UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF FULL LENGTH OLEOSIN cDNA CLONE FROM OIL PALM (*Elaies guineensis* Jacq.) KERNEL

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ISOLATION AND CHARACTERIZATION OF FULL LENGTH OLEOSIN cDNA CLONE FROM OIL PALM (*Elaies guineensis* Jacq.) KERNEL

By

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

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ISOLATION AND CHARACTERIZATION OF FULL LENGTH OLEOSIN cDNA CLONE FROM OIL PALM (*Elaies guineensis* Jacq.) KERNEL

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Chairman : Associate Professor Chuah Teong Guan, PhD

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Oleosins are embedded proteins in oil bodies. They have a structural role in stabilizing the triacylglycerol (TAG) / cytosol oil body interface in oil body biogenesis. A putative lipase attachment site on the oleosins implicates its involvement in the process of lipolysis (Hsieh and Huang, 2004). This lipolytic activity which produces free fatty acid (FFA) results in rancidity and impairment of oil palm quality. Oleosins also have been proposed as a carrier for the expression and purification of recombinant pharmaceuticals peptide. Besides, oleosins may also act as a natural emulsifying and stabilizing agent at an oil/water interface.

A full length cDNA clone coding for oleosin from oil palm kernel was isolated through RACE-PCR (Rapid amplification of cDNA ends) technique. It has 381 bp of coding region with 45 bp of 5' untranslated (UTR) region and 343 bp of 3' UTR region. The longest open reading frame encodes a protein of 127 amino acids. The deduced protein

sequence of oil palm oleosin cDNA exhibited high homology to the low molecular weight isoform of oleosin from *Sesamum indicum* (68%), *Oryza sativa* (67%), *Hordeum vulgare* (66%) *Zea mays* (65%) and *Bromus secalinus* (63%).

The analysis on the amino acid composition of the deduced protein sequence of oil palm kernel oleosin showed that valine, leucine and alanine are the most predominant residues with low levels of asparagine, histidine, methionine and tryptophan. Valine, leucine and alanine are classified as nonpolar residues which occur mostly in the interior of a protein, out of contact with the aqueous solvent.

The expression profile obtained in the Northern analysis showed that the oil palm oleosin expressed in both kernel and embryo tissues but no detectable signal in mesocarp, leaf and germinating seedling tissues. This result indicated that oleosins are found only in tissues that undergo dehydration. Thus, the oleosin transcript from oil palm is tissue-specific and its expression is tightly regulated.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi kerperluan untuk ijazah Master Sains

PEMENCILAN DAN PENCIRIAN KLON cDNA LENGKAP OLEOSIN DARI KERNEL KELAPA SAWIT (*Elaeis guineensis* Jacq.)

Oleh

SHARIZA JAMEK

FEBRUARI 2008

Pengerusi : Profesor Madya Chuah Teong Guan, PhD

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Oleosin merupakan protein yang bergabung dengan jasad minyak. Struktur oleosin memainkan peranan dalam menstabilkan permukaan antara triasilgliserol/ sitosol dengan jasad lemak. Oleosin dikatakan mempunyai tapak perlekatan bagi enzim lipase yang terlibat dalam proses lipolisis (Hsieh and Huang, 2004). Proses lipolisis ini menghasilkan asid lemak bebas yang akan menjejaskan kualiti minyak kelapa sawit. Selain itu, oleosin juga dikatakan berpotensi untuk menghasilkan peptida gabungan farmaseutikal. Malahan, ia juga boleh bertindak sebagai agen penstabil dan pengemulsi semulajadi bagi permukaan antara air dan minyak.

Sehubungan itu, satu klon cDNA yang mengkodkan jujukan penuh oleosin telah berjaya dipencilkan daripada transkrip isirung kelapa sawit dengan menggunakan kaedah PCR-RACE (rapid amplification of cDNA ends). Ia mempunyai kawasan pengkodan sebanyak 381 bp dengan diapit oleh kawasan tidak mengkodkan sebanyak 45 bp pada hujung 5’

and 343 bp pada hujung 3'. Rangka bacaan terbuka yang paling panjang mengkodkan protein yang terdiri daripada 127 asid amino. Jujukan asid amino oleosin kelapa sawit didapati menunjukkan homologi yang tinggi dengan isoform oleosin berat molekul rendah daripada *Sesamum indicum* (68%), *Oryza sativa* (67%), *Hordeum vulgare* (66%), *Zea mays* (65%) dan *Bromus secalinus* (63%).

