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On the nature of categories of chains in amylopectin and their connection to the super helix model

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Abstract

Amylopectin of waxy maize and amylose-free potato starch (PAPS) were analysed for their compositions of categories of unit chains. The ratio of A:B-chains was best obtained by debranching of limit dextrins of the amylopectins. Although, an exact ratio was difficult to obtain without adjustments, the discrepancies were small and the ratio was not dependent on the original length of the chains. From the knowledge of the external chain length and the chain distribution of the ϕ,β-limit dextrins, the original distributions of A- and B-chains were traced and the chains were grouped into characteristic categories. Clustered chains were represented by a degree of polymerisation (DP) from 9 to 34. A-chains overlapped with 'fingerprint' B_{fp}-chains (DP 15–20) and constituted probably building blocks inside clusters. The shortest A-chains (A_{fp}) at DP 6–8 and all long chains (DP ≥ 35, including long A-chains present in PAPS) were amorphous. In a proposed two-directional backbone model, the clusters are connected to a backbone that extends in a (almost) perpendicular direction and is formed by the amorphous chains. The model makes it possible to construct a super-helical structure from a single amylopectin macromolecule.

Keywords: Waxy maize starch; Potato amylopectin starch; Amylopectin structure

1. Introduction

The unit chains of the major starch component amylopectin are classified in several ways. The basic categories were defined by Peat, Whelan, and Thomas (1952) as A-chains that are not carrying other chains (through the C-6 position) and B-chains that are carrying other chains (A- and/or B-chains). In addition, each macromolecule has a single C-chain that carry the sole reducing end-group. In most experimental designs, the C-chain is not distinguished from the B-chains. The unit chains are also grouped into short chains, with a degree of polymerisation (DP) 6–35, and long chains (DP > 35) (Hizukuri, 1985). In many starch samples, the latter group consists of two or more subgroups (Hizukuri, 1986). The amylopectin component inside starch granules (the native form of starch) crystallises into either A- or B-type structures (Imberty, Buléon, Tran, & Pérez, 1991). A-type starches generally possess somewhat shorter chain lengths (CL) than B-type starches (Hizukuri, 1985; Hizukuri, Kaneko, & Takeda, 1983).

The unit chains of amylopectin were suggested to be organised into clusters by Nikuni (1978) and French (1972). On the basis of a periodicity in length, Hizukuri (1986) suggested that two clusters of short chains are interconnected by B2-chains of DP 40–50, whereas B3-chains (DP 70–75) span over three clusters, etc. Hizukuri (1986) also defined B1-chains as being short chains that build up the clusters together with the A-chains, which he assumed are represented by the shortest chains (a subgroup of the short chains at approximately DP 6–16, depending on the sample). Later, Hanashiro, Abe, and Hizukuri (1996) compared the unit chain distribution of several amylopectins with that of arrowhead and found a periodicity at intervals of DP 12. Chains with DP 6–12 were suggested to represent the A-chains, whereas the rest of the short chains were B1 (DP 13–24) and B2 (25–36). Long chains (DP > 37) were classified as B2 or B3 (and thus some uncertainty with the older classification existed). From analyses of chains involved in dextrins obtained by α-amylolysis Bertoft (1991) and Bertoft and Koch (2000) subdivided the B1-chains into B1a and B1b that seem comparable to the later definitions of B1 and B2, respectively. Ong, Jumel, Tokarczuk, Blanshard, and Harding (1994) divided

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the experimentally obtained unit chain profile into a series of individual Gaussian distributions and found two populations of A-chains at DP 10 (A1) and 13–15 (A2). Four groups of B-chains were comparable to those originally described by Hizukuri (1986). Blennow, Engelsen, Munck, and Møller (2000) also used a Gaussian distribution approach with a result in accordance with Hizukuri's division from 1986 (with a single group of A-chains). Thus, the classification of chains in amylopectins remains unclear and apparently depends on the method used (size exclusion or anion-exchange chromatography) and the treatment of the obtained unit chain distributions.

The molar ratio of A:B chains has been estimated from the unit chain profile of amylopectins, assuming that the A-chains are shorter than B-chains. A survey of the literature shows that, in general, A-crystalline starches (maize, rice, wheat, sweet potato) tend to have a higher ratio of A:B-chains (1.3–2.3) than B-crystalline starch (potato, 0.8–1.6) (Hanashiro, Tagawa, Shibahara, Iwata, & Takeda, 2002; Hizukuri, 1986; Ong et al., 1994). There is, however, an alternative possibility to determine the ratio, namely from the unit chain distribution of the limit dextrin (LD) of the amylopectin. LD:s have their external chains (in B-chains the segment extending from the outermost branch point to the non-reducing end, in A-chains the entire chain) shortened into small characteristic stubs. β-Amylase gives β-LD:s, in which the A-chains are reduced into maltose and maltotriose stubs (depending on if the chain contains an even or odd number of residues, respectively) (Peat et al., 1952; Summer & French, 1956). Thus, the amount of maltose + maltotriose is the amount of A-chains. The external chain length (ECL) of the B-chains is 1–2 and very small amounts of the shortest possible B-chain may also be maltotriose. Phosphorylase *a* gives ϕ-LD:s in which all A-chains are maltotetraosyl stubs (Walker & Whelan, 1960). The shortest B-chain is maltopentaose, because their ECL is three (proposed by indirect evidence by Bertoft (1989)). When a ϕ-LD is further treated with β-amylase into a ϕ,β-LD, all A-chains become maltosyl stubs and all B-chains are longer than that (Bertoft, 1989). The benefit to use LD is that the A:B chain ratio is obtained regardless the original lengths of the chains. A survey of published ratios obtained from LD:s of amylopectins of both A- and B-crystalline starches showed no large differences between them. The ratio varies between 0.7 and 1.5, being ~1.2 at average in both crystalline types (Asaoka, Okuno, & Fuwa, 1985; Bertoft, 1991; Bertoft & Koch, 2000; Enevoldsen & Juliano, 1988; Nilsson, Bergquist, Nilsson, & Gorton, 1996; Robin, 1981; Yun & Matheson, 1993; Zhu & Bertoft, 1996). With the same wheat sample, Hizukuri and Maehara (1990) obtained a higher ratio (1.7) with native amylopectin, than when using the β-LD (1.3). Similar results were obtained by Yuan, Thompson, and Boyer (1993) with mutant maize starches, among which A-crystalline types (*wx* and *duwx*) showed higher ratio if analysed from the native amylopectin than when the β-LD was used, whereas B-crystalline types (*aewx*) showed similar or even lower ratio with native samples.

