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GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE REACTIONS AND 'EDIBLE OIL SYNTHESIS IN OIL PALM *(Elaeis guineensis)* TISSUE

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Acyltransferase enzymes are used in three of the four steps of the Kennedy pathway for storage lipid formation. Their specificities, especially those of the first two reactions involving glycerol-3-phosphate acyltransferase (GPAT) and 1-acylglycerol-3-phosphate acyltransferase (LPAAT), determine the acyl quality of triacylglycerol (TAG) to a significant extent. Therefore, we determined the characteristics of the acyltransferases in oil palm (Elaeis guineensis), one of the world's most important agricultural species and the most productive oil crop. Two tissue sources were used. Calli were established and used for in situ manipulation and labelling studies as well as a source of microsomal fractions for enzyme measurements. In addition, acetone powder was prepared from oil palm fruits (14-18 weeks after pollination) for enzyme purification. High speed particulate fractions isolated from mesocarp acetone powder or calli were incubated with [^{14}C]glycerol 3-phosphate and the formation of Kennedy pathway intermediates followed. Conditions were optimized with regard to substrate concentrations, etc. and the overall rate manipulated using temperature. GPAT was solubilized from particulate fractions of the acetone powder and calli. Optimal solubilization of GPAT activity using CHAPS treatment was achieved at 0.5% (w/v) concentration. Details of the purification procedure and properties of the solubilized enzyme are discussed.

INTRODUCTION

In recent years, interest in fatty acid synthesis and its relationship to the assembly of triacylglycerols (TAGs) in oil palm has increased appreciably. This is due largely to the realization that oil palm is the most productive oil crop. Due to increased availability of palm oil, it is thought desirable to be able to manipulate acyl quality in a precise fashion and so produce additional modified oils for market diversification and also to serve as feedstock for non-edible oil utilization.

In higher plants, TAGs are often stored as oil bodies in the seed and are formed in a manner essentially similar to that outlined for animals by Kennedy (Kennedy, 1961). The acylation reactions within the pathway are known to occur on the endoplasmic reticulum (Harwood and Griffiths, 19921. In the course of TAG biosynthesis, acyl groups are esterified at the *sn*-1, *sn*-2 and sn-3 positions of the glycerol backbone by sn-glycerol-3-phosphate acyltransferase (GPAT), 1 - acyl - *sn* - glycerol - 3 phosphate acyltransferase (LPAAT) and 1,2-diacylglycerol acyltransferase (DAGAT), respectively (Stymne and Stobart, 1987). The specificities of these acyltransferases play an important role in determining the final TAG composition. The formation of TAG and plant membrane lipids are likely to be controlled by a number of factors including the size and composition of the endogenous pool of acyl-CoAs, the developmental stage of the tissue and the activities and selectivities of key enzymes in the pathway (Ichihara, 1984; Stymne and Stobart, 1987). GPAT (EC 2.3.1.15) catalyzes the transfer of a fatty acid from the acyl donor to the sn-1 position of glycerol 3-phosphate to yield 1-acylglycerol-3-phosphate (LPA) (Roughan and Slack, 1982). LPAAT (EC 2.3.1.51) transfers a second fatty acid to the sn-2 position to produce phosphatidate (PA) (Griffiths et al., 1985). In plants, the first acylation step occurs at much slower rates than the second (Bafor et al., 1990). In this way, GPAT limits the amount of LPA available and, therefore, controls the rate of PA (and TAG) production. To produce novel crop varieties with economically valuable food and non-food uses (Topfer et al., 1995), knowledge about GPAT as the initial enzyme in the Kennedy pathway (which limits carbon entry to this pathway) is obviously needed.

As a first step to the above long-term goals, purification of GPAT is needed and yet, despite its importance, the endoplasmic reticulum GPAT has not been completely purified from any plant (Murata and Tasaka, 1997). Partly, this deficiency is because the enzyme is an integral membrane protein. In this paper, we describe *our* achievements in solubilizing and purifying GPAT from 14-week mesocarp acetone powders and calli. We believe that the information provided in this paper will be useful for future efforts to genetically manipulate oil palm.

MATERIALS AND METHODS

Plant Materials

Oil palm calli were obtained *from* Dr John Eeuwens of Unifield T.C.Ltd., Cambridge and maintained by subculturing every five weeks on Murashige and Skoog medium supplemented with 1-napthaleneacetic acid (11μM). Acetone powders were prepared from oil palm fruits (14-18 weeks after pollination) harvested by PORIM, Malaysia.