Analisis terhadap komposisi jujukan asid amino oleosin kelapa sawit menunjukkan kehadiran valina, leusina dan alanina sebagai residu utama di samping residu-residu lain seperti asparagina, histidina, metionina dan triptofana. Valina, leusina dan alanina adalah tergolong sebagai residu-residu tidak polar yang kebanyakkannya hadir di bahagian dalam sesebuah protein dan tidak berinteraksi dengan cecair akuas.

Hasil analisis pemblotan Northern menunjukkan oleosin kelapa sawit hanya diekspreskan di dalam tisu isirung dan embrio. Tiada signal dikesan di dalam tisu mesokap, daun dan anak benih cambah. Ini menunjukkan oleosin hanya terdapat di dalam tisu yang mengalami pendehidratan. Oleh itu, transkrip oleosin dari kelapa sawit bersifat spesifik kepada tisu tertentu dan pengekpresannya adalah dikawalatur.

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Finally, I dedicate this thesis to my beloved family, in particular, my parents, without their love, support and encouragement, it would not be possible for me.

I certify that an Examination Committee met on 28 February 2008 to conduct the final examination of Shariza Jamek on her Master of Science thesis entitled "Isolation and Characterization of Full Length Oleosin cDNA Clone From Oil Palm Kernel (*Elaies Guineensis* Jacq.) in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date: 14th August 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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SHARIZA JAMEK

Date: 27th June 2008

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LIST OF ABBREVIATIONS/ NOTATIONS

α	Alpha
β	Beta
%	Percentage
º C	Degree centigrade
bp	Base pair
ci	Curie
C-terminal	Carboxyl terminal
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid
dCTP	2'-deoxy-cytidine-5'-triphosphate
dNTP	Deoxynucleotides
DEPC	Diethyl pyrocarbonate
DMSO	Dimethylsulphonyl oxide
EtBR	Ethidium bromide
EDTA	Ethylenediaminetetraacetic acid
g	Gram
HCI	Hydrochloride acid
kDa	Kilodalton
LiCI	Lithium chloride
M	Molar
mM	Milimolar
MOPS	3-(N-morpholino) propanesulfonic acid
mRNA	Messenger RNA

MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Council
NaCI	Sodium chloride
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
N-terminal	Amino terminal
OD	Optical density
ORF	Open reading Frame
PCR	Polymerase chain reaction
RACE	Rapid amplification of cDNA Ends
RNA	Ribonucleic acid
RNase	Ribonuclease
RT	Reverse transcriptase
rpm	Revolutions per minute
TAE	Tris-acetate EDTA
UTR	Untranslated region
µl	Microliter
µg	Microgram
UV	Ultra violet
X-gal	5- bromo-4-chloro-3-indolyl- β-D-galactopyranoside
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

1.1 Background

The oil palm (*Elaeis guineensis* Jacq.) is a perennial tree crop which is widely grown commercially in South East Asia, Equatorial America, Africa and South Pacific. It has been known as the highest oil yielding crop in the world compared to any other oil crop. The typical yields of oil palm is about 3.68 tonnes of oil per hectare compared to rapeseed of 0.59 tonnes per hectare, sunflower seed (0.42) and soybean (0.36) (Davidson, 2006).

In Malaysia, oil palm currently covers 4.17 million hectares of land with 3.9 tonnes of oil yield per hectare. As the biggest producer and exporter of palm oil and palm oil products, Malaysia currently accounts for 51% of world palm oil production and 62% of world exports. The export earnings of oil palm products rose to a record RM 31.8 billion (MPOC, 2006).

Palm oil has a wide application. About 80% of palm oil goes into food and 20% used in the non-food sector. The four main traditional uses of palm oil in food sectors are as cooking/frying oils, shortening, margarine and confectionary fats. In the non-food sector, palm oil is used in the production of soaps and detergent, pharmaceutical products, cosmetics and oleo-chemical products. Carotenoids and vitamin E (tocopherols and tocotrienols) are two minor components in palm oil which are important nutritionally.

