UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERISATION OF cDNA CLONES ENCODING ADP-GLUCOSE PYROPHOSPHORYLASE (AGP) FROM SAGO PALM (METROXYLON SAGU)

AU SIAN LOONG

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ISOLATION AND CHARACTERISATION OF cDNA CLONES ENCODING ADP-GLUCOSE PYROPHOSPHORYLASE (AGP) FROM SAGO PALM (*METROXYLON SAGU*)

By

AU SIAN LOONG

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master Science in the Faculty of Food Science and Biotechnology
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

ISOLATION AND CHARACTERISATION OF cDNA CLONES ENCODING ADP-GLUCOSE PYROPHOSPHORYLASE (AGP) FROM SAGO PALM (*METROXYLON SAGU*)

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January 2001

Chairman: Suhaimi bin Napis, Ph.D.

Faculty: Food Science and Biotechnology

In general, the biosynthetic steps required for starch biosynthesis involves three major enzymes: ADP-glucose pyrophosphorylase (AGP), starch synthase (SS) and starch branching enzyme (SBE). Many studies have indicated that AGP is an important control point of flux through the pathway of starch biosynthesis in many plant species. All plant AGPs are composed of two subunit types, large (lAGP) and small (sAGP) subunits that give rise to an $\alpha_2\beta_2$ heterotetrameric native enzyme. The presence of both subunits are crucial for the stability and activity of the enzyme.

One full length and three partial AGP cDNA clones have been isolated and characterised from sago palm leaves and pith by a PCR amplification technique. Three of the clones (*agpl19*, *agpp10* and *agpp12*) encode AGP large subunits; the fourth clone (*agpl1*) encodes a small subunit. The complete cDNA of *agpl1* has been isolated from a mature leaf cDNA library by a PCR screening technique. Semi-quantitative RT-PCR analysis

revealed that *agpl19* was leaf specific while *agpp10* and *agpp12* were pith specific. *agpl1* was found to be present in leaves as well as pith tissue.

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

PENGASINGAN DAN PENCIRIAN KLON cDNA UNTUK ADP-GLUCOSE PYROPHOSPHORYLASE (AGP) DARI POKOK SAGU (*METROXYLON SAGU*)

Oleh

AU SIAN LOONG

Januari 2001

Pengerusi: Suhaimi bin Napis, Ph.D.

Faculty: Sains Makanan dan Bioteknologi

Secara umumnya, langkah-langkah yang diperlukan untuk menghasilkan kanji melibatkan tiga jenis enzim yang utama: ADP-glucose pyrophosphorylase (AGP), starch synthase (SS), dan starch branching enzyme (SBE). Banyak pengajian telah menunjukkan bahawa AGP adalah pusat pengawal "flux" yang penting dalam proses pembentukan kanji bagi kebanyakkan jenis tumbuhan. Semua AGP tumbuhan terdiri daripada dua jenis, iaitu subunit besar (lAGP) and subunit kecil (sAGP). Kedua-dua jenis subunit itu membentuk enzim $\alpha_2\beta_2$ heterotetrameric. Pengwujudan kedua-dua jenis subunit itu adalah penting untuk kestabilan and keaktivitian enzim tersebut.

Empat klon cDNA tidak lengkap untuk AGP telah diasingkan dari daun pokok sagu melalui teknik PCR amplifikasi. Tiga daripada klon (*agpl19*, *agpp10* dan *agpp12*) mengkodkan subunit AGP besar; manakala klon keempat (*agpl1*) mengkodkan subunit AGP kecil. Selain daripada itu, cDNA lengkap untuk *agpl1* telah diasingkan dari "cDNA library" untuk daun pokok sagu yang matang melalui teknik PCR. "Semi-

quantitative RT-PCR" telah menunjukkan bahawa *agpl19* hanya boleh didapati dari daun. Manakala, *agpp10* dan *agpp12* hanya boleh didapati dari batang sagu. *agpl1* boleh didapati dari daun dan juga batang sagu.