Chemicals

L-[U-^{14}C]Glycerol 3-phosphate, ammonium salt (specific activity = 5.59 GBq/mmol) was obtained from Amersham Pharmacia Biotech U.K. Ltd. (Amersham, Buckinghamshire, U.K.). All other chemicals and solvents were of the best available grades and purchased from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.), BDH (Poole, Dorset, U.K.), Boehringer Mannheim (Mannheim, Germany), Whatman International Ltd. (Maidstone, Kent, U.K.), Pall Filtron (Northborough, MA, USA) and Pharmacia Biotech (Uppsala, Sweden).

Lipid Extraction

Lipid products were extracted using a method with acidified water saturated butanol, adapted from Bjerve et al. (1974). An equal volume (2ml) of a mixture, containing lml butan-1-ol, 0.95ml distilled water and 0.05ml

acetic acid was added to the aqueous reaction mixture as one phase. After vortexing, this was followed immediately by addition of an equal volume (2ml) of butan-1-ol which was also vortex mixed. The phases were separated by spinning at 1000*g* for 7min in a bench top centrifuge and the upper butanol phase transferred to a clean glass tube for storage. The remaining aqueous phase (3ml) was extracted with an equal volume of artificial upper phase [made by mixing the above solvents (Bjerve et al., 1974) with an equal volume of water]. The upper phases were combined and stored under nitrogen. This procedure was designed to assure quantitative extraction of lysophospholipid. Radioactivity was determined in aliquots of the combined butanol extracts.

Lipid Analysis

Thin layer chromatography (TLC) was performed using me-coated silica-gel plates activated before use for one hour at 110°C. Polar lipids were separated using a solvent system of chloroform/methanol/acetic acid/ water (170:30:20:7, by vol.). Non-polar lipids were separated with a solvent system of petroleum ether (60-80, bp)/diethyl ether/acetic acid (80:20:1, by vol.). Lipids were separated using a one dimensional double development technique.

Lipid Identification

Neutral and polar lipids on TLC were visualized by spraying with 8-anilino-1-napthalenesulphonic acid (ANSA) in anhydrous methanol (0.2%, w/v) and viewing under UV light. Identification of individual lipids was by using specific colour reagents (Rates, 19721 and co-chromatography with authentic standards. In some instances, TLC plates were exposed to x-ray film for few days to record the individual bands before radioactivity determination.

Radioactivity Determination

Incorporation of radioactivity from radiolabelled precursors into individual lipid classes was determined using a Beckman 129 Rackbeta liquid scintillation counter. In some instances, bands revealed by ANSA were scraped and collected in plastic vials prior to addition of Optifluor scintillant. All samples were corrected for sample quenching using an external standard channels ratio method.

Preparation of High Speed Particulate Fractions

Acetone powder was made by homogenizing 1g fresh mesocarp with 5ml ice-cold acetone. After standing at 4°C for approximately 30min, the homogenate was filtered through a high speed vacuum (Pharmacia LKB). The powder was stored at -80°C. All the following steps were carried out at 4°C. Approximately, 50g of mesocarp acetone powder were ground for 30s using a precooled homogenizer in 250ml of Buffer A containing 0.32M sucrose in 20mM potassium phosphate buffer (pH 7.21. The same ratio of tissue (g): Buffer B (ml) containing 50mM HEPES (pH 7.2), 330mM sorbitol, 1mM $MgCl_2$, 3mM EDTA, 5mM β-mercaptoethanol, 0.1% BSA, 0.2% ascorbate and 1% PVPP was used for oil palm calli. Protease inhibitors (aminocaproic acid, benzamidine-HCl and phenylmethylsulfonylfluoride) were added to the buffers at final concentrations of 5mM, 1mM and 1mM, respectively. The homogenate was spun at 5000xg for 20min and the supernatant (with the floating fat layer removed) spun again at 18 OOOxg for 30min. The recovered 18 OOOxg supernatant was centrifuged at 105 OOOxg for one hour to yield a high speed particulate pellet. Pellets were resuspended in Buffer A or Buffer C (50mM HEPES, pH 7.2, 330mM sorbitol, 1mM dithiothreitol) using a pre-cooled glass homogenizer at a concentration of 2-4μg ul^{-1} protein buffer. Bradford reagent (Sigma) was used to assay the protein content. Bovine serum albumin was used as a standard. Aliquots were frozen at -80°C until required.

