

The Fractionation of the Fatty Acid Synthetase Activities of Avocado Mesocarp Plastids

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1. The range of fatty acids formed by preparations of ultrasonically ruptured avocado mesocarp plastids was dependent on the substrate. Whereas [^{14}C]palmitate and [^{14}C]oleate were the major products obtained from [$1\text{-}^{14}\text{C}$]acetate and [$1\text{-}^{14}\text{C}$]acetyl-CoA, the principal product from [$2\text{-}^{14}\text{C}$]malonyl-CoA was [^{14}C]stearate. 2. Ultracentrifugation of the ruptured plastids at 105000g gave a supernatant that formed mainly stearate from [$2\text{-}^{14}\text{C}$]malonyl-CoA and to a lesser extent from [$1\text{-}^{14}\text{C}$]acetate. The incorporation of [$1\text{-}^{14}\text{C}$]acetate into stearate by this fraction was inhibited by avidin. 3. The 105000g precipitate of the disrupted plastids incorporated [$1\text{-}^{14}\text{C}$]acetate into a mixture of fatty acids that contained largely [^{14}C]palmitate and [^{14}C]oleate. The formation of [^{14}C]palmitate and [^{14}C]oleate by disrupted plastids was unaffected by avidin. 4. The soluble fatty acid synthetase was precipitated from the 105000g supernatant in the 35-65%-saturated- $(\text{NH}_4)_2\text{SO}_4$ fraction and showed an absolute requirement for acyl-carrier protein. 5. Both fractions synthesized fatty acids *de novo*.

It was concluded from our earlier study (Weaire & Kekwick, 1975) that the major site of fatty acid biosynthesis in the mesocarp of the avocado pear (*Persea americana*) is the plastid fraction. Whereas the major product of incorporation of [$1\text{-}^{14}\text{C}$]acetate into fatty acids by the intact plastids is oleate, ultrasonic disintegration resulted in the emergence of stearate-synthesizing activity, which predominated after a short period of ultrasonic treatment. Ultracentrifugation of the plastid sonicate yielded a supernatant which incorporated [$1\text{-}^{14}\text{C}$]acetate and [$2\text{-}^{14}\text{C}$]malonyl-CoA into a mixture of fatty acids, 90% of which was stearate. This supernatant resembled in some respects the soluble fatty acid synthetase activity attributed by Yang & Stumpf (1964) to the cytoplasm of the avocado mesocarp. In view of the apparent change of the products of fatty acid synthetase activity on rupture of the plastids, and of the similarity of the fatty acid synthetase activity of the 105000g supernatant of the disrupted plastids to that found by Yang & Stumpf (1964) in the 140000g supernatant of the whole avocado mesocarp homogenate, an attempt has been made to resolve the fractions concerned in the synthesis of palmitate, oleate and stearate in the avocado plastids.

Some of the work described has already been outlined in a preliminary communication (Weaire & Kekwick, 1973).

Materials and Methods

Special chemicals

The source of the radiochemicals and the methods of preparations of CoA esters and the *Escherichia coli* acyl-carrier protein were as given previously (Weaire & Kekwick, 1975). Avidin was obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K., the concentration being calculated on the basis that 1 unit of avidin binds 1 μg of biotin and that 1 mol of avidin binds 3 mol of biotin.

Avocado preparations

Avocado pears were obtained from local retailers and were used on the day of purchase. The plastids were prepared by the procedure given by Weaire & Kekwick (1975) and were disintegrated in a Mullard ultrasonic disintegrator at 360 W for 5 s. Subsequent ultracentrifugation and fractionation with $(\text{NH}_4)_2\text{SO}_4$ was carried out at 4°C.

