UNIVERSITI PUTRA MALAYSIA

CARBON FLUX ANALYSIS OF LIPID BIOSYNTHESIS PATHWAYS IN OIL PALM (*ELAEIS GUINEENSIS JACQ. TENERA*)

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia , in Fulfilment of the Requirement for the Degree of Master of Science

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DEDICATION

I dedicate this thesis to my parents and my family.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: **Associate Professor Ong King Kok, Ph.D.**

Faculty: **Medicine and Health Sciences**

The aim of this study was to investigate the carbon flux through lipid biosynthesis pathways in the oil palm (*Elaeis guineensis* Jacq. *tenera*) using metabolic control analysis (MCA). Three types of tissue from oil palm, namely liquid culture, plastid and mesocarp were used. The results showed mesocarp tissue was the most suitable tissue for carbon flux analysis because it incorporated the radioactive precursors mainly into triacylglycerol (TAG). Further analysis on different stages of fruit development was carried out using mesocarp tissue at 12, 15 and 20 weeks after anthesis (WAA). It was confirmed that 20-WAA mesocarp tissue was the best stage of fruit development for metabolic flux studies because it reflected biosynthesis of storage lipid. Three modes of radiolabel introduction into the oil palm fruits were investigated, namely injecting the radiolabel into the fruits still attached to the palm, injecting the radiolabel into the loose fruit and injecting the radiolabel into incubation mixture containing mesocarp tissue slices. The level of radioactivity in fruits attached to the palm was lower than the other two modes of radiolabel introduction. Carbon flux of lipid biosynthesis pathways was modulated by temperature and the inhibitor 2-bromooctanoate. Radiolabels [1-^{14}C]

acetate and [U-^{14}C] glycerol were used to monitor the carbon flux through the lipid biosynthesis pathways. Temperature caused a constraint in the distribution of radioactivity at the level of diacylglycerol acyltransferase (DAGAT). Therefore, DAGAT may be a regulatory enzyme. 2-Bromooctanoate inhibited the carbon flux of lipid biosynthesis pathways. The overall results of MCA suggested that the control of carbon flux in the oil palm may be distributed over two blocks of the lipid metabolic pathway, namely the fatty acid biosynthesis block and TAG formation block. Acetyl-CoA carboxylase (ACCase) plays an important role in fatty acid biosynthesis block while DAGAT plays an important role in the TAG formation block. The molecular structure of ACCase was investigated using immunoblotting with streptavidin and screening of ACCase gene in 15-WAA oil palm mesocarp cDNA library. Immunoblots with streptavidin showed the presence of large molecular weight (approximately 180 kDa) multifunctional ACCase and smaller molecular weight (approximately 58 kDa) multisubunit ACCase in oil palm mesocarp. Screening for ACCase gene in 15-WAA oil palm mesocarp cDNA library showed several strong signals corresponding to the putative β-carboxyl transferase subunit of ACCase.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS FLUKS KARBON TAPAK JALAN BIOSINTESIS LIPID DI DALAM POKOK SAWIT (*ELAEIS GUINEENSIS* JACQ. *TENERA*)

Oleh

EMILY QUEK MING POH

Mac 2002

Pengerusi: **Profesor Madya Ong King Kok, Ph.D.**

Fakulti: **Perubatan dan Sains Kesihatan**

Matlamat kajian ini adalah untuk mengkaji fluks karbon melalui tapak jalan biosintesis lipid di dalam pokok sawit (*Elaeis guineensis* Jacq. *tenera*) dengan menggunakan analisis kawalan metabolik (MCA). Tiga jenis tisu daripada pokok sawit iaitu kultur cecair, plastid dan mesokarpa telah digunakan. Hasil menunjukkan bahawa tisu mesokarpa merupakan tisu yang sangat sesuai untuk analisis fluks karbon kerana ia menukarkan prekursor radioaktif kebanyakannya ke dalam triasilgliserol (TAG). Analisis lanjutan ke atas peringkat perkembangan buah yang berlainan telah dilakukan dengan menggunakan tisu mesokarpa pada 12, 15 dan 20 minggu selepas pendebungaan (WAA). Tisu mesokarpa pada 20 WAA telah disahkan sebagai peringkat perkembangan buah yang terbaik untuk kajian fluks metabolik kerana ia mengimbas biosintesis penyimpanan lipid. Tiga cara kemasukan penanda radioaktif ke dalam buah sawit iaitu menyuntik penanda radioaktif ke dalam buah yang masih di pokok, menyuntik penanda radioaktif ke dalam buah yang dipetik dan menyuntik penanda radioaktif ke dalam larutan eraman yang mengandungi hirisan tisu mesokarpa telah dikaji. Aras aktiviti radioaktif dalam buah yang masih di pokok adalah lebih