GPAT Assay

Incubations were carried out in 15ml Pyrex culture tubes for one hour with gentle shaking in a 30°C water bath. The standard incubation mixture contained L-[U-^{14}C]glycerol 3-phosphate (0.05μCi, 100nmol), 100μM oleoyl-CoA and 100μM palmitoyl-CoA, 1% BSA (fraction V,

fatty acid free) and 10mM EDTA. Reactions were made up to a final volume of 0.25ml with either 35mM HEPES (pH 7.2 to 8.2) or 0.1M potassium phosphate (pH 6.8 to 7.2) and the reactions were stopped at appropriate time points with equal volume of 0.15M acetic acid. Lipid products were extracted as described in lipid extraction.

GPAT Solubilization

High speed particulate fractions equivalent to a protein concentration of 2mg ml^{-1} were incubated with six solubilizing agents (CHAPS, Triton X-100, Tween 20, Tween 40, Tween 80 and urea) at different concentrations in a 50mM potassium phosphate buffer pH 7.2 containing 0.15M potassium chloride, 5mM aminocaproic acid and 1mM benzamidine-HCl. Solubilization was carried out for 30 to 60min at 4°C with gentle stirring. After the incubation, solubilized material was isolated by centrifuging at 105 000xg for one hour. The supernatant (containing solubilized GPAT) and pellet were assayed to measure their specific activities and recoveries. The supernatant was kept at 4°C and used within 24-hour for further purification of the enzyme.

GPAT Purification

All steps were carried out at 4°C. The solubilized material was applied to a column (1 x 15cm, Pharmacia) packed with diethylaminoethyl cellulose (DE52, Whatman) equilibrated with 5 vol. of 20mM Tris-HCl (pH 8.0) containing CHAPS (0.25%, w/v), 5mM amino caproic acid and 1mM benzamidine-HCl (Buffer D). Unbound protein was eluted with Buffer D (3 column vol.). The column was eluted stepwise with 40, 80 and 100mM NaCl in Buffer D. Fractions containing acyltransferase activity were combined and concentrated with a Macrosep Centrifugal Concentrator 10K (Filtron) and PD10 (Pharmacia). After purification, the protein fraction was applied to a second column (1.6 x 16cm, Pharmacia) packed with DE52 (Whatman) equlibrated with 5 vol. of Buffer D. Unbound protein was eluted with Buffer D (3 column vol.). Enzyme activity was eluted with a linear gradient from 0 to 100mM NaCl in Buffer D (flow rate 60ml hr^{-1}) using a HiLoad system (Pharmacia). The eluate was collected in 3ml fractions from which GPAT activity was determined. Peak fractions were pooled and concentrated as above. The concentrated fractions were directly applied to a Sephadex G 100 column (1 x 15cm) which had been equilibrated with 5 vol. of Buffer D. The column was eluted with buffer D (flow rate 18ml hr^{-1}). The eluate was collected in 1ml fractions from which GPAT activity was determined. Fractions with acyltransferase activity were pooled and concentrated as above, prior to addition of 20% (v/v) glycerol for storage at -80°C.

RESULTS AND DISCUSSION

The work presented here is primarily concerned with the ability of high speed particulate fractions from acetone powders of oil palm mesocarp or from oil palm cultures to synthesize storage lipid in vitro. Microsomal fractions from oil seeds possess all the necessary enzyme activities for *de novo* biosynthesis of TAG (Frentzen, 1986) and can, thus, be used as convenient in vitro systems. The use of acetone powders is a well-tried technique for preparing stable enzymes from lipid-rich tissues or membrane fractions. It was considered particularly useful for the present study because palm material had to be transferred to Cardiff in good condition. From the calli, high speed particulate fractions can be prepared fresh and these would have the usual properties and contain endoplasmic reticulum and other membrane fragments.