Fatty acid synthesis

The incubation mixture used for the study of fatty acid synthesis contained, in addition to the avocado preparation and radioactive substrates, details of which are given for each experiment, 30 μmol of

NaHCO₃, 20 μmol of ATP, 2 μmol of MnCl₂, 50 nmol of CoA, 200 nmol of NADH, 200 nmol of NADPH, made up to a total volume of 1.5 ml with a solution that contained 0.3 M-sucrose, 20 mM-Tris-HCl buffer, pH 7.2, 20 mM-potassium phosphate buffer, pH 7.2, 10 mM-KCl, 0.5 mM-EDTA and 0.75 mg of bovine serum albumin/ml. The rate of incorporation of the ¹⁴C-labelled substrate into fatty acids was proportional to the protein concentration of the incubation medium when allowance was made for the added bovine serum albumin and the total incorporation was linear for the first 60 min of incubation, which was carried out at 30°C.

Analysis of radioactive fatty acids

Details of the procedure for the g.l.c. analysis of fatty acids and the assay of radioactivity have been given previously (Weaire & Kekwick, 1975).

Protein analysis

Protein was measured by the biuret procedure after removal of interfering pigments, as described by Weaire & Kekwick (1975).

Results

Plastid preparations from the avocado mesocarp were disintegrated by ultrasonic treatment, and the ruptured plastids were centrifuged at 105 000g for 20 min. The incorporation of [¹⁻¹⁴C]acetate, [¹⁻¹⁴C]acetyl-CoA and [²⁻¹⁴C]malonyl-CoA into fatty acids by the sonicate and the derived precipitate and supernatant was assayed. Table 1 shows the radioactive fatty acids that were formed from [¹⁻¹⁴C]acetyl CoA and [¹⁻¹⁴C]acetate by the sonicate and the two derived fractions. Although the total sonicate incorporated the two substrates into the range of fatty acids found in the avocado plastid lipids (for

composition see Weaire & Kekwick, 1975) the relative incorporations of the two radioactive substrates into the different fatty acids differed somewhat. The proportion of the total radioactivity of [¹⁻¹⁴C]acetyl-CoA incorporated into fatty acids found in palmitate (27%) was consistently somewhat lower than that of [¹⁻¹⁴C]acetate so incorporated (37%) but the proportion of the [¹⁻¹⁴C]acetyl-CoA incorporated into oleate (47%) was consistently higher than that of the [¹⁻¹⁴C]acetate (32%) so incorporated. Although these differences were reproducible their significance was not clear, particularly when it is seen that the precipitate incorporated these two substrates into a similar range of fatty acids and that the proportion of the radioactivity derived from each substrate present in the various fatty acids agreed closely. A more striking and much more significant difference is seen in the fatty acids formed from these two substrates by the 105 000g supernatant, about 65% of the total radioactivity of [¹⁻¹⁴C]acetyl-CoA or [¹⁻¹⁴C]acetate incorporated into fatty acids being found in stearate. Table 2 shows that the principal fatty acid formed from [²⁻¹⁴C]malonyl CoA by all three preparations was stearate (small amounts of [¹⁴C]palmitate and oleate were also formed by the 105 000g precipitate). Although the stearate-forming 105 000g supernatant had a much greater specific synthetic activity with [²⁻¹⁴C]malonyl-CoA than with [¹⁻¹⁴C]acetate as substrate, the corresponding activity of the parent sonicate and the 105 000g precipitate was greater with [¹⁻¹⁴C]acetate as substrate. These observations suggested that centrifugation of the plastid sonicate had resulted in a partial fractionation of two fatty acid synthetase activities; a particulate system utilizing [¹⁻¹⁴C]acetate and [¹⁻¹⁴C]acetyl-CoA to form a mixture of fatty acids in which oleate and palmitate predominated, and a soluble system utilizing [²⁻¹⁴C]malonyl-CoA in preference to [¹⁻¹⁴C]acetyl-CoA and [¹⁻¹⁴C]acetate to form a mixture of fatty acids of which 90% was stearate.

