Biochem. J. (1985) **226**, 551–556
Printed in Great Britain

Localization of chloroplastic fatty acid synthesis *de novo* in the stroma

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(*Received 29 August 1984/Accepted 6 November 1984*)

The synthesis of fatty acids *de novo* from [2-^{14}C]malonyl-CoA was studied in fractions from lettuce (*Lactuca sativa*) and pea (*Pisum sativum*) chloroplasts. When lettuce chloroplasts were subjected to osmotic lysis, disintegration through a Yeda press and high-speed centrifugation, essentially all of the fatty-acid-synthetic activity was found to be soluble. The distribution of the activity in various chloroplast fractions was similar to that of soluble marker enzymes such as ribulose-1,5-bisphosphate carboxylase and $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase. Marked differences were apparent in the quality of products from fatty acid synthesis *de novo* in the various fractions of chloroplasts. Thus soluble fractions produced predominantly stearate, whereas those containing membranes produced a greater proportion of palmitate. In pea chloroplasts, osmotic lysis released almost all of the fatty acid synthetase into the stromal fraction. In this instance, no major alterations in the products of fatty acid synthesis were observed. The fatty-acid-synthetic activity of the stromal fraction was still soluble after prolonged ultracentrifugation. The results show clearly the soluble nature of fatty acid synthesis *de novo* in lettuce and pea chloroplasts. Thus fatty acid synthesis measured in microsomal fractions from such plant tissues is not due to the presence of chloroplastic membranes.

Recently there has been considerable interest in the subcellular site of fatty acid synthesis by plant tissues (Harwood & Stumpf, 1972; Ohlrogge *et al.*, 1979). Fractions from the cells shown to synthesize fatty acids include mitochondria, microsomes, plastids and soluble fractions probably equivalent to the cytoplasm (Bolton & Harwood, 1977; Roughan *et al.*, 1979). In particular, plastids have been extensively studied, since they represent a logical subcellular site for anabolic reactions such as fatty acid synthesis. Chloroplasts represent the sole site for photosynthetic reactions and synthesize many amino acids and other molecules of low M_r (Givan & Harwood, 1976). So far as fatty acid synthesis *de novo* is concerned, it is clear that, in photosynthetic tissues, the plastid is the major, perhaps the exclusive, site (Ohlrogge *et al.*, 1979).

In previous experiments studying fatty acid synthesis from [^{14}C]malonyl-CoA by microsomal preparations from germinating peas (*Pisum sativum*), results from partial-proteolysis experiments indicated that two sorts of membrane fragment were active (Sanchez *et al.*, 1982). Thus treatment of microsomal fractions with increasing amounts of rat pancreatic trypsin-like proteinase resulted in progressive loss of fatty-acid-synthesizing activity. However, the maximum inhibition obtained was in the region of 50%. The remaining activity could have been located on different membranes from the susceptible enzymes (Sanchez *et al.*, 1982). Because of the reported high activity of chloroplast preparations, including thylakoid fractions (Weaire & Kekwick, 1975), it was suggested that one of the active membranes could have been derived from plastids. It was in order to see whether isolated chloroplast membrane preparations were capable of any synthesis of fatty acids *de novo* that the present study was carried out.

Materials and methods

Pea (*Pisum sativum* L., cv. Feltham First) seeds were obtained from Asmer Seeds, Leicester, U.K., and were imbibed in distilled water at 20°C for 24h, then grown in trays of vermiculite in the laboratory. Lettuce (*Lactuca sativa*) was obtained fresh from the local market, then washed and soaked for 30min in distilled water.

[2-^{14}C]Malonyl-CoA (sp. radioactivity 2.146GBq/mmol) and $NaH^{14}CO_3$ (sp. radioactivity 37MBq/mmol) were purchased from Amersham International, Amersham, Bucks., U.K.

Acyl-carrier protein was purified from *Escherichia coli* as far as the DEAE-cellulose-column stage (Sauer *et al.*, 1964). DL-Glyceraldehyde-3-phosphate solution was prepared from the diethyl acetal (monobarium) salt and assayed for the D-isomer as described in *Sigma Technical Bulletin no. 1–78*. The Yeda Press was obtained from Linca Scientific Instruments, P.O. Box 3138, Tel-Aviv, Israel. Other chemicals were of the best available grades and were obtained from Sigma or BDH (both of Poole, Dorset, U.K.).