Characterization of GPAT Activity

Studies utilizing high speed particulate fractions from acetone powders of oil palm fruits (14-18 weeks) in *vitro* showed that incorporation of radioactivity from [^{14}C]glycerol 3-phosphate into lipids was high in young tissues (*Table* I). Nevertheless, high speed particulate fractions from calli were three times more active than acetone powders on a protein basis. However, due to limitations in the amount of oil palm cultures available, most of the characterization of GPAT activity was carried out

TABLE 1. A COMPARISON OF HIGH SPEED PARTICULATE FRACTION ACTIVITIES FROM OIL PALM TISSUES

Sample	Activity (nmol mid mg^{-1} protein)
Acetone powder of oil palm fruits	
14 weeks after pollination	1.42 ± 0.07
15 weeks after pollination	1.14 ± 0.06
18 weeks after pollination	0.48 ± 0.04
Oil palm cultures	3.93 ± 0.19

Data as means (n=3) ± s.d.

using 14-week oil palm mesocarp acetone powders. Radiolabel from [U-^{14}C]G3-P was incorporated into LPA, PA, diacylglycerol (DAG) and TAG in the presence of exogenous palmitoyl-CoA and oleoyl-CoA (Figure *1*). Temperature, as an important environmental parameter, was altered to investigate its effect on qualitative and quantitative aspects of TAG synthesis in oil palm tissues. In oil palm cultures, a rise of

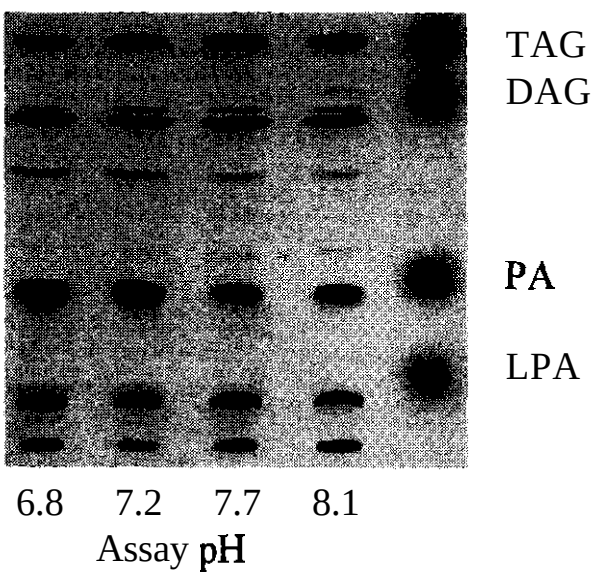

Figure 1. Autoradiograph of lipids extracted from incubations carried out for one hour at 30°C with shaking and which contained [^{14}C]G3-P (0.05μCi, 100 nmole), palmitoyl-CoA (25 nmole), oleoyl-CoA (25 nmole) and 50μg microsomal protein from 14-week mesocarp acetone powders in 0.1M potassium phosphate (pH as indicated) containing 1% BSA and 10mM EDTA in a final volume of 0.25ml. Lipids were extracted using a modified Bjerve procedure. The plate was exposed to Kodak x-ray film for four days.

10°C from 20°C caused changes in the labelling of complex lipids from [^{14}C]acetate, particularly *increased* the rate of TAG labelling, and more saturated fatty acid components accumulated (results not shown). These studies showed that lipid metabolism can be manipulated conveniently by temperature. Use of [^{14}C]G3-P for 14-week mesocarp acetone powder high speed particulate fractions showed that a rise of 10°C from 20°C did not affect significantly the total activity of the enzymes of the Kennedy pathway but that there was a significant decline at 40°C (*Table 2*). This change in temperature changed the pattern of labelling with an increase in the relative labelling of TAG at the expense of LPA. The effects of temperature during incubation presumably reflect changes in the properties of the individual enzymes of the Kennedy pathway.

Palmitate and oleate are the two most abundant fatty acids in oil palm (Rossell *et* al., 19851. Therefore, to study GPAT in cytoplasm, it was decided to add them in the incubations as their acyl-CoA esters. Figure 2 shows that palmitoyl-CoA, oleoyl-CoA or a combination of palmitoyl-CoA and oleoyl-CoA affected the incorporation of radioactivity from [^{14}C]G3-P into lipids. In the case of palmitoyl-CoA supplied as a single acyl-CoA source, acyltransferase activities increased with its level up to a concentration of 0.2mM, although higher concentrations may have been slightly inhibitory. The highest overall activity was found with the combination of oleoyl-CoA (0.1mM) and palmitoyl-CoA (0.05 to 0.25mM). Under these conditions, the total activity increased up to a palmitoyl-CoA concentration of 0.25mM. Clearly, although palmitoyl-CoA could be used by all the