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Finally, I would like to take this opportunity to acknowledge my parents, brother and sister for their unremitting love, inspiration and support throughout the years of my study.

I certify that an Examination Committee met on 11th January 2001 to conduct the final examination of Au Sian Loong on his Master of Science thesis entitled "Isolation and Characterisation of cDNA Clones Encoding ADP-Glucose Pyrophosphorylase (AGP) from Sago Palm (*Metroxylon sagu*)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Raha Abdul Rahim, Ph.D.
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairperson)

Suhaimi bin Napis, Ph.D.
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Tan Siang Hee, Ph.D.
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Harikrishna a/l Kulavesingam, Ph.D.
Associate Professor
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

MOHD. GHAZALI MOHAYIDIN, Ph.D,
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia

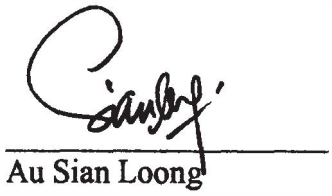
Date: 5 MAR 2001

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science.

MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor
Deputy Dean of Graduate School
Universiti Putra Malaysia

Date: 12 APR 2001

I hereby declare that the thesis is based on my original work except for equations 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.



Au Sian Loong

Date: 1-3-2001

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ABBREVIATIONS

ADP-Glc	ADP-glucose
AGP	ADP-glucose pyrophosphorylase
lAGP	large AGP subunit
sAGP	small AGP subunit
DBE	debranching enzyme
F6P	fructose-6-bisphosphate
1,6-FBP	fructose-1,6-bisphosphate
FBPase	fructose-1,6-bisphosphatase
G1P	glucose-1-phosphate
G6P	glucose-6-phosphate
GBSS	granule-bound starch synthase
PFK	phosphofructokinase
PFP	pyrophosphate:fructose-6-phosphate-1-phosphotransferase
3-PGA	3 phosphoglycerate
PPi	inorganic pyrophosphate
Pi	inorganic phosphate
Rib-1,5-P2	ribulose-1,5-bisphosphate
RuBP	ribose-1,5-bisphosphate
RPP	reductive pentose phosphate
SBE	starch branching enzyme
S6P	sucrose-6-phosphate

SPS	sucrose-6-phosphate synthase
SS	starch synthase
SSS	soluble starch synthase
TP	triose phosphate
UDP-Glc	UDP-glucose

CHAPTER I

INTRODUCTION

Back in the 70's and 80's, the introduction of many new food products and ingredients was attributed to the manipulation of existing processes or formulations through product development. Other than creative process modification or ingredient formulation, another frontier with regard to new functions lies with the raw ingredient. This was made possible with the ability to isolate genes encoding virtually any enzyme and the ability to transform these genes into almost any species. For example, recombinant rennins (chymosins) are already a backbone of the cheese industry.

While protein bioengineering has paved the way for genetically modified products, polysaccharides and lipids will closely follow. Since DNA provides the direct genetic blueprint for proteins, protein bioengineering is based on genetic manipulation of the direct gene products. On the other hand, polysaccharides and lipids are not direct gene products, their bioengineering will require a higher degree of scientific understanding. Polysaccharides and lipids are assembled from precursors by a series of enzymatic steps. Therefore, in theory, it should be possible to engineer at polymer levels by a combination of metabolic precursor levels and the selected expression of key regulatory enzymes within a given pathway (Wasserman et al., 1995). As a result, to devise rational strategies for bioengineering, the metabolic pathway and molecular biology of the enzymes involved must be thoroughly understood.

Sago palm (*Metroxylon sagu*) of Malaysian and Oceanic origin has an enormous potential for starch production. Unquestionably, sago starch possesses several advantages that could not be found in other starches. Sago starch produces sizing pastes of lower viscosity at a given concentration, lower retrogradation and is less inclined to gelate under cool conditions and is thus easier to handle (Sim, 1977). Besides, sago starch could be a good source for the production of novel carbohydrates designed for a particular application. To date, no reports have been published on the understanding of the biochemical and molecular biology of starch biosynthesis in sago palm. Therefore, at this point, isolation and characterisation of cDNA clones encoding ADP-glucose pyrophosphorylase (AGP) is a preliminary step in the commencement of a molecular biology study of sago palm starch metabolism and catabolism.

