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# FATTY ACID METABOLISM BY A PARTICULATE FRACTION FROM GERMINATING PEAS

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**Abstract**—The incorporation of fatty acids into lipid fractions was studied using a high-speed particulate fraction from germinating peas (*Pisum sativum* cv Feltham First). The acids were incorporated principally into the acyl-CoA and polar lipid fractions, with unsaturated fatty acids labelling the latter fraction at higher rates than saturated substrates. α-Hydroxylation also occurred. Oleate from oleoyl-CoA or oleoyl-acyl carrier protein was incorporated into polar lipids faster than from ammonium oleate. The incorporation of fatty acids into polar lipids was dependent on the presence of CoA and ATP in the medium and on the consequent generation of acyl-CoA's. Time-course studies and experiments when two fatty acid substrates were added consecutively confirmed the role of acyl-CoA's in the transfer of acyl groups to phospholipids. Although CoA was necessary when acyl-CoA's had to be generated, high concentrations were found to inhibit the rate of acyl transfer. The results are discussed in terms of the 'switching mechanism' for controlling the fate of fatty acids in the plant cell.

## INTRODUCTION

The microsomal fraction from germinating peas contains a *de novo* fatty acid synthetase, a palmitate elongase and a stearate elongase [1, 2]. The elongation systems are able to utilize fatty acyl substrates when these were supplied exogenously as phosphoglycerides, especially as phosphatidylcholine [3, 4]. During the course of our studies on these reactions we noted that the levels of CoA and acyl carrier protein were extremely important in controlling both the rate of synthesis of fatty acids and also the lipid class to which they were esterified [5, 6].

The results from the above experiments [5] were interpreted as being consistent with the so-called 'switching mechanism' proposed by Stumpf and co-workers (cf. refs. [7, 8]) by which newly synthesized fatty acids are transferred to complex lipids through the mediation of CoA and the formation of acyl-CoA's. This process has assumed more importance recently in view of accumulating evidence that plant tissues synthesize fatty acids and acyl lipids through a co-operation of organelles (cf. refs. [7–11]). Thus, for example the chloroplast is capable of the photosynthetic production of palmitate and oleate from mitochondria-derived acetate [12] and the oleoyl-acyl carrier protein (oleoyl-ACP) donates its acyl group to CoA by the combined action of a stromal acyl-ACP hydrolase [13] and an envelope long-chain acyl-CoA synthetase [14]. The acyl group can then be rapidly transferred to phospholipids of the endoplasmic reticulum [15, 16] where further modifications may take place [9, 10].

The pea microsomal fraction is a useful system with which to study fatty acid metabolism since it contains enzymes for fatty acid synthesis and elongation in addition to the various hydrolases, thioester synthetases and transferases necessary for transfer of newly-formed fatty acids into lipids. Thus, one can study the sequence of metabolic reactions involved in the 'switching mechanism' using a single sub-cellular fraction. Because our previous report, concerning the acyl products formed during fatty acid synthesis by pea microsomes, had provided indirect evidence in favour of the 'switching mechanism' [5] we have now extended the work by the use of exogenous fatty acids and acyl-thioesters. The results of such experiments are reported here.

## RESULTS AND DISCUSSION

Several radiolabelled fatty acids and acyl-thioesters were tested in order to study the incorporation of acyl residues into different lipid fractions (Table1). All of the six fatty acids tested were incorporated into acyl-thioester and acyl lipid fractions with only small amounts of substrate fatty acids remaining at the end of the incubation. This experiment was carried out under the conditions usually used for fatty acid synthesis [5] and a 4 hr incubation period represented equilibrium conditions for the labelling of the various fractions (see below). However, the relative rates of incorporation of individual fatty acids into the fractions over shorter times gave a similar pattern.