From the above overview, it is obvious that a consensus regarding the nature of the categories of chains in amylopectin has not been achieved. The purpose of the present investigation was therefore to critically re-examine the ratio of A:B-chains and the subcategories of these chains in two amylose-free starches, namely waxy maize starch (WMS) and potato amylopectin starch (PAPS), which represent typical A- and B-crystalline granules, respectively. The collective construction of the amylopectin macromolecule by these chains is important to understand and is discussed on the basis of what is known about the semicrystalline architecture of the starch granules (Imberty et al., 1991; Jenkins, Cameron, & Donald, 1993). The structure of amylopectin is also discussed in light of reports that suggest that it exist as a left-handed super-helix inside the granules (Oostergetel & Bruggen, 1993; Waigh, Donald, Heidelbach, Riekel, & Gidley, 1999).

2. Materials and methods

2.1. Starches and enzymes

PAPS was a kind gift from Lyckeby Stärkelsen, Sweden, and WMS was from AVEBE, Belgium. WMS was defatted by Soxhlet extraction in hot aqueous 85% methanol overnight before use. Rabbit muscle phosphorylase *a* (EC 2.4.1.1), specific activity 24 U/mg, was from Sigma, Germany. The following enzymes were purchased from Megazyme, Ireland: Barley β-amylase (EC 3.2.1.2) with a specific activity of ca. 1400 U/mg, isoamylase from *Pseudomonas* sp. (EC 3.2.1.68) with ca. 280 U/mg, and pullulanase from *Klebsiella planticola* (EC 3.2.1.41) with 42 U/mg.

2.2. Production of limit dextrins

Starch granules (100 mg) were dissolved in 90% dimethylsulphoxide (DMSO, 3 ml) by stirring on a boiling water bath ca. 10 min and then at room temperature overnight. The sample was diluted with warm (~70 °C) water (32.7 ml) before addition of 1.1 M sodium phosphate buffer, pH 6.8 (3.6 ml), 2.8 mM EDTA (sodium salt, 1.7 ml) and a freshly prepared solution of 0.9 U/ml phosphorylase *a* (9 ml). The final concentration of amylopectin was 2 mg/ml. The mixture was stirred overnight at room temperature and then boiled for 5 min. An aliquot (1 ml) was diluted with water (2 ×) and centrifuged for 20 min at 3000 × *g* using a Centriplus Centrifugal Filter Device with MWCO 10.000 (Millipore, Finland). The carbohydrate content in the filtrate (containing glucose 1-phosphate) and the total carbohydrate content before centrifugation was measured with the phenol-sulphuric

acid reagent (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and used to calculate the percentage degree of phosphorolysis (1st ϕ). The rest of the sample was concentrated by rotary evaporation and then dialysed against water for 2 days, after which the remaining content of glucose 1-phosphate was estimated using the Centrifugal Filter Device. The sample concentration was adjusted to 2 mg/ml and the phosphorolysis was repeated. The degree of phosphorolysis was estimated as above (2nd ϕ) after subtraction of the remaining small amounts of glucose 1-phosphate found after the dialysis. The ϕ-limit value of amylopectin was calculated from: ϕ-limit = [1 − (1st ϕ)/100] × (2nd ϕ) + (1st ϕ). Finally, the sample was concentrated and dialysed.

One part of the ϕ-limit dextrins (ϕ-LD) was lyophilised and the other part was used to produce ϕ,β-LD as follows: the sample volume was adjusted to give a dextrin concentration of 2 mg/ml thereafter 0.33 volumes of 0.01 M NaOAc buffer, pH 6.0, and a volume of β-amylase corresponding to 4 U/mg of the substrate was added. After incubation at room temperature overnight the sample was boiled and the β-amylolysis limit was obtained using the centrifugal filtration technique described above. The ϕ,β-limit value = [1 − (ϕ-limit)/100] × (β-limit) + (ϕ-limit). After dialysis, the sample of ϕ,β-LD was lyophilised.

To produce β-LD, starch granules (50 mg) were dissolved as described above and diluted to 5 mg/ml. The sample was then treated with buffer and β-amylase as described above for the production of ϕ,β-LD.

2.3. Enzymatic analyses

Starch granules or lyophilised limit dextrins were dissolved in DMSO as above and diluted to 4 mg/ml. The starch or ϕ-LD solution (0.45 ml) was diluted with 0.1 M NaOAc, pH 3.5 (0.05 ml) and isoamylase (1 μl) was added. After overnight incubation, the sample was boiled and analysed chromatographically as described below. β- or ϕ,β-LD solutions (0.9 ml) were diluted with 0.01 M NaOAc buffer, pH 5.5 (0.1 ml), and then pullulanase (2 μl) was added. After overnight incubation and boiling, an aliquot (0.45 ml) of the sample was further treated with isoamylase as described above.

2.4. Chromatographic techniques

2.4.1. Gel-permeation chromatography (GPC)

Debranched samples (0.2 ml) were applied to a column (1 × 90 cm) of Superdex 75 (Pharmacia) and eluted with 0.1 M KCl at 0.5 ml/min. Fractions (0.5 ml) were collected and their carbohydrate content was analysed with the phenol-H_2SO_4 reagent (Dubois et al., 1956). The column was calibrated with dextrins of known DP (Bertoft & Spoof, 1989).

2.4.2. Anion-exchange chromatography (HPAEC)

The debranched samples were also analysed on a Dionex (USA) HPLC system (series 4500i) equipped with a BioLC gradient pump and pulsed amperometric detection (PAD). The samples were filtered and injected (25 μl) onto a CarboPac PA-100 column (250 × 4 mm) in combination with a similar guard column and eluted at 1 ml/min. A gradient was made by mixing eluent A (150 mM NaOH) and eluent B (150 mM NaOH containing 500 mM NaOAc) as follows: from 0 to 9 min a gradient of B from 15 to 36%; 9 to 18 min from 36 to 45%; and 18 to 110 min from 45 to 100%. The PAD response was calibrated to quantitative carbohydrate content by the method of Koch, Andersson, and Åman (1998) using fractions of debranched PAPS collected from GPC. Chains of DP > 55 were not resolved but quantitatively approximated by a continuous area division of the chromatogram. For samples containing a lot of maltotetraose (ϕ-LD:s), maltose (ϕ,β-LD:s), or maltose and maltotriose (β-LD:s), the PAD signal was initially recorded at a 10 × less sensitive output range (3000 nA instead of 300 nA).