Table 1. Comparison of fatty acid synthesis from [¹⁻¹⁴C]acetate and [¹⁻¹⁴C]acetyl-CoA by fractions from sonicated plastids

Mixtures containing [¹⁻¹⁴C]acetate (0.5 μmol, 0.5 μCi) or [¹⁻¹⁴C]acetyl CoA (0.56 μmol, 1.19 μCi) were incubated with the standard mixture of cofactors given in the Materials and Methods section (CoA was omitted from mixtures containing acetyl-CoA) together with either sonicated plastids (0.98 mg of protein), 105 000g precipitate (0.63 mg of protein) or 105 000g supernatant (0.17 mg of protein) in a total volume of 1.5 ml for 1 h at 30°C.

Preparation	Fatty acid Substrate	Substrate incorporated (nmol/h per mg of protein)				
		C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2} + C _{18:3}
Sonicated plastids	[¹⁻¹⁴ C]Acetate	44	3.0	30	40	2.4
	[¹⁻¹⁴ C]Acetyl-CoA	24	2.7	24	52	1.7
Precipitate (105 000g)	[¹⁻¹⁴ C]Acetate	29	3.4	11	36	1.5
	[¹⁻¹⁴ C]Acetyl-CoA	13	15	7.1	33	2.8
Supernatant (105 000g)	[¹⁻¹⁴ C]Acetate	16	2.8	63	15	2.0
	[¹⁻¹⁴ C]Acetyl-CoA	7.0	1.1	31	5.8	0.6

Table 2. Comparison of fatty acid synthesis from [1-¹⁴C]acetate and [2-¹⁴C]malonyl-CoA by fractions from sonicated plastids
 Mixtures containing [1-¹⁴C]acetate (5.0 μmol, 0.5 μCi) and the standard mixture of cofactors (see the Materials and Methods section), and mixtures containing [2-¹⁴C]malonyl-CoA (0.25 μmol, 0.5 μCi) with the standard mixture of cofactors, but excluding ATP, CoA and MnCl₂, were incubated with the total sonicate (1.2mg of protein), the 105000g supernatant (0.17 mg of protein) or the 105000g precipitate (0.85 mg of protein) in a total volume of 1.5 ml for 1 h at 30°C.

Fraction	Fatty acid ... Substrate	Substrate incorporated (nmol/h per mg of protein)				
		C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2} +C _{18:3}
Sonicated plastids	[1- ¹⁴ C]Acetate	22	1.1	14	9.6	0.46
	[2- ¹⁴ C]Malonyl-CoA	1.5	0.57	19	2.8	0.67
Precipitate (105000g)	[1- ¹⁴ C]Acetate	15.4	1.0	6.7	15	0.66
	[2- ¹⁴ C]Malonyl-CoA	4.3	0.9	13	6.3	0.91
Supernatant (105000g)	[1- ¹⁴ C]Acetate	6.0	1.6	31	2.8	1.2
	[2- ¹⁴ C]Malonyl-CoA	11	3.2	210	3.5	5.6

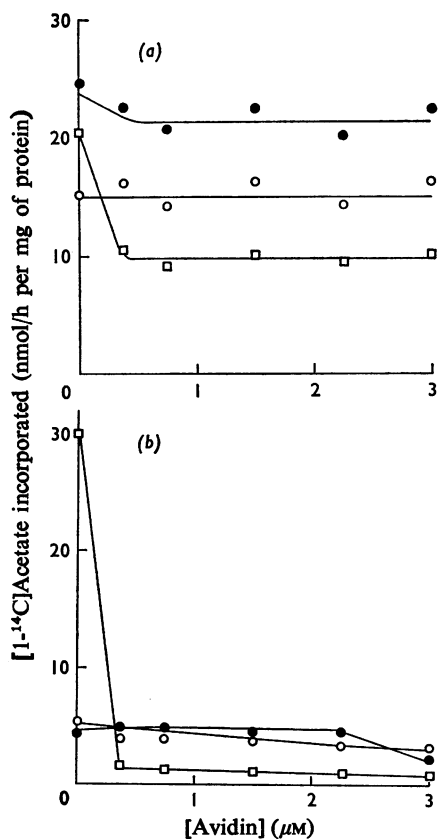


Fig. 1. Effect of avidin on the synthesis of fatty acids from [1-¹⁴C]acetate by (a) sonicated avocado plastids and (b) 105000g supernatant of sonicated avocado plastids