Chloroplast isolation

All procedures were performed at 4°C unless otherwise stated. Lettuce chloroplasts were isolated by homogenizing washed deveined outer leaves in ice-cold buffer [50mM-sodium phosphate (pH6.5)/10mM-NaCl/330mM-sorbitol] with a 5–10s burst of a Polytron tissue homogenizer. The homogenate was then filtered through six layers of Miracloth (Calbiochem–Behring Corp.) and the filtrate centrifuged to 4000*g* with immediate braking. The pellet was gently resuspended in ice-cold low-salt washing medium [50mM-sodium phosphate (pH7.4)/10mM-NaCl/330mM-sorbitol; cf. Andersson & Akerlund, 1978] and again centrifuged to 4000*g* with immediate braking. The resultant pellet contained approx. 95% chloroplasts. Intactness was between 50–70% as determined by phase-contrast microscopy. Intact pea chloroplasts were isolated by the procedure of Mills & Joy (1980), except that bovine serum albumin was omitted from the Percoll solution. Briefly this involved homogenization of 14-day-old-pea leaf and stem tissue in ice-cold buffer [50mM-Tricine/KOH (pH7.9)/330mM-sorbitol/2mM-EDTA/1mM-$MgCl_2$].

Approx. 30ml of homogenate per centrifuge tube was underlayered with 11–12ml of Percoll solution [50mM-Tricine/KOH (pH7.9)/40% (v/v) Percoll/300mM-sorbitol] and centrifuged for 5min at 1800*g*. The supernatant was removed by aspiration to leave a chloroplast pellet. This was judged to contain 90–95% intact chloroplasts by phase-contrast microscopy.

Chloroplasts were osmotically lysed by resuspending the pellets in 50mM-sodium phosphate (pH7.4)/10mM-NaCl/33mM-sorbitol for 30min on ice. This fraction constituted the total chloroplast fraction and was free of intact chloroplasts as determined by phase-contrast microscopy.

Sub-chloroplastic fractionation

The osmotically lysed chloroplasts were centrifuged at 4000*g* for 1min to separate the 'stromal' fraction from the pellet of class II chloroplasts. During washing experiments this was repeated twice and the supernatants from three such washes retained to give three successive stromal fractions; the final pellet was also retained as washed class II chloroplasts.

Lettuce class II chloroplasts were fractionated by a procedure similar to that described by Andersson & Akerlund (1978). After resuspension in lysis buffer (above), class II chloroplasts were disintegrated by passing them twice through a Yeda press at a N_2 gas pressure of 7MPa. The resultant suspension was centrifuged for 30min at 40000*g*, the supernatant retained, and the pellet resuspended in more lysis buffer.

Enzyme and protein assays

Fatty acid synthetase activity was assayed in a reaction mixture containing NADH (0.67μmol), NADPH (0.67μmol), ATP (4.5μmol), *E. coli* acyl-carrier protein (1mg) and 0.05μCi of [2-^{14}C]-malonyl-CoA. Between 0.2 and 0.5ml of the various fractions were added with potassium phosphate buffer, pH7.0, to a volume of 1.0ml (0.05M final concn.). Incubations were carried out in triplicate at 25°C for 2 or 4h with continuous shaking and the reaction was terminated by the addition of 0.1ml of 60% (w/v) KOH. Hydrolysis was carried out for 30min at 70°C. After acidification with 0.3ml of 20% (w/v) H_2SO_4, 4ml of chloroform were added, the aqueous layer removed and the chloroform phase washed with 3ml of water before being evaporated to dryness under N_2. Fatty acid methyl esters were prepared by transmethylation with 1ml of 2.5% (v/v) H_2SO_4 in anhydrous methanol at 70°C for 2h. Samples of the light-petroleum (b.p. 60–80°C) extract were then taken for radioactivity measurement and analysis by g.l.c. The methyl esters were separated in 15% (w/v) EGSS-X on Chromosorb W AW (80–100 mesh) columns (1.5m × 0.4cm internal diameter) with a Pye 104 gas chromatograph coupled to Panax gas-flow proportional counter. Pentadecanoate was used as internal standard. All radioactive samples were counted for radioactivity in a scintillant consisting of PCS (Amersham–Searle; The Radiochemical Centre, Amersham, Bucks., U.K.)/xylene (2:1, v/v). The efficiency of counting was corrected for each sample by the external-standards method.

Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) was assayed by measuring incorporation of $^{14}CO_2$ under N_2 into acid-stable products as described by Somerville & Ogren (1980). The enzyme in the various fractions was first heat-activated by the method of Kawashima *et al.* (1971) by heating at 50°C for 20min. $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13) was assayed spectrophotometrically by measuring the formation of NADPH at 340nm. The method employed was that of Byers (1982) for yeast, except that $NADP^+$ was used instead of NAD^+.

Latent Ca^{2+}-dependent ATPase is reported to be cold-labile (Kamienetzky & Nelson, 1975), and lettuce chloroplasts were therefore isolated at a compromise temperature of 10°C when ATPase activity was to be measured. Activity was assayed by the method of Kamienetzky & Nelson (1975) after an initial heat activation of the various fractions at 64°C for 4min in the presence of 3mM-dithiothreitol. P_i release from ATP was determined by the method of Baginski *et al.* (1967).

Other assays

Protein was determined (after first removing interfering pigments) by the modified Lowry procedure of Schacterle & Pollack (1973). A 1ml portion of protein-containing solution was mixed with 0.2ml of 48% (final concn. 8%, w/v) trichloroacetic acid and 1.2ml of diethyl ether. The top layer was removed after centrifugation at 10000*g* for 1min. After vigorous agitation of the protein solution it was re-sedimented at 20000*g* for 10min. The pellet was redissolved in 1M-NaOH and portions of this taken for assay by the method of Schacterle & Pollack (1973). Bovine serum albumin fraction V (Sigma Chemical Co.) was included as internal standard in some determinations and was used also for calibration of assays.

Total chlorophyll was determined by the method of Arnon (1949).

Results and discussion

The fractionation and coincidence of lettuce chloroplastic fatty acid synthesis *de novo* with chloroplast marker enzymes is summarized in Table 1. On osmotic lysis, approx. 30% of fatty-acid-synthetic activity is released into the stromal fraction. Only 7.7% of the ribulose-1,5-bisphosphate carboxylase activity is released by this relatively gentle disruption procedure. This is to be expected for this carboxylase in view of reports of its association with the outer surface of the lamellar membrane (Miller & Staehelin, 1976). Considering the solubility of $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase (Heber *et al.*, 1967), it is surprising that only 10.0% of the total chloroplast activity is released on osmotic lysis. However, a yield of only 11.6% of protein suggests poor release of the stromal contents by this procedure (stromal protein usually constitutes about half of the chloroplast protein). This sort of phenomenon has been observed in other chloroplast preparations on osmotic lysis (Nikolau *et al.*, 1981; Mohan & Kekwick, 1980). The latent Ca^{2+}-dependent ATPase found in plant chloroplasts is associated with the thylakoid membranes (Kamienetzky & Nelson, 1975). This is confirmed by the relatively minor release of total chloroplast activity on osmotic lysis (or disintegration in the Yeda press).

Table 1. *Distribution of fatty acid synthesis, marker enzymes, protein and chlorophyll in fractions from lettuce chloroplasts*

The chloroplast fraction was isolated, osmotically lysed and the particulate (class II chloroplasts) and soluble (stroma) fractions prepared as described in the Materials and methods section. The class II chloroplasts were subjected to Yeda-press treatment and the resultant suspension centrifuged at 40000*g* for 30min. Details of enzymic and other assays are given in the Materials and methods section. Results are means±S.D. for independent experiments (each carried out in triplicate) the number of which is shown in parentheses. Activities are compared with those of the original chloroplast fraction after osmotic lysis. For latent Ca^{2+}-dependent ATPase measurements, chloroplasts were isolated and fractionated by the same methods, but at a temperature of 10°C with 50mM-imidazole buffer instead of sodium phosphate. Endogenous lipid was measured by determining the total fatty acyl content by g.l.c. Original activities on a mg-of-protein basis were: fatty acid synthesis (59.3 ± 6.6 pmol·h^{-1}·mg^{-1}), RuBPCase (4.70 ± 0.26 μmol·h^{-1}·mg^{-1}), (NADP)GAPDH (3.05 ± 0.22 μmol·h^{-1}·mg^{-1}), latent Ca^{2+}-dependent ATPase (0.67 ± 0.12 μmol·h^{-1}·mg^{-1}), chlorophyll (92 μg·mg^{-1}), endogenous lipid (312 μg·mg^{-1}). Abbreviations used: RuBPCase, ribulose-1,5-bisphosphate carboxylase; (NADP)GAPDH, NADP-linked glyceraldehyde-3-phosphate dehydrogenase; tr, <0.5%.