3. Results and discussion

3.1. Chain length distribution of amylopectin

Starch granules of waxy maize are typical A-crystalline (Gernat, Radosta, Anger, & Damaschun, 1993), whereas the granules of potato amylopectin starch possess the B-type pattern (McPherson & Jane, 1999). Fig. 1 shows the unit chain distributions of the amylopectins obtained by GPC. The resolution of the chromatograms allowed only a division of the chains into long and short at DP ~ 35. The proportion of the former was much higher in PAPS than in WMS, which is characteristic for B-type starches (Hizukuri, 1985). Fig. 2 shows the same samples analysed by HPAEC-PAD by which the resolution is considerably higher. Chains longer than DP 55 were not resolved as peaks, however, and the amount of chains in that area had to be estimated

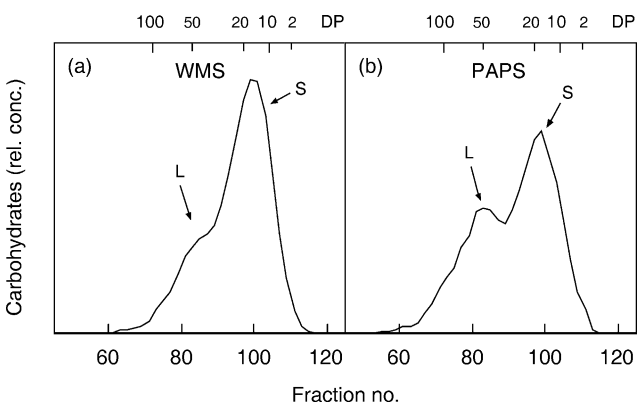

Fig. 1. Distribution on Superdex 75 of the chains of debranched (a) WMS and (b) PAPS. The groups of short (S) and long (L) chains are indicated.

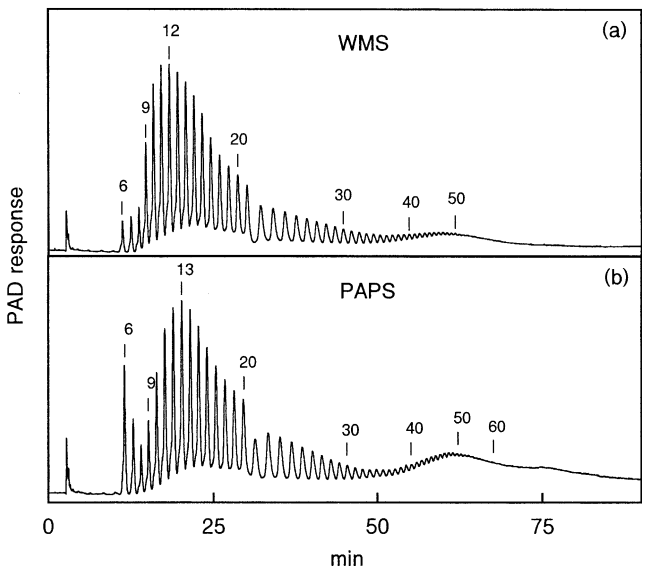

Fig. 2. Chain length distribution profiles with HPAEC-PAD of (a) WMS and (b) PAPS. Numbers indicate the degree of polymerisation.

approximately by a continuous division of the chromatogram into successive areas for each chain length (CL). The average CL thus obtained is compared with that obtained by GPC in Table 1 and was found to be in good agreement. The CL estimated for the short chains (SCL) was slightly higher by HPAEC, whereas that for the long chains (LCL) became slightly lower when the division was made at DP 35. These small differences were considered as acceptable.

The long chains of PAPS were resolved as two groups by HPAEC and corresponded with the division into B2-chains (CL 35–60) and B3-chains (CL > 60) according to Hizukuri (1986). In WMS practically only B2-chains were present (Fig. 2). In neither sample, the short chains could be subdivided into A- and B1-chains. The shortest chains with CL 6–8 possessed, however, typical profiles. That of WMS is common for many, but not all, A-crystalline starches. That of PAPS, with a minimum at CL 8, is characteristic for potato, but is also possessed by some other amylopectins (Hanashiro et al., 1996; Koizumi, Fukuda, & Hizukuri, 1991; Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000). The profiles of short chains were regarded to be fingerprints of the amylopectin source

Table 1
Chain lengths in waxy maize starch and potato amylopectin starch analysed by GPC (Superdex 75) or HPAEC (CarboPac PA-100)

Chain length[a]	WMS		PAPS	
	GPC	HPAEC	GPC	HPAEC
CL	17.6	18.1	21.2	21.2
SCL	14.2	15.7	14.6	15.8
LCL	51.0	48.6	56.8	54.7

[a] The average chain length of the whole sample (CL), short chains (SCL, DP ≤ 35) and long chains (LCL, DP > 35).

(Koizumi et al., 1991). The group at CL 6–8 was probably A-chains and are here called 'fingerprint chains' (A_{fp}).

3.2. Chain lengths and distribution in limit dextrins

The limit dextrins of the amylopectin samples were debranched and analysed by GPC (Fig. 3). Isoamylase was used to debranch the ϕ-LD:s, in which CL < 4 should not be found, whereas pullulanase, which attacks maltosyl chain stubs more efficiently (Hizukuri & Maehara, 1990) was used for the β-amylase treated dextrins. WMS limit dextrins were apparently debranched by this treatment, but a small fraction of the β- and ϕ,β-LD:s of PAPS was resistant to pullulanase and eluted at the void volume of the gel (Fig. 3b and Table 2). This fraction represented only a few percent of the total sample and was susceptible to the attack by isoamylase, because it disappeared upon the addition of the latter enzyme. The remaining stubs of the A-chains were detected in the chromatograms as a more or less poorly resolved peak at DP 2–10. The rest of the chromatograms showed the distribution of the remaining internal parts of the B-chains. As for the original amylopectin chains, the internal parts of the B-chains were resolved as short (BS) and long (BL). In addition, the BL-chains were found in clearly larger amounts in PAPS than in WMS, suggesting that the original division of long and short (B)-chains remained after the removal of the external parts.

