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From sucrose to starch granule to starch physical behaviour: a focus on rice starch

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Abstract

The diversity of the physical and consequently the functional behaviour of starches, isolated from different rice varieties, is related to their specific structures. The latter are directly related to the starch biosynthetic pathway. To fully take advantage of the different functionalities of starches from different rice varieties and to design tailor-made starches, it is important to gain insight into biosynthesis–structure–physical behaviour–functionality relations. In a first part of this review, starch composition is described with a focus on rice starch. Secondly, current knowledge on starch biosynthesis is discussed. This more specifically includes (i) the function of the rice biosynthetic enzymes (i.e. adenosine diphosphate glucose pyrophosphorylases, synthases, branching and debranching enzymes), (ii) the effect of mutations on rice starch structure and, (iii) models for amylose and amylopectin synthesis. Thirdly, starch structure [i.e. from granule (2–100 μm), to growth ring (120–500 nm), blocklet (20–500 nm), amorphous and crystalline lamellae (9 nm), and amylopectin and amylose chain levels (0.1–1.0 nm)] is dealt with. Finally, relations between rice starch structural aspects [i.e. amylopectin (core) chain length distributions] and physical behaviour (i.e. gelatinisation and amylopectin retrogradation as measured by differential scanning calorimetry) are studied.

Keywords: Rice starch; Composition; Biosynthesis; Structure; Physical behaviour; Functionality

1. Introduction

Rice (*Oryza sativa L.*) is one of the most important food crops of the world. Besides being consumed as such, its separate fractions are used for various industrial applications. Rice bran and polish, which are the most important minor constituents, are rich sources of protein, oil and dietary fiber. Rice starch, the major constituent of rice and subject of this review, is used, e.g. as thickening, stabilising or filling agent in many food applications.

The biodiversity of rice is larger than that of other cereal grains (e.g. maize and wheat). Thousands of rice varieties exist. From an industrial point of view, this large biodiversity is important as it allows isolation of rice starches with different functionalities. Several studies demonstrated that differences in physical behaviour

Abbreviations: ADP, adenosine diphosphate; ADPGlc, adenosine diphosphate glucose; ADPGlcPP, adenosine diphosphate glucose pyrophosphorylase; *ae*, amylose extender; AFM, atomic force microscopy; AM, amylose; *amf*, amylose free; AP, amylopectin; ATP, adenosine triphosphate; BE, branching enzyme; BEI, branching enzyme I; BEIIa, branching enzyme IIa; BEIIb, branching enzyme IIb; B_l, long B amylopectin chains; B_s, short amylopectin chains; *bt-1*, brittle-1; *bt-2*, brittle-2; CL, average chain length; CT, cystosine and thymine; DBE, debranching enzyme; D-enzyme, disproportioning enzyme; DP, degree of polymerisation; DP_n, number average degree of polymerisation; DP_w, weight average degree of polymerisation; DSC, differential scanning calorimetry; *du*, dull; ECL, average external chain length; FAM, free amylose; Frc 6P, fructose-6-phosphate; GBSS, granule bound starch synthase; Glc 1P, glucose-1-phosphate; Glc 6P, glucose-6-phosphate; *lam*, low amylose; ΔH_{retro}, amylopectin retrogradation enthalopy (on dry matter starch basis); $\Delta H_{AP\,retro}$, amylopectin retrogradation enthalpy (on amylopectin basis); ICL, average internal chain length; LAM, lipid complexed amylose; MOS, malto-oligosaccharides; PB, protein body; PBI, protein body I; PBII, protein body II; PG, phytoglycogen; 3-PGA, 3-phosphoglycerate; P_i, inorganic orthophosphate; PP_i, inorganic pyrophosphate; Pre-AP, pre-amylopectin; *sbe1*, starch branching enzyme I; SAXS, small-angle X-ray scattering; SCLCP, side-chain liquid-crystalline polymer; *sh*, shrunken; *sh-2*, shrunken-2; SS, starch synthase; SSI, starch synthase I; SSIIa, starch synthase IIa; SSIII, starch synthase III; SSIIIa, starch synthase IIIa; SSIIIb, starch synthase IIIb; *sug-1*, sugary-1; TEM, transmission electron microscopy; T_o, gelatinisation onset temperature; T_p, gelatinisation peak temperature; T_c, gelatinisation conclusion temperature; $T_{c\,retro}$, amylopectin retrogradation conclusion temperature; UDPGlc, uridine diphosphate glucose; UDPGlcPP, uridine diphosphate glucose pyrophosphorylase; UTP, uridine triphosphate; WSP, water soluble polysaccharides; *wx*, waxy.

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and functionality of rice starches are related to starch structure (Fujita et al., 2003; Lai, Lu, & Lii, 2000; Lai, Shen, Yeh, Juliano, & Lii, 2001; Lin, Yeh, & Lii, 2001; Lu, Chen, & Lii, 1997; Nakamura et al., 2002; Noda, Nishiba, Sato, & Suda, 2003; Patindol & Wang, 2002; Qi, Tester, Snape, & Ansell, 2003; Vandeputte, Derycke, Geeroms, & Delcour, 2003; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003a, b; Wang & Wang, 2002; Yao, Zhang, & Ding, 2002). The different starch structures originate from the varying activity levels of the isoforms of starch biosynthetic enzymes.

Within the past decade, much information has been and is still being gathered on how mutations (i.e. under-expressions of isoforms of biosynthetic enzymes), alter rice starch structure, and consequently starch physical behaviour and functionality. In what follows, a basic overview is given of the current knowledge on rice starch composition, biosynthesis, structure and relations between starch structure and physical behaviour. In a first part, the major and minor components of rice starch are described. In a second part, the biosynthesis of rice starch is reviewed. Both the role of biosynthetic enzymes, the effects of mutations on rice starch structure and models for amylose and amylopectin synthesis are described. In a third part, we look at the starch structure from granules (2–100 μm), to growth rings (120–500 nm), to blocklets (20–500 nm), to amorphous and crystalline lamellae (9 nm), to amylopectin and amylose chains (0.1–1.0 nm) with a focus on rice starch. The different models of starch structure that have been brought about and often have been refined, are evaluated. Finally, relations between rice starch structural aspects [amylopectin (core) length distribution] and physical behaviour (i.e. gelatinisation and amylopectin retrogradation as measured by differential scanning calorimetry) are studied. While the papers by Denyer, Johnson, Zeeman, and Smith (2001), James, Denyer, and Myers (2003), Kossmann and Lloyd (2000), Myers, Morell, James, and Ball (2000), Nakamura (2002), and Smith (2001) and the most recent chapter on starch by Fitzgerald (2003) in the Rice Chemistry and Technology (3rd edition) generally cover the enzyme systems involved in starch biosynthesis in more depth, the present effort brings together current views on rice starch composition and biosynthesis as well as on rice starch structure and relations between rice starch structure and physical behaviour.

2. Starch composition

2.1. Amylose

Starch consists mainly of amylose and amylopectin. Amylose is an essentially linear polymer of α-(1 → 4)-linked D-glucopyranosyl units with few (<0.1% according to Ball et al., 1996) α-(1 → 6) linkages. It has a number average degree of polymerisation (DP_n) of 800–4920, average chain lengths (CL) of 250–670 and β-amylolysis limits of 73–95% (Morrison & Karkalas, 1990). Rice starch amyloses have DP_n values of 920–1110 (with little variety difference between Indica and Japonica amylose), CL of 250–370 and β-amylolysis limits of 73–84%. They are slightly branched with 2–5 chains on average (Takeda, Hizukuri & Juliano, 1986). Wheat (DP_n 1300) and maize (DP_n 930) amyloses have a similar DP_n, whereas potato (DP_n 4920) and tapioca (DP_n 2600) amyloses have higher DP_n values (Champagne, 1996; Takeda et al., 1986). Takeda, Tomooka, and Hizukuri (1993) found ratios of branched to linear rice amylose molecules of 0.22:0.78 by mole and 0.32:0.68 by weight with DP_n values of 1180 and 740, respectively. The branched amylose molecule has been suggested to have a structure intermediate between that of linear amylose and amylopectin and is consequently often referred to as intermediate material (Takeda et al., 1993).

Typical levels of amyloses in starches are 15-25% (Manners, 1979). However, waxy (*wx*) rice (Sano, 1984) and, e.g. *wx* maize (Shure, Wessler, & Fedoroff, 1983) and *wx* wheat (Nakamura, Yamamori, Hirano, Hidaka, & Nagamine, 1995) starches are virtually amylose free. On the contrary, mutants with high levels of amylose are also known. Amylose extender (*ae*) mutants of maize (Boyer & Preiss, 1978) have amylose contents in a range of 50–85% (Manners). In rice, amylose contents are classified as waxy (0–2% amylose), very low (5–12%), low (12–20%), intermediate (20–25%) or high (25–33%) (Juliano et al., 1981). The *ae* mutants of rice have amylose contents in a range of 35–40% (Juliano, 1992). In determining amylose contents, the existence of both lipid complexed amylose (LAM) and free amylose (FAM) (major fraction) must be taken into account (Morrison, Tester, Snape, Law, & Gidley, 1993). LAM may be present in the native starch (Morrison et al., 1993), but is possibly also formed during hydrothermal treatment or gelatinisation of the starch (Biliaderis, Page, Maurice, & Juliano, 1986). Furthermore, amylose forms complexes with, e.g. iodine and alcohols.

2.2. Amylopectin

Amylopectin consists of α-(1 → 4) linked D-glucosyl chains and is highly branched with 5–6% α-(1 → 6)-bonds (Buléon et al., 1998). It has a DP_n of 4700–12,800, CL values of 17–24 and β-amylolysis limits of 55–60% (Morrison & Karkalas, 1990). The individual chains may vary between 10 and 100 glucose units (Manners, 1979). Rice starch amylopectins have a DP_n of 8200–12,800, CL of 19–23, β-amylolysis limits between 49–59% (Takeda, Hizukuri, & Juliano, 1987), average external chain lengths (ECL) of 11.3–15.8 and average internal chain lengths (ICL) of 3.2–5.7 (Lu et al., 1997). Compared to amylopectins of Japonica rice starch, waxy and non-waxy amylopectins of Indica rice starch have lower DP_n values (Indica: DP_n 4700–5800, Japonica: DP_n 8200–12,800), but higher CL (Indica: CL 21–22, Japonica: CL 19–20) (Takeda et al., 1987), higher ECL (Indica: ECL 13.2–15.8, Japonica: ECL 11.8–12.6) and higher ICL values

(Indica: ICL 4.8–5.7, Japonica: ICL 3.2–4.6) (Lu et al., 1997). Waxy Japonica rice starches have the lowest CL (17–19) (Morrison & Karkalas, 1990). For wheat and maize, e.g. DP_n values of 5000–9400 and 10,200, CL values of 19–20 and 22 and β-amylolysis limits of 56–59 and 60% have been reported (Shibanuma, Takeda, Hizukuri, & Shibata, 1994; Morrison & Karkalas, 1990).

Recently, Takeda, Shibahara and Hanashiro (2003) reported the DP_n of amylopectins from starches of different botanical orgins to be in the range of 9600–15,900. Moreover, they revealed the presence of large (DP_n 13,400–26,500), medium (DP_n 4400–8400) and small (DP_n 700–1200) species.

Amylopectin is generally defined in terms of a cluster model (Nikuni, 1978; Robin, Mercier, Charbonnière, & Guilbot, 1974; Robin, Mercier, Duprat, Charbonnière, & Guilbot, 1975) with polymodal chain length distribution (Hizukuri, 1986) and a non-random nature of branching (Thompson, 2000) (details in Section 4.5.).