TABLE 2. EFFECT OF TEMPERATURE ON THE INCORPORATION OF RADIOACTIVITY FROM [^{14}C]GLYCEROL 3-PHOSPHATE INTO LIPIDS BY HIGH SPEED PARTICULATE FRACTIONS FROM 14-WEEK MESOCARP ACETONE POWDERS

Temp.		Lipid class labelling (%)			
(°C)	Incorporation (nmol min^{-1} mg^{-1})	LPA	PA	DAG	TAG
20	1.01 ± 0.03	42 ± 1	14 ± 1	7 ± 1	37 ± 2
30	1.07 ± 0.05	23 ± 3	8 ± 1	10 ± 3	58 ± 2
40	0.80 ± 0.04	18 ± 2	8 ± 1	9 ± 1	65 ± 2

Data are expressed as means of duplicate incubations ± maximum/minimum of duplicate values

acyltransferases of the Kennedy pathway (and hence stimulate total labelling), the saturation concentration was reached at 0.2mM. In the case of oleoyl-CoA supplied as a single acyl-CoA source, acyltransferase activity increased slowly with its level up to a concentration of 0.25mM, although the total activity was much lower than that for palmitoyl-CoA. This showed that GPAT was unable to use oleate as well as palmitate. Increasing the levels of oleoyl-CoA up to a concentration of 0.2mM in the presence of palmitoyl-CoA increased the total activity. Increasing the concentration of oleoyl-CoA above 0.2mM resulted in an inhibitory effect. Thus, as for palmitoyl-CoA alone, there was some evidence for substrate inhibition (possibly a detergent effect) above 0.2mM. The preferential use of palmitate over oleate by the oil palm GPAT in *vitro* is interesting in view of the enrichment of palm TAG with palmitate at the *sn*-1 position.

Other optimal assay conditions for microsomal fractions are shown in ***Table 3. The*** data for glycerol 3-phosphate were fitted to the Michaelis-Menten equation to give apparent K_m values. These experiments identified the best tissue sources and optimal assay conditions for further purification experiments.

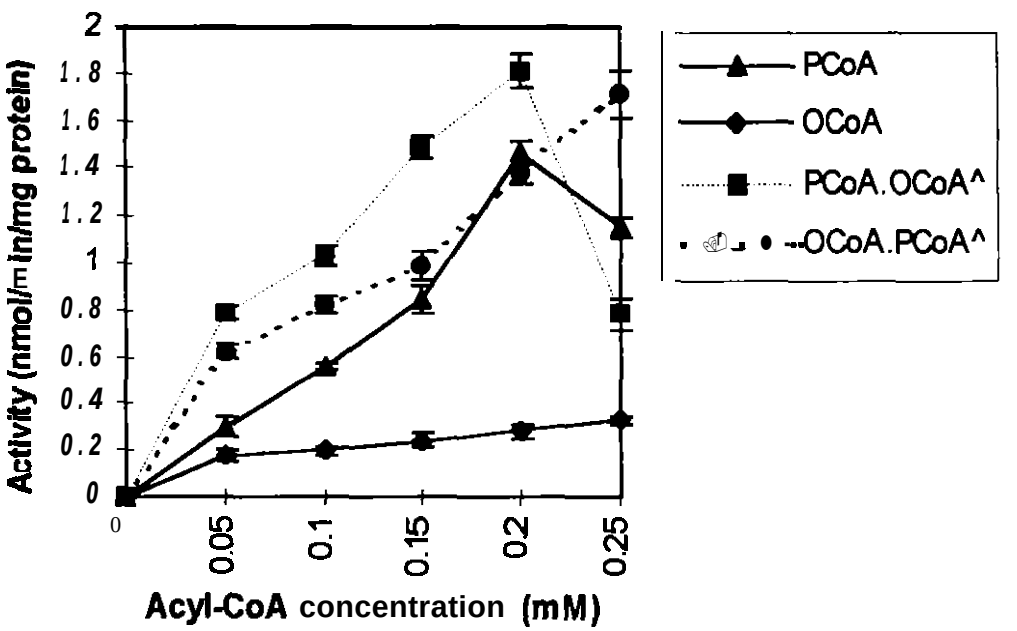

Figure 2. Effect of acyl-CoA concentration OR incorporation of radioactivity from [^{14}C]G3-P into lipids by high speed particulate fractions from 14-week mesocarp acetone powder. Data as means ± s.d. (n=3), PCoA (16:0-CoA: as indicated), OCoA (18:1-CoA: as indicated), PCoA.OCoA^ (16:0-CoA: 0.1mM; 18:1-CoA: as indicated) and OCoA.PCoA^ (18:1-CoA : 0.1mM; 16:0-CoA: as indicated).