Figs. 4 and 5 show the chain distributions obtained by HPAEC-PAD and the major features found by GPC were also now distinguished. To be able to quantify the peaks representing A-chains in β-LD (maltose and maltotriose),

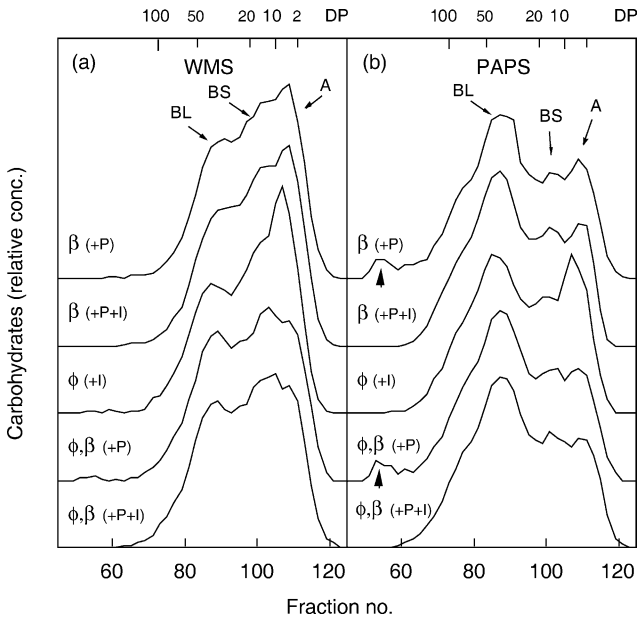

Fig. 3. Unit chain distribution on Superdex 75 of limit dextrins of (a) WMS and (b) PAPS. β-, ϕ-, and ϕ,β-LD:s were debranched with pullulanase (+P), isoamylase (+I), or successively with pullulanase and isoamylase (+P + I). Different chain categories are indicated. Thick arrowheads indicate material resistant to pullulanase attack.

Table 2
Comparison of chain length parameters calculated from limit dextrins

Parameter	WMS					PAPS				
	β-LD	β-LD	ϕ-LD	ϕ,β-LD	ϕ,β-LD	β-LD	β-LD	ϕ-LD	ϕ,β-LD	ϕ,β-LD
Debranching enzyme[a]	P	P + I	I	P	P + I	P	P + I	I	P	P + I
Resistant material (%)[b]	0.1	0	0.3	0.4	0	2.7	0	0.2	2.2	0.1
CL of limit dextrin[c]	8.0	7.6	9.4	7.8	7.7	9.6	9.5	11.6	9.1	9.0
ECL[d]	12.1	12.5	12.2	11.8	11.9	13.7	13.7	13.1	13.6	13.7
ICL[e]	5.0	4.6	4.9	5.3	5.2	6.6	6.5	7.1	6.6	6.5
TICL[f]	12.0	11.5	12.0	12.1	12.0	18.3	18.4	17.8	17.4	16.7
NC:B[g]	2.0	2.1	2.0	1.9	2.0	2.4	2.5	2.2	2.3	2.2

[a] Debranching with isomylase (I), pullulanase (P), or successively with pullulanase and isoamylase (P + I).
[b] Weight % resistant to debranching obtained by GPC.
[c] From HPAEC.
[d] External chain length of amylopectin (ECL) = CL × (% limit value/100) + (ECL of limit dextrin).
[e] Internal chain length (ICL) = CL − ECL − 1.
[f] Total internal chain length obtained from HPAEC.
[g] Average number of chains per B-chain = TICL/(ICL + 1).

ϕ-LD (maltotetraose), and ϕ,β-LD (maltose), the PAD signal was detected at a 10 × less sensitive output range as indicated in the figures. The structure of the ϕ- and ϕ,β-LD, in which the external chain stub of the B-chains contain three and one D-glucosyl residues, respectively (which is one residue shorter than earlier suggested (Walker & Whelan, 1960)) was proposed on the basis of indirect evidence showing that the ϕ,β-limit value tends to be slightly higher than the β-limit (Bertoft, 1989). The fact that the shortest possible B-chain, maltopentaose in ϕ-LD

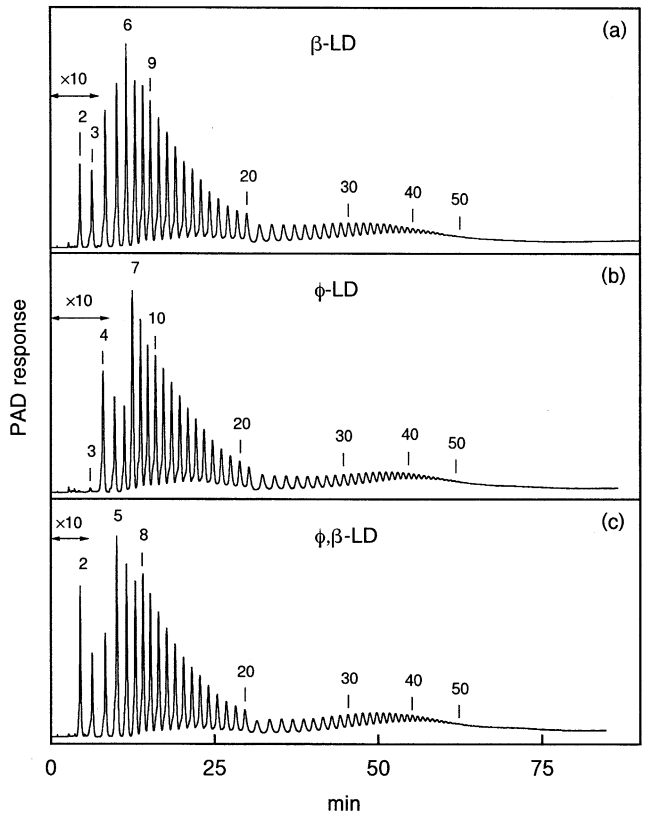

Fig. 4. Chain length distribution profiles with HPAEC-PAD of (a) β-, (b) ϕ-, and (c) ϕ,β-limit dextrins of WMS. Numbers indicate degree of polymerisation. The time interval at which the PAD response was recorded at 10 × lower sensitivity is indicated.