2.3. Minor components

The main minor components in starch, which are either at the surface or inside the starch granules, are lipids and proteins. Cereal starches contain up to ~1% lipids and ~0.25% proteins (Baldwin, 2001; Swinkels, 1985). Non-waxy rice starches (12.2–28.6% amylose) contain 0.9–1.3% lipids comprising 29–45% fatty acids and 48% lysophospholipids (Azudin & Morrison, 1986). Waxy rice starches (1.0–2.3% amylose) contain negligible amounts of lipids (Azudin & Morrison, 1986). Starch proteins are mostly either (i) storage proteins or (ii) biosynthetic or degradative enzymes (Baldwin, 2001). Rice storage proteins exist mainly as protein bodies (PB), i.e. PB I (mainly prolamin) or PB II (mainly gluteline) (Resurreccion, Li, Okita, & Juliano, 1993). Biosynthetic or degradative enzymes are most probably entrapped within the starch granules following starch synthesis (Denyer et al., 1995). Besides lipids and proteins, phosphorus is an important non-carbohydrate component of rice starch. In waxy rice starches, it is mainly present as phosphate-monoesters (0.003% on dry basis), whereas non-waxy rice starches predominantly contain phospholipid phosphorus (0.048% on dry basis) (Hizukuri, Kaneko, & Takeda, 1983; Jane, Kasemsuwan, Chen, & Juliano, 1996). Other mineral components of starch are calcium, potassium, magnesium and sodium in their ionic form.

3. Starch biosynthesis

The starch biosynthetic pathway plays a distinct role in plant metabolism. In the plastids of higher plants, either transient or reserve starch is formed. The former is synthesised in leaves and serves as a temporary carbohydrate reserve. It is accumulated in chloroplasts during the day and serves as a major source for cytosolic sucrose synthesis at night (Shannon & Garwood, 1984). Sucrose is then transported to the storage organs of plants, such as seeds, fruits, tubers and roots. Subsequently, precursors for starch biosynthesis are formed in the apoplast and cytosol of cells. Eventually, reserve starch is biosynthesised in amyloplasts.

3.1. Precursors for starch biosynthesis

The formation of adenosine diphosphate glucose (ADPGlc) is the first committed step of the starch biosynthetic pathway (Rahman, Batey, Cochrane, Appels, & Morell, 2000). However, before ADPGlc is formed, the action of apoplastic (Emes et al., 2003; Hirose, Takano, & Terao, 2002) and cytoplastic (James et al., 2003; Kossmann & Lloyd, 2000; Smith, 2001; Smith, Denyer, & Martin, 1997) enzymes is required. Fig. 1 describes the main enzyme reactions.

Sucrose, the primary substrate, is degraded by three different mechanisms. Firstly, sucrose is hydrolysed into glucose and fructose by an apoplastic invertase (EC 3.2.1.26) (1 in Fig. 1). Although this enzyme may be partly involved in starch accumulation, its main involvement is in the proliferation of rice endosperm cells (Hirose et al., 2002). The formed glucose and fructose are transported into the cytosol by a hexose transporter (2 in Fig. 1). Glucose is converted into glucose 6-phosphate (Glc 6P) and fructose into fructose 6-phosphate (Frc 6P) by a hexokinase (EC 2.7.1.1) (3 in Fig. 1) and a fructokinase (EC 2.7.1.4) (4 in Fig. 1), respectively. Frc 6P is converted into Glc 6P by the action of a glucose phosphate isomerase (EC 5.3.1.9) (5 in Fig. 1).

Secondly, sucrose is transported into the cytosol via a sucrose transporter (6 in Fig. 1) (Furbank et al., 2001; Scofield etal., 2002). Subsequently, the conversion of sucrose into glucose and fructose is catalysed by cytoplastic invertase (EC 3.2.1.26) ($1'$ in Fig. 1).

Thirdly, sucrose in the cytosol is converted into fructose and uridine diphosphate glucose (UDPGlc) by sucrose synthase (EC 2.4.1.13) (7 in Fig. 1), which, compared to apoplastic and cytosolic invertase, is most likely the predominant enzyme during endosperm development (e.g. wheat: Riffkin, Duffus, & Bridges, 1995). UDPGlc is then further metabolised to Glc 1P by action of UDPGlc pyrophosphorylase (UDPGlcPP) (EC 2.7.7.9) (8 in Fig. 1). The formed Glc 1P serves as substrate for cytosolic phosphoglucomutase (EC 2.7.5.1) (9 in Fig. 1) to form Glc 6P. Both Glc 1P and Glc 6P are translocated into the amyloplasts via phosphate translocators (10 in Fig. 1; Kammerer et al., 1998; Tetlow, Bowsher & Emes, 1996). In wheat endosperm, the main hexose unit is Glc 1P (Tetlow, Blissett, & Emes, 1994), whereas it is Glc 6P in pea embryos (Hill & Smith, 1991). To the best of our knowledge, it is unknown whether mainly Glc 1P or Glc 6P is transported to the amyloplast in the case of rice. Glc 6P in the amyloplast

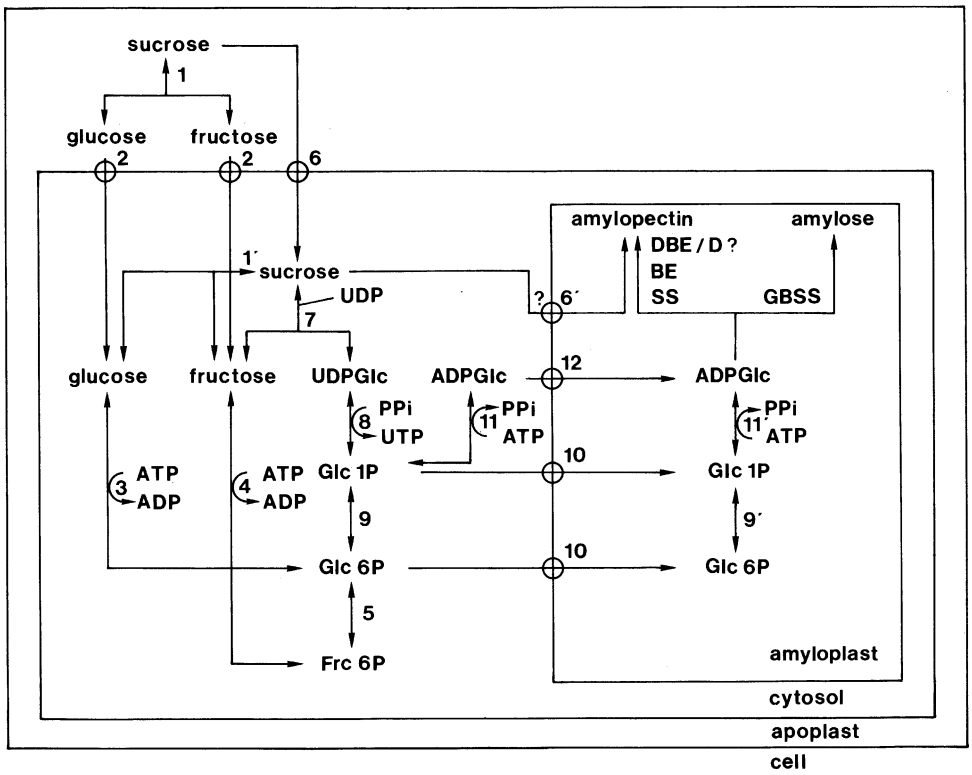

Fig. 1. The starch biosynthetic pathway in non-photosynthetic cells. (1) apoplastic invertase (EC 3.2.1.26), (1′) cytoplastic invertase (EC 3.2.1.26) (2) hexose transporter, (3) hexokinase (EC 2.7.1.1), (4) fructokinase (EC 2.7.1.4), (5) glucose phosphate isomerase (EC 5.3.1.9), (6) sucrose transporter, (6′) sucrose transporter, (7) sucrose synthase (EC 2.4.1.13), (8) uridine diphosphate glucose pyrophosphorylase (EC 2.7.7.9), (9) cytosolic phosphoglucomutase (EC 2.7.5.1), (10) phosphate translocator, (11) cytosolic adenosine diphosphate glucose pyrophosphorylase (EC 2.7.7.27), (11′) amyloplastic adenosine diphosphate glucose pyrophosphorylase (EC 2.7.7.27), (12) adenosine diphosphate glucose translocator; ADP, adenosine diphosphate; ADPGlc, adenosine diphosphate glucose; ATP, adenosine triphosphate; BE, branching enzyme (EC 2.4.1.18); D, disproportionating enzyme (D-enzyme) (EC 2.4.1.25); DBE, debranching enzyme [isoamylase (EC 3.2.1.68); pullulanase (EC 3.2.1.41)]; Frc 6P, fructose 6 phosphate; GBSS, granule bound starch synthase (EC 2.4.1.21); Glc 1P, glucose 1 phosphate; Glc 6P, glucose 6 phosphate; PP_i, inorganic pyrophosphate; SS, starch synthase (EC 2.4.1.21); UDP, uridine diphosphate; UDPGlc, uridine diphosphate glucose; UTP, uridine triphosphate; DBE, debranching enzyme.

is then converted into Glc 1P by amyloplastic phosphoglucomutase (EC 2.7.5.1) (9′ in Fig. 1). Glc 1P subsequently serves as a substrate for amyloplastic ADPGlc pyrophosphorylase (ADPGlcPP) (EC 2.7.7.27) (11′ in Fig. 1). Subsequently, ADPGlc is formed.

Remarkably, Mukerjea and Robyt (2003) recently suggested that, besides ADPGlc, sucrose might be the immediate and specific substrate (thus no UDPGlc and ADPGlc intermediates) for an enzyme system [starch synthase (SS) + branching enzyme (BE)] catalysing the synthesis of a highly branched component in starch. It is possible that sucrose is transported into the amyloplast via a sucrose transporter (6′ in Fig. 1). The rationale behind this view is as follows. The level of α-limit dextrin in 12 starches of different botanical origin is 0.51–8.47% w/w (with 8.47% for normal rice starch) (Mukerjea & Robyt, 2003). Incubation of these starches with [^{14}C]-sucrose resulted in incorporation of [^{14}C]-glucose. Subsequent α-amylolysis of the starches resulted in a high percentage (16.1–84.1%) of high molecular weight α-limit dextrins (Mukerjea & Robyt, 2003). On the contrary, incubation of the same starches with ADPGlc or UDPGlc did not result in a significant amount of α-limit dextrin (only 1–2%) (Mukerjea & Robyt, 2003). In conlusion, the highly branched component in starch is thus most likely formed via sucrose and not via ADPGlc.

3.2. Enzymes involved in starch biosynthesis

3.2.1. Adenosine diphoshate glucose pyrophosphorylase (ADPGlcPP) (Table 1)

ADPGlcPP (EC 2.7.7.27) catalyses the conversion of Glc 1P and adenosine triphosphate (ATP) into ADPGlc and inorganic pyrophosphate (PP_i) (11 and 11′ in Fig. 1). This tetrameric enzyme generally consists of large and small subunits that show variations in composition within plant organs and between species (James et al., 2003; Nakamura & Kawaguchi, 1992; Smith-White & Preiss, 1992). Genes for large and small subunits in the rice protein have been described by Anderson et al. (1991). It is suggested that ADPGlcPP of rice endosperm has at least one small and two large subunits (Nakamura, 2002).

There are two possible outcomes: (i) in cereal endosperms (with exception of wheat), ADPGlcPP is mainly cytosolic (11 in Fig. 1) and partially plastidial (11′ in Fig. 1) [e.g. rice (Sikka et al., 2001), barley (Thorbjørnsen, Villand,

Table 1
Roles of adenosine diphosphate glucose pyrophosphorylases and starch synthases in rice starch biosynthesis and effect of important mutations on rice starch biosynthesis and structure

Enzyme	Role in rice starch biosynthesis	Mutant	Effect of mutation on rice starch biosynthesis and structure	Reference
ADPGlcPP large subunit	ADPGlc transport	*Shrunken (Sh)*	↑[a] or ↓[b] amylose content, amylopectin with sligthly shorter chains	Asoaka et al. (1993)
ADGlcPP small subunit	No data available	*Brittle-2 (Bt-2)*in maize	No data available	No data available
ADPGlc translocator	No data available	*Brittle-1 (Bt-1)*in maize	No data available	No data available
SSI	Synthesis of very short chains of amylopectin	SSI deficient	↑[a] [DP 6–7], ↓[b] [DP 8–12]	Nakamura (2002) and Umemoto et al. (2002)
		Amylose extender (*ae*)[c] lowered SSI activity	Pleiotropic effect with BEIIb	Nishi et al. (2001)
SSIIa	Elongation A and B_1 chains of amylopectin	SSIIa deficient	↑[a] [DP ≤ 11], ↓[b] [12 ≤ DP ≤ 24]	Umemoto et al. (2002), Nakamura (2002)
SSIII	Most likely formation of long B_1 and B_2 chains of amylopectin	SSIII deficient	No data available	Nakamura (2002)
GBSSI	AM synthesis	*Waxy (wx)*	amylose free	Sano (1984) and Yano et al. (1988)
		Dull (du)	↓[b] amylose content	Asaoka et al. (1993)
		Opaque (op)	↓[b] amylose content	Mikami et al. (1999)
	AP chain elongation ?			Takeda et al. (1987) Lu et al. (1997) Villareal et al. (1997)

ADPGlc, adenosine diphosphate glucose; ADPGlcPP, Adenosine diphosphate glucose pyrophosphorylase; BEI, branching enzyme I; BEIIa, branching enzyme IIa; BEIIb, branching enzyme IIB; GBSS, granule bound starch synthase; SSI, starch synthase I; SSIIa, starch synthase IIa; SSIIb, starch synthase IIb; SSIII, starch synthase III.