Like all oils, triacylglycerols (TAGs) are the major component in palm oil with over 95% of palm oil comprising a mixture of TAG. Each TAG is made up of a glycerol backbone esterified with three fatty acids (Sambanthamurthi *et al*., 2000). Composition of TAG in oil palm is a major determinant of oil quality. Degradation of TAG by lipase (triacylglycerol acylhydrolase) will produce monoacylglycerol (MAG), diacylglycerol (DAG) and free fatty acid (FFA). This lipolytic activity which produces FFA results in rancidity and impairment of oil quality.

Oil bodies are intracellular plant organelles that contain TAGs. They occur abundantly in oil seeds. A major group of oil body associated proteins called oleosins has been proposed to act as the recognition signal for the specific binding of lipase to the oil bodies (Hsieh and Huang, 2004). Therefore, it is necessary to understand the correlation of oleosins with the lipolytic action of lipase which consequently decreases the quality of palm oil. Basic information regarding to the molecular aspects of oil palm oleosins will be useful for verifying the proposed role of oleosins as a receptor for lipase binding.
Previous study by Yong et al. (1999) reported only a partial nucleotide sequence of an oleosin from oil palm kernel. In view of this, there is a need to get a full length of the oleosin sequence in order to get more information regarding the molecular aspects of oil palm oleosins.

Besides oleosin manipulation, there is also interest in oleosins as they act as a natural emulsifying and stabilizing agents at the oil/water interface. This suggests a possible biotechnological application for oil palm oleosins in the stabilization of emulsion systems, in industries such as food processing, pharmaceutical manufacture, and oil

spillage treatment. Study by Li et al. (2002) reported that surface-oriented, amphipathic N- and C- terminal domains of oleosins protein may play an important role in emulsion formation. This is showed when once the N-and C-terminal domains were removed by protease digestion, the resulting rapeseed central domain was relatively poor emulsifying and stabilizing agent. In relation to this, this study which aim to isolate the full length cDNA clone of oleosin from oil palm kernel is seen as a first step in order to identify the central, N- and C-terminal domain of oil palm oleosin before further studies in revealing the physical behaviour among those domains (e.g. the stability of the resultant emulsions) can be done.

Rooijen and Moloney, (1995) have proposed oleosins as a carrier for the expression and purification of recombinant pharmaceutical peptides and industrial enzymes. Hence, the isolation of the full length of oleosin cDNA sequence will permitted further genetic manipulation to be applied in order to express the recombinant forms of oil palm oleosin and produced the desired recombinant protein.

1.2 Objectives of the Study

The main objectives of this study are to isolate the cDNA clone for oleosin from oil palm (*Elaeis guineensis* Jacq.) and to analyse the expression profile of oleosin.

The specific objectives are:

1. To isolate the full length cDNA clone of oleosin from oil palm kernel.
2. To characterize the DNA sequence of oleosin from oil palm kernel.
3. To analyse the expression profile of oleosin in different types of tissue in oil palm.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Plants store lipids to provide energy and carbon supports for germination and seedling growth. Lipids are stored in the form of triacylglycerol (TAG), which is composed of three fatty acyl chains esterified to a glycerol backbone (Tzen *et al*., 1993). The TAGs are present in small discrete intracellular organelles called oil bodies. Each oil body contains a TAG matrix surrounded by a monolayer of phospholipids (PL) embedded with proteins termed oleosins (Tzen and Huang, 1992). Oleosins form a steric barrier, preventing the PL layers of adjacent oil bodies from coalescing. In addition to playing the above structural role, oleosins may also serve as recognition signals on the surface of oil bodies for the binding of newly synthesized lipase during germination (Huang, 1996).

2.2 Storage Lipid

In plants, seeds are the major sites of lipid storage. The lipid storage may be accumulated in one or both of the main types of seed storage tissue; endosperm (kernel) or embryo. In species such as coriander, castor bean and carrot, the endosperm (kernel) is the main site of storage lipid accumulation. In oilseeds such as sunflower, rapeseed and linseed, the major sites of storage lipid accumulation are in the cotyledons of the embryo tissue. In tobacco, both embryo and endorsperm tissues store considerable quantities of lipid (Murphy, 1993).