Fraction	Distribution (% of total chloroplast fraction)						
	Fatty acid Synthesis	RuBPCase	(NADP)GAPDH	Latent Ca^{2+}-dependent ATPase	Protein	Chlorophyll	Endogenous lipid
	(4)	(3)	(2)	(2)	(4)	(1)	(1)
Particulate (class II chloroplasts)	67.2±7.4	83.4±15.8	84.4±2.8	95.5±2.8	90.4±8.1	92.1	95.0
Soluble (stromal fraction)	29.7±10.5	7.7±1.0	10.0±3.4	1.5±1.1	11.6±2.7	7.9	3.9
Yeda-press fractions							
40000*g* pellet	2.6±1.1	5.9±0.5	5.3±4.2	89.7±5.7	56.2±4.8	92.1	96.0
40000*g* supernatant	62.7±6.4	79.2±15.4	82.0±0.1	3.7±1.0	35.3±3.3	tr.	1.0

The more severe treatment of the Yeda press causes destacking of thylakoids with the formation of membrane vesicles (Andersson & Akerlund, 1978). The total fatty-acid-synthetic activity *de novo* released into soluble fractions by distintegration in the Yeda press was over 92%. This was a similar fractionation to that of ribulose-1,5-bisphosphate carboxylase and NADP-linked glyceraldehyde-3-phosphate dehydrogenase, two marker enzymes for chloroplast stroma (Wildman, 1963; Heber *et al.*, 1967) and is in good agreement with the results of Mudd & McManus (1962). On Yeda-press treatment a further 35% (46% in all) of the chloroplastic protein is solubilized, yet there is essentially no further release of latent Ca^{2+}-dependent ATPase activity, chlorophyll or endogenous lipid.

The high proportion of total fatty-acid-synthetic activity associated with broken (class II) chloroplast fractions and released by Yeda-press treatment implied that there was extensive resealing of chloroplasts (and formation of envelope vesicles) after osmotic shock (Table 1). To check this by another method, we subjected the lettuce class II chloroplasts to repeated washing (see the Materials and methods section). Repeated washing caused significant release of protein, fatty-acid-synthetic activity and $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase activity (Table 2). The amounts of the two activities in the various fractions were approximately the same, although somewhat more fatty-acid-synthetic activity remained associated with the washed chloroplast fraction than NADP-linked glyceraldehyde-3-phosphate dehydrogenase. The results in Table 1 indicated that the enzymes involved with fatty acid synthesis were loosely associated with thylakoid membranes and, in contrast with Yeda-press treatment (Table 1), were not fully released by osmotic-shock treatment and repeated washing.

A similar osmotic-shock treatment and repeated washing of pea chloroplasts yielded rather analogous results (Table 3). Some 94% of the total recovered fatty-acid-synthetic activity from [^{14}C]malonyl-CoA was released from the chloroplasts, and all detectable $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase was localized in the first two soluble fractions. Again, as with lettuce, Yeda-press treatment of class II pea chloroplasts released all of the remaining fatty-acid-synthetic activity into the soluble fraction (results not shown). These results from lettuce and pea chloroplasts explain fully the observations reported in the literature that some fatty acid synthesis is associated with chloroplast membranes (e.g. Weaire & Kekwick 1975; Kannangara

Table 2. *Effect of successive washing on protein release from lettuce chloroplasts*

Osmotically lysed lettuce chloroplasts were subjected to three successive washings as described in the Materials and methods section. Results are the means ± S.D. of triplicate measurements. Fatty acid synthesis in original chloroplast fractions was 62.4 ± 3.2 pmol/h per mg of protein and (NADP)GAPDH activity was 2.5 ± 0.7 μmol/h per mg of protein. For the abbreviation, see Table 1.