The incubation mixture contained 0.5 mmol of [1-¹⁴C]-acetate (0.5 μCi) and either 1.0 mg of total sonicate protein (a) or 0.37 mg of 105000g supernatant protein (b), in a total volume of 1.5 ml, which contained the standard mixture of cofactors given in the Materials and Methods section, and was incubated for 1 h at 30°C. ○, C_{16:0}; ●, C_{18:1}; □, C_{18:0}.

Further evidence concerning the possible presence of two fatty acid synthetase activities in disrupted plastid preparations was obtained by the use of avidin to inhibit the formation of malonyl-CoA from acetyl-CoA. Fig. 1 shows that the incorporation of [1-¹⁴C]acetate into stearate by the whole sonicate was selectively inhibited by avidin, the formation of [¹⁴C]oleate and [¹⁴C]palmitate being unaffected. The distribution of the radioactivity from [1-¹⁴C]acetate among the fatty acids was similar to that obtained when [1-¹⁴C]acetate was incubated with the 105000g precipitate in the absence of inhibitor. When the effect of avidin on the incorporation of [1-¹⁴C]acetate into fatty acids by the 105000g supernatant was studied, it was found that the formation of [¹⁴C]-stearate characteristic of this fraction was almost completely inhibited by 0.37 μM-avidin. However, as observed with the whole sonicate, the formation of [¹⁴C]palmitate and [¹⁴C]oleate, which was small in this fraction, was only inhibited to 50% by an eight-fold increase in avidin concentration. As would be expected, the incorporation of [2-¹⁴C]malonyl-CoA was unaffected by avidin.

Although the formation of [1-¹⁴C]stearate from [1-¹⁴C]acetate by the whole sonicate was selectively inhibited by avidin, omission of bicarbonate from the incubation medium resulted in an almost equal decrease in the formation of all ¹⁴C-labelled fatty acids (Table 3). It is, however, noteworthy (Table 3) that 1 mM-arsenite effected a selective inhibition of the incorporation of [¹⁴C]acetate into stearate.

The properties of the soluble fatty acid synthetase activity of 105000g supernatant were investigated. Fig. 2(a) shows that the incorporation of [1-¹⁴C]acetate and [2-¹⁴C]malonyl-CoA into fatty acids by this fraction was affected differently by the addition of ATP. The incorporation of [1-¹⁴C]acetate was stimulated by the addition of increasing amounts of ATP but, although the incorporation of [2-¹⁴C]-malonyl-CoA was increased by the addition of small amounts of ATP, concentrations in excess of 2 mM

Table 3. Effect of addition of arsenite and omission of bicarbonate on the biosynthesis of fatty acids by sonicated avocado plastids

Plastids were prepared and disintegrated by the standard procedure. Incubations contained the standard incubation mixture together with 1.4 mg of sonicate protein in a total volume of 1.5 ml.

Factor added or omitted	Fatty acid ... Total	[1- ¹⁴ C]Acetate incorporated (nmol/mg of protein)				
		C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2} +C _{18:3}
None	50.0	15.5	1.52	20.0	13.9	0.9
Bicarbonate omitted	15.7	6.6	0.52	4.44	3.3	0.33
1 mM-Arsenite added	30.0	15.1	1.7	5.0	7.0	0.4

Table 4. Fractionation of fatty acid synthetase activity in the supernatant (105 000g) obtained from avocado plastids

Mixtures containing either the 105 000g supernatant of the plastid sonicate (0.71 mg of protein), or the 0–35%-sadt.-(NH₄)₂SO₄ precipitate (0.19 mg of protein), the 35–65%-sadt.-(NH₄)₂SO₄ precipitate (0.21 mg of protein), or the 65–85%-sadt.-(NH₄)₂SO₄ precipitate (0.21 mg of protein) therefrom, together with [2-¹⁴C]malonyl-CoA (0.25 μM, 0.05 μCi), 30 μmol of HCO₃⁻, 0.2 μmol of NADH, 0.2 μmol of NADPH, 2.0 μmol of MnCl₂, in a total volume of 1.5 ml were incubated for 1 h at 30°C.