[a] ↑, increase.

[b] ↓, decrease.

[c] Nishi et al. (2001) showed that both branching enzyme IIb (BEIIb) and starch synthase I (SSI) activities were lower in *ae* rice mutants.

Denyer, Olsen, & Smith, 1996), maize (Denyer, Dunlap, Thorbjørnsen, Keeling, & Smith, 1996) endosperm] and the formed cytosolic ADPGlc is translocated to the amyloplast via an ADPGlc translocator or *Brittle-1* protein (12 in Fig. 1) (Pozueta-Romero, Frehner, Viale, & Akazawa, 1991; Shannon, Pien, Cao, & Liu, 1998) or (ii) in other cereal starch-storing organs and in non-cereal tissues (endosperms and other starch-storing organs), ADPGlcPP is solely plastidial [e.g. potato tuber (Naeem, Tetlow, & Emes, 1997; Sweetlove, Burrell, & Rees, 1996)] (Beckles, Smith, & Rees, 2001; James et al., 2003).

The study of ADPGlcPP and adenylate translocator deficient mutants has provided evidence for the importance of ADPGlcPP in starch biosynthesis. In maize endosperms, *brittle*-2 and *shrunken*-2 mutants, lesions within the small and large subunit genes, respectively, are associated with a reduction in starch content (Tsai & Nelson, 1966; Kossman & Lloyd, 2000). Furthermore, the *brittle*-1 mutation in maize reduces the amount of starch produced in the endosperm and results in the accumulation of ADPGlc in the cytosol (Sullivan, Strelow, Illingworth, Phillips, & Nelson, 1991). As for rice endosperms, Asaoka, Okuno, Yano, and Fuwa (1993) reported that *shrunken* mutants of rice either showed an increase or decrease in amylose content and the amylopectin had slightly shorter chains. To the best of our knowledge, no data are available on the ADPglcPP small subunit and the ADPGlc translocator of rice.

Finally, in contrast to ADPGlcPP of barley, wheat and some maize endosperms, ADPGlcPP of rice endosperm is an allosteric enzyme. It is activated by 3-phosphoglycerate (3-PGA) and inhibited by orthophosphate (P_i) (Sikka et al., 2001). Ballicora et al. (1995) suggested that the ADPGlcPP large subunit modulates the sensitivity of the small subunit to allosteric regulation by P_i and 3-PGA. The major function of the small ADPGlcPP subunit was proposed to be catalysis (Ballicora et al., 1995). These allosteric features are suggested to be important with regard to control of the rate of starch synthesis (Smith et al., 1997).

3.2.2. Starch synthase (SS) (Table 1)

Succeeding the ADPGlcPP catalytic reaction, starch synthase (SS) (EC 2.4.1.21) catalyses the transfer of the glucosyl unit from ADPGlc to the reducing end of a glucose chain via an α, 1 → 4 linkage (Mukerjea, Yu, & Robyt, 2002) (Fig. 1). Five isoforms of SS have been distinguished in rice (Nakamura, 2002): soluble starch synthases SSI, SSIIa, SSIIIa, SSIIIb and granule-bound starch synthase (GBSS) or waxy (*wx*) protein. Soluble starch synthases have a higher affinity for ADPGlc than GBSS and are mainly responsible for amylopectin elongation. However, some

'soluble' starch synthases are partially 'granule bound' in e.g. maize (Harn et al., 1998; Mu-Forster et al., 1996) and potato tubers (Edwards et al., 1995).

SSI in rice most likely contributes to the synthesis of very short amylopectin chains as SSI deficient rice mutants have decreased amounts of amylopectin chains with DP 8–12, whereas they are enriched in amylopectin chains of DP 6–7 (Nakamura, 2002). Moreover, and, as discussed below, *ae* mutants of rice have both reduced branching enzyme IIb (BEIIb) activity and SSI activity (Nishi, Nakamura, Tanaka, & Satoh, 2001).

Umemoto, Yano, Satoh, Shomura, and Nakamura (2002) and Nakamura et al. (2002) suggested that the SSIIa gene is one of the important genes (other genes may play additional roles) and consequently SSIIa an important biosynthetic enzyme determining starch structure and consequently its properties. Evidence for the importance of the SSIIa gene was based on the fact that the SSIIa gene, the *alk(t)* gene [controlling the varietal difference in the alkali (1.3% KOH, at room temperature) disintegration of starch granules], the *gel(t)* gene (controlling the difference in the gelatinisation in 4 M urea solution at 30 °C), and the *acl(t)* gene [controlling the difference in amylopectin chain length distribution, i.e. the ratio of short (DP ≤ 11) to intermediate size (12 ≤ DP ≤ 24) amylopectin chains] all map to the same locus on the chromosome 6 in the rice genome. The SSIIa biosynthetic enzyme was suggested to have a role in elongating A and B_1 amylopectin chains (Nakamura et al., 2002). Nakamura et al. (2002) suggested Indica and Japonica rice varieties to have L-type and S-type amylopectin, respectively. L-type amylopectin (Indica rice) has less short (DP ≤ 11) and more intermediate size (12 ≤ DP ≤ 24) chains than S-type amylopectin (Japonica rice). The SSIIa activity determined the ratio of these chain lengths and is defective in S-type amylopectin and thus in Japonica varieties (Nakamura et al., 2002; Umemoto et al., 2002).

Finally, although SSSIII deficiency has not yet been reported in rice, it is likely that the enzyme plays a distinct role in the formation of long B_1 and B_2 amylopectin chains (Nakamura, 2002).

Granule bound starch synthase I (GBSSI), the product of the *wx* gene, is bound to the starch granules and generally has an apparent molecular weight of ca. 60 kDa [rice (Villareal & Juliano, 1986); maize (Shure et al., 1983), pea (Denyer et al., 1995)]. It is mainly responsible for amylose synthesis. The well known *wx* or glutinous rice mutants (Sano, 1984) lack GBSS as they carry the recessive *wx* gene and are consequently virtually amylose free. Similar mutants are the *wx* mutants of maize (Shure et al., 1983), the amylose free (*amf*) mutants of potato and the low amylose *(lam)* mutants of pea (Denyer et al., 1995).

The rice amylose content is determined by the (activity) level of GBSSI, which in turn depends on the three functional alleles at the *wx* locus: (i) the Wx^a allele, (ii) the Wx^b allele, derived from the Wx^a allele during domestication of rice (Sano, 1984), and (iii) the waxy opaque (Wx^{op}) allele, also derived from the Wx^a allele, independently of the Wx^b gene (Mikami, Aikawa, Hirano, & Sano, 1999). The Wx^{op} allele is expressed in opaque rice, which, unlike *wx* rice, contains ~10% amylose. Sano (1984) described the differential regulation of waxy gene expression in rice endosperm. The Wx^a and Wx^b alleles regulate the level of GBSS. Rice plants with the Wx^a allele (Indica rice varieties) produce more GBSS than Japonica rice varieties with the Wx^b allele. Consequently, Indica rices have higher amylose contents than Japonica rices (Villareal & Juliano, 1989). The mutation in Wx^b is a single-base substitution form G to T at the 5′ splice site of the first intron of GBSSI, changing the sequence at the splice site from AG*G*TATA to AG*T*TATA (Fitzgerald, 2003; Isshiki et al., 1998; Mikami et al., 1999; Olsen & Purugganan, 2002; Wang et al., 1995).

More recently, it is suggested that a microsatellite, i.e. a simple sequence repeat of 8–20 cytosine and thymine (CT) dinucleotide repeats in the 5′ untranslated region of exon 1 of the GBSSI gene (Ayres et al., 1997; Bligh, Till, & Jones, 1995), discriminates between different types of amylose. Fitzgerald (2003) summarised the observed relationships between the amylose content and the number of CT repeats (Ayres et al., 1997; Bao, Corke, & Sun; 2002; Bergman, Delgado, Mc Clung, & Fjellstrom, 2001; Bligh et al., 1995; Olsen & Purugganan, 2002): (i) rices with 18–19 CT repeats are low amylose temperate Japonica rices with the Wx^b allele, (ii) rices with 14–20 CT repeats are intermediate amylose tropical Japonica with the Wx^a allele (14–20 CT repeats), (iii) rices with 8, 10 or 11 CT repeats are intermediate or high amylose Indica rices carrying the Wx^a allele (8, 10 or 11 CT repeats) and (iv) rices with 17 CT repeats have evolved in several phylogenetic categories, including waxy, and carry either the Wx^a or the Wx^b allele.

However, the levels of amylose in rice are also controlled by the *dull* locus. *Dull*-mutants of rice have reduced amounts of GBSS and consequently have lower amylose contents than normal amylose rice starches (Isshiki, Nakajima, Satoh, & Shimamoto, 2000; Yano, Okuno, Satoh, & Omura, 1988).

Finally, GBSS is most likely also involved in synthesis of long amylopectin chains (Denyer, Clarke, Hylton, Tatge, & Smith, 1996). Evidence includes (i) that *wx* rice starch is depleted in very long amylopectin chains (Hizukuri, Takeda, Maruta, & Juliano, 1989) and (ii) that high amylose (Indica) rice starches contain higher proportions (up to 14.2%) of long (CL 85–180) amylopectin chains (Takeda et al., 1987). Villareal, Hizukuri, and Juliano (1997) also showed that amylopectin from high amylose rice starches contains a long amylopectin chain fraction with weight average degree of polymerisation (DP_w) > 120. Surprisingly, and in spite of the longer CL than in ordinary amylopectin, the amylose extender *(ae)* mutant (see below in paragraph on starch branching enzymes) of IR36 rice starch does not have very long amylopectin chains [B_4 chains with $DP_w > 200$)] (Villareal et al., 1997). Similarly, Lu et al. (1997) found Indica varieties with high amylose contents to have

Table 2
Roles of branching and debranching enzymes in rice starch biosynthesis and effect of important mutations on rice starch biosynthesis and structure

Enzyme	Role in rice starch biosynthesis	Mutant	Effect of mutation on rice starch biosynthesis and structure	Reference
BEI	Role in synthesis of B_1, B_2, B_3 chains of amylopectin	*Starch-branching enzyme 1 (sbe1)*	↓[b] [12 ≤ DP ≤21], ↓[b] [DP ≥ 37], ↑[a] [DP ≤10], ↑[a] [24 ≤ DP ≤34]	Nakamura (2002) and Satoh et al. (2003)
BEIIa	Support of BEI and/or BEIIb function	BEIIa deficient	No significant change in amylopectin chain length distribution	Nakamura (2002)
BEIIb	Role in synthesis of A chains of amylopectin	*Amylose extender (ae)*	↓[b] [DP ≤ 17], ↑[a] [18 ≤ DP ≤ 36], ↑[a] [DP ≥ 38] 'apparently' high amylose content	Asaoka et al. (1993), Nishi et al. (2001) and Yano et al. (1988)
Isoamylase + Pullanase	Debranching amylopectin	*Sugary-1 (sug-1)*	Synthesis of (i) sugary-amylopectin: ↑[a] [DP < 12], ↓[b] [DP13–24] vs. wild type amylopectin and (ii) phytoglycogen	Kubo et al. (1999), Nakamura et al. (1996) and Wong et al. (2003)
Isoamylase	Debranching amylopectin	*Isoamylase-1*	Synthesis of (i) water insoluble modified amylopectin and (ii) water soluble polyglucan	Fujita et al. (2003)

BEI, branching enzyme I; BEIIa, branching enzyme IIa; BEIIb, branching enzyme IIb.
[a] ↑, increase.
[b] ↓, decrease.