Fraction	Distribution (% of activity of total chloroplast fraction)		
	Fatty acid synthesis	(NADP)GAPDH	Protein
Supernatant			
I	51.0 ± 1.6	52.3 ± 3.5	21.9 ± 3.7
II	28.8 ± 2.8	31.8 ± 1.6	9.4 ± 1.6
III	3.3 ± 0.5	4.1 ± 1.9	9.4 ± 0.5
Washed particulate fraction (Class II chloroplasts)	10.9 ± 0.5	3.0 ± 2.6	55.9 ± 8.4

Table 3. *Distribution of fatty acid synthesis in various fractions from pea chloroplasts*

Details of incubations and analysis were as described for pea in the Materials and methods section. Results are means ± S.D. for three independent experiments each carried out in triplicate. For other details and definition of (NADP)GAPDH, see Table 1. Abbreviation: n.d., not detected. Activities in the total chloroplast fraction were 82.8 ± 8.1 pmol/h per mg of protein for fatty acid synthesis and 6.7 ± 1.2 μmol/h per mg of protein for (NADP)GAPDH.

	Distribution (% of activity in total chloroplast fraction)	
	Fatty acid synthesis	(NADP)GAPDH
Supernatant		
I	85.7 ± 1.6	98.9 ± 1.8
II	8.9 ± 5.6	8.8 ± 5.7
III	2.6 ± 1.3	n.d.
Washed particulate fraction (Class II chloroplasts)	5.5 ± 0.8	n.d.

et al., 1973). In fact, such activity does not appear to be an integral feature of the membranes and can be released by appropriate treatment.

In order to check that none of the 'soluble' fatty-acid-synthetic activity released from chloroplasts was located on small membrane fragments (such as those from envelopes or thylakoids), we subjected the pea chloroplast stromal fraction to high-speed centrifugation (Table 4). It will be seen clearly that although about 16% of the total glyceraldehyde-3-phosphate dehydrogenase activity recovered was resedimented by such treatment, negligible amounts of fatty acid synthesis were found in the particulate fraction.

Comparing the release of enzymes in lettuce and pea chloroplasts on osmotic-shock treatment and subsequent washing, the enzymes of pea chloroplasts seem much more readily released. This phenomenon is most likely explained by the original higher intactness of the pea chloroplast fraction (see the Materials and methods section), the total lettuce chloroplast fraction possibly exhibiting between 30 and 50% stromal depletion.

The products of fatty acid synthesis *de novo* in various fractions of chloroplasts are shown in Table 5. In pea chloroplasts there was no significant difference in the pattern of fatty acids formed between the original chloroplast preparation and the stromal fraction. The major labelled fatty acids were stearate (50–56%), palmitate (24–28%) and myristate (10–12%), with smaller proportions of oleate, dodecanoate and palmitoleate.

With lettuce chloroplasts (Table 5), in agreement with previous observations with isolated chloroplasts and [^{14}C]malonyl CoA as substrate (cf. Stumpf, 1980), the main products were stearate (48%) and palmitate (37%), with myristate, palmitoleate and oleate present as minor products only. As expected, polyunsaturated fatty acids were hardly synthesized at all, their formation in isolated chloroplasts being dependent on intact organelles and, therefore, permeable precursors as well as other special conditions (cf. Jones & Harwood, 1980). In lettuce preparations, on osmotic lysis and separation of the stroma from lamellar material, some differences in the pattern of radiolabelled products was seen. In agreement with the results of Weaire & Kekwick (1975), who used avocado (*Persea americana*) mesocarp plastids, the stromal fraction produced a greater percentage of stearate under the same conditions of incubation as for the total chloroplast fraction.

Table 4. *Effect of high-speed centrifugation on the distribution of fatty acid synthesis and glyceraldehyde-3-phosphate dehydrogenase activities in fractions from pea chloroplast stroma*

Fatty acid synthesis was measured by the incorporation of radioactivity from [2-^{14}C]malonyl-CoA as described in the Materials and methods section. Preparation of pea chloroplasts, stromal fraction and assay of NADP-linked glyceraldehyde-3-phosphate dehydrogenase were carried out as described in the Materials and methods section. Results represent means ± S.D. for an experiment carried out in triplicate. (NADP)GAPDH is defined in Table 1. Activities in the original stroma fraction were 70.9 pmol/h per mg of protein for fatty acid synthesis and 6.6 μmol/h per mg of protein for (NADP)GAPDH.

