



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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(*METROXYLON SAGU*)**

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By

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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
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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

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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 kebanyakan 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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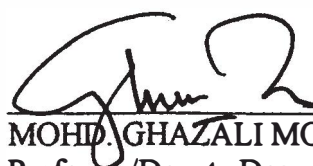
I certify that an Examination Committee met on 11th January 2001 to conduct the final examination of Au Sian Loong on his Master of Science thesis entitled “Isolation and Characterisation of cDNA Clones Encoding ADP-Glucose Pyrophosphorylase (AGP) from Sago Palm (*Metroxylon sagu*)” in accordance with 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:

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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

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TABLE OF CONTENTS

		Page
	ABSTRACT	ii
	ABSTRAK	iv
	ACKNOWLEDGEMENTS	vi
	APPROVAL SHEETS	vii
	DECLARATION FORM	ix
	LIST OF TABLES	xiii
	LIST OF FIGURES	xiv
	ABBREVIATIONS	xvi
CHAPTER		
I	INTRODUCTION	1
II	LITERATURE REVIEW	3
	Botany of the Sago Palm	3
	Starch Accumulation in Sago Palm	4
	Composition of Sago Trunk	5
	Characteristics and Quality of Sago Palm Starch	6
	Starch	7
	The Basic Structure of Starch and the Starch Granule	8
	The Biosynthesis of Starch	11
	From Source to Sink	12
	Conversion of Soluble Metabolites into Starch	15
	Major Enzymes Involved in Starch Biosynthesis	19
	ADP-Glucose Pyrophosphorylase (AGP)	19
	Starch Synthases (SS)	26
	Starch Branching Enzyme (SBE)	32
	New Models of Amylopectin and Amylose Synthesis	33
	Modifying Starch Biosynthesis	35
	Production of Novel Carbohydrates	36
	Production of Starches with	
	Altered Chemico-physical Properties	37
	Production of More Starch	38
III	MATERIALS AND METHODS	39
	RNA Isolation	39
	RNA Isolation from Sago Leaf	39
	RNA Isolation from Sago Pith	40
	Genomic DNA Isolation	41
	PCR Cloning of AGP cDNAs	42
	Removal of Contaminating	
	Genomic DNA from RNA	42
	First Strand cDNA Synthesis	43



PCR Amplification with Degenerate Primers	43
Purification of the Band of Interest from Agarose Gel	44
Cloning of PCR Product	44
Analysis of Putative Clones	46
Plasmid Isolation	46
Screening for Plasmid with Insert	47
Sequencing	47
cDNA Library Construction	48
First Strand cDNA Synthesis	48
cDNA Amplification by LD PCR	49
Cloning into λ TriplEx2 Vector	49
Packaging of the Ligated cDNA	51
Titering the Unamplified Library	52
Library Amplification	53
Titering the Amplified Library and Determine the Percentage of Recombinant Clones	53
PCR Screening of cDNA Library	54
Amplification of cDNA	54
Pooling of Phage	54
PCR Analysis	55
Converting λ TriplEx2 to pTriplEx2	56
Sequencing of Putative Clones	57
Semi-Quantitative RT-PCR Analysis	57
First Strand cDNA Synthesis	57
Quantitation of cDNA Synthesised	58
PCR Analysis with Gene Specific Primers	59
Confirmation of the Specificity of PCR Products	60
 IV	
RESULTS AND DISCUSSION	63
Sago Palm RNA	63
Sago Palm Matured Leaf cDNA Library	65
PCR Cloning of Sago Palm AGP cDNAs	66
Comparison Among PCR Clones AGP cDNAs	71
PCR Screening of Full Length <i>agpl1</i>	73
Nucleotide Sequence of Full Length <i>agpl1</i>	74
Deduced Amino Acid Sequence of Full Length <i>agpl1</i>	77
Comparison of Full Length <i>agpl1</i> with <i>E.coli glgC</i>	81
Expression of <i>agpl1</i> , <i>agpl19</i> , <i>agpp10</i> and <i>agpp12</i> in Sago Palm Tissues	85
Southern Blotting Analysis	90
 V	
CONCLUSION	93
 BIBLIOGRAPHY	95
APPENDIX	109



LIST OF TABLES

Table 1	A comparison of sago palm AGP subunits with wheat AGP subunits.	72
Table 2	A comparison of sago palm AGP subunits with potato AGP subunits	73