If individual fatty acids have to be activated to acyl-thioesters (probably acyl-CoA's) in order for them to be transferred to complex lipids [7] then the results are in keeping with this idea, since oleoyl-CoA was a more effective precursor of radioactive polar lipids than ammonium oleate. The results also agree with the observed specificity of acyl-CoA synthetase from the chloroplast envelopes of spinach leaves [14]. This enzyme was more active with unsaturated substrates and less active with

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Table 1. Incorporation of different fatty acids into lipid classes by pea microsomal fraction

| Radiolabelled precursor | No. of expts | Distribution of radioactivity (% total [$^{14}$C]lipids) | | | | |
|---|---|---|---|---|---|---|
| | | Acyl-CoA's | Acyl-ACP's | FFA | NL | PL |
| Laurate | 2 | 59 ± 17* | 6 ± 1* | 8 ± 2 | 15 ± 13 | 12 ± 1*** |
| Myristate | 1 | 35 | 2 | 11 | 15 | 37 |
| Palmitate | 4 | 33 ± 2 | 2 ± 1 | 6 ± 2 | 14 ± 3 | 45 ± 5 |
| Stearate | 5 | 32 ± 4 | 2 ± 1 | 21 ± 3** | 11 ± 3 | 34 ± 4* |
| Oleate | 2 | 19 ± 4** | 2 ± 1 | 8 ± 1 | 13 ± 4 | 58 ± 7 |
| Linoleate | 2 | 16 ± 4** | 2 ± 1 | 7 ± 2 | 8 ± 6 | 67 ± 10* |
| Oleoyl-CoA | 2 | 11 ± 2** | 3 ± 1 | 6 ± 3 | 6 ± 3* | 74 ± 2*** |
| Oleoyl-ACP | 1 | 4 | 20 | 1 | 2 | 72 |

Assays were carried out with complete incubation system (see Experimental) for 4 hr. Analysis of the lipid classes was by the method of Mancha *et al.* [28]. Fatty acids were added as their ammonium salts.

Abbreviations: acyl-ACP, acyl-acyl carrier proteins; PL, polar lipids; NL, neutral lipids; FFA, non-esterified fatty acids.

Results are expressed as means ± s.e. for individual experiments carried out in triplicate. Patterns were compared with those for palmitate and statistical analyses made by Student's *t* test for paired experiments. * $P < 0.1$; ** $P < 0.025$; *** $P < 0.01$.

stearate, in agreement with the relatively high levels of stearic acid remaining at the end of our assays (Table 1). Interestingly, oleoyl-ACP was a better precursor of radiolabelled phospholipids than ammonium oleate and, since oleoyl-ACP is probably unable to donate oleate directly to such lipids [15], an acyl-CoA:acyl-ACP acyl transferase may be involved.

Within the polar lipid fraction, all major phospholipids (cf. ref. [3]) were labelled, but phosphatidylcholine contained 60–70% of the total radioactivity of this fraction as expected from previous results on polar lipid labelling in pea and other plant tissues (cf. refs. [7, 17]). α-Hydroxy fatty acids which are formed from exogenous fatty acids by the microsomal fractions were only present in small amounts in the polar lipid fraction shown in Table 1. This was presumably because of the presence of pyridine nucleotides in the assay system (see below).

As expected, in view of the accumulation of newly-synthesized medium chain fatty acids as acyl-ACP's in the pea microsomal fraction [3], lauric acid was incorporated to a significant level in this fraction (Table 1). Furthermore, this result agreed with the esterification of laurate into acyl-ACP's which was observed in developing oil seeds [18]. However, all other precursors were also incorporated in detectable amounts into the acyl-ACP fraction. This suggests that a long chain acyl-ACP synthetase (albeit with low activity) may be present in pea microsomes as reported for a similar fraction from leek [19].

The presence of acyl-thioesterase activity in the microsomal fraction also leads to the liberation of fatty acids from acyl-thioesters (Table 1). Acyl-CoA thioesterase activity has been reported in several plant systems (e.g. refs. [15, 20–22]), may consist of more than one enzyme [13] and has a broad substrate specificity [23]. The activity from spinach leaves is localized in the chloroplast envelope [23] and proplastid envelope membranes are probably present in our microsomal fraction. On the other hand, the acyl-ACP thioesterase from avocado has the oleoyl derivative as its principal substrate [13] and is a soluble enzyme.