CHAPTER II

LITERATURE REVIEW

Botany of The Sago Palm

The sago palm belongs to the *Lepidocaryoid* subfamily of *the Arecaceae (Palmeae)*. Sago palm falls in the genus of *Metroxylon*, the name of the palm genus *Metroxylon* is derived from Greek, 'metra' meaning "pith" and 'xylon' meaning "xylem". Probably, the economically most important species are *M. sagu* Rottb., and *M. rumphii* Mart., of which the later has spines on petioles, spathes and even leaflets.

Sago palms are hapaxanthy, meaning "once-flowering". Hapaxanthy pass through a vegetative stage during which carbohydrates are accumulated in the plant. Following this is the final reproductive stage where the plant's food reserves are expended on the production of the inflorescence, flowers and then fruits, after which the plant dies. This phenomenon usually takes place in the eighth year. Apparently, the appearance of an inflorescence is preceded by a number of leaves of rapidly decreasing size that heralds the end of the life cycle of the palm. The sago palm flowers are borne spirally in pairs on a tertiary axis. Of each pair of flowers, one is male and the other complete but only functionally female. Since the anthers shed their pollen before the female flowers are receptive, sago palm can be considered as an obligatory cross-pollinator, and this could lead to very diverse offspring.

Sago palms are also a tillering or suckering (soboliferous) perennial. Just as all other palms, whether planted as a seedling or as a sucker, at first the plant forms a rosette of leaves. During the first year of development, the palm forms a number of buds in the axils of lower leaves that develop into shoots or suckers which may develop into new palms.

Starch Accumulation in Sago Palm

Sago starch is accumulated in the trunk during the vegetative phase. Trunk formation is supposed to start when the photosynthetic apparatus, the fronds, attains full size. Starch accumulation is assumed to begin at the start of the trunk formation as well. In the trunk when their fronds are in full sunshine, starch accumulation over longer period progresses linearly. At flowering, the photosynthetic apparatus diminishes due to decreasing leaf size, and at fruiting, it disappears. This would lead to a starch accumulation curve as in Figure 1.