10.1–10.9 weight % extra long chains (DP > 100). Thirdly, GBSS catalyses the incorporation of ADP-[^{14}C] glucose into amylopectin chains of starch isolated from sweet potato roots (Baba, Yoshii, & Kainuma, 1987), pea embryos (Denyer, Clarke, et al., 1996) and potato tubers (Denyer, Clarke, et al.). Nevertheless, a dramatic shift in the incorporation of [^{14}C]-glucose from amylopectin to amylose was observed when malto-oligosaccharides (MOS) were added to isolated granules from pea embryos and potato tubers (Denyer, Clarke, et al.) (details on amylose synthesis in Section 3.4.).

3.2.3. Starch branching enzyme (BE) (Table 2)

After elongation of the glucan chains by starch synthases, starch branching enzymes (BE) (EC 2.4.1.18) or Q-enzymes catalyse (i) the hydrolysis of α, 1 → 4 bonds and (ii) the transfer of the released reducing end to a C_6-glucosyl unit resulting in an α, 1 → 6 branch point (Fig. 1). In rice (Yamanouchi & Nakamura, 1992), maize (Guan & Preiss, 1993) and barley (Sun, Sathish, Ahlandsberg, Deiber, & Jansson, 1997) at least three isoforms of BE are distinguished: BEI, BEIIa and BEIIb. BEI preferentially branches amylose, whereas BEII preferentially branches amylopectin (Guan & Preiss, 1993; Rydberg, Andersson, Andersson, Åman, & Larsson, 2001).

Recently, it was shown that starch-branching enzyme 1 (*sbe1*) mutants of rice have (i) decreased levels of amylopectin chains with DP 12–21 and DP ≥ 37, (ii) increased levels of amylopectin chains with DP ≤ 10 and, (iii) slightly increased levels of amylopectin chains with DP 24–34 (Satoh et al., 2003). The results suggested that BEI has an important role in the synthesis of B_1, B_2 and B_3 amylopectin chains (Nakamura, 2002; Satoh et al.).

Nakamura (2002) showed that BEIIa deficiency does not significantly change the amylopectin chain length profile and suggested that BEIIa supports the function of BEIIb and/or BEI in the rice endosperm.

Amylose extender (*ae*) mutants of, e.g. rice (Mizuno et al., 1993; Nishi et al., 2001) and maize (Boyer & Preiss, 1978) are BEIIb deficient. Nishi et al. (2001) reported that *ae* mutants of rice have (i) decreased levels of amylopectin chains with DP ≤ 17 (the greatest reduction in DP 8–12), (ii) increased levels of intermediate (DP 18–36) and long (DP ≥ 38) amylopectin chains and (iii) 'apparently' high amylose contents caused by the abnormal amylopectin structure (i.e. increased amounts of longer amylopectin chains) (Nishi et al., 2001). In addition, the *ae* mutation lowered the SSI activity. Therefore, it was suggested that BEIIb has a distinct role in the transfer of short chains, which are then most probably extended by starch synthase to form A and B_1 chains of the rice amylopectin cluster structure (Nishi et al., 2001).

3.2.4. Starch debranching enzyme (DBE) (Table 2)

Following starch branching, starch debranching enzymes (DBE) (EC 3.2.1.10) catalyse the hydrolysis of α, 1 → 6 bonds (Fig. 1). Two debranching enzymes with different substrate specificity have been distinguished: (i) isoamylase (EC 3.2.1.68) and (ii) pullulanase (EC 3.2.1.41). Isoamylase debranches both glycogen, phytoglycogen and amylopectin, but does not attack pullulan (Nakamura, 1996). In contrast, pullulanase attacks both pullulan and amylopectin, but not glycogen and phytoglycogen. For a long time, one was convinced that debranching enzymes were only involved in starch degradation but not in starch biosynthesis. Although the specific roles of isoamylase and pullulanase have not yet been completely elucidated, *sugary-1*(Kubo et al., 1999; Wong et al., 2003) and *isoamylase-1* mutants (Fujita et al., 2003) of rice endosperms provided evidence that both DBE are involved in biosynthesis of rice endosperm amylopectin (Fujita et al.; Kubo et al., 1999; Nakamura et al., 1996; Wong et al.). *Sugary-1* mutants of rice endosperm, which have very low isoamylase activities (or absent) and decreased pullulanase activities (Kubo et al., 1999), contain more *sugary*-amylopectin and water-soluble phytoglycogen

rather than wild type amylopectin (Wong et al., 2003). The *sugary*-amylopectin is located at the outer regions of the rice endosperm, has more short amylopectin chains (DP < 12), but less amylopectin chains of DP 13–24 than wild-type amylopectin (Wong et al., 2003). In contrast, phytoglycogen is found within the inner regions of the rice endosperm and is highly branched (> sugary-amylopectin > wild type amylopectin) (Wong et al., 2003).

Moreover, *isoamylase1*-mutants of rice endosperm are isoamylase deficient, but have pullulanase activities similar to those in wild type rice. These mutants consist of 84% water-insoluble modified amylopectin and 16% water-soluble polyglucan (WSP) (Fujita et al., 2003). The modified amylopectin contains more short amylopectin chains (DP 5–12) than wild type amylopectin (Fujita et al., 2003). The WSP resembles phytoglycogen in the *sugary-1* mutants of rice endosperm, though it is distributed over the whole endosperm and not only present in the inner regions (Fujita et al., 2003).

Based on their own and other studies regarding DBE, Fujita et al. (2003) suggest a unique role for isoamylase in amylopectin biosynthesis, and a complementary role for pullulanase. They suggest two isoamylase threshold levels: <6% isoamylase activity (i.e. *isoamylase-1* rice mutants) and <1% isoamylase activity (i.e. *sugary-1* rice mutants). In the case of *isoamylase-1* rice mutants, pullulanase cannot prevent the formation of water-insoluble modified amylopectin (84%) and water-soluble polyglucan (16%). On the other hand, the role of pullulanase is pronounced in the *sugary*-1 rice mutants. The authors suggest that low pullulanase activity in the *sugary*-1 rice mutants leads to more phytoglycogen, whereas higher pullulanase activity results in the formation of more *sugary*-amylopectin (less branched than phytoglycogen) (Fujita et al., 2003).

3.3. Models for amylopectin biosynthesis

Amylopectin biosynthesis occurs at the surface of starch granules with their single reducing end groups towards the centre or hilum of the granules (French, 1984). Today, it is clear that, within the starch biosynthetic pathway, the subtle interplay between SS, SBE and possibly DBE results in the synthesis of amylopectin of a complex structure. As is clear from Section 3.2, the main role of SS is to catalyse elongation of amylopectin chains, whereas that of BE is to catalyse the introduction of α, 1 → 6 bonds in amylopectin chains, resulting in branch points. On the other hand, the role of DBE is to catalyse the hydrolysis of some of the formed α, 1 → 6 bonds within the amylopectin chains.

In the past decade, three models have been developed that are very helpful for understanding the biosynthesis of amylopectin. The first is the 'glucan trimming model' by Ball and coworkers (Ball et al., 1996; Mouille et al., 1996; Myers et al., 2000). They believe that debranching enzymes have an active role in the starch biosynthetic pathway. In contrast, Smith and coworkers (Smith, 2001; Zeeman et al., 1998) are convinced that isoamylase is only involved indirectly in amylopectin synthesis. They developed the 'water soluble polysaccharide clearing model'. Recently, Manners (2004) also stated that DBE are only indirectly involved in amylopectin synthesis. However, recent research on starch biosynthesis with good reason focuses on the role of the different isoforms of SS, SBE and DBE within the construction of the amylopectin cluster structure. The other models do not fully explain the specific contribution of the individual isoforms to the fine structure of amylopectin. A third model by Nakamura (2002) helps to develop of a clear cut model explaining the distinct function of individual isozymes of SS and BE in terms of construction of the cluster structure.

According to the first model, i.e. the 'glucan trimming model' (Ball et al., 1996; Mouille et al., 1996; Myers et al., 2000), amorphous and crystalline lamellae (details in Section 4.4) are discontinuously synthesised (Fig. 2A–B). The discontinuous synthesis starts from a 'trimmed' amorphous lamella (1 in Fig. 2A). Starch synthases elongate very short glucan chains in this amorphous lamella until they reach an appropriate length for branching to occur. Next, intensive branching generates phytoglycogen structures, i.e. pre-amylopectin (2 in Fig. 2A). This pre-amylopectin is then 'trimmed' by debranching enzymes. Following crystallisation, mature amylopectin is formed (3 in Fig. 2A). The debranching enzymes are essential when a semi-crystalline amylopectin structure is to be obtained in mature amylopectin. Additional information is presented in Fig. 2B. Starch synthases catalyse the transfer of the glucose unit of ADPGlc to a primer in order to form a linear precursor. Subsequently, pre-amylopectin is formed by the action of branching enzymes. This pre-amylopectin is 'trimmed' by debranching enzymes and crystallises to form mature amylopectin. Debranching enzyme deficiency results in continued action of starch synthases and branching enzymes on a population of polysaccharides that fail to crystallise and lead to phytoglycogen (PG). Both PG and AP are then end products of a diverging pathway. Furthermore, MOS are released from pre-amylopectin by debranching enzymes. Disproportionating enzymes (D-enzymes) (EC 2.4.1.25), which catalyse the hydrolysis of α, 1 → 4 bonds and the transfer of the released reducing end to a separate chain through a new α, 1 → 4 linkage, then most probably transfer linear chains from MOS directly back into pre-amylopectin (Myers et al., 2000). These enzymes also participitate in the generation of ADPGlc from MOS (Myers et al., 2000). Ball and coworkers therefore consider D-enzyme in addition to SS, BE and DBE as a potential direct determinant of chain length distribution. They suggest that D-enzyme is required for amylopectin synthesis, as D-enzyme deficiency in *Chlamydomonas reinhardtii* leads to accumulation of MOS (Colleoni et al., 1999). In contrast, Smith and co-workers revealed a critical role of D-enzyme

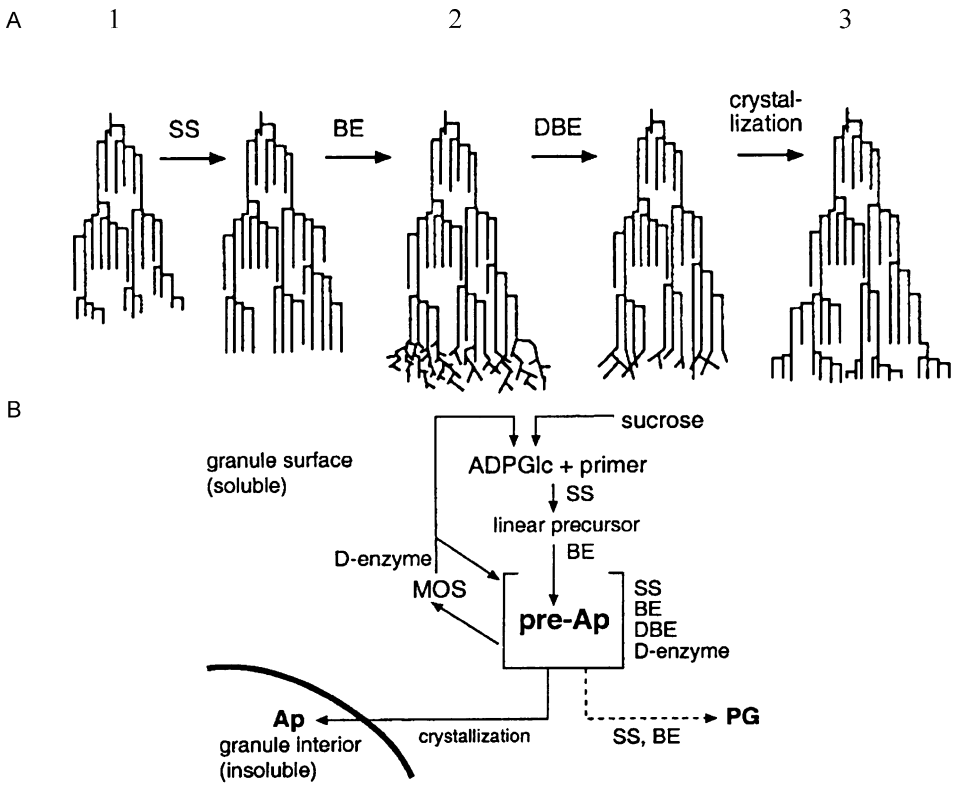

Fig. 2. (A–B) Glucan trimming model for amylopectin biosynthesis as proposed and drawn by Ball and coworkers (Ball et al., 1996; Mouille et al., 1996; Myers et al., 2000). (A) Amylopectin biosynthesis involving elongation of a trimmed amorphous lamella (1) by SS, branching by BE, selective debranching of pre-amylopectin (2) by DBE and the formation of mature Ap (3) following crystallisation (Myers et al., 2000). (B) Schematic representation of the glucan trimming model (discussion in text) (Myers et al., 2000). ADPGlc, adenosine diphosphate glucose; Ap, amylopectin; BE, branching enzyme; DBE, debranching enzyme; D-enzyme, disproportioning enzyme; MOS, malto-oligosaccharides; PG, phytoglycogen; pre-A_P, pre-amylopectin; SS, starch synthase.

in starch breakdown by a knock-out mutation in *Arabidopsis* (Critchley, Zeeman, Takaha, Smith, & Smith, 2001).