(a) Distribution of fatty acid synthetase

	Total protein (mg)	[2- ¹⁴ C]Malonyl-CoA incorporated (nmol/h per mg of protein)	Total activity (nmol incorporated/h)
Sonicated plastid supernatant (105 000g)	28.0	104	2910
0–35%-sadt.-(NH ₄) ₂ SO ₄ precipitate	7.50	Not detectable	—
35–65%-sadt.-(NH ₄) ₂ SO ₄ precipitate	13.3	160	2130
65–85%-sadt.-(NH ₄) ₂ SO ₄ precipitate	8.28	Not detectable	—

(b) Distribution of radioactivity among fatty acids formed by fractions

Fraction	Fatty acid ...	Percentage of total radioactivity in fatty acids		
		C _{16:0}	C _{18:0}	C _{18:1} +C _{18:2} +C _{18:3}
Sonicated plastid supernatant (105 000g)		3.8	91	5.2
35–65%-sadt.-(NH ₄) ₂ SO ₄ precipitate		3.5	91	5.5

were inhibitory. From this it might be inferred that some at least of the [1-¹⁴C]acetate was not incorporated into fatty acids via malonyl-CoA, for it is difficult to envisage why ATP should not inhibit the incorporation of malonyl-CoA formed endogenously from added [1-¹⁴C]acetyl-CoA in the same way that it inhibited the incorporation of exogenous [2-¹⁴C]-malonyl-CoA.

Fig. 2(b) shows that the incorporation of [2-¹⁴C]-malonyl-CoA into fatty acids by the 105 000g supernatant was to some extent dependent on acetyl-CoA, and it is therefore improbable that this fraction contained appreciable amounts of malonyl-CoA decarboxylase.

To obtain a preparation of the soluble fatty acid synthetase on which the effect of cofactors could be investigated, the 105 000g supernatant was fractionated with (NH₄)₂SO₄. Table 4(a) shows that, with [2-¹⁴C]malonyl-CoA as substrate, about 75% of the fatty acid synthetase activity of the 105 000g

supernatant was precipitated in the 35–65%-sadt.-(NH₄)₂SO₄ fraction, and this precipitate formed the same range of fatty acids from [2-¹⁴C]malonyl-CoA as the parent supernatant (Table 4b); that is, stearate was the principal fatty acid formed.

Analysis of the cofactor requirement for the synthesis of fatty acids by this (NH₄)₂SO₄ fraction showed (Table 5) an absolute requirement for both NADH and NADPH and an almost absolute requirement for acyl-carrier protein. Mn²⁺ ions, found to stimulate fatty acid synthesis by intact plastids (Weaire & Kekwick, 1975), had a somewhat inhibitory effect on fatty acid synthesis by this fraction. As would be expected from the observation that stearate was the principal fatty acid formed, fatty acid synthesis by this fraction was much more sensitive to inhibition by arsenite than was that by the parent 105 000g supernatant.