Since the first experiments (Table 1) had been carried out with the complete incubation medium (as used for fatty acid synthesis measurements), the effect of individual cofactors on acyl transfer was tested. Because the incorporation of stearate into stearoyl-ACP was small (Table 1) the effect of ACP was not tested. In the absence of cofactors, considerable amounts of stearate were oxidized at the α-carbon to produce 2-hydroxystearate. Accordingly, hydroxy fatty acids are shown separately from the other polar lipids which consisted, therefore, entirely of phosphoglycerides (Table 2). In the presence of CoA and ATP, stearoyl-CoA was formed and labelling of the acyl lipid fractions was increased considerably. This change also caused a decrease in the labelling of 2-hydroxystearate and in the label remaining as stearic acid. The formation of 2-hydroxystearate was decreased to very low levels in the presence of pyridine nucleotides which, otherwise, had little effect on the transfer of stearate into other lipid fractions. The decrease of unesterified stearic acid in the presence of cofactors may, in addition to having been due to acyl-CoA synthetase activity, have been due to a decrease in acyl-CoA thioesterase activity as described for leek microsomes [20] in the presence of nucleotides.

Since the above results were in keeping with a role for acyl-CoA's in the transfer of fatty acids into the complex lipids of the microsomal fraction, we next examined the transfer of stearate with time. Two substrates were compared—ammonium stearate and stearoyl-CoA. The results are shown in Fig. 1. When ammonium stearate was used in the absence of cofactors a small transfer of [$^{14}$C]stearate into complex lipids and acyl-thioesters was seen which increased with time. However, by far the major reaction was the α-hydroxylation of stearate (Fig. 1A). When ATP and CoA were added a rapid formation of stearoyl-CoA was seen, followed by labelling of the polar and neutral lipid fractions (Fig. 1B) which had reached a steady state after *ca* 2 hr. Finally, the transfer of stearate from stearoyl-CoA into acyl lipids was very rapid and had reached equilibrium by 15 min (Fig. 1C). The generation of unesterified fatty acids in the latter case and the

Table 2. The effect of the addition of different cofactors on the incorporation of stearate into different lipid fractions

| Addition (final concn) | Distribution of radioactivity (% total [$^{14}$C]lipids) | | | | |
|---|---|---|---|---|---|
| | Acyl-CoA's | FFA | NL | OH-FA | PL |
| None | 3±tr | 34±9 | 2±1 | 48±3 | 13±2 |
| CoA (0.5 mM) | 2±tr | 28±2 | 4±1 | 54±2 | 12±2 |
| CoA (0.5 mM), ATP (3 mM) | 26±3 | 7±1 | 10±1 | 13±2 | 44±3 |
| CoA (0.5 mM), ATP (3 mM), MgCl (1 mM) | 28±3 | 12±2 | 10±1 | 8±3 | 42±6 |
| CoA (0.5 mM), ATP (3 mM), MgCl (1.0 mM), NADH (0.67 mM), NADPH (0.67 mM) | 26±3 | 19±2 | 9±1 | 2±1 | 44±5 |

The microsomal fraction was incubated (2–4 mg protein/ml) in 0.03 mM potassium phosphate buffer, pH 7, at 25° for 4 hr. Analysis of lipid fractions was as described in the Experimental. For abbreviations and other details see the legend to Table 1. Results are the means ± s.e.s of three independent experiments carried out in triplicate.

Abbreviations: tr, trace (< 0.5%); OH-FA, hydroxy fatty acids.