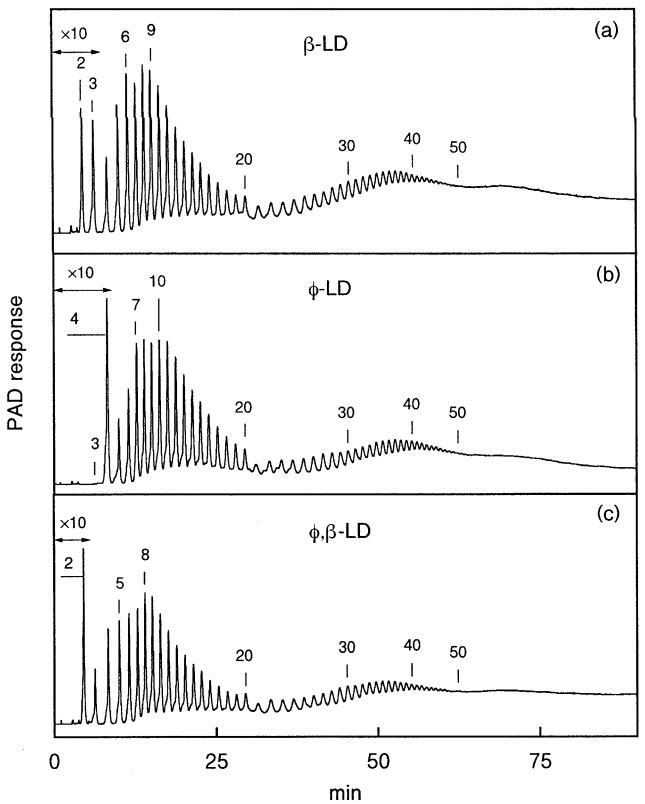

Fig. 5. Chain length distribution profiles with HPAEC-PAD of (a) β-, (b) ϕ-, and (c) ϕ,β-limit dextrins of PAPS. Numbers indicate degree of polymerisation. The time interval at which the PAD response was recorded at 10 × lower sensitivity is indicated.

and maltotriose in ϕ,β-LD, was found in the debranched mixtures suggested finally direct evidence for the proposed structures by Bertoft (1989).

The CL of the limit dextrins estimated from HPAEC are given in Table 2. The uncomplete debranching of β- and ϕ,β-LD:s with pullulanase was reflected as slightly higher CL-values compared to those obtained by a successive debranching with pullulanase and isoamylase. The average lengths of the chains of PAPS LD:s were approximately two residues longer that in WMS LD:s. The differences between the three types of limit dextrins reflected fairly well the theoretical differences that should be obtained if the A:B chain ratio is one (when ECL of β-LD is 2.0, ϕ-LD is 3.5, and ϕ,β-LD is 1.5).

From the difference of the CL-values of the LD:s and the original amylopectins, the β-amylolysis and phosphorolysis limits were obtained. In Table 3, these values are compared with those estimated by the novel centrifugal filtration technique used during the production of the limit dextrins. The estimation of limit values is very sensitive to small variations in the experimentally derived results. This is perhaps best reflected in the β-limit of WMS that was 55.8% when using the CL obtained when only pullulanase was used for debranching of the β-LD, but was 57.9% when both the pullulanase and isoamylase were used. Yet the difference in CL calculated from the two HPAEC chromatograms was only 0.4 (Table 2). With this in mind, the values obtained by the centrifugal filtration method, which was simple to perform, agreed well with those calculated from the differences of CL-values. Altogether, the limit values were of the same order as formerly reported for WMS (Klucinec & Thompson, 2002; Yun & Matheson, 1993) and PAPS (Nilsson et al., 2001).

From the knowledge of the ECL of the limit dextrins (assuming the ratio of A:B chains is one), the limit values, and the original CL, the ECL of the original amylopectin can be estimated (Bertoft, 1989; Manners, 1989). Within experimental errors, the ECL values calculated from different limit dextrins agreed (Table 2). WMS possessed an ECL-value ~12.0 and that of PAPS was ~1.5 residues longer. The average length of the internal segments between branch points (ICL) was also ~1.5 residues longer in PAPS. Though this suggests a less tight branching in PAPS at average, the actual distribution of branches within and between clusters cannot be told.

Table 3
Limit values of WMS and PAPS obtained by the centrifugal filtration (cf) technique or from the differences in CL between amylopectin and the limit dextrins

Parameter	WMS			PAPS		
	cf	CL	CL	cf	CL	CL
Debranching enzyme[a]	–	P or I	P + I	–	P or I	P + I
β-Limit (%)	52.9	55.8	57.9	54.6	54.9	55.2
ϕ-Limit (%)	47.2	48.0	–	42.9	45.2	–
ϕ,β-Limit (%)	57.7	57.0	57.7	56.9	57.1	57.7
β-Limit of ϕ-LD (%)	19.8	17.2	18.5	24.6	21.6	22.7

[a] ϕ-LD:s were debranched with isoamylase (I), β- and ϕ,β-LD:s with either pullulanase (P) or both enzymes (P + I) before analysis with HPAEC in order to obtain CL.

The major part of the distribution profile of the limit dextrins reflects the distribution of the inner part of the B-chains together with their short external chain stubs (in β-LD they have DP ≥ 4, though a few are DP 3, in ϕ-LD ≥ 5, and in ϕ,β-LD ≥ 3). By subtracting the external stubs, the total internal chain length (TICL), defined as including all branch-point residues along the B-chain (Bertoft, 1991), is obtained. The value was considerably longer in PAPS than in WMS (Table 2), which reflected the higher amounts of long B-chains and the presence of B3-chains in PAPS (Fig. 5). The average number of side-chains along a B-chain (NC/B) can be calculated from NC/B = TICL/(ICL + 1).

The B-chains of WMS carried at average two other chains (that could be either A-, B- or both types), whereas the B-chains of PAPS carried 2.2–2.5 chains (Table 2). It is, however, not possible to decide the actual NC-distribution between the different categories of B-chains (B1, B2 and B3) from these average values.