LIST OF FIGURES

Figure 1	Assumed rate of starch accumulation in a sago palm trunk on clayish soil	5
Figure 2	Amylograph of sago palm starch	7
Figure 3	The structure of amylopectin	9
Figure 4	Photosynthetic carbohydrate metabolism in leaves of C3 plants	13
Figure 5:	Schematic representation of pathways leading to starch in source (mesophyll) and sink cells	17
Figure 6	Steps of starch biosynthesis	18
Figure 7	Total RNA from matured leaves	64
Figure 8	Total RNA from young leaves	65
Figure 9	Total RNA from pith	65
Figure 10	PCR amplification of AGPs cDNA	67
Figure 11	AGP cDNA in pBluescript SK ⁺	67
Figure 12	The nucleotide sequence of partial <i>agpl1</i> and its deduced amino acid sequence	68
Figure 13	The nucleotide sequence of partial <i>agpl19</i> and its deduced amino acid sequence	69
Figure 14	The nucleotide sequence of partial <i>agpl10</i> and its deduced amino acid sequence	69
Figure 15	The nucleotide sequence of partial <i>agpp12</i> and its deduced amino acid sequence	70
Figure 16	Comparison of deduced sago palm AGP subunits amino acid sequences	71
Figure 17	PCR amplification of inserts	74
Figure 18	The nucleotide sequence of full length <i>agpl1</i> and its deduced amino acid sequence	75

Figure 19	Aligned amino acid sequences of sago palm (<i>agpl1</i>) wheat, rice, potato, barley, pea and watermelon AGP small subunit	78
Figure 20	Alignment of the sago palm <i>agpl1</i> N-terminal sequence with that of the purified spinach leaf 51 kDa AGP amino acid sequence obtained by Edman degradation	81
Figure 21	Comparison of the deduced <i>agpl1</i> amino acid sequence with <i>E.coli glgC</i>	82
Figure 22	Semi-quantitative RT-PCR analysis	86
Figure 23	Expression levels of <i>agpl1</i> , <i>agpl19</i> and <i>agpp</i> in different tissues	87
Figure 24	Specificity confirmation of semi-quantitative RT-PCR analysis	88
Figure 25	Expression levels of <i>agpl1</i>	89
Figure 26	Expression levels of <i>agpl19</i>	89
Figure 27	Expression levels of <i>agpp</i>	89
Figure 28	Digested genomic DNA prepared by single CsCl selective sedimentation	91
Figure 29	Genomic DNA isolated with double CsCl selective sedimentation	92
Figure 30	Digested genomic DNA prepared by double CsCl Selective sedimentation	92
Figure 31	λ <i>HindIII</i> DNA marker (Promega)	109
Figure 32	1 kb DNA ladder (Fermentas)	110
Figure 33	100 bp DNA ladder (Fermentas)	111

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
PPF	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 *Areceaceae* (*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 hapaxanthly, meaning "once-flowering". Hapaxanthly 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.

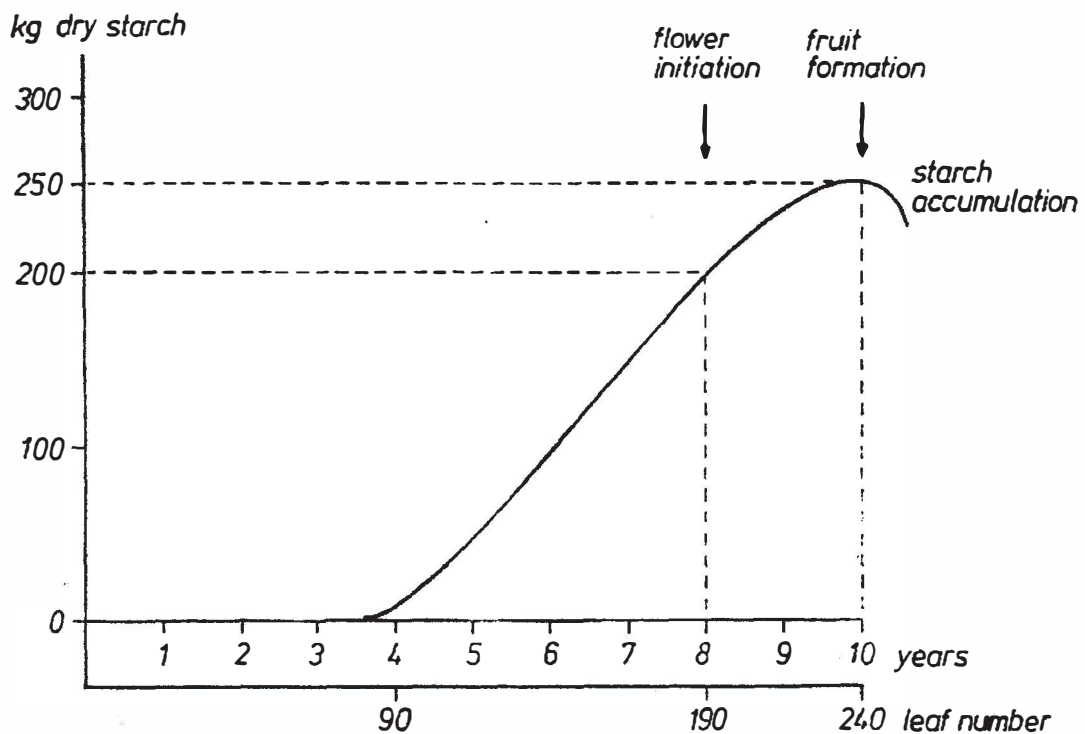


Figure 1: Assumed Rate of Starch Accumulation in a Sago Palm Trunk on Clayish Soil.
(Source: Flach, 1977)

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.