The storage lipids of seeds usually consist of TAGs that accumulate during the maturation phase of the embryo and/ or the endosperm. The TAGs in most seeds contain the same acyl groups that are found in membrane lipids. These are predominantly palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2), and linolenate (18:3) (Voelker and Kinney, 2001).

2.3 Storage Lipid Synthesis

In plants, the reactions for *de novo* fatty acid synthesis are located in plastids (Figure 2.1). Sucrose, which is imported by the embryo from maternal tissues, is metabolized by pathways in the cytosol, plastids and endoplasmic reticulum to form starch and oil.

Sucrose, the most common imported carbon in fatty acid synthesis can readily cross the membrane of the cell but is unable to traverse the innermost of the two envelope membranes of the plastid. Therefore, the imported sucrose needs to be metabolized further before it can be used for intra-plastid fatty acid synthesis via acetyl-CoA (Murphy, 1993). The action of invertase to imported sucrose, produced a glycerol-6-phosphate (G-6-P). This G-6-P will be later converted to pyruvate by glycolytic pathway. Acetyl-CoA generated from the pyruvate is then used as the source of two-carbon units for the synthesis of fatty acids.

The synthesis of fatty acids requires the combined action of the enzyme complexes, acetyl-CoA carboxylase and fatty acid synthase. The fatty acids are then exported to the cytosol and activated to their acyl-CoA derivatives. The acyl chains are incorporated into glycerolipids through acylation of glycerol-3-phosphate (G-3-P) by the combined action

of the acyltransferases of the Kennedy pathway, which are located in the endoplasmic reticulum (ER). Diacylglycerol (DAG) forms the branch point between membrane phospholipids (PC) and storage triacylglycerol (TAG) synthesis (Hills, 2004).