3.3. Estimation of A:B-chain ratio from limit dextrins

As noted above, a division into A- and B-chains was not possible to make on the basis of the unit chain profiles of the amylopectins obtained by either GPC or HPAEC (Figs. 1 and 2). Instead, the chain distributions of the limit dextrins were used. For a β-LD, the length of the external chain stubs is dependent on whether the chain segment originally had an even or odd number of D-glucosyl residues. The A-chains appear, therefore, as maltose and maltotriose after debranching of the β-LD, and the ratio of these carbohydrates reflects the ratio of even and odd numbered chains. As shown in Table 4, the β-LD of WMS possessed slightly more maltotriosyl than maltosyl chain stubs and the ratio of even:odd A-chains was 0.97. The ratio was thus close to 1:1 in agreement with other reports on different amylopectins (Bathgate & Manners, 1966; Enevoldsen & Juliano, 1988; Yun & Matheson, 1993), but slightly lower than the ratios (1.13–1.22) reported by Robin (1981). If assumed that the sum of maltose and maltotriose equals the amount of A-chains, the ratio of A:B chains was 1.06 in WMS. This value corresponded with those (0.7–1.3) reported for WMS when based on the debranched β-LD (Inouchi, Glover, & Fuwa, 1987; Manners & Matheson, 1981; Robin, 1981; Yuan et al., 1993; Yun & Matheson, 1993), but was in sharp contrast to the value (2.3) estimated directly from the debranched native WMS (Yuan et al., 1993).

B-chains of the β-LD possess 1 or 2 residues as their external chain stubs. From studies on waxy rice amylopectin (Umeki & Yamamoto, 1975), it can be concluded that B-chains with the shortest possible TICL, which is two,

Table 4
Estimation of A- and B-chains in limit dextrins

Parameters	WMS			PAPS		
	β-LD	ϕ-LD	ϕ,β-LD	β-LD	ϕ-LD	ϕ,β-LD
Uncorrected values[a]						
Maltose	25.36	–	48.50	30.35	–	55.45
Maltotriose	26.13	1.78	3.02	29.45	0.14	1.82
Maltotetraose (as A-chains)	–	48.18	–	–	54.59	–
A-chains	51.49	48.18	48.50	59.80	54.59	55.45
A:B	1.06	0.93	0.94	1.49	1.20	1.24
Even A:odd A[b]	0.97	–	–	1.03	–	–
Correction by method (1)[c]						
B-chains as maltotriose	0.77	–	–	−0.90	–	–
A-chains	50.72	–	–	60.70	–	–
A:B	1.03	–	–	1.54	–	–
Correction by method (2)[d]						
B-chains as maltotriose	0.62	–	1.24	0.84	–	1.68
A-chains as maltotriose	25.51	1.78	1.78	28.61	0.14	0.14
A-chains	50.87	49.95	50.28	58.96	54.73	55.59
A:B	1.04	1.00	1.01	1.44	1.21	1.25
Even A:odd A[b]	0.99	–	–	1.06	–	–

All values are mole% or molar ratios obtained by HPAEC after debranching with isoamylase (ϕ-LD) or pullulanase and isoamylase (β-LD and ϕ,β-LD).
[a] The amount of A- and B-chains were estimated assuming that A-chains have DP 2 + 3 in β-LD, 4 in ϕ-LD, and 2 in ϕ,β-LD.
[b] The ratio of A-chains with an even or odd number of D-glucosyl residues.
[c] The amount of A- and B-chains were corrected by method (1), in which some B-chains in β-LD are assumed to have DP 3 (see text for details).
[d] The amount of A- and B-chains were corrected by method (2), in which some A-chains in ϕ-LD are assumed to have DP 3 (see text for details).

should appear as DP 3 or 4 in the β-LD. Therefore, half the number of these chains are included into the maltotriose peak. If statistically half of the A-chains have an odd number of residues and appear as maltotriose, the other half is represented by the maltose. The difference should equal the B-chains in the maltotriose peak. These B-chains represented ~3% of the maltotriose or 0.77 mole% of all chains in WMS (Table 4). Thus, the amount of A-chains could be corrected as 2 × the amount of maltose, which here is called 'correction method (1)', and resulted in a small decrease of the A:B ratio from 1.06 to 1.03. A similar method was used by Enevoldsen and Juliano (1988), though their basis of calculation was the amount of maltotriose.

The uncorrected A:B ratio of PAPS β-LD was 1.49 and the ratio of even:odd A-chains was slightly higher than 1:1. Thus, more maltose than maltotriose was found and the amount of B-chains involved in the maltotriose peak became negative when a correction based on method (1) was made (Table 4). The assumption that half of the A-chains have an even (or odd) number of residues appears, therefore, too simplified. The amount of B-chains as maltotriose cannot be concluded and, as a result, the estimated amount of A-chains based on β-LD will be uncertain.

Though β-LD:s are more commonly used, ϕ- and ϕ,β-LD:s constitute alternatives with the benefit that all A-chains, regardless if they have an even or odd number of residues, are reduced into maltotetraosyl and maltosyl stubs, respectively. The amount of A-chains (maltotetraose) of the ϕ-LD was lower than that found in β-LD of both WMS and PAPS (Table 4). When the LD was transformed into the ϕ,β-LD, the amount of A-chains (represented by maltose) agreed fairly well with that found for the ϕ-LD, and the comparatively low ratio of A:B chains was confirmed. Lii and Lineback (1977) reported also slightly higher ratios obtained from β-LD in comparison with ϕ,β-LD from wheat, triticale and rye samples. Possible explanations for the different results are that (i) all A-chains are not reduced into maltotetraosyl stubs when treated with phosphorylase, or (ii) the ratio of even:odd A-chains is higher than 1:1 so that the maltotriose peak obtained from β-LD hides more B-chains than expected. A careful examination of the unit chain profile of WMS ϕ-LD (Fig. 4b) indicated a small peak at DP 3 (detected at 10 × less sensitive output signal). An even smaller, trace amount was found for PAPS ϕ-LD (Fig. 5b). Possibly, a tiny amount of the A-chains was reduced into maltotriosyl stubs by the phosphorylase. In 'correction method (2)' these stubs were therefore included to the A-chains of the ϕ-LD, which increased the A:B ratio of WMS from 0.93 to 1.00, but had only a negligible effect on PAPS (Table 4). In ϕ,β-LD, all of the shortest B-chains are expected to be maltotriose. Because the maltotriose already produced by the phosphorylase (being A-chains) will not be shortened further by the additional β-amylolysis, the maltotriose peak of ϕ,β-LD should be a mixture of A- and B-chains. The ratio of A:B chains in ϕ,β-LD was therefore also corrected by 'method (2)'. In PAPS ϕ,β-LD the proportion of A-chains in the maltotriose peak was small,

but in WMS they represented ~60%. When subtracted from the maltotriose peak, the remaining amount represented the 'true' shortest B-chains. This amount was rather similar in WMS and PAPS (1.24 and 1.68 mole%, respectively).