The series of experiments described above suggested the presence of two separable fatty acid synthetase

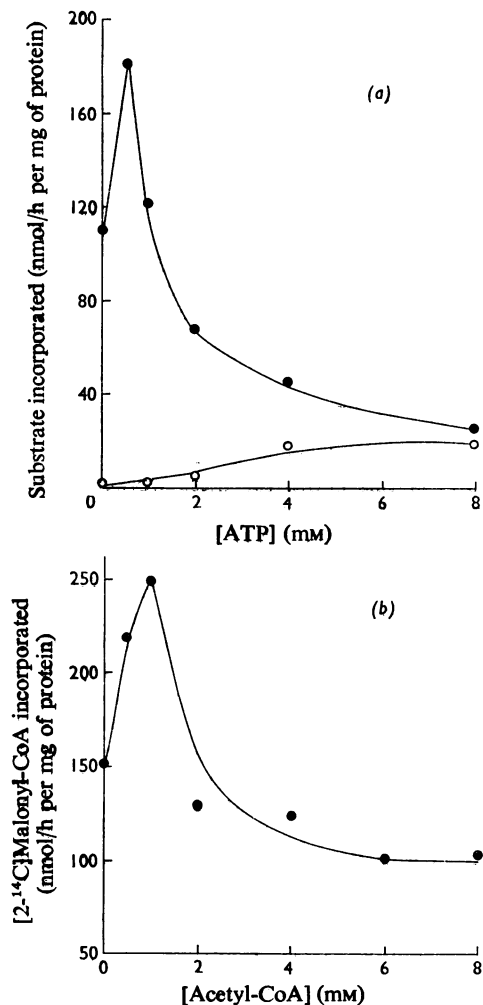


Fig. 2. (a) Effect of ATP on the incorporation of [2-¹⁴C]malonyl-CoA (●) and [1-¹⁴C]acetate (○), and (b) effect of acetyl-CoA on the incorporation of [2-¹⁴C]malonyl-CoA, into fatty acids by the 105 000g supernatant of disrupted plastids

Incubations contained either 500 nmol of [2-¹⁴C]malonyl-CoA (0.041 μCi) or 400 nmol of [1-¹⁴C]acetate (0.5 μCi). Incubations containing [1-¹⁴C]acetate contained the complete cofactor mixture given in the Materials and Methods section and those containing [2-¹⁴C]malonyl-CoA had the same cofactor mixture except that CoA and NaHCO₃ were omitted. The total volume of the mixture was 1.5 ml and it was incubated for 1 h at 30°C.

systems in the avocado plastid. Since it appeared that the formation of stearate was relatively unimportant in the intact plastid it was desirable to ascertain whether the isolated stearate-synthesizing system formed fatty acids *de novo* or whether it merely

Table 5. Cofactor requirement for, and effect of arsenite on, synthesis of fatty acids from [2-¹⁴C]malonyl-CoA by 35–60%-sadt.-(NH₄)₂SO₄ precipitate from sonicated plastid supernatant

The complete system contained 250 nmol of [2-¹⁴C]malonyl CoA, 35–60%-sadt.-(NH₄)₂SO₄ precipitate from the 105 000g supernatant of sonicated plastids (0.33 mg of protein), *E. coli* acyl-carrier protein (0.2 mg of protein), 30 μmol of HCO₃⁻, 10 μmol of ATP, 2 μmol of Mn²⁺, 50 nmol of CoA, 200 nmol of NADH, 200 nmol of NADPH made up to a total volume of 1.5 ml, which was incubated for 1 h at 30°C.

Factor omitted	[2- ¹⁴ C]Malonyl-CoA incorporated (nmol/h per mg of protein)
Complete system	167
Acyl-carrier protein	1.24
NADH	0.00
NADPH	0.00
Mn ²⁺	184
HCO ₃ ⁻	105
Complete system + 1 mM-arsenite	16.3

utilized preformed fatty acids for chain elongation with malonyl-CoA. Such preformed fatty acids might have remained associated with the synthetase during the (NH₄)₂SO₄ fractionation. The [¹⁴C]stearate formed from [1,3-¹⁴C]malonyl-CoA by the (NH₄)₂SO₄ fraction was subjected to decarboxylation by the Schmidt (1923) procedure, and the results were compared with similar decarboxylations of [¹⁴C]oleate and [¹⁴C]palmitate synthesized from [1-¹⁴C]acetate by the original sonicate. As shown in Table 6 the ratio of radioactivity present in the carboxyl carbon to that present in the rest of the molecule was consistent with synthesis *de novo* both of oleate and palmitate from [1-¹⁴C]acetate by the whole sonicate and of stearate from [1,3-¹⁴C]malonyl-CoA and [1-¹⁴C]acetate by the 105 000g plastid supernatant.