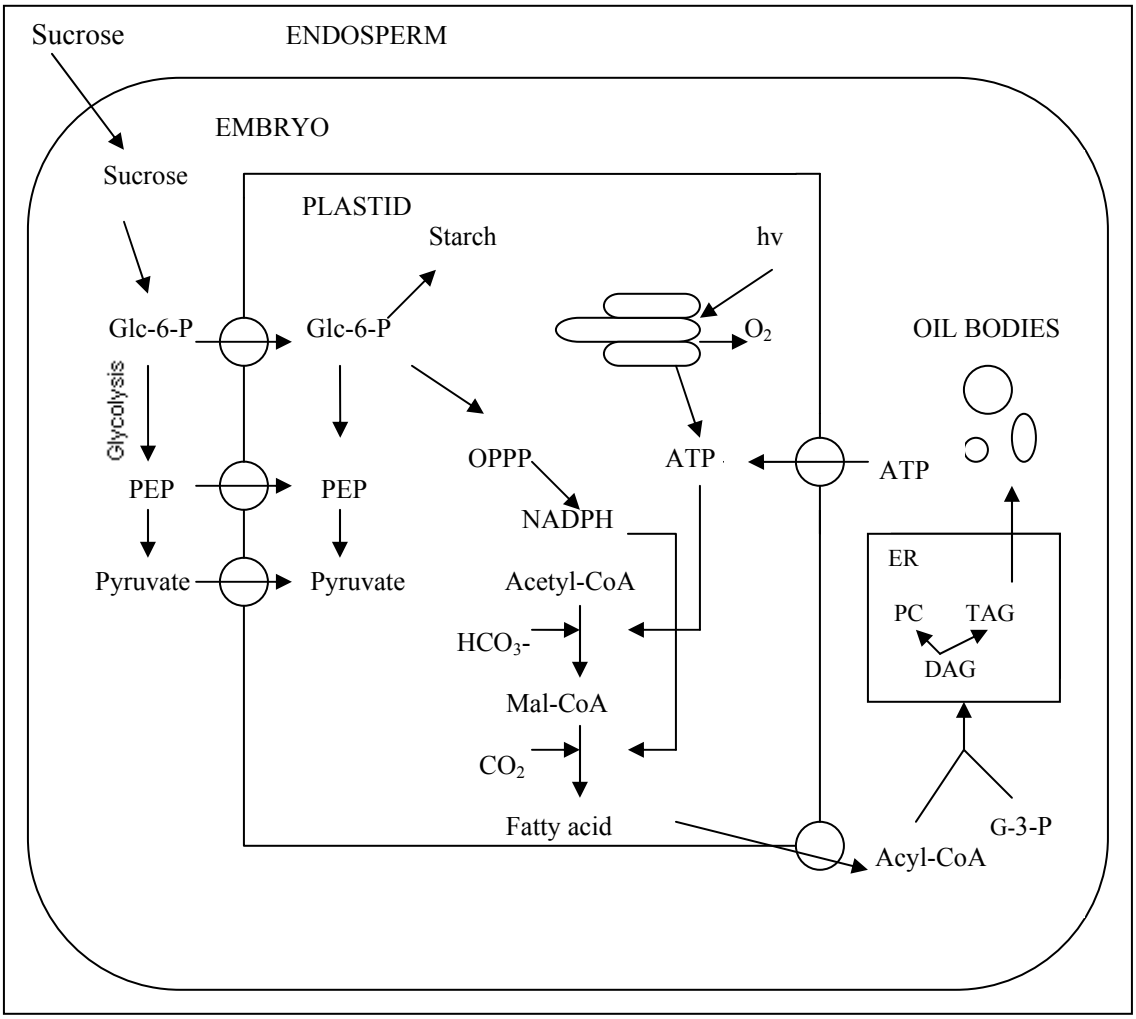

Figure 2.1: Simplified diagram showing the metabolic pathways of storage-product synthesis in a seed (Hilss, 2004).
Abbreviations used: G-6-P, Glycerol-6-phosphate; DAG, Diacylglycerol; G-3-P, Glycerol-3-phosphate ; PEP, phosphoenolpyruvate ; Mal-coA, malonyl-CoA; TAG, triacylglycerol; ER, Endoplasmic reticulum OPPP, oxidative pentose phosphate pathway; PC, acyltransferases phospholipids.

2.4 Oil Bodies

Diverse organisms store lipids in subcellular particles as food reserves. These lipid particles are called oil bodies. They can be found in the seeds, pollens, flowers, roots and stems of flowering plants, the spores and vegetative organs of non flowerings plants (Huang, 1996).

2.4.1 Oil Bodies in Seed

Oil bodies are intracellular plant organelles, which occur abundantly in oil seeds. They have spherical shape and posses diameters ranging from 0.2– 2.5 μM, depending on the species (Huang, 1992). They consist of a matrix of TAGs, which is surrounded by phospholipids and proteins called oleosins as depicted in Figure 2.2.

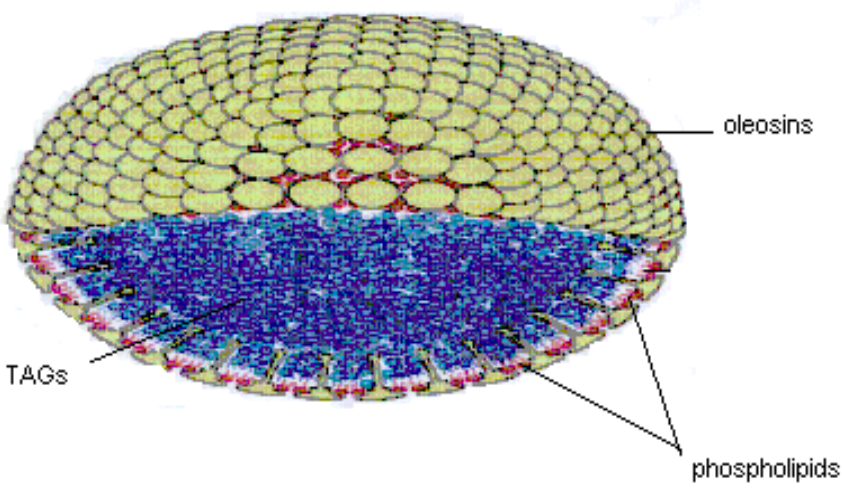

Figure 2.2: Model of an oil body. Oleosins, TAGs and phospholipids are drawn approximately to scale, whereas the diameter of oil body has been reduced 24 times to magnify the surface structure (Hsieh and Huang, 2004).