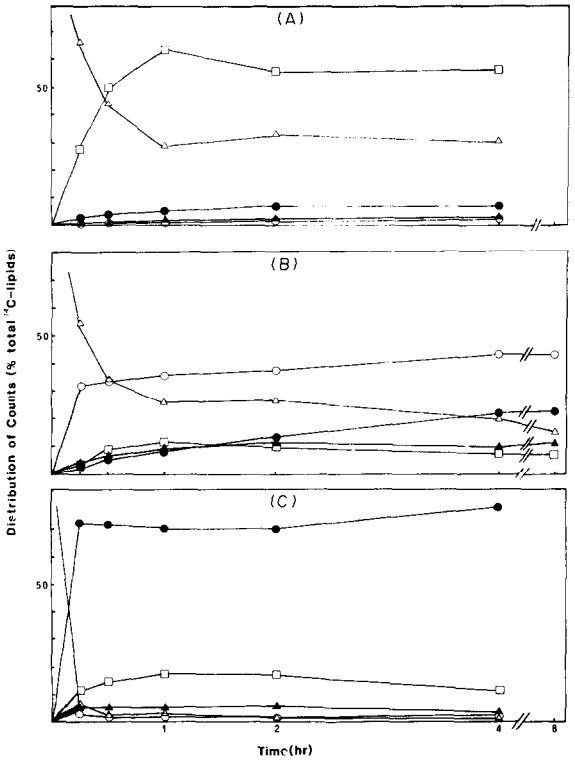


Fig. 1. Time-course of the incorporation of stearate into different lipid fractions. Incubations were carried out in 0.03 M potassium phosphate buffer pH 7 as described in the Experimental. Samples were taken at the times indicated and incorporation into (○) acyl-CoA's, (●) polar lipids, (△) unesterified fatty acids, (▲) neutral lipids and (□) hydroxy fatty acids determined. Means ± s.e. are shown. (A) [1-$^{14}$C]Stearic acid (0.1 μCi) was added as the ammonium salt and no further additions made. (B) 0.05 μCi ammonium [1-$^{14}$C]stearate was used as substrate. ATP (1 mM) and CoA (1 mM) were also present. (C) 0.1 μCi [1-$^{14}$C]stearoyl-CoA was used as substrate and no further additions made. Results are the average of duplicates, (A) and (C), or of triplicates (B) with s.d.s of less than 5% and a microsomal protein concentration of 3.2 mg/ml.

formation of hydroxystearate [which probably requires an unesterified fatty acid substrate (cf. ref. [24])] undoubtedly reflect the acyl-CoA thioesterase activity of the microsomal fraction which was referred to above. Taken together, the results in Fig. 1 agree well with the observations on phosphatidylcholine labelling from stearate substrates in leek microsomes [20] and are in agreement with the postulate that acyl-CoA's are involved in the transfer of fatty acids to complex lipids [7, 15, 22].

We had previously noted that the CoA levels in incubations played a critical role in the transfer of newly-synthesized fatty acids into different lipid classes as well as affecting the rate of total synthesis [5, 6]. Accordingly, we tested the effect of CoA concentrations at two different ATP levels (Table 3). It will be seen from the results that the transfer of stearate to polar lipids was impaired by high concentrations of CoA. At 1 mM ATP this decrease was accompanied by an increase in the accumulation of radioactivity in stearoyl-CoA, possibly because CoA acted as an end-product inhibitor of the acyl transfer reaction. At 5 mM ATP, the generation of stearoyl-CoA appeared to be rate-limiting and there was less metabolism of substrate at high CoA concentrations.

Because the accumulation of radioactivity in different lipid fractions was time-dependent (Fig. 1) and because high concentrations of CoA reduced the labelling of phospholipids (Table 3), we tested whether CoA would impair acyl transfer to phospholipids over an extended time period. The addition of exogenous CoA (2 mM) was found to markedly slow the rate of transfer of [$^{14}$C]oleate from carrier-free [$^{14}$C]oleoyl-CoA into phospholipids. However, because there was the possibility that dilution of substrate could have taken place in the presence of CoA, due to the generation of oleoyl-CoA from endogenous oleic acid, the experiment was repeated using 0.5 mM [$^{14}$C] oleoyl-CoA. It will be seen clearly in Fig. 2 that exogenous CoA slowed the transfer of oleate from oleoyl-CoA into phospholipids in keeping with the results in Table 3. Under these conditions generation of unlabelled oleoyl-CoA was very small (calculated from the level of unesterified oleic acid) and could only account, at maximum, for a 5% dilution of substrate. There is no doubt, therefore, that CoA produces a pronounced inhibition of