rendah berbanding dengan dua cara kemasukan penanda radioaktif yang lain. Fluks karbon dalam tapak jalan biosintesis lipid telah dimodulasikan oleh suhu dan perencat 2-bromooktanoat. Penanda radioaktif [1-^{14}C] asetat dan [U-^{14}C] gliserol telah digunakan di dalam kajian ini untuk memantau fluks karbon melalui tapak jalan biosintesis lipid. Suhu menyebabkan rencatan dalam penyebaran aktiviti radioaktif pada peringkat diasilgliserol asiltransferase (DAGAT). Oleh itu, DAGAT mungkin merupakan enzim pengawalatur. 2-Bromooktanoat merencat fluks karbon dalam tapak jalan biosintesis lipid. Hasil keseluruhan MCA mencadangkan kawalan fluks karbon dalam buah sawit disebarkan melalui dua blok dalam tapak jalan metabolik lipid iaitu blok biosintesis asid lemak dan blok pembentukan TAG. Asetil-CoA karboksilase (ACCase) memainkan peranan penting dalam blok biosintesis asid lemak manakala DAGAT memainkan peranan penting dalam blok pembentukan TAG. Stuktur molekular ACCase telah dikaji dengan menggunakan pemblotan imuno dengan streptavidin dan penyaringan gen ACCase dalam koleksi cDNA 15-WAA mesokarpa sawit. Pemblotan imuno dengan streptavidin menunjukkan kehadiran protein berberat molekul besar (kira-kira 180 kDa) iaitu ACCase pelbagai-fungsi dan protein berberat molekul kecil (kira-kira 58 kDa) iaitu ACCase pelbagai-subunit di dalam mesokarpa sawit. Penyaringan gen ACCase dalam koleksi cDNA 15-WAA mesokarpa sawit telah menunjukkan beberapa signal yang menyamai subunit β-karboksil transferase ACCase.

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LIST OF ABBREVIATIONS

ACCase	Acetyl-CoA carboxylase
ACP	Acyl carrier protein
ADP	Adenosine biphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BC	Biotin carboxylase
BCCP	Biotin carboxyl carrier protein
BCS	Biodegradable counting scintillant
BSA	Bovine serum albumin
BUCA	Bottom-up metabolic control analysis
C12:0	Lauric acid
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C16:0-ACP	Palmitoyl-ACP
C18:0-ACP	Stearoyl-ACP
C18:1-ACP	Oleoyl-ACP
C_{16}	16 carbons
C_{18}	18 carbons
Ci	Curie
CoA	Coenzyme A
CO_2	Carbon dioxide
cpm	Counts per min
CPO	Crude palm oil
CT	Carboxyl transferase
DAG	Diacylglycerol
DAGAT	Diacylglycerol acyltransferase

DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FAS	Fatty acid synthase
FFA	Free fatty acid
HCl	Hydrochloric acid
HCO_3^-	Ion bicarbonate
HEPES	N-[2-Hydroxylethyl]piperazine-N'-2-ethanesulphonic acid
H_2SO_4	Sulphuric acid
IPTG	Isopropylthio-β-D-galactoside
KAS	β-Ketoacyl-ACP synthetase
KCl	Potassium chloride
kDa	Kilo Dalton
$KHCO_3$	Potassium bicarbonate
KOH	Potassium hydroxide
LB	Luria-Bertani
LPC	Lysophosphatidylcholine
MAG	Monoacylglycerol
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
$MnCl_2$	Manganous chloride
MCA	Metabolic control analysis
MES	2-[N-morpholino]ethanesulphonic acid
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate reduced form
$NaH^{14}CO_3$	Sodium [^{14}C] bicarbonate
NaOH	Sodium hydroxide
PAGE	Polyacrylamide gel electrophoresis
pfu	Plaque forming unit
pH	Hydrogen potential
PKO	Palm kernel oil

PL	Phospholipid
POD	Peroxidase
P_i	Inorganic phosphate
PP_i	Pyrophosphate
ppm	Part per million
PVDF	Polyvinylidene difluoride
SD	Standard deviation
SDS	Sodium dodecyl sulphate
sn	Stereospecific number
SOC	Sodium citrate
SPSS	Statistical package for social sciences
SSC	Sodium-citrate saline
TAG	Triacylglycerol
TBS	Tris-buffered saline
TBST	TBS-Tween 20
TCA	Tricarboxylic acid
TDCA	Top-down metabolic control analysis
TLC	Thin layer chromatography
V	Volt
var.	Variety
v/v	Volume/volume
WAA	Weeks after anthesis
w/v	Weight/volume
X-gal	5-Bromo-4-chloro-3-indolyl-β-D-galactoside

CHAPTER 1

INTRODUCTION

Recent advances in biotechnology, such as in genomics, proteomics, DNA microarray and bioinformatics, have enabled plants to be modified to produce novel products. These novel products include biodegradable plastics (Poirier *et al.*, 1992), antibodies (Hiatt *et al.*, 1989; Ma and Hein, 1995) and interferon (De Zoeten *et al.*, 1989).

