions isolated from expanding pea leaves and developing safflower seeds, but some as-yet-unidentified inhibitory factor(s) prevented the detection of active enzyme in total homogenates or in other subcellular fractions.

DISCUSSION

Elovson & Vagelos (1968) reported that purification of holo-ACP synthase from *E. coli* was hindered by a marked instability of the enzyme. They were able to minimize the stability problems by inclusion of high concentrations of reduced CoA plus Mg^{2+}. A (54 °C) heat-treatment step was subsequently used during purification of the stabilized enzyme. Spinach leaf holo-ACP synthase was also unstable, but this could only be partially overcome by Mg^{2+} plus DTT. CoA was not tested as a stabilizing agent. The spinach protein was still very labile when heated or during column chromatography. Addition of ethylene glycol, glycerol, EDTA or a wide range of proteinase inhibitors failed to stabilize the plant enzyme further.

Holo-ACP synthase from *E. coli* did not bind to DEAE-cellulose (Elovson & Vagelos, 1968). Anion-exchange chromatography of spinach leaf holo-ACP synthase on DEAE-cellulose at pH 7.8 resolved the activity into one fraction that did not bind and possibly two fractions that were absorbed. Although this separation might be an artefact due to proteolysis or association of the enzyme with other macromolecules, it could also represent different forms of the protein. Results of analysis by gel-permeation chromatography are also suggestive of multiple forms of the protein. It has been clearly demonstrated that higher plants contain at least two forms of ACP (Hoj & Svendsen, 1984; Ohlrogge & Kuo, 1984) and multiple forms of biosynthetic enzymes which use ACP as substrate (see, e.g., Guerra & Ohlrogge, 1986). The reasons for this multiplicity are not yet clear.

Before the present study, holo-ACP synthase had been partially purified and characterized only from *E. coli* (Elovson & Vagelos, 1968; Prescott *et al.*, 1969). Several

Table 1. Subcellular localization of holo-ACP synthase

Enzyme	*Spinacia* leaves						Developing *Ricinus* endosperm					
	Total activity*	Distribution (%)				Recovery† (%)	Total activity*	Distribution (%)				Recovery† (%)
		P_5	P_{27}	P_{100}	S_{100}			P_5	P_{27}	P_{100}	S_{100}	
Pyrophosphate:fructose-6-phosphate 1-phosphotransferase	90	0	1	2	97	100	704	2	3	2	96	103
RuBisCo	100	38	16	8	28	90	100	56	13	4	27	100
Succinate dehydrogenase	600	18	68	15	0	101	2400	9	72	11	0	93
Catalase	18	17	31	6	55	109	103	8	41	9	42	100
NADH:cytochrome *c* reductase	400	4	2	91	0	97	900	1	2	94	0	97
Holo-ACP synthase	350	6	5	2	104	116	28	6	15	1	78	100

* Total activity measurements are nmol/min per g fresh wt., except for catalase, the activity of which is given in Lück units, holo-ACP synthase, the activity of which is given in pmol/min per g fresh wt., and RuBisCo, the activity of which is given in arbitrary units measured immunochemically.
† Recovery was calculated by dividing the activity in each of the fractions by the activity in the original homogenate.

of the catalytic characteristics of the bacterial and plant enzymes, including pH optimum, substrate specificity and bivalent-cation requirement, were quite similar. Kinetic constants were 0.5 μM, 150 μM and 3 mM for apo-ACP, CoA and $MgCl_2$ respectively for the *E. coli* enzyme, and 2 μM, 72 μM and 3 mM for the spinach enzyme. The relatively high K_m of spinach holo-ACP synthase for apo-ACP may be due to structural differences between the bacterial protein used as the substrate in these studies and plant apo-ACP. Prescott *et al.* (1969) reported that structural alterations resulting from partial proteolysis of apo-ACP served to increase the K_m of *E. coli* holo-ACP synthase approx. 4-fold. It should be noted, however, that for several plant enzymes *E. coli* ACP is as effective a substrate as plant ACP (Ohlrogge, 1987).