According to the second model, i.e. the 'water soluble polysaccharide clearing model' (Smith, 2001; Zeeman et al., 1998) (Fig. 3A–B), SS and SBE synthesise amylopectin rather than pre-amylopectin. However, both enzymes also catalyse the formation of water soluble polysaccharides (WSP) (Fig. 3A). Consequently, water-soluble polysaccharide synthesis competes with amylopectin synthesis (Myers et al., 2000). In this model, DBE, such as isoamylase, are 'scavenging' enzymes, which prevent the elaboration of MOS (Smith, 2001). Isoamylase deficiency results in accumulation of MOS as phytoglycogen (Fig. 3B).

The third model, i.e. the 'two-step branching and improper branch clearing model', was developed by Nakamura (2002) for synthesis of amylopectin in rice endosperm (Fig. 4). Despite species-specific variations, the author suggested that the model may be valid for other amylopectin synthesising organs as well (Nakamura, 2002). The first branching step involves the branching in the amorphous lamellae. BE, i.e. BEI and to a less extent BEIIa and BEIIb, catalyse the transfer of chains derived from longer chains of the preceding cluster to a B_2 chain or the longest chain of the previous cluster (Fig. 4I and II). The branched chains are then elongated by SS, i.e. SSIII or in addition by SSI and SSIIa (Fig. 4III). The core skeleton for the new cluster is formed. DBE are crucial in the removal of 'ill-placed' branches (James et al., 2003) (Fig. 4II).

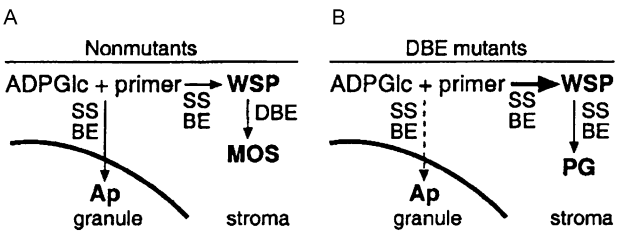

Fig. 3. (A–B) Water soluble polysaccharide clearing model for amylopectin biosynthesis as proposed by Smith and coworkers (Smith, 2001; Zeeman et al., 1998) but drawn by Ball and coworkers (Myers et al., 2000). (A) Non-DBE mutants: competition between amylopectin formation and WSP synthesis. Debranching enzymes prevent the elaboration of malto-oligosaccharides. (B) DBE mutants: accumulation of malto-oligosaccharides as phytoglycogen. ADPGlc, adenosine diphosphate glucose; Ap, amylopectin; BE, branching enzyme; DBE, debranching enzyme; MOS, malto-oligosaccharides; PG, phytoglycogen; SS, starch synthase; WSP, water soluble polysaccharide.

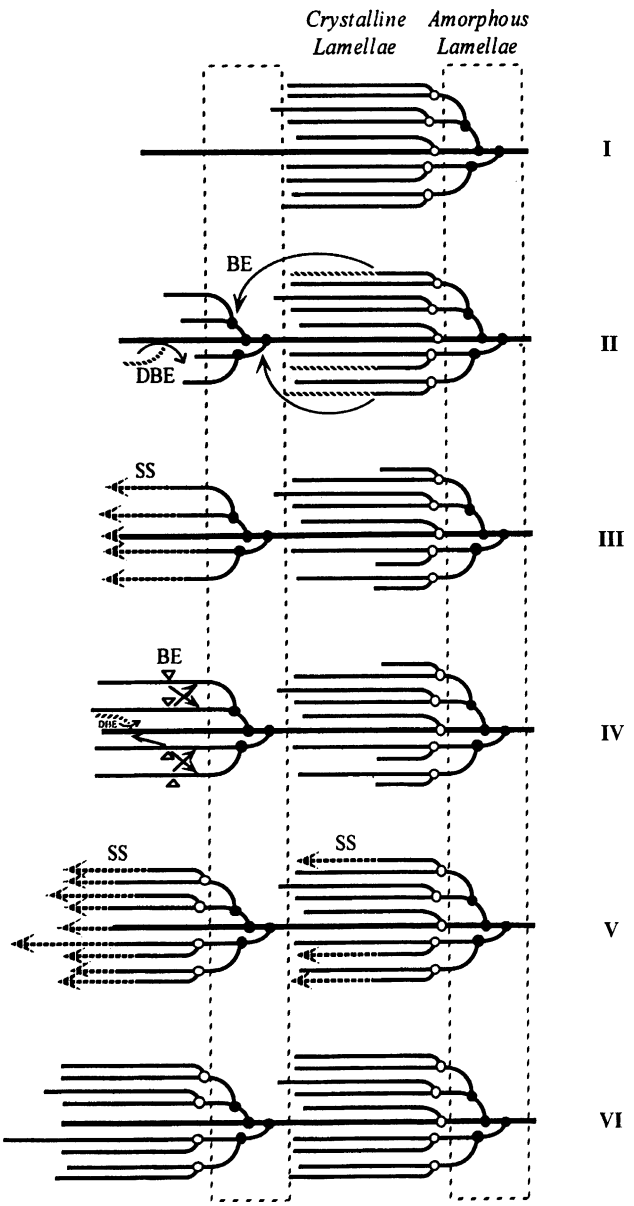

Fig. 4. Model for the synthesis of an amylopectin cluster in rice endosperm (Nakamura, 2002). BE, branching enzyme: mainly BEI and to a less extent BEIIa and BEIIb in (II), mainly BEIIb in (IV); DBE, debranching enzyme; SS, starch synthase: mainly SSIII and possibly in addition SSI and SSIIa in (III), mainly SSIIa in the newly formed left cluster in (V), mainly SSI in the existing cluster in (V).

When the chains reach a length of around DP 12 (Fig. 4III), the second branching step takes place. It involves the branching in the crystalline lamellae. The triangle and arrows show the sites for cleavage and subsequent branching of chains by the predominant BEIIb (Fig. 4IV and V). Next, SSIIa is considered to play a major role in elongating the newly formed short chains, although SSI might be partly involved in the process by elongating very short chains (Fig. 4V). Similarly, DBE are crucial in the removal of 'ill-placed' branches (James et al., 2003). The synthesis of the new cluster is completed (Fig. 4VI). In line with suggestions by Jane et al. (1997), α, 1 → 6 branches are located both in the amorphous (closed circles) and crystalline (open circles) lamellae of the cluster for A-type starches (Fig. 4VI).

3.4. Models for amylose biosynthesis

Amylose biosynthesis is linked to and depends on amylopectin biosynthesis (Denyer et al., 2001). In the past decade, three different models have been developed for the amylose biosynthesis (Figs. 5 and 6A–B). In a first model by Smith and coworkers (Smith, 2001; Zeeman et al., 1998; Zeeman, Smith, & Smith, 2002), GBSS uses MOS as substrate, implying MOS primed synthesis of amylose by GBSS. In contrast, in the two models proposed by Ball and coworkers (Ball, van de Wal, & Visser, 1998; van de Wal et al., 1998), amylopectin is the substrate and competes with MOS as acceptor substrate for GBSS, implying amylopectin primed amylose synthesis.

In the first model by Zeeman et al. (1998) (Fig. 5), a MOS diffuses through the polysaccharide matrix (amylopectin matrix) and reaches the GBSS catalytic site. The immobilized GBSS elongates the chain progressively and pushes the 'amylose' chain inside the granule. Branching of the growing amylose chains towards the reducing end occurs through random collision with a branching enzyme. Next, a novel MOS reaches the GBSS catalytic site. This releases the amylose molecule and primes a novel amylose chain.

In the second model (van de Wal et al., 1998, Fig. 6A), an amylopectin chain is the primer for amylose formation. The GBSS elongates the formed chain. Amylose cleavage then occurs downstream mediated by a branching enzyme (or by a starch hydrolase) within the granule. To achieve several rounds of amylose synthesis on an amylopectin primer, GBSS would have to extend to another molecule from a novel accessible non-reducing end, implying total freedom of movement.

In the third model (van de Wal et al., 1998, Fig. 6B) and similarly to the second mechanism, an amylopectin chain is the primer for amylose formation. Other than in the second model, however, GBSS is considered to be directly responsible for cleavage of the amylose chain (at site H within GBSS). The cleavage of amylose would be triggered by steric hindrance encountered during the progress of the growing amylose chain.

These models rule out the possibility that amylose biosynthesis occurs at the granule surface. Also Kuipers, Jacobsen, & Visser (1994) and Tatge, Marshall, Martin, Edwards, & Smith (1999) showed that it is more likely that amylose is synthesised within the amylopectin matrix, more specifically, behind the zone of amylopectin biosynthesis (at the surface of the granules). Kuipers et al. (1994) showed that reduced GBSS gene expression in transgenic potato tubers results in amylose formation in a restricted zone, i.e. the hilum or core, of the granules. The size of this zone

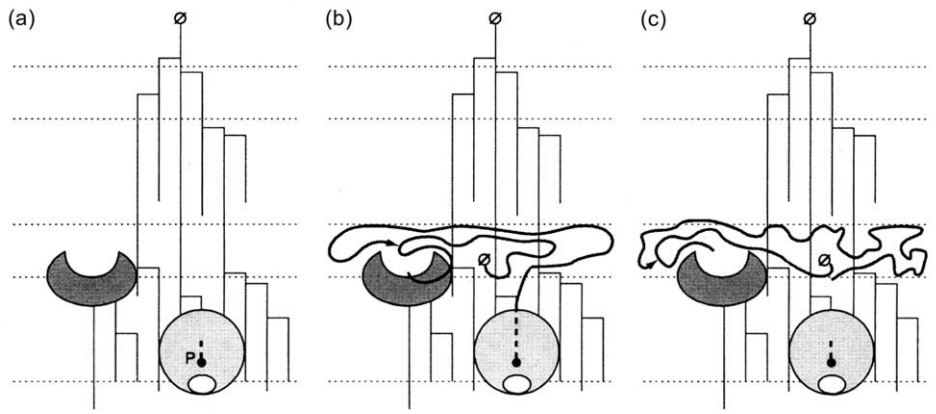

Fig. 5. Malto-oligosaccharide primed amylose biosynthesis as proposed by Smith and coworkers (Zeeman et al., 1998) but drawn by Ball and coworkers (Ball et al., 1998). (a) A malto-oligosaccharide (short broken line) has reached the granule bound starch synthase (GBSS) (circle) with catalytic site P within the amylopectin matrix; crescent shaped structure: branching enzyme. (b) The immobilised GBSS elongates the chain, pushing it inside the granule. A random collision with a branching enzyme in the path of the growing amylose chain results in branching of the molecule towards the reducing end (indicated by arrow). (c) A novel malto-oligosaccharide (short broken line) has reached the catalytic site, resulting in release of the amylose molecule and priming of a novel chain. ●, non-reducing end; ϕ, reducing end.