Activity	Distribution of activity (% of total recovered)		Recovery of activity (% of that in original stroma fraction)
	105000*g* pellet	105000*g* supernatant	
Fatty acid synthesis	4.4 ± 0.2	95.6 ± 0.2	123 ± 14.0
(NADP)GAPDH	15.5 ± 3.0	84.5 ± 3.0	111 ± 4.0

Table 5. *Products of fatty acid synthesis from [2-^{14}C]malonyl-CoA in chloroplast fractions of lettuce and pea*

Fractions were prepared as described in Table 1. Results are expressed as means ± S.D. for four (lettuce) and three (pea) independent experiments, each carried out in triplicate. Abbreviations: tr., trace (< 0.5%); n.d., none detected. Fatty acids are designated with the number before the colon indicating the carbon chain length and that afterwards showing the number of double bonds. The double-bond positions were not determined in these experiments, but previous data obtained with lettuce and pea showed that $C_{16:1}$ was palmitoleic acid and $C_{18:1}$ was oleic acid.

	Fraction	Fatty acid ...	Distribution of radioactivity (% of total ^{14}C-labelled fatty acids)					
			$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$
Pea	Total chloroplast fraction		3.7 ± 2.2	11.1 ± 5.3	27.6 ± 5.1	2.7 ± 2.1	50.8 ± 3.1	6.4 ± 5.3
	Stroma		3.1 ± 0.9	10.7 ± 6.7	24.5 ± 4.3	2.7 ± 1.3	55.4 ± 5.4	4.9 ± 2.8
Lettuce	Total chloroplast fraction		n.d.	6.1 ± 3.1	37.3 ± 5.9	3.5 ± 2.2	47.5 ± 5.0	6.7 ± 1.1
	Class II chloroplast		1.3 ± 0.7	7.7 ± 3.3	56.3 ± 4.7	6.0 ± 5.1	23.4 ± 7.3	5.6 ± 1.9
	Stroma		tr.	2.6 ± 0.3	14.6 ± 1.8	tr.	81.4 ± 2.9	1.3 ± 1.1
	40000*g* supernatant (After Yeda-press treatment)		n.d.	n.d.	10.5 ± 3.6	n.d.	75.9 ± 3.5	13.6 ± 0.1

This was at the expense of production of palmitoleate, palmitate and oleate. The proportional increase in stearate labelling may have been due to a relative increase in the activity of a C_{16}-fatty-acid-specific β-oxoacyl acyl-carrier protein synthase noted by Shimikata & Stumpf (1982) to be present in stromal fractions. The change in pattern of fatty acids made could also have been due to the presence (or absence) of associated enzymes such as acyltransferases. However, studies with various fractions from pea did not reveal any large changes in the labelling of lipid classes that would have indicated such acyltransferase activity (Sanchez & Harwood, 1981). Lettuce chloroplasts do not synthesize as much oleate *in vitro* as do many other plant chloroplasts (cf. Burton & Stumpf, 1966; Jones & Harwood, 1980). It was therefore not surprising that oleate labelling from [^{14}C]malonyl-CoA was poor in all fractions (Table 5).

As seen in Table 5, loss of the C_{16}-fatty-acid-specific β-oxoacyl acyl-carrier protein synthase on osmotic-shock treatment caused a decrease in the relative labelling of C_{18} fatty acids by the washed class II fraction. Stearoyl-acyl-carrier protein (Δ^9) desaturase has been purified as a soluble enzyme from safflower (*Carthamus tinctorius*) seeds (McKeon & Stumpf, 1982). From Table 5 (and also data from Weaire & Kekwick, 1975) it appears that this enzyme may be loosely associated with thylakoid membranes. Thus the stromal fraction after osmotic-shock treatment hardly synthesized any labelled oleate, whereas the supernatant fraction after Yeda-press lysis showed 13.6% of the total radioactivity in oleate. Since the latter supernatant showed a proportional increase in [^{14}C]stearate formed, increased stearoyl-acyl-carrier protein substrate for the desaturase was probably available and the proportion of oleate labelled was higher than in class II or total chloroplast fractions. Thus, in general, the differences in labelling patterns of various chloroplast fractions may be a result of the balance between substrate availability and enzyme complement in each particular fraction.

The results described here for pea and lettuce chloroplasts show that the fatty-acid-synthetic activity *de novo* measured by incorporation from [^{14}C]malonyl-CoA is soluble (i.e. located in the stroma). Although some fatty-acid-synthetic activity appeared to be loosely associated with membrane fractions (and, presumably *in vivo* with the thylakoids), there was no evidence that chloroplast membranes could contribute to the activity observed in microsomal fractions from pea (e.g. Sanchez & Harwood, 1981) and other plant tissues (e.g. Cassagne & Lessire, 1978).

The financial support of the Science and Engineering Research Council is gratefully acknowledged.

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