TABLE 3. OPTIMAL INCUBATION CONDITIONS FOR MEASUREMENT OF GPAT ACTMTY IN HIGH SPEED PARTICULATE FRACTIONS OF 14-WEEK OIL PALM MRSOCARP ACETONE POWDER

Condition	Optimal value
Time (min)	up to 60
Protein (mg ml-')	up to 0.3
pH	6.8 to 7.2
EDTA (mM)	15
BSA (%, w/v)	0.75 to 1
Temperature (°C)	30
Acyl-CoA (mM)	0.2
G3P (mM)	(0.67) (1.11")

The standard incubation conditions are described in Materials and Methods. Values for apparent K_ms with G3-P as fitted to the Michaelis-Menten equation are shown in parentheses and (") for microsomal fractions of oil palm cultures.

Solubilization of GPAT

As a first step in membrane-bound enzyme purification, alternative methods of solubilization were tested for their ability to release GPAT activity from microsomal fractions. The presence of enzyme activity as solubilized membrane protein was measured in the supernatant fractions following centrifugation at 105 000xg. This is a standard criterion of solubilization (Neugebauer, 1990).

A variety of methods were tested of which some of the more successful are shown in *Table* 4. GPAT activity was solubilized by treatment of high speed particulate fractions with 2M urea and both recovery and specific activity for the enzyme was improved if the 2M urea treatment was preceded by a 0.15M KC1 treatment. However, total recoveries of activity in the urea treatment alone and urea treatment of salt-washed high speed particulate fractions were low, being only 13% and 24% of the original high speed particulate fraction activities, respectively. Detergent solubilization with CHAPS (0.5%) resulted in better recovery. The specific activity of the enzyme at 2.9nmol min^{-1} mg^{-1} protein was 145% the original microsomal specific activity and there was a 44% recovery. The CHAPS-solubilized GPAT activity was stable for at least 24hr at 4°C and up to 72hr in the presence of protease inhibitors at the standard concentrations (GPAT Solubilization).

Purification of Solubilized GPAT

The CHAPS-solubilized GPAT was then partly purified using preparations from 14-week oil palm mesocarp and calli. We were anxious to compare both preparations in order to ensure that from calli was similar in characteristics. This was particularly in view of the fact that the microsomal fraction from calli was more active and, from past experience, was likely to be more stable to storage (Rutter et *al.*, 1997).

TABLE 4. SOLUBILIZATION OF HIGH SPEED PARTICULATE FRACTION GPAT USING EITHER UREA OR DETERGENT TREATMENT

Fraction	Specific activity (nmol min^{-1} mg^{-1} protein)	Total activity (nmol min^{-1})	Recovery (% control activity)
Microsomal	1.99 ± 0.18	5.04 ± 0.17	100
solubilized GPAT			
2 M urea (U)	0.90 ± 0.10	0.66 ± 0.10	13
2 M U + 0.15 M KCl	1.70 ± 0.40	1.22 ± 0.30	24
0.25% CHAPS	2.60 ± 0.10	2.00 ± tr	40
0.5% CHAPS	2.90 ± 0.10	2.20 ± tr	44
0.75% CHAPS	2.40 ± 0.30	1.80 ± 0.20	36
1% CHAPS	1.40 ± 0.10	1.10 ± tr	22

The high speed particulate &actions were prepared from 14-week oil palm mesocarp acetone powder. Data are expressed as means of duplicate incubations ± maximum/minimum of duplicate values and tr (trace) (< 0.05).