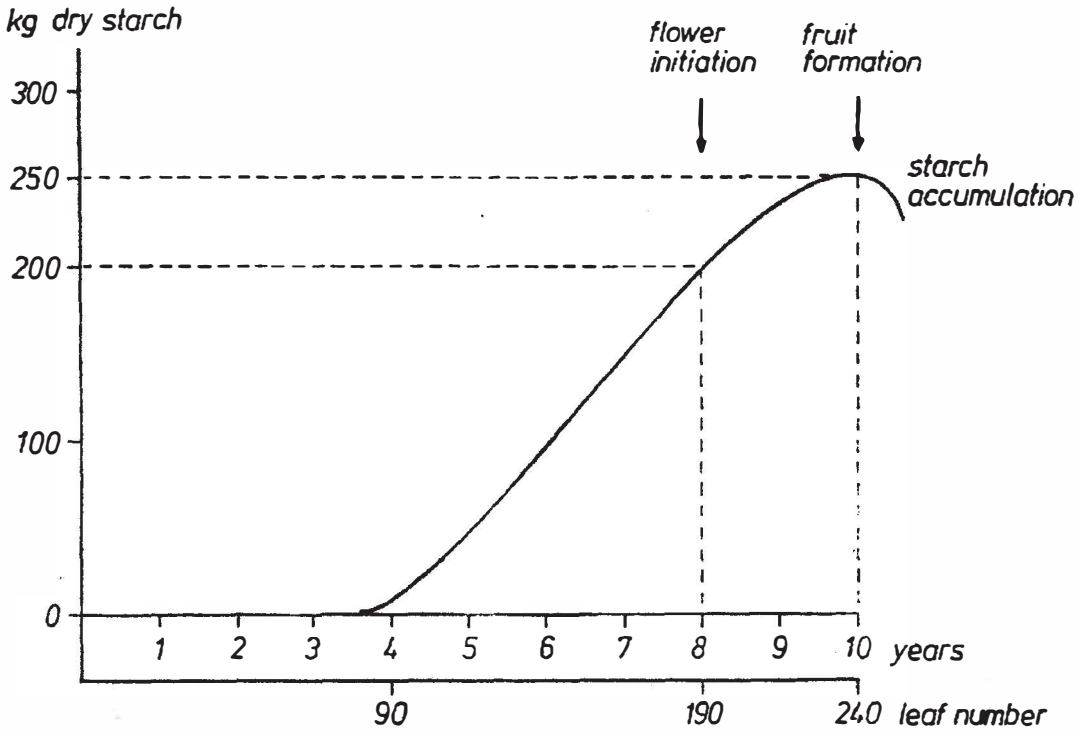

Figure 1: Assumed Rate of Starch Accumulation in a Sago Palm Trunk on Clayish Soil.
(Source: Flach, 1977)

Composition of Sago Trunk

The weight of a sago trunk varies from 800 kg to 1250 kg; bark or cortex varies from 32-25.7%; and pith weight varies from 643 to 850 kg. The cortex or bark of the trunk contains very little starch. The pith contains 20.2-29% starch and 50-66% water, the remainder being 13.8-21.3%. Based on dry matter, the pith contains 54-60% starch and 46-40% of other dry matter (Flach, 1983).

Characteristics and Quality of Sago Palm Starch

The granular size of sago palm starch ranges from about 80 μm to 5 μm, with an average of about 30 μm. About 90% of the starch has a particle size of between 20 and 40 μm. All other starches are smaller except for potato starch which, is the same (Flach, 1983).

Sago starch consists of 27% amylose and 73% amylopectin (Ito *et al.*, 1979). Where the high amylose content is comparable to that of corn starch.

Sago starch has several advantages over other starch (Sim, 1977):

1. Sago starch produces sizing pastes of lower viscosity at a given concentration than pastes from maize and potato.
2. Sago pastes are less inclined to gelate under cooling than maize pastes, and are therefore easier to handle.
3. Sago pastes show low retrogradation; their stability in viscosity is high when kept for long periods at near boiling point, provided they are boiled for two hours before use.

The properties of sago starch in relation to other starches can be shown in Figure 2.