Table 3. The effect of CoA and ATP on the incorporation of stearate into different lipid classes

| Addition (final concn) | Distribution of radioactivity (% total [$^{14}$C]lipids) | | | | |
|---|---|---|---|---|---|
| | Acyl-CoA's | FFA | NL | OH-FA | PL |
| ATP (1 mM), CoA (0.1 mM) | 19 ± 1 | 26 ± 7 | 8 ± 1 | 9 ± 2 | 38 ± 8 |
| ATP (1 mM), CoA (0.5 mM) | 29 ± 1 | 28 ± 7 | 7 ± tr | 10 ± 1 | 26 ± 6 |
| ATP (1 mM), CoA (2.0 mM) | 32 ± 2 | 27 ± 5 | 5 ± tr | 13 ± 3 | 23 ± 8 |
| ATP (1 mM), CoA (5.0 mM) | 37 ± 2 | 33 ± 10 | 3 ± 1 | 12 ± 1 | 15 ± 3 |
| ATP (5 mM), CoA (0.1 mM) | 19 ± 6 | 33 ± 9 | 4 ± 2 | 9 ± 2 | 35 ± 6 |
| ATP (5 mM), CoA (0.5 mM) | 20 ± 8 | 37 ± 7 | 5 ± 3 | 9 ± 1 | 29 ± 6 |
| ATP (5 mM), CoA (2.0 mM) | 20 ± 6 | 43 ± 6 | 5 ± 3 | 8 ± 2 | 24 ± 8 |
| ATP (5 mM), CoA (5.0 mM) | 23 ± 1 | 52 ± 8 | 5 ± 3 | 8 ± 2 | 12 ± 4 |

Incubations were carried out as described in the legend to Table 2. Results are the means ± s.e.s of two independent experiments carried out in triplicate. For abbreviations see the legends to Tables 1 and 2.

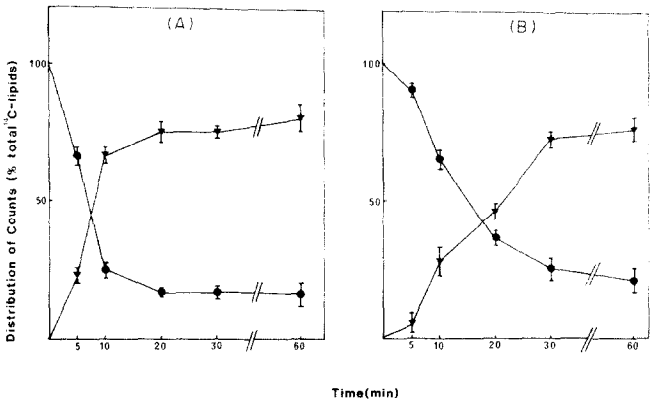


Fig. 2. The effect of exogenous CoA on the transfer of oleate from oleoyl-CoA to polar lipids. Incubations were carried out in 0.03 M potassium phosphate buffer, pH 7 with 2.1 mg microsomal protein/ml and 1 mM ATP as described in the Experimental. 0.05 $\mu$Ci [1-$^{14}$C]oleoyl-CoA was used per tube as substrate and the incorporation into (▼) polar lipids and other lipid fractions (less than 6% of total) determined. Disappearance of substrate (●) with time was also measured as described in the Experimental. (A) Control; (B) in the presence of 2 mM CoA. Means ± s.e.s ($n$ = 2) are shown.

acyl transfer into phospholipids under the experimental conditions used.

Although the data described so far all fit with the hypothesis that acyl-CoA's are involved as mediators in the transfer of fatty acids into acyl lipids [7], the small amount of transfer in the absence of exogenous CoA (Table 2) raised the possibility that transfer could take place without the use of acyl-CoA's. So, to shed more light on this point, an experiment was set up in which two radiolabelled precursors, [$^{14}$C]palmitate and [$^{14}$C]stearate, were used consecutively (Table 4). In both incubations, the pattern of fatty acids incorporated into acyl lipids was dictated by that of the acyl-CoA's and not that of the unesterified fatty acids. The second precursor, in each case, was rapidly incorporated into acyl-CoA's and, more slowly, into complex lipids. Thus, it seems that radiolabelled fatty acids when added to the medium are activated to acyl-CoA's before incorporation into lipids as discussed above.