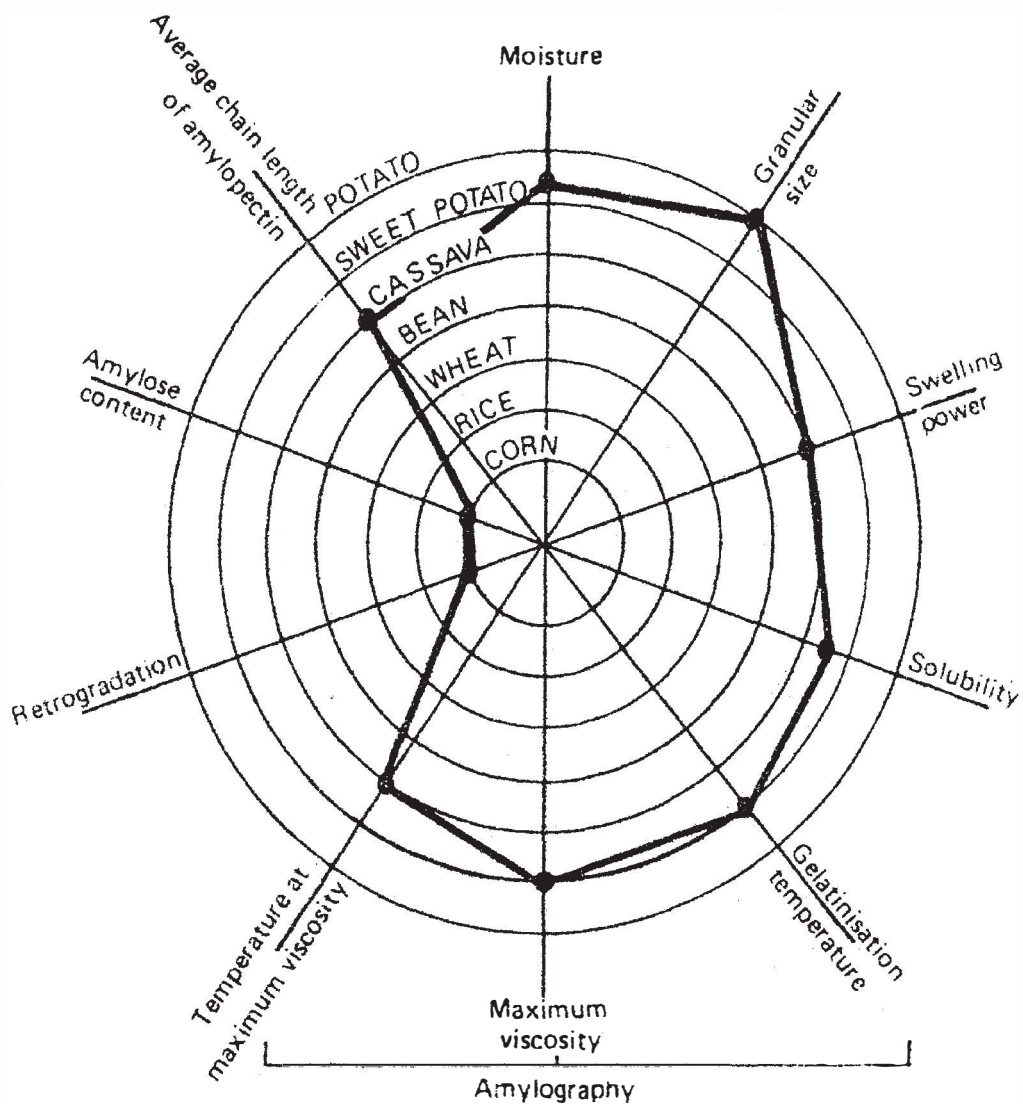


Figure 2: Amylograph of Sago Palm Starch.
(Source: Flach, 1977)

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 $\alpha(1-4)$ -linked glucose residues, each approximately 1000 residues long. Amylose is usually branched at a low level (approximately one branch per 1000 residues) by $\alpha(1-6)$ 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).