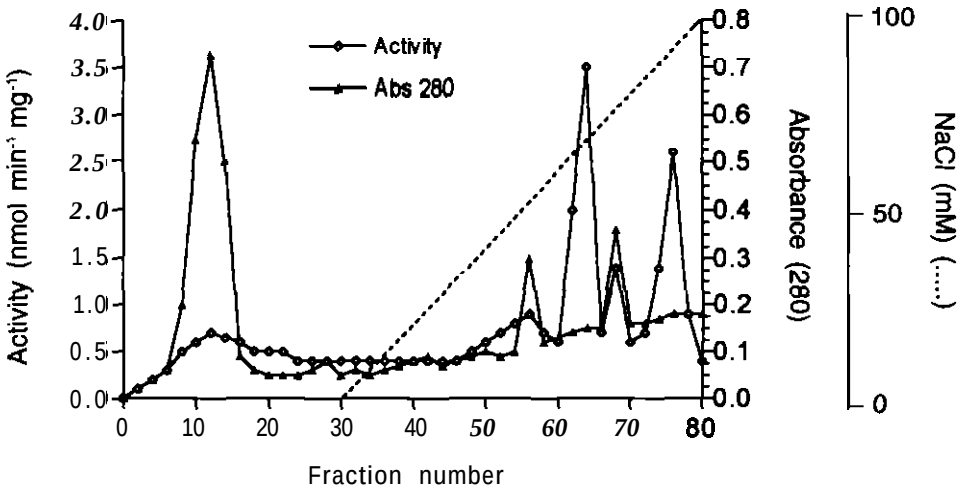

Figure 3. Elution profiles of GPAT activity after ion-exchange chromatography (DE52). GPAT activity was solubilized from 14-week mesocarp acetone powders. Fractions were collected and assayed for GPAT activity.

Ion-exchange chromatography of solubilized extracts obtained by treating the microsomal fractions with 0.5% CHAPS was carried out with a salt gradient on DE52. GPAT activity eluted in two peaks at 60mM (peak 1) and 80mM NaCl (peak 2) (*Figure* 3). The two peaks were concentrated and then chromatographed on a Sephadex G-100 gel filtration. But no activity was recovered from peak 2 (above). Peak 1 of the ion-exchange column gave GPAT activity eluting in a single peak after the void volume (Figure 4).

Table 5 shows the recovery of each purification step. The active GPAT fractions produced in ion-exchange and gel permeation chromatography were concentrated and analysed using SDS-PAGE to assess the number of protein bands present (Figure *5*). There were two major proteins that stain with Coomassie blue, haying approximate molecular masses of 97 and 55 kDa, respectively, as calculated from the R, values of the molecular weight markers.

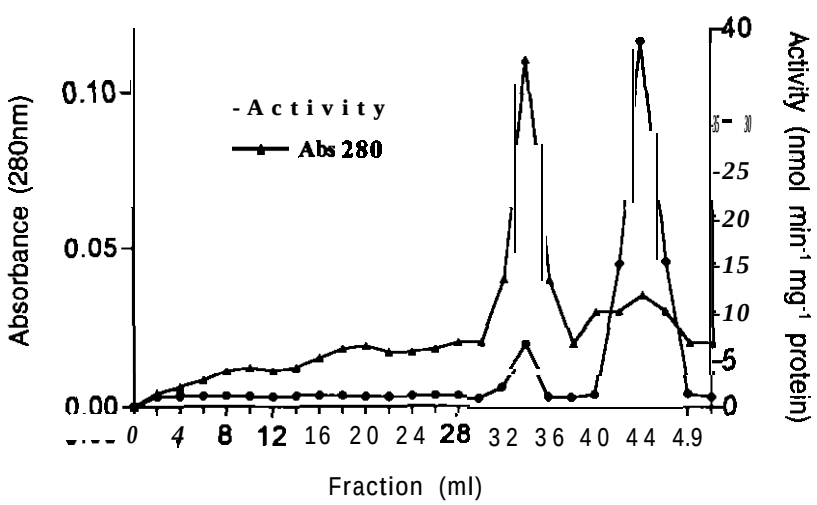

Figure 4. Elution profile of GPAT activity after gel permeation chromatography (Sephadex G-100). Fractions were collected and assayed for GPAT activity.