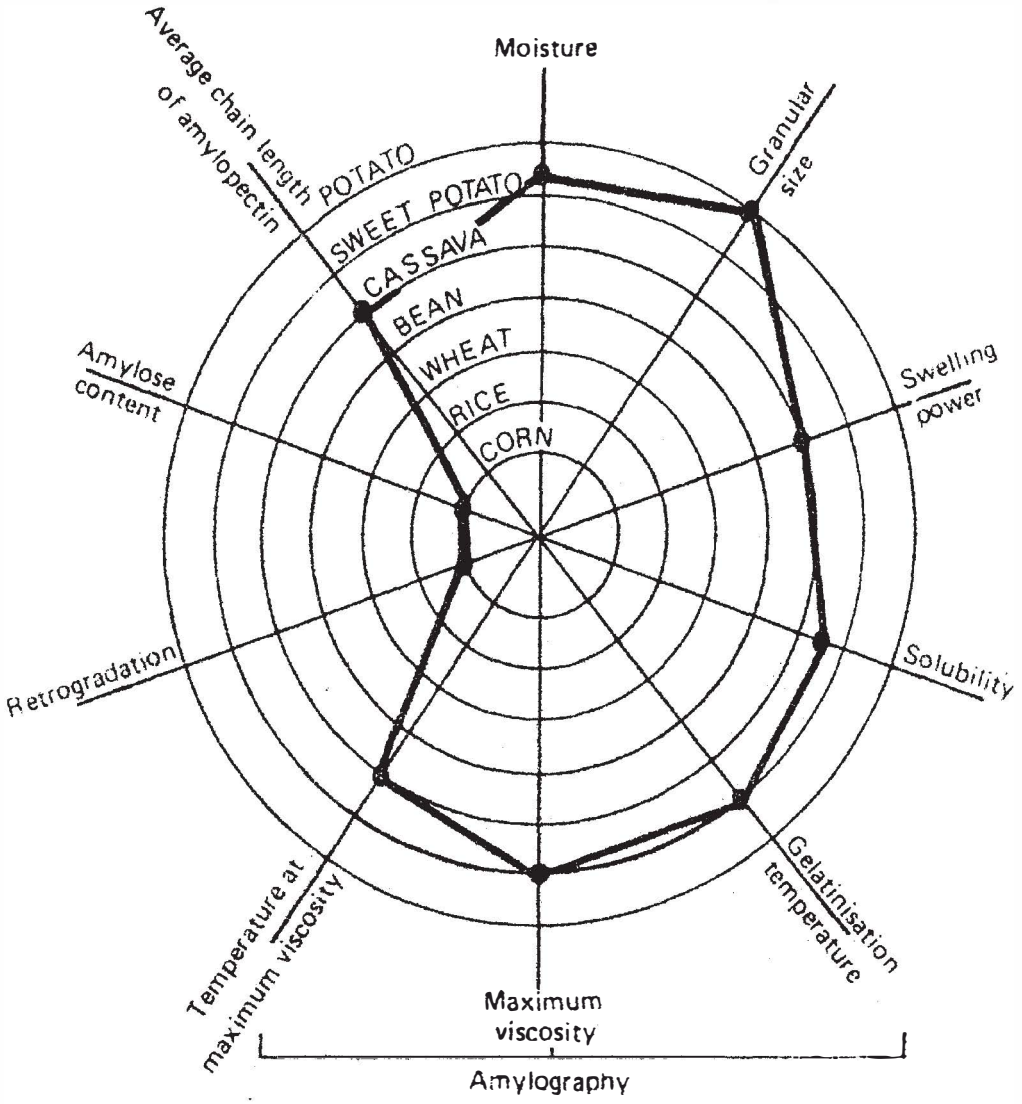

Figure 2: Amylograph of Sago Palm Starch.
(Source: Flach, 1977)

Starch

Starch is the major reserve carbohydrate of plants and function as a short-term as well as long-term store of carbon and energy. It is also one of the most well characterised plant products, with virtually every cell in every higher plant able to

produce it (John, 1992). In the plastids of plant cells, starch is always found in the form of granules. Starch granules (also called starch grains) consists of different glucose polymers arranged into a three-dimensional, semicrystalline structure. Therefore, the studies of starch biosynthesis involve not only the production of the composite glucans but also their arrangement into an organised form within the starch granule (Martin and Smith, 1995).

The Basic Structure of Starch and the Starch Granule

The starch granule is a three-dimensional, semicrystalline structure, which consists of different glucose polymers and can be chemically fractionated into two types of glucan polymer: amylose and amylopectin. Amylose is essentially a linear polymer consisting of α(1-4)-linked glucose residues, each approximately 1000 residues long. Amylose is usually branched at a low level (approximately one branch per 1000 residues) by α(1-6) linkages and makes up about 30% of starch (Martin and Smith, 1995). However, this proportion may vary considerably with the plant species, cultivar, plant organ, maturity and to some extent, the growth conditions of the plant (Shannon and Garwood, 1984). Once extracted from plants and in solution, amylose forms hydrogen bonds between molecules, resulting in rigid gels. However, depending on the concentration, degree of polymerisation, and temperature, it may crystallise and shrink after heating (Shewmaker and Stalker, 1992).