The amount of B-chains as maltotriose in β-LD should be half of that found in ϕ,β-LD. Correction method (2) was therefore extended to estimate the amount of these chains in the β-LD. In the β-LD of WMS the amount of B-chains in the maltotriose peak was 0.62 mole% according to method (2), which agreed with the amount found by 'method (1)' (Table 4). The rest of the maltotriose peak represented the A-chains with odd number of residues. The ratio of even:odd A-chains increased slightly and became very close to 1:1, whereas the ratio of A:B chains slightly decreased so that it agreed with the values obtained with ϕ- and ϕ,β-LD:s as closely as one could wish. In the β-LD of PAPS method (2) resulted in a similar estimation of the amount of the shortest B-chains as found in WMS. This decreased the number of odd A-chains, and thus the proportion of even A-chains became higher. As with WMS, the A:B ratio decreased for β-LD, though it remained at a higher level than in ϕ- and ϕ,β-LD. Unfortunately, the reason remained unclear.

In conclusion, short B-chains in the form of maltotriose tend to overestimate the A:B chain ratio based on β-LD:s, whereas A-chains in the form of maltotriose seem to be produced by phosphorylase and result in an underestimation of the ratio. When the latter chains are accounted for, at least a partial correction of the ratio is possible. Nevertheless, the discrepancies are comparatively small and, most probably, the ratio obtained from limit dextrins is more correct than if estimating the A- and B-chains directly from unit chain profiles of original amylopectins.

3.4. Reconstruction of categories of chains in amylopectin

In Fig. 6, the B-chain profiles of the ϕ,β-LD:s of WMS and PAPS are compared on a molar basis. As already noticed, the different groups of B-chains were also clearly distinguished after the removal of the external parts, which suggested that the latter were of fairly similar length in all B-chain categories. The distribution of the B1-chains suggested that the shortest chains of DP 3–7 constituted a subgroup. WMS possessed a peak at DP 5 and a distribution very similar to that earlier found in waxy rice starch and named short B1a-chains (Bertoft & Koch, 2000). These chains were suggested to build up the inner parts of the smallest branched units (building blocks) of the clusters in waxy rice. It was interesting to notice that this group of chains had a different profile in PAPS. Moreover, Shi and Seib (1995) published the chain distributions from β-LD:s of different maize mutants (*wx*, *duwx*, *aewx*, *aeduwx*), from which the same group of chains is distinguished. Possibly, therefore, the profiles in Fig. 6 are characteristic fingerprints of the building block chains in amylopectins. This subgroup of B1-chains is therefore here called 'fingerprint B-chains' (B_{fp}), in analogy to A_{fp}-chains.

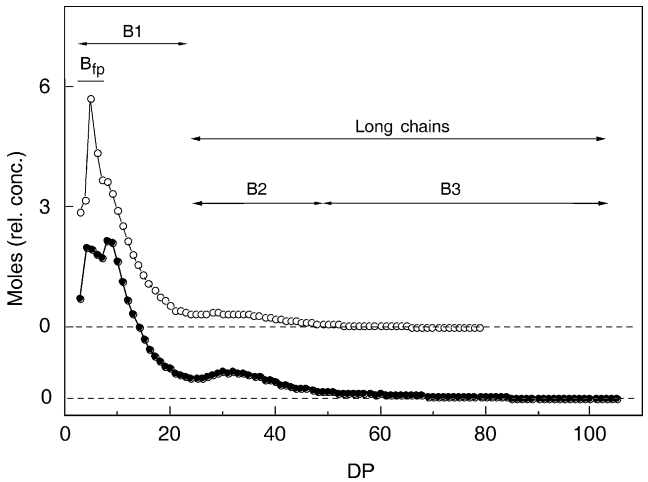

Fig. 6. Molar distribution of the B-chains (DP ≥ 3) of the ϕ,β-limit dextrins of WMS (○) and PAPS (●). Groups of different chains are shown.

Though the ϕ,β-LD is used to analyse the A:B-chain ratio and the distribution of the internal parts of the B-chains, it is also of greatest interest to know the original lengths of these chains. Inouchi et al. (1987) suggested that all external segments of the B-chains are of the same lengths and this was, as noted above, supported in this work. Therefore, it should be possible to trace theoretically the original length distribution by addition of the experimentally found ECL-value subtracted by one (the sole external residue in the B-chains) to each chain. As shown in Fig. 7a, the traced position of the B-chains fitted almost perfectly the experimentally obtained unit chain profile of the original WMS sample at DP ≥ 16. From DP 39–49 the molar concentration of reconstructed B-chains was slightly higher than originally. Though this was within experimental errors, a similar, but more pronounced, result was found for waxy rice amylopectin, which possibly was explained by the existence of external segments of some B-chains that were slightly longer than the average value (Bertoft & Koch, 2000).

The A-chains were traced as the difference between the original unit chain profile and the reconstructed B-chain profile. When correction method (2) was applied to adjust the number of B-chains of DP 3 in the ϕ,β-LD, the majority of the reconstructed A-chains possessed a smooth distribution around DP 12 (Fig. 7a). A minor population appeared at DP 17–18. Its significance is uncertain, but a similar minor population was found in the waxy rice amylopectin (Bertoft & Koch, 2000). Thus, the method used here supported the suggestion by Hizukuri (1986) and others that the A-chains generally are the shortest chains of amylopectin. At DP 14–18 A-chains were, however, mixed with the group of B_{fp}-chains. The number of A-chains in this overlapping area represented 20% of all the A-chains.