Another point of interest concerning the role of CoA in lipid metabolism concerns the stearate elongase activity of the pea microsomal fraction. This fraction synthesizes very long chain fatty acids by addition of 2C units from malonyl-CoA [2]. In leek microsomes such a reaction was demonstrated using stearoyl-CoA as a substrate [25] but attempts to elongate stearoyl-CoA using pea microsomes were unsuccessful. Instead, stearoyl-phosphatidylcholine seemed to be an effective substrate [3] though as discussed in that paper and later [17], cleavage of the ester bond must take place for elongation since the addition is at the carboxyl end of the acyl chain. The accumulation of very long chain fatty acids in the acyl lipid fractions and not as acyl CoA's [5, 6] was in agreement with the involvement of phospholipids in stearate elongation. In the present

Table 4. Effect of palmitate or stearate on the transfer of the other fatty acid into lipid classes by pea microsomal fraction

| | | Distribution of radioactivity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Acyl CoA's | | | Complex Lipids | | | FFA | | |
| | Time (min) | (% total) | 16:0 (% FAs) | 18:0 | (% total) | 16:0 (% FAs) | 18:0 | (% total) | 16:0 (% FAs) | 18:0 |
| (a) Pre-incubation with [$^{14}$C]stearate followed by [$^{14}$C]palmitate addition | 0 | 26±4 | — | 100 | 37±5 | — | 100 | 37±4 | — | 100 |
| | 5 | 23±1 | 33 | 67 | 17±2 | 8 | 92 | 60±1 | 55 | 45 |
| | 10 | 24±2 | 39 | 61 | 17±3 | 11 | 89 | 60±4 | 44 | 56 |
| | 20 | 25±tr | 33 | 67 | 19±3 | 11 | 89 | 56±3 | 48 | 52 |
| | 40 | 25±3 | 40 | 60 | 26±1 | 16 | 84 | 49±5 | 42 | 58 |
| | 180 | 27±2 | 48 | 52 | 37±4 | 23 | 77 | 36±7 | 46 | 54 |
| (b) Pre-incubation with [$^{14}$C]palmitate followed by [$^{14}$C]stearate addition | 0 | 44±1 | 100 | — | 10±tr | 100 | — | 46±2 | 100 | — |
| | 5 | 28±tr | 81 | 19 | 20±tr | 92 | 8 | 52±tr | 14 | 86 |
| | 10 | 23±3 | 70 | 30 | 21±3 | 89 | 11 | 56±1 | 11 | 89 |
| | 20 | 28±2 | 68 | 32 | 21±1 | 89 | 11 | 51±3 | 10 | 91 |
| | 40 | 29±1 | 63 | 37 | 25±1 | 76 | 24 | 45±1 | 9 | 91 |
| | 180 | 26±3 | 59 | 41 | 30±4 | 62 | 38 | 44±8 | 7 | 93 |

Incubations were carried out as described in the Experimental except that acyl carrier protein and pyridine nucleotides were omitted. The microsomal fraction was pre-incubated with radiolabelled fatty acid for 45 min before addition of a second radiolabelled fatty acid and the beginning of the time-course. Carrier-free fatty acids were used and these were added as the ammonium salts (66 000 dpm [$^{14}$C]stearate; 56 000 dpm [$^{14}$C]palmitate). Samples were removed at the time intervals indicated and analysed as detailed in the Experimental. Figures represent the means ±s.e.s for two independent experiments.

series of experiments no elongation of any exogenous fatty acid or acyl-thioester was ever detected even when conditions were such as to allow rapid rates of elongation from [$^{14}$C]malonyl-CoA. However, both exogenous palmitate and exogenous stearate were rapidly α-hydroxylated. These α-hydroxy fatty acids were probably produced by the same α-oxidation system which was responsible for the formation of odd-chain length fatty acids by the pea microsomal fraction [5]. It may also be the same enzyme which was previously reported in the high speed supernatant from germinating peas [26] since α-oxidation activity in plant systems has been reported to be present in both microsomal and soluble fractions [24].