Palm oil has become an important edible oil over the last few decades with a production of about 18.2 million tonnes in 1996-2000 from 3.7 million tonnes in 1976-1980 when it supplied a mere 7.1% of the world's oils and fats (Basiron, 2000). It now contributes 23% to the world's oils and fats production, making it the second-most produced oil after soybean oil (Gunstone, 2001). The oil palm is the highest-yielding oil crop in the world, surpassing the coconut (the next highest-yielding oil crop) by about 50-100% and other oil crops by even more (Basiron, 2000).

Malaysia is the largest producer of palm oil with 60% of the world production (Chow, 1997). Palm oil is predicted to become the major oil in the world by 2012 (Oil World, 1999), but the stiff competition from other oils has made it necessary to diversify its use. In addition, novel higher value-added products can be made producible by the oil palm by employing biotechnology methods such as recombinant DNA technology and genomics (Cheah, 1999). However, this requires a detailed understanding of the basic plant metabolism.

Plant lipid biosynthesis has been much studied in recent years (Browse and Somerville, 1991; Harwood, 1999; Ohlrogge and Jaworski, 1997; Slabas and Fawcett, 1992) but there remains much more to be learnt. To manipulate the oil palm for novel oils such as high oleate (Cheah, 1997) would require substantially more information on the regulation of lipid biosynthesis.

As metabolic pathways are under multi-step control, unraveling a single step or an individual enzyme is insufficient to understand the entire metabolism. Indeed, the converse is needed – to have an overall picture of the metabolic pathway before the particular steps can be understood. This can be achieved by applying the metabolic control analysis (MCA).

In most plants, including the oil palm, the storage lipids are mainly triacylglycerols (TAGs) (Harwood, 1980; Murphy, 1993). The metabolic pathways to TAG involve acetyl-CoA as the immediate carbon source, and information on the metabolic flux will be useful in modeling the carbon flux through pathways. This work was therefore to investigate the carbon flux in lipid biosynthesis by the oil palm using a radiolabel. It is hoped that the information gained may be useful in diverting the carbon to the formation of higher-value products by genetic manipulation.

To increase the production of a metabolite(s), it is necessary to manipulate a reaction, or a set of reactions, over another. However, the manipulation may still not result in production of the metabolite(s) if the necessary control mechanisms are not in place. Therefore, a comprehensive understanding of the entire cellular metabolism is needed.

Currently, attempts are being made to design cellular metabolism in order to maximize output of the desired metabolites. But the requisite prelude to this is quantification of the metabolite flux by MCA.

The objectives of this research were to:

1) Investigate carbon flux through the oil palm lipid biosynthesis pathway(s).
2) Apply MCA for a better understanding of the overall quantitative control structure of the lipid biosynthesis pathway(s).

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm

Oil palm is a perennial oil-bearing crop that has an economic life of about 25 years (Gascon *et al.*, 1989; Basiron, 2001). It begins to bear fruit two to three years after planting (Basiron, 2001). It is a unique crop that yields two types of oil, crude palm oil (CPO) from the mesocarp of the fruit and palm kernel oil (PKO) from the seed or kernel. These two oils have different physical and chemical properties. CPO contains mainly palmitic acid (C16:0) and oleic acid (C18:1), the two most common fatty acids in nature while PKO contains mainly lauric acid (C12:0).

The oil palm fruit is a sessible drupe which is usually oval in shape. The length of the fruit is 2.5 to 5.0 cm and 2.5 cm in diameter. It may weigh from 3 to 30 g (Godin and Spensley, 1971; Gascon *et al.*, 1989).

The oil palm fruit consists of the mesocarp, the shell or endocarp and the kernel as shown in Figure 2.1. The mesocarp or pulp of the ripe oil palm fruit is yellowish-orange in colour. It is oily and fibrous (Vaughan, 1970). The outer layer of oil palm is called exocarp or epicarp. It shows variation in colour through yellow, orange, red, brown and black according to the variety (Cobley and Steele, 1976).