(as measured by iodine staining) was suggested to depend on the GBSS protein level (Kuipers et al., 1994). Tatge et al. (1999) showed that, during potato tuber development, the blue core volume increases as the granule volume increases and the extent of the increase is greater in potatoes with higher GBSS levels. Factors that determine the rate of amylose biosynthesis are e.g. the amount of GBSS, the availability of ADPGlc and MOS and the physical

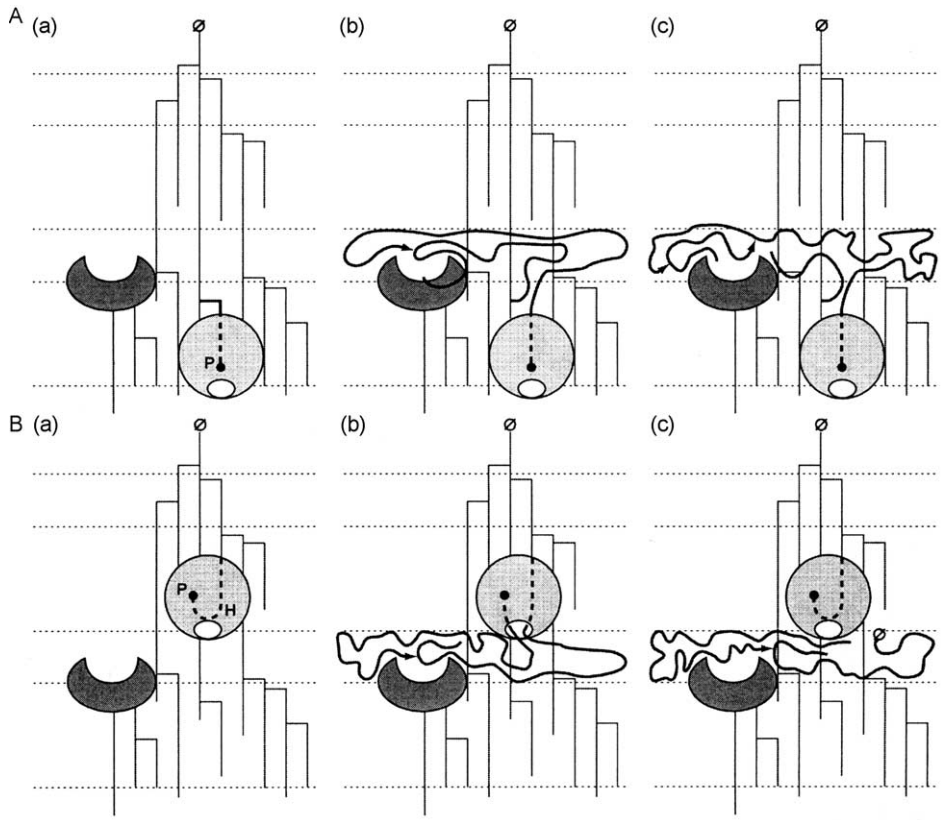

Fig. 6. (A–B) Amylopectin primed amylose biosynthesis as proposed and drawn by Ball and coworkers (Ball et al., 1998; van de Wal et al., 1998). (A): (a) Granule bound starch synthase (full circle) within the amylopectin matrix, one of the amylopectin side chains (broken line) is the primer for amylose synthesis, crescent shaped structure: branching enzyme. (b) GBSS elongates the chain, pushing it inside the granule. A random collision with a branching enzyme in the path of the growing amylose chain results in branching of the molecule (as indicated by the arrow). (c) Branching enzyme mediated cleavage and release of the amylose molecule. The next round of biosynthesis depends on the avalability of a new non-reducing end. (B): (a) and (b) similar to (a) and (b) of (A). (c) Hydrolase activity at site H within GBSS and release of the amylose molecule. ●, non-reducing end, ϕ: reducing end.

space within the amylopectin matrix (Smith, Zeeman, & Denyer, 2001).

Finally, although it is assumed that most of the amylose is located within the amorphous regions of the granule, these models support the idea that some amylose is also present within the semi-crystalline areas of the granule (Jenkins & Donald, 1994).

4. Starch structure

4.1. Starch structure at the 2–100 μm scale: granules

The shape (round, oval, polyhedral), particle size (2–100 μm) and particle size distribution (unimodal, bimodal, trimodal) of starch granules are typical of the botanical origin. In rice, several polyhedral small (2–7 μm) granules are produced in one amyloplast. They form parts of compound granules. In contrast, only one granule is produced in one amyloplast of, e.g. maize (polygonal or round, 10–15 μm), wheat and potato tubers (oval, 15–100 μm). Many starches have a unimodal distribution of granules, whereas those from *Triticicae* have a bimodal distribution comprising large A-type (lenticular, 10–35 μm) and small B-type (spherical, 1–8 μm) granule populations (Stoddard, 1999). Starch granules show 'birefringence' when microscopically viewed using polarized light. The refraction of polarized light by the crystalline regions in starch result in a 'Maltese cross' characteristic of a radial orientation of the macromolecules (Buléon, Colonna, Planchot, & Ball, 1998). However, the center or hilum of starch granules is less organised than the rest of the granule. Moreover, the pheripheral amylopectin helices do not point to a single focus, but to an inner ellipsoid (Waigh et al., 1997).

4.2. Starch structure at the 120–500 nm scale: growth rings

Starch granules consist of alternating semi-crystalline and amorphous growth rings, as observed with optical microscopy (French, 1984; Jenkins & Donald, 1994) (Fig. 7a). They grow by apposition from the hilum of the granule. The number and size of the growth rings depend on the botanical origin of the starch (French, 1984) and on the amount of carbohydrates available at the time of biosynthesis. French (1984) suggested that the semi-crystalline growth rings have a thickness between 120 and 400 nm. According to Cameron and Donald (1992), the amorphous growth ring is at least as thick as the semi-crystalline one. Based on atomic force microscopy (AFM) measurements, Dang and Copeland (2003) suggested that the growth rings in rice are approximately 400 nm apart.

4.3. Starch structure at the 20–500 nm scale: blocklets

In contrast to the general acceptance of growth rings in starch granules, the level of order between the growth rings and the lamellae, i.e. blocklets, is not as commonly mentioned in the literature (Buléon et al., 1998). Based on scanning electron microscopy observations, Gallant, Bouchet, and Baldwin (1997) suggested that both

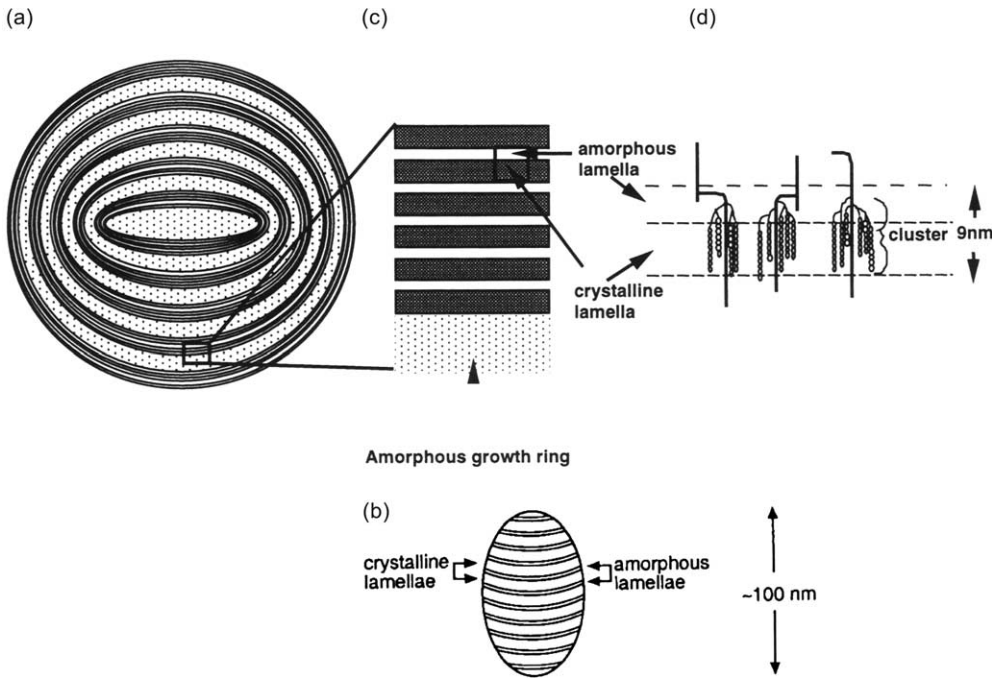

Fig. 7. Schematic representation of the starch granule: (a) amorphous and semi-crystalline growth rings in a starch granule, (b) blocklet, (c) amorphous and crystalline lamellae in a stack and part of an amorphous growth ring, (d) aligned double helices (from amylopectin side chains) within a crystalline lamella and amylopectin branch points within an amorphous lamella [(a),(c), and (d): Donald et al., 2001; (b): proposed by Gallant et al. (1997) but drawn by Ball and coworkers (Myers et al., 2000)].

semi-crystalline and amorphous growth rings are subdivided into large (20–500 nm in diameter, e.g. 80–120 nm in wheat starch) and small (25 nm in diameter in wheat starch) spherical blocklets, respectively. In this view, one blocklet in the semi-crystalline growth ring contains several amorphous and crystalline lamellae (Fig. 7b). On average, two end-to-end blocklets would constitute a single semi-crystalline growth ring. Dang and Copeland (2003) suggested that the cross striations within the growth rings of rice starch (as observed by AFM) correspond to the blockets of amorphous and crystalline lamellae. These blocklets have an average size of 100 nm in diameter and are proposed to contain 280 amylopectin side chain clusters.

4.4. Starch structure at the 9 nm scale: amorphous and crystalline lamellae

One semi-crystalline growth ring (120–400 nm thick) consists of a stack, i.e. about 16 repeats, of alternating amorphous (2–5 nm thick) and crystalline (5–6 nm thick) lamellae (Cameron & Donald, 1992; French, 1984) (Fig. 7c). Independent of the botanical origin of the starch, repeat distances of an amorphous and crystalline lamellae are about 9 nm (Jenkins, Cameron, & Donald, 1993; Oostergetel & van Bruggen, 1989). Amorphous lamellae contain branch points of the amylopectin side chains and possibly some amylose, whereas semi-crystalline lamellae are constituted of amylopectin double helices (Fig. 7d). Amorphous growth rings are at least as thick as semi-crystalline growth rings (Cameron & Donald, 1992) and contain amylose and probably less ordered amylopectin (Morrison, 1995). Wrinkled pea (Oostergetel & van Bruggen, 1989) and some high amylose maize starches (Oostergetel & van Bruggen; Shamai, Bianco-Peled, & Shimoni, 2003; Vermeylen, Goderis, Reynaers, & Delcour, 2004) display transmission electron microscopy (TEM) and/or small angle X-ray scattering (SAXS) characteristics pointing to a less prominent lamellar organisation than that of *wx* and regular starches.

4.4.1. Helical lamellar model

Oostergetel and van Bruggen (1993) suggested that the crystalline lamellae are twisted into a superhelical structure for potato starch (Fig. 8A–B). Waigh, Donald, Heidelbach, Riekel, & Gidley (1999) consequently suggested that the amylopectin branch points lay within an amorphous lamellar superhelix and that the superhelical structure is built up by the amylopectin helices as the basic building blocks, with a width of approximately 4–5 helices and approximately 18–40 adjacent double helices in a helical pitch (9 nm). Gallant et al. (1997) supported the helical lamellar model. However, they suggested that helical lamellae are formed in blocklets of limited lateral extent.

4.5. Starch structure at the 0.1–1.0 nm scale: amylopectin and amylose chains

As amylopectin builds up the starch granule structure, the main focus in this part is amylopectin.