TABLE 5. PURIFICATION OF GPAT

Purification step	Total activity nmol min^{-1}	Protein µg	Specific activity nmol min^{-1} mg^{-1}	Yield %	Purification fold
Microsomal	17.19[a]	6 250"	2.75'	100"	1"
Fraction	6.84	8 450	0.81	100	1
Solubilized	9.11"	2 206"	4.13'	53"	2"
GPAT	3.08	2 316	1.33	45	2
Ion-exchange	8.09"	420"	19.25"	47"	7'
(DE52)	2.51	720	3.48	37	4
Gel permeation	3.69'	20[a]	184.25[a]	21"	67[a]
(Sephadex G-100)	1.53	40	38.17	22	47

Oil palm culture profiles are indicated by (a) whilst the others are from 14-week mesocarp acetone powder. Specific activity is per mg protein

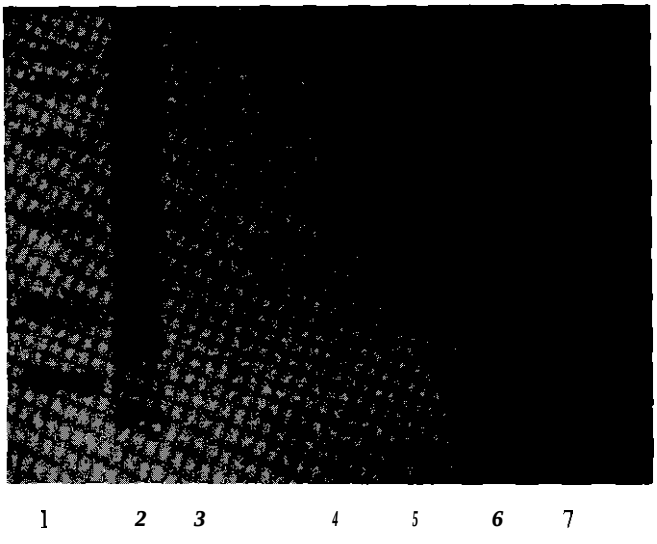

***Figure** 5. SDS-PAGE of GPAT fractions. Lanes 1 and 7 represent molecular-mass marker proteins which were (top to bottom) 97.4, 66.2, 45, 31. 21.5 and 14.4kDa. Lane 2:10µg protein of 0.5% (w/v) CHAPS solubilized GPAT from 14-week mesocarp acetone powders; Lanes 3 and 4:2µg of fractions after DE52 of mesocarp and calli preparation, respectively; Lanes 5 and 6:2µg of fractions after Sephadex G-100 of mesocarp and calli preparation, respectively.*

CONCLUSION

Purification of enzymes of the Kennedy pathway in plants has proved to be difficult due to the fact that their location is in the complex environmentoftheendoplasmicreticulum. Membrane-bound enzymes must be removed from the microsomal fraction in active form in order for further purification to be achieved. Optimal solubilization of GPAT activity using CHAPS treatment was achieved at a concentration of 0.5% (w/v). Detergentsolubilization with CHAPS was selected rather than with urea and Tween (20, 40 and 80) due to the facts that CHAPS interferes very little with protein *assay* and that the solubilized activity is moderately stable for at least 24hr at 4°C. Furthermore, previous work with avocado GPAT showed that CHAPS-solubilized material did not require reconstitution for activity (Eccleston and Harwood, 1995).

We basically used two further steps (ion-exchange and gel permeation chromatography) to achieve good purification ofsolubilized GPAT. Two major peptide bands were found in the purified fractions after gel permeation chromatography, having approximately molecular masses of 97 and 55kDa, respectively. In comparison, GPAT from the chloroplasts of pea (*Pisum sativum*) and spinach has a molecular mass of slightly more than 42kDa (Bertrams and Heinz, 1981). GPATs from the chloroplasts of squash (*Cucurbita moschata*) cotyledons were purified to homogeneity at about 30 and 40kDa, respectively (Nishida *et al.*, 1987). Purification of GPAT from a microsomal fraction of avocado mesocarp revealed the presence of three major proteins, with molecular masses of 70, 60 and 54kDa, respectively (Eccleston and Harwood, 1995). Thus, it seems likely that the band of 55kDa corresponded to a GPAT although confirmation of this identity requires further experiments.

Further purification and characterization of the oil palm GPAT should be possible using immunological and protein sequencing approaches. Furthermore, in order to utilize micro-

somal GPAT in genetic manipulation, it may be possible to screen, isolate and characterize the gene using the amino acid sequence information of plastid GPATs of other plants. When the cytoplasmic GPAT is cloned, engineering of its gene should allow us to modify the fatty acid composition of TAG and/or its total rate of formation. These are long-term goals of the work.

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