By using gel-permeation chromatography and rate-zonal sedimentation in sucrose density gradients, Elovson & Vagelos (1968) estimated an M_r of 50000 for *E. coli* holo-ACP synthase. Spinach leaf holo-ACP synthase was eluted from a Sephacryl S-200 gel-permeation column later than ovalbumin, suggesting that it is smaller than the bacterial enzyme. Further studies using a different chromatographic matrix will be necessary to accurately determine the M_r of the plant protein.

Many nuclear-encoded organellar proteins contain prosthetic groups. Only in a few instances, however, has the question of the site of addition been addressed, and as yet no consistent pattern has emerged. It has been reported that the attachment of haem to mitochondrial proteins takes place either in the intermembrane space [cytochrome *c* (Enosawa & Ohashi, 1968)] or within the matrix (Harbin & Dailey, 1985). Pre-apo-(aspartate aminotransferase) is translocated into the mitochondrial matrix, then proteolytically processed to the mature apoprotein before the addition of pyridoxal phosphate (Sharma & Gehring, 1986). Catalase maturation involves transport of monomers into the peroxisomes, after which haem is added and the tetrameric holoenzyme is formed (Lazarow & de Duve, 1973). Takahashi *et al.* (1986) showed that the addition of the iron–sulphur cluster to ferredoxin occurs within isolated chloroplasts. In contrast, the synthesis of FAD apparently takes place in the cytoplasm, and pre-apo-(ferredoxin:NADP reductase) can associate with FAD outside the chloroplast, forming an active pre-holo-reductase (Carrillo, 1985). It is presumably this form of the enzyme which is transported across the plastid envelope to the thylakoid membranes.

In spinach leaves ACP is located exclusively within the plastids (Ohlrogge *et al.*, 1979). Ohlrogge (1987) reported that a larger precursor of apo-ACP was synthesized *in vitro* from spinach leaf mRNA. A presumptive *N*-terminal transit peptide must then be removed during the uptake and proteolytic processing of the precursor by the chloroplasts (Schmidt & Mishkind, 1986). These results raised the question of when the phosphopantetheine prosthetic group is attached during the processes of synthesis, transport and maturation. We have addressed this question by assaying holo-ACP synthase activity in subcellular fractions isolated from young spinach leaves and developing *Ricinus* endosperm. Active fatty acid synthesis *de novo* takes place in both of these tissues (for membane lipid biosynthesis in the expanding leaves and for storage lipid biosynthesis in the developing seeds). In both tissues, holo-ACP synthase activity was recovered primarily in the cytosol, although low levels of activity were associated with the plastid and mitochondrial fractions.

A certain amount of holo-ACP synthase activity in the plastids could allow replacement of the prosthetic group removed by non-specific phosphohydrolases, or by ACP hydrolase, an enzyme which in *E. coli* catalyses this removal (Powell *et al.*, 1969; Vagelos & Larrabee, 1967). The occurrence of this enzyme in plants has not yet been examined. Together with holo-ACP synthase, these enzymes potentially provide a turnover cycle for the ACP prosthetic group. In *E. coli*, however, the prosthetic group appears very stable. An elegant recent study of phosphopantetheine turnover showed that, during exponential growth, there is less than one turnover cycle per ACP molecule per generation (Jakowski & Rock, 1984). There is as yet no information on turnover of the prosthetic group of plant ACPs.

Although it is not possible to discount the low levels of organellar holo-ACP synthase activity, we conclude that the large amount of activity in the cytosol is responsible for attachment of the prosthetic group during synthesis of ACP *de novo*. On the basis of these and previously published data, we propose the following sequence in plant tissues: (1) pre-apo-ACP is synthesized on free ribosomes in the cytoplasm; (2) the prosthetic group is added by holo-ACP synthase while still in the cytoplasm; (3) then the pre-holo-ACP is recognized by the plastids, taken up and proteolytically processed to mature holo-ACP.

Names of vendors are included for the benefit of the reader and do not imply endorsement or preferential treatment by the United States Department of Agriculture (USDA). This work was supported in part by the Co-operative State Research Service, USDA, under agreement 84-CRCR-1-1402. We acknowledge the technical assistance of Mr. D. L. Elmore and the helpful advice of Dr. D. J. Guerra.

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