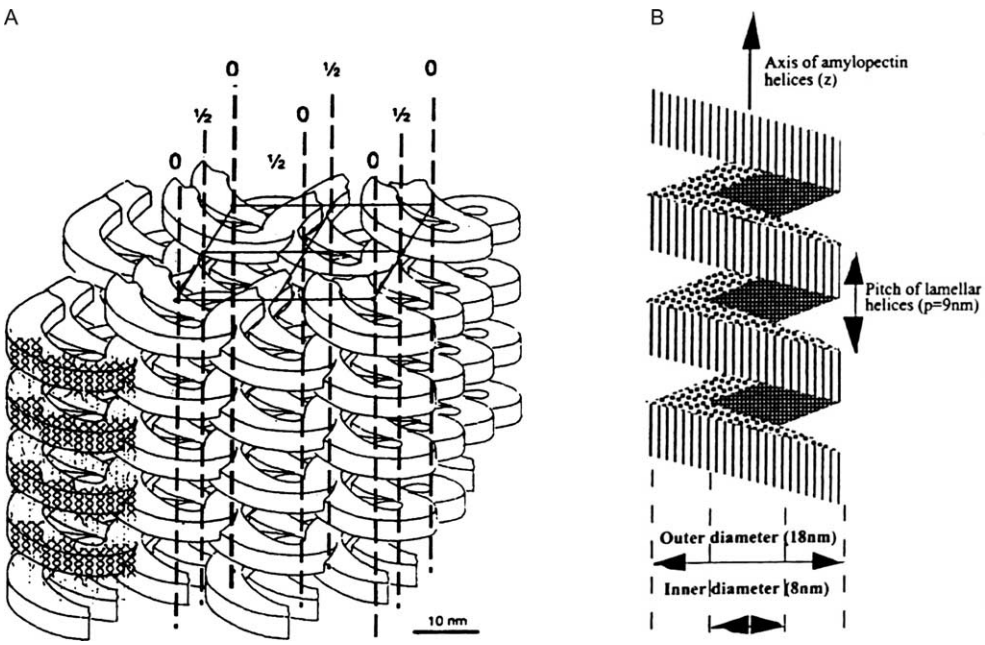

Fig. 8. Helical lamellar model of crystalline lamellae in starch (Oostergetel & van Bruggen, 1993) (A) Semi-crystalline lamellae form a continous network of left handed superhelices (diameter 18 nm, pitch 9 nm). Neigbouring helices are shifted relative to each other by half the helical pitch (indicated by 0 and 1/2) (B) Schematic representation of the helical lamellar model (Waigh et al., 1999).

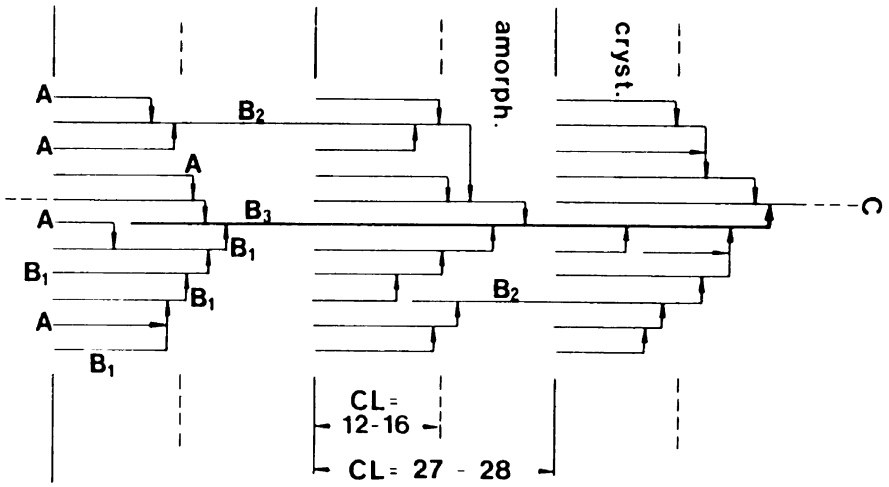

Fig. 9. The cluster model of amylopectin proposed by Hizukuri (1986) but redrawn by Morrison and Karkalas (1990). ϕ, reducing chain-end; solid lines, (1 → 4)-α-D-glucan chain; arrows, α-(1 → 6) linkage; C.L., average chain length.

4.5.1. Amylopectin cluster model with polymodal chain length distribution and non-random nature of branching

Peat, Whelan, and Thomas (1956) defined the basic structure of amylopectin in terms of linear A-, B- and C-chains. A-chains (outer chains) are attached through their potential reducing end to B-chains. The latter are linked in the same way and carry one or more A-chains. The C-chain contains the single reducing group of the amylopectin molecule and carries other chains. Several models of amylopectin have been developed over the years. One of them, i.e. the cluster model, is widely accepted today (Nikuni, 1978; Robin et al., 1974, 1975). Based on the A-, B-, C-chain terminology of Peat et al. (1956), Hizukuri (1986) refined the cluster model (Fig. 9). Amylopectin has a polymodal distribution with A (CL 12–16) and B chains, i.e. B_1 (CL 20–24), B_2 (CL 42–48), B_3 (CL 69–75) and B_4 (CL 104–140) chains. A and B_1 chains form a single cluster, whereas B_2, B_3 and B_4 extend into 2, 3 and more than 4 clusters. Recently, Hanashiro, Tagawa, Shibahara, Iwata, and Takeda (2002) suggested that C chains are very similar among botanical sources with range in size from 10 to 130 glucose units, with most being around 40 glucose units (Hanashiro et al., 2002). The following composition was proposed for waxy rice amylopectin: A (CL 13), B_1 (CL 22), B_2 (CL 42), B_3 (CL 69), B_4 (CL 101) (Hizukuri, 1986).

Moreover, 80–90% of these amylopectin chains constitute a single cluster (A and B_1 chains with CL 12–14), whereas 10–20% form inter-cluster connections (B_2, B_3 and B_4 chains with CL 41–42). Recently, Qi et al. (2003) observed that low gelatinisation temperature waxy rice starches contain two major chain fractions upon debranching with isoamylase (DP 16 and DP 51 on average), while the high gelatinisation temperature starches contain three fractions (DP 16, DP 19 and DP 40 on average).

The ratio of A to B chains of amylopectin depends on the botanical origin of the starch and is typically of the order $<1{:}1$ to $>2{:}1$ on a molar basis or $<0.5{:}1$ to $>1{:}1$ on a weight basis (Morrison & Karkalas, 1990; Tester, Karkalas, & Qi, 2004). Enevoldsen and Juliano (1988) reported waxy and non-waxy (low amylose) rice amylopectin to have similar molar ratios of A to B chains (1.1–1.5).

Finally, although far less documented, amylopectins of starches from the same and different botanical origin (i) may have a different branching pattern (cf. infra) and (ii) have non-randomly distributed amylopectin branch points (Thompson, 2000).

4.5.2. Model for the structure of A-type and B-type starches

The semi-crystalline nature of normal amylose content and wx starches (15–45% according to Zobel, 1988) is generally ascribed to amylopectin, as the amylopectin side chain branches (outer chains) intertwine and form double helices, which are packed into lamellar crystallites. It is accepted that the crystalline domains of starch granules are composed of A chains and the exterior parts of B chains. Moreover, amylose can be leached from the granule without loss of crystallinity (French, 1984). Native starch granules are characterised by either an A-type (cereal starches of, e.g rice, wheat, maize), a B-type (tuber and root starches of, e.g. potato and tapioca, retrograded starches of any botanical origin, high amylose cereal starches such as high amylose maize starch), or a C-type (leguminosae starches of, e.g pea, bean and tropical starches of e.g. cassava starch) X-ray diffraction pattern (Katz, 1928). C-type starches are mixtures of A-type and B-type starches (Buléon et al., 1998).

Imberty, Chanzy, Pérez, Buléon, and Tran (1988) and Imberty and Pérez (1988) proposed a model for the structure of A-type and B-type starches. Electron diffractograms from micron-sized, needle shaped crystals, revealed a monoclinic ($a = 2.12$ nm, $b = 1.17$ nm, $c = 1.07$ nm, $\gamma = 123.5°$) and hexagonal unit cell ($a = b = 1.85$ nm, $c = 1.04$ nm, $\gamma = 120°$) for the A-type and B-type structures, respectively. A-type structures are densily packed with

only 4 water molecules in the unit cell, whereas B-type structures are more open with 36 –42 water molecules per unit cell. Moreover, the double helix structure for both types are similar: left-handed parallel stranded double helices with double helices repeats within 2.1 nm (6 glucosyl residues). Besides A-, B-, and C-type starches, V-type diffractions patterns exist, which reveal the presence of complexed amylose.

Buléon et al. (1997) studied X-ray diffraction patterns of wild-type and mutant *C. reinhardtii* starches. Both strains containing no defective SS and mutants carrying a disrupted GBSS were A-type starches with high degree of crystallinity. In contrast, SSII deficient mutants had a B-type diffraction pattern with low crystallinity. Furthermore, mutants carrying only SSI were C-type starches. This study implied that SSII is involved in the synthesis of crystalline structures in starch, whereas a new type of amylopectin is formed in the case SSI is the only SS.

Whether starches are of the A-, B- or C-type, depends on several factors.

Firstly, Hizukuri et al. (1983) postulated that CL is the major determinant of crystalline polymorphism among native starches. Amylopectins of B-type starches have higher proportions of longer amylopectin chains than A-type starches (Hizukuri et al., 1983). Indeed, mild acid hydrolysis of regular A- and B-type starches (e.g. Bogracheva et al., 1999; Jacobs, Eerlingen, Rouseu, Colonna, & Delcour, 1998; Jane, Wong, & McPherson, 1997) indicates that B-type starches have thicker crystallites, and hence longer external amylopectin chains.

Secondly, it was envisaged that, besides the differences in CL, the branching patterns of the different type of starches may also differ. Several studies affirm this reasoning. Lateral distances between various double helices in A- and B-type crystalline unit cells differed (Imberty, Buleon, Tran, & Pérez, 1991). Moreover, computer simulations on pairs of double helices coupled to one another through an internal α-(1–6) linkage revealed that internal chain lengths determine the lateral distance between the double helices in stable conformations (O'Sullivan & Pérez, 1999).

Thirdly, analyses on acid resistant starch fractions of A- and B-type starches indeed indicated a different branching pattern for both starch types (Jane et al., 1997). Moreover, Gérard, Planchot, Colonna, and Bertoft (2000) suggested that amylopectin clusters with numerous short amylopectin chains and short ICL crystallize into the A-type crystal polymorph. In contrast, amylopectin clusters with fewer but longer chains and longer ICL crystallize into a B-type crystal polymorph. The different branching density in amylopectin clusters crystallized into the A- or B-polymorph has recently been confirmed (Hanashiro et al., 2002; Takeda et al., 2003).