In conclusion, therefore, the above results provide further evidence for the mediation of acyl-CoA's in the transfer of fatty acids to complex lipids. The use of the pea microsomal fraction has proved especially useful in this regard because all the necessary enzymes, including those of *de novo* fatty acid synthesis, are present in the same subcellular preparation. Where comparisons with other plant tissues are available, the results described here are in good agreement and provide further support for the existence of a 'switching mechanism' [7] which controls the final destination of fatty acids in plant tissues.

## EXPERIMENTAL

*Materials.* Pea (*Pisum sativum* cv Feltham First) seeds were purchased from Asmer Seeds, Leicester, U. K., and were surface-sterilized and germinated as previously described [3]. After 24 hr germination pea seeds were homogenized and the microsomal fraction was isolated as previously detailed [3]. Enzyme marker analysis showed that the microsomal fraction contained *ca* 80% of the total recovery of typical endoplasmic reticulum enzymes, such as glucose-6-phosphatase, UDPase or unspecific esterase, but only small amounts of mitochondrial enzymes and no detectable chlorophyll.

[1-$^{14}$C]Lauric acid (specific radioactivity, 0.55 GBq/mmol), [U-$^{14}$C]palmitic acid (14.9 GBq/mmol), [1-$^{14}$C]palmitic acid (2.2 GBq/mmol), [1-$^{14}$C]stearic acid (2.1 GBq/mmol), [1-$^{14}$C]oleic acid (2.2 GBq/mmol), [1-$^{14}$C]linoleic acid (2.1 GBq/mmol), [1-$^{14}$C]stearoyl-CoA (1.8 GBq/mmol) and [1-$^{14}$C]oleoyl-CoA (2.1 GBq/mmol) were purchased from Amersham International, Amersham, U.K. [1-$^{14}$C]Myristic acid (specific radioactivity 1.7 GBq/mmol) was a kind gift from Dr. N. J. Russell of this department and [1-$^{14}$C]oleoyl-ACP was a kind gift from Professor P. K. Stumpf, University of California, Davis, U.S.A. All fatty acid substrates were checked for purity by GC and acyl thioesters by TLC before use. Acyl carrier protein was isolated from *Escherichia coli* [27].

*Incubations.* Unless otherwise stated the reaction mixtures contained NADH (0.67 μmol), NADPH (0.67 μmol), ATP (3 μmol), *E. coli* ACP (1 mg), 200 000 dpm of carrier-free radiolabelled substrate and 0.4 ml (2–4 mg protein) of microsomal fraction made up to a total vol. of 1 ml with KPi buffer, pH 7.0 (0.03 M final concn). Incubations were carried out at 25° in triplicate with continuous shaking for 4 hr. Fatty acids were converted to their ammonium salts and suspended in $H_2O$ by bath-sonication before use.

*Lipid analysis.* Analysis of the reaction products was carried out by the method of ref. [28]. Analysis was also carried out by extracting acyl lipids [29] and separating them by TLC on Si gel G plates with petrol (bp 60–80°)–$Et_2O$–HOAc (50:50:1) as first solvent followed (after drying at room temp. under $N_2$) by $CHCl_3$–MeOH–HOAc–$H_2O$ (170:30:20:7) run up to the monoacylglycerol marker band. Lipid bands were located under

UV after spraying with 0.005% aq. Rhodamine 6G and eluted and counted for radioactivity as previously described [3, 6]. Quench corrections were made by the external standard channels-ratio method. Lipid bands were routinely identified by co-chromatography with authentic markers but all the major lipids present in the pea microsomal fraction had been completely identified previously [3]. Fatty acid analysis by radio-GC was carried out as previously described [5, 6]. Identities of individual fatty acids had been previously established [3, 4, 26] and routine identifications were made by reference to standards.

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