Discussion

The relation between substrate utilized and fatty acid product of the plastid sonicate shown in Tables 1 and 2 is similar to that found for a homogenate of the whole avocado mesocarp by Yang & Stumpf (1964). The principal product obtained by Yang & Stumpf (1964) from acetate or acetyl-CoA was palmitate and that from malonyl-CoA was stearate. Further, Yang & Stumpf (1964) found that the stearate-synthesizing activity was present in the 140 000g supernatant of the homogenate and considered that this soluble system was cytoplasmic in origin. The grounds on which we attribute this soluble fatty acid synthetase to the plastids have already been outlined (Weaire & Kekwick, 1975) and the similarity of the

Table 6. Distribution of radioactivity between carbon atoms of fatty acids formed from [1-¹⁴C]acetate and [1,3-¹⁴C]malonyl-CoA by preparations from sonicated avocado plastids

Incubations were carried out by using the cofactor mixtures and under the conditions given in Table 2. Radioactivity present in the carboxyl carbon was obtained from CO₂ produced by the Schmidt (1923) reaction; predicted values are those expected if synthesis from the radioactive substrate is *de novo*.

Preparation	Substrate	Fatty acid	Total radioactivity (d.p.m.)	Radioactivity in CO ₂ (d.p.m.)	Predicted radioactivity in CO ₂ (d.p.m.)	Recovery (% of predicted)
Plastid sonicate	[1- ¹⁴ C]Acetate	C _{16:0}	13950	1425	1745	81.6
Plastid sonicate	[1- ¹⁴ C]Acetate	C _{18:0}	7240	656	805	81.5
Plastid sonicate	[1- ¹⁴ C]Acetate	C _{18:1}	14080	1236	1561	79.2
Supernatant (105000g)	[1,3- ¹⁴ C]Malonyl-CoA	C _{18:0}	2590	243	283	84.5
Precipitate (105000g)	[1- ¹⁴ C]Acetate	C _{18:1}	8750	781	972	80.5

properties of the soluble stearate-synthesizing system, obtained from the plastid sonicate described above, to the soluble synthetase obtained from the 140000g supernatant of the whole mesocarp homogenate by Overath & Stumpf (1964) confirms this view. Fractionation of the 140000g supernatant of the mesocarp homogenate by Overath & Stumpf (1964) yielded a precipitate with 20–60% satd. (NH₄)₂SO₄ which apparently contained the synthetase, but to obtain activity it was necessary either to add a heat-stable component present in the 60–90% satd. (NH₄)₂SO₄ fraction, or a fraction from *E. coli* now known to contain the acyl-carrier protein. The implication of the acyl-carrier protein in fatty acid biosynthesis by the avocado mesocarp was subsequently confirmed by the isolation of this substance from the tissue by Simoni *et al.* (1967). The co-factor requirements of the preparation of Overath & Stumpf (1964) in respect of acyl-carrier protein, NADH and NADPH were very similar to those of the 35–65% satd. (NH₄)₂SO₄ fraction from the 105000g supernatant of the total plastid sonicate described above. There was, however, a difference in the products of the two preparations. Overath & Stumpf (1964) found that the principal fatty acid formed from malonyl-CoA by their 140000g supernatant was stearate, but the principal product of the derived 20–60% satd. (NH₄)₂SO₄ fraction was palmitate. As Table 3 shows, no such difference was observed between the fatty acids synthesized by the 105000g supernatant of the plastid sonicate and the 35–65% satd. (NH₄)₂SO₄ fraction derived therefrom; the proportions of the radioactive fatty acids formed by the two preparations were almost identical and the major product was stearate.