4.5.3. Chiral side-chain liquid-crystalline polymeric model

The most recent model of starch structure is the chiral side-chain liquid-crystalline polymeric model proposed by Donald and coworkers (Waigh et al, 2000). It considers starch to be a side-chain liquid-crystalline polymer (SCLCP) (Fig. 10A–B). A SCLCP consists of (i) a flexible backbone, (ii) rigid units or mesogens and, (iii) flexible spacers. In the case of starch, these three components correspond with (i) an amylopectin backbone, (ii) amylopectin double helices, and (iii) the segments of the amylopectin side chains that decouple the helical forming units from the amylopectin backbone, respectively (Donald, 2001). Furthermore, there are two different liquid crystalline mesophases: the glassy nematic and the plasticised smectic mesophases. Dry starch (≤5% w/w) is in the glassy nematic state (Fig. 10A), whereas hydrated starch, i.e. hydration at room temperature, has a side-chain liquid-crystalline smectic structure (Fig. 10B). Even though the amylopectin helices have their long axes aligned in the dry glassy nematic state, they do not form lamellae. In contrast, water addition causes the lamellae to self-assemble as the amylopectin side chains and main chains are plasticised and their mobility is increased. In hydrated starch, the amylopectin double helices in the lamellae are in a crystalline smectic hexagonal phase and the amorphous backbone and flexible spacers are in a highly plasticised liquid phase. Both a 9 nm repeat of amorphous and crystalline lamellae and a 1.6 nm interhelix spacing

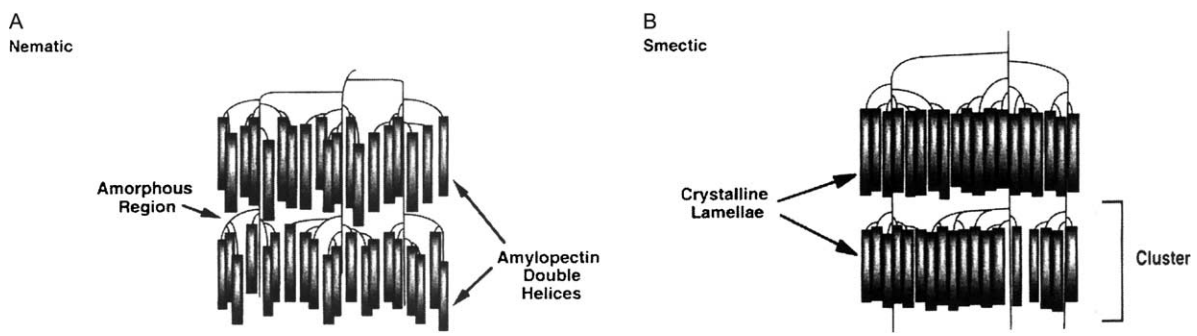

Fig. 10. Side-chain liquid-crystalline polymeric model (Donald et al., 2001). (A) Dry starch (≤5% water) is in the nematic state: amylopectin double helices (mesogens) do not form lamellae. The amorphous region contains the flexible spacers. (B) Hydrated starch is in the smectic state: amylopectin double helices are arranged within crystalline lamellae and the amorphous backbone and flexible spacers are in a highly plasticised liquid phase.

(only in B-type starch) appear in the hydrated starch (as measured by Small Angle X-ray Scattering), but disappear on dehydration. This increased order is due to an increased degree of plasticisation and increased flexibility of the spacers. Waigh et al. (2000) suggest A-type starches to have shorter mesogens, but longer flexible spacers than B-type starches, which have long mesogens and short spacers. The 9 nm spacing remains. The tendency for the mesogens to order is in opposition to the entropy of the amylopectin backbone. To maximise the entropy of the amylopectin backbone, the mesogens are forced away to either a nematic or smectic structure as long as the flexible spacers allow sufficient decoupling.

5. From rice starch structure to physical behaviour

The varying activity levels of one or several isoform(s) of the starch biosynthetic enzymes, result in formation of rice starches with varying structure, such as different amylopectin chain length distribution, average external and internal chain lengths and core chain length distribution. Consequently, starches with varying physical behaviour and functionality are obtained. Important structural aspects that relate rice starch structure to gelatinisation and amylopectin retrogradation behaviour as measured by differential scanning calorimetry (DSC) are highlighted in this paragraph.

5.1. Rice starch gelatinisation related to starch structure

With regard to amylopectin chain length distribution, three recent studies (Nakamura et al., 2002; Noda et al., 2003; Vandeputte et al., 2003a) revealed that very short (DP $<$ 12) amylopectin chains related negatively, while somewhat longer ($12 < \mathrm{DP} < 24$) amylopectin chains related positively to gelatinisation onset (T_o), peak (T_p) and conclusion (T_c) temperatures of rice starches as measured by DSC. Studies on (i) mutant (Wong et al., 2003), (ii) transformant (Fujita et al., 2003) and (ii) wild (Wang & Wang, 2002) rice starches affirmed this observation. Whether amylopectin chains have a positive or negative influence on gelatinisation temperature depends on the way they are packed into the lamellar structure of the starch granules. Short amylopectin chains may destabilize the lamellar structure in several ways. Indeed, Gidley and Bulpin (1987) found that at least 10 glucose units are needed for double helix formation of maltooligosaccharides. Although double helix formation may occur at slightly lower DP when maltooligosaccharide are 'immobilized' by linkage to the amylopectin backbone, very short amylopectin chains most likely have a reduced tendency to form double helices. Starches with higher relative amounts of very short amylopectin chains will thus have lower molecular and crystalline order and a non-optimized packing within the crystalline lamellae. Consequently, they will most likely have lower gelatinisation T_o, T_p, T_c. Additionally, the 5–7 nm thicknesses of crystalline lamellae reported in TEM and SAXS studies corresponds to optimal external chain lengths of DP 15–20, considering that glucose in a double helical conformation has a 'length' of 0.35 nm. Amylopectin chains of DP 18–21 were hence envisaged to span the complete crystalline lamella (Jane et al., 1999). Shorter chains would not be able to adequately 'fill' the crystalline lamellae, which may be viewed to (i) decrease the local thickness of the lamellar crystallite, (ii) decrease its density (Vermeylen et al., 2004) and (ii) introduce crystal defects (McPherson & Jane, 1999). Either interpretation can be assumed to decrease the melting temperature of crystallites.

The tendency to add defects to lamellar crystals is, however, not restricted to chains which are too short. Too long chains may also introduce defects, and when crystallization is under thermodynamic control, they are therefore rejected by the crystallites (Wunderlich, 1976). Indeed, the less rigorous lamellar organization of B-type starches, has been suggested to result in part from longer chains (DP $\sim$ 45), which are quite abundant in B-type starches, frustrating the double helix packing in crystalline lamellae (Vermeylen et al., 2004). However, for sets of uniform A-type rice starches, some authors (Patindol & Wang, 2002; Wang & Wang, 2002) noticed a positive correlation between relative amounts of very long amylopectin chains (DP $>$ 37) and starch T_o, T_p, T_c. While it is tempting to suggest that these chains form longer double helices (not necessarily crystallites) that require higher temperatures to dissociate, this hypothesis is unlikely, because, for regular (and *wx*) starches such chains are generally accepted to be branched entities connecting multiple amylopectin clusters (Hizukuri, 1986). The branched nature of these chains would virtually impede the formation of long, uninterrupted double helices. The above explanations for rice starches (Patindol and Wang, 2002; Wang & Wang, 2002) are in contrast to observations for *aewx* maize starches (Shi & Seib, 1995; Yuan, Thompson, & Boyer, 1993). In *aewx* maize starches, greater proportions of longer amylopectin chains (DP $>$ 30 in Yuan et al., 1993; DP $>$ 16 in Shi & Seib, 1995) may indeed increase gelatinisation temperatures through formation of longer double helices, as the population of A chains in *aewx* maize starches contain some unusually long chains (molar A:B ratio of 1.5 for *aewx* maize starch vs. 0.9–1.1 for *wx* and *duwx* maize starches) (Yuan et al., 1993).

In addition to linking amylopectin chain length distribution to the gelatinisation behaviour of starches, further information can be gained from the core chain length distributions of debranched rice β-limit dextrins. Reddy, Ali, & Bhattacharya (1993) found that the chain length profile of β-limit dextins was more or less constant in *wx* and regular rice varieties. The ratios of short B (B_S) to long B (B_L) amylopectin chains were similar. However, different levels of external chains (obtained by debranching amylopectin) were observed. In their study, they stated that

the internal structure of amylopectins (inside the outer branch points) is essentially similar in all varieties and that the differences between starches are evoked by differences in external chain lengths. This would mean that the internal structure of the amylopectins, i.e. inside the outer branch points, is essentially the same in all varieties. However, in contrast to the above, recent work by Vandeputte and coworkers (unpublished results), reveals differences in the core chain length distributions of debranched β-limit dextin profiles of *wx* and regular rice amylopectins.

Recently, Qi et al. (2003) studied the molecular structure of low and high gelatinisation temperature waxy rice starches. Debranched high T_p waxy rice starches contain three fractions (F_3: DP 16, F_2: DP 19 and F_1: DP 40), whereas debranched low T_p waxy rice starches only have two fractions (F_3: DP 16 and F_1: DP51). As the chain length distributions of the debranched β-limit dextrins of the high and low T_p waxy rice starches are similar ($F_{3\beta}$: DP5, $F_{2\beta}$: DP 13, $F_{1\beta}$: DP 19), Qi et al. (2003) suggest that the high vs. low T_p are dictated by the exterior amylopectin chains. The higher proportion of exterior chains, i.e. F_3 and especially F_2, were suggested to be responsible for the high T_o, T_p, T_c and ΔH.

5.2. Rice amylopectin retrogradation related to starch structure

Similarly to gelatinisation, well defined populations of amylopectin chains either favour (DP 12–22) or inhibit (DP 6–9; DP > 25) amylopectin retrogradation (Lin et al., 2001; Lu et al., 1997; Vandeputte et al., 2003b). This has to do with their ability or lack thereof to form double helices during retrogradation. As described above, at least 10 glucose units are required for malto-oligosaccharides to form double helices (Gidley & Bulpin, 1987). Lai et al. (2000) suggested that the minimum DP for rice starch amylopectin retrogradation is DP 12 because of the effect of steric hindrance in an amylopectin cluster. Very short amylopectin chains with DP < ~12 thus (i) most likely have a reduced tendency to form double helices during retrogradation or (ii) at the very most co-crystallise with amylose and/or longer amylopectin chains. Starches with a higher proportion of amylopectin chains with DP < 12 most likely less easily regain a partial crystalline polymer system resulting in lower $T_{o\ retro}, T_{p\ retro}, T_{c\ retro}, \Delta H_{retro}$, and/or $\Delta H_{AP\ retro}$. For sets of uniformly A-type rice starches, Patindol and Wang (2002) noticed a positive correlation between the relative amounts of very long amylopectin chains (DP > 37) and starch $T_{o\ retro}$ and ΔH_{retro}. Similarly to gelatinisation, the hypothesis that such chain may form longer double helices during retrogradation is unlikely.

Yao, Zhang, and Ding (2003) confirmed the importance of ECL on amylopectin retrogradation, as monitored by pulsed nuclear magnetic resonance. Partial β-amylolysis of rice amylopectin, pregelatinised starch and flour with either reagent or food grade β-amylase reduced the external amylopectin chain lengths. When the average external chain length (ECL) of the rice amylopectin was <DP 11.6, the amylopectin retrogradation was essentially inhibited.

6. Conclusions and perspectives

To design tailor-made starches in the future, it is important to gain insight into biosynthesis–structure–physical behaviour–functionality relations. The biosynthetic pathway from sucrose to starch granule is complex. Sucrose is transported to the cells of storage organs of plants (such as rice seeds). Within the cytosol of cells, it is converted to (i) mainly ADPGlc and (ii) to a lesser extent to Glc 1 P and/or Glc 6 P. The cytosolic ADPGlc is translocated to the amyloplasts via ADPGlc translocators. Glc 1P and/or Glc 6 P are translocated to the amyloplasts via phosphate translocators and are subsequently converted to ADPGlc. The ADPGlc within the amyloplasts, orginated from the above described pathways, is used for starch (i.e. amylose and amylopectin) synthesis within the amyloplasts of cells. Amylose and/or amylopectin synthesis is catalysed by several isoforms of rice starch synthases, branching enzymes and debranching enzymes, each with a specific function. Different models for both amylose and amylopectin synthesis have been suggested by Ball and coworkers (Ball et al., 1996; Myers et al., 2000; Mouille et al., 1996; van de Wal et al., 1998) and Smith and coworkers (Smith, 2001; Zeeman et al., 1998). A recently developed model for amylopectin synthesis in rice endosperm was suggested by Nakamura (2002). Eventually starch granules with a complex structure are synthesised. Starch granules (2–100 μm) are composed of alternating semi-crystalline (120–500 nm) and amorphous (120–500 nm) growth rings. The former contain amylose and probably less ordered amylopectin. Every semi-crystalline growth ring is composed of repeats of alternating amorphous (2–5 nm) and crystalline (5–6 nm) lamellae (together 9 nm), which consist of branch points of the amylopectin side chains (and possibly some amylose) and amylopectin double helices, respectively. In addition, there may be a level order between growth rings and lamellae, i.e. blocklets (20–500 nm), which contain several amorphous and crystalline lamellae. Specific structural aspects, such as amylopectin (core) chain length distributions result in starch with a specific physical behaviour and functionality.

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