The results presented above indicate that there may be two distinct fatty acid synthetase systems in the avocado mesocarp plastids; a particulate system forming palmitate and oleate from acetate or acetyl-CoA, and a soluble system forming stearate from malonyl-CoA. The observations of Delo *et al.* (1971)

and of Ernst-Fonberg & Bloch (1971) have some bearing on this point, for these workers obtained evidence for the presence of two fatty acid synthetase systems in *Euglena gracilis*. Whereas etiolated (dark-grown) heterotrophic cultures of the alga were found to contain only a particulate acyl-carrier protein-independent fatty acid synthetase which formed mainly palmitate, the green autotrophic cells contained in addition a soluble fatty acid synthetase, present in the 105000g supernatant of the disrupted cells, which was precipitated in the 35–75% satd. (NH₄)₂SO₄ fraction. The soluble fatty synthetase was acyl-carrier-protein-dependent and synthesized mainly stearate, but the preferred substrate for both *Euglena* fatty acid synthetase systems was malonyl-CoA. The formation of the soluble fatty acid synthetase by greening cultures of *Euglena* was found by Ernst-Fonberg & Bloch (1971) to be inhibited by chloramphenicol, and it was therefore considered probable that protein for this system was synthesized on the 70S ribosomes of the chloroplast.

Although the avocado mesocarp cannot be considered to be a typical photosynthetic tissue the plastids isolated therefrom do contain chlorophyll and do appear to have a higher degree of organization than those from an obviously non-photosynthetic tissue such as those of cauliflower buds (Weaire & Kekwick, 1975). Activities concerned in photosynthesis can be detected in the avocado mesocarp plastids. A low ribulose diphosphate carboxylase activity has been detected (S. B. Mohan & R. G. O. Kekwick, unpublished work), and the plastids catalyse the Hill (1939) reaction (P. J. Weaire & P. E. Visser, unpublished work). On these grounds a comparison of the characteristics of the fatty acid synthetase activity of the avocado mesocarp plastids with those of normal chloroplasts may be justified. The synthetase system described above is similar to that obtained from butter-lettuce chloroplasts by Brooks & Stumpf (1966). Whereas extracted lettuce chloroplasts formed mainly palmitate and oleate

from acetate, ruptured chloroplasts formed stearate from malonyl-CoA. Similarly, the stroma of spinach chloroplasts was found by Kannangara *et al.* (1973) to form stearate from acetate and the lamellae from these chloroplasts formed a considerable proportion of oleate and palmitate from the same substrate.

The significance of, and the relation between, the two fatty acid systems is not clear. The particulate system may respond to illumination, for both Brooks & Stumpf (1966), with butter-lettuce chloroplasts, and Boardman & Stumpf (1970), with spinach chloroplasts, found increased oleate synthesis in the light. It might be inferred from the work of Nagai & Bloch (1965) who isolated a stearyl acyl-carrier protein desaturase system from *Euglena*, that the role of the soluble stearate-forming system is to produce stearate for subsequent desaturation. Time-course studies reported earlier (Weaire & Kekwick, 1975) do not, however, indicate a precursor-product relation between the [^{14}C]stearate and [^{14}C]oleate formed by whole plastids but it is possible that the small amounts of [^{14}C]stearate detected may be present in a metabolically inactive pool. The presence of a soluble stearate-forming system is clearly not peculiar to avocado chloroplasts but it is not clear whether it is an artifact of preparation which *in vivo* forms part of the acetate-utilizing particulate system synthesizing palmitate and higher unsaturated fatty acids. The particulate fatty acid synthetase system appears to be a tightly bound complex but whether its relative inactivity with malonyl-CoA and its insensitivity to avidin arise from the conformation of its constituent enzymes or

result from the functioning of a pathway not involving malonyl-CoA is not clear. This problem and that of the role of the soluble stearate-synthesizing system may be elucidated when the enzyme components of the particulate system have been successfully resolved.

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