A similar reconstruction of the A- and B-chains was made for PAPS. For an ECL value of 13, a close to perfect fit between reconstructed B-chains and the original unit

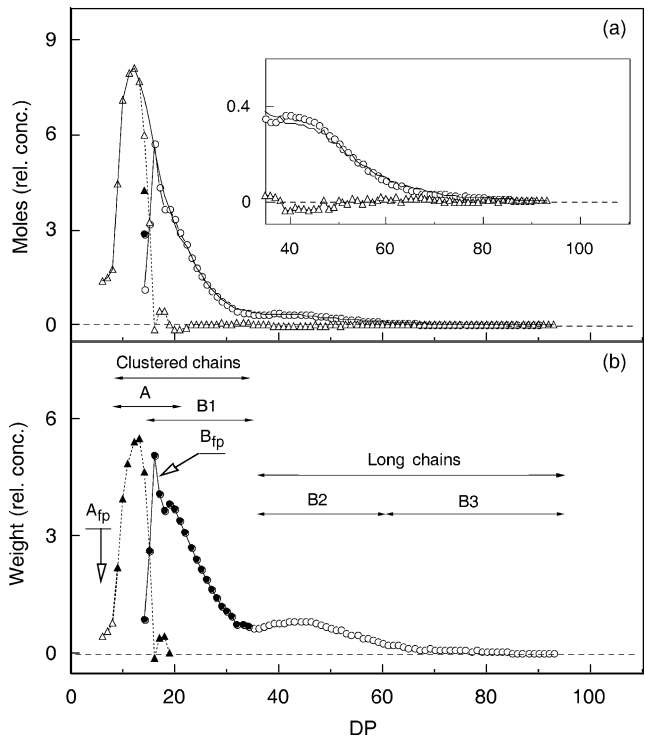

Fig. 7. Theoretical reconstruction of (a) the molar distribution and (b) the weight distribution of unit chains in WMS. In (a) the experimentally obtained distribution (—) is compared with reconstructed distributions of A- (Δ) and B-chains (○). Filled symbols at DP 14 show the number of A- and B-chains without correction with method (2) (see text). In (b) filled symbols show chains involved in clusters and in crystalline areas through their external segments, and open symbols show amorphous chains. Characteristic chain categories are shown.

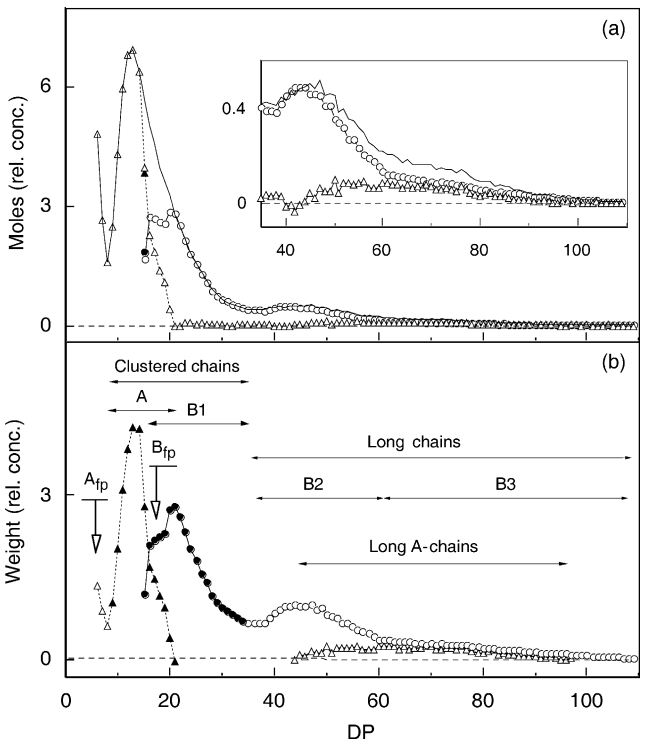

Fig. 8. Theoretical reconstruction of (a) the molar distribution and (b) the weight distribution of unit chains in PAPS. In (a) the experimentally obtained distribution (—) is compared with reconstructed distributions of A- (Δ) and B-chains (○). Filled symbols at DP 15 show the number of A- and B-chains without correction with method (2) (see text). In (b) filled symbols show chains involved in clusters and in crystalline areas through their external segments, and open symbols show amorphous chains. Characteristic chain categories are shown.

chain profile was found at DP 21–44 (Fig. 8a). Also in this sample, ~20% of the A-chains overlapped with the B-chains at DP 15–20. Interestingly, at DP 45–90 the number of reconstructed B-chains was lower than the original profile suggested. The difference between the two curves, therefore, resulted in a reconstructed population of long A-chains in this sample. In Table 5, the relative molar amount of different chain categories are summarised. Though the long A-chains represented only 2.6% of all chains, they constituted a significant part (~19%) of the population of long chains in the potato amylopectin. It could be argued that the long A-chains are artefacts due to the method of reconstruction used. This can, however, at least partly be controlled. If all A-chains in a sample are short, then the experimentally found number of short and long chains, and thus the ratio of S:L, in the limit dextrin is expected to be the same as in the native amylopectin. As shown in Table 6, this was the case for WMS, but for PAPS the ratio was higher in the ϕ,β-LD. Therefore, some originally long chains in PAPS were reduced into short when producing the limit dextrin. Also, if all A-chains are short, then the number of short B-chains in the LD should be equal to the difference between the number of S- and A-chains in the amylopectin. The ratio of BS:BL should then equal (S–A):L. The latter ratio in PAPS was lower, which, therefore, also suggested that a population of long A-chains existed in that sample.

The categories of chains thus identified in WMS and PAPS are shown in Figs. 7b and 8b, respectively, on

Table 5
Relative amount (mole%) of chain categories in WMS and PAPS

Chain category[a]	WMS	PAPS
S	92.95	86.14
L	7.05	13.62
A	50.28	55.59
BS	42.55	33.13
BL	7.17	11.28
A_{fp}	4.61	9.05
Clustered A	45.67	43.94
Long A	–	2.60
B_{fp}	19.81	12.45
B2	6.65	8.83
B3	0.52	2.45

[a] The chain length ranges are shown in Figs. 7 and 8: S, short chains of DP < 35 and L, long chains (DP ≥ 35) from debranched native samples; A, A-chains from ϕ,β-LD and calculated by 'correction method (2)'; BS, short B-chains (= B1); BL, long B-chains (= B2 and B3); A_{fp}, 'fingerprint' A-chains of DP 6–8; clustered A, A-chains included in clusters; long A, A-chains with DP ≥ 35; B_{fp}, fingerprint B-chains that in ϕ,β-LD are of DP 3–7; B2, DP 35–60; and B3 = DP > 60.

Table 6
Molar ratios of chain categories in WMS and PAPS

Ratio[a]	WMS	PAPS
S:L[b]	13.2	6.3
S_{LD}:L_{LD}[c]	12.9	7.9
BS:BL	5.9	2.9
(S−A):L	6.0	2.2
(A_{fp} + long A):BL	0.6	1.0
A_{fp}:B2	0.7	1.0
Long A:B3	–	1.0
Clustered A:all A	0.9	0.8
Clustered A:BS	1.1	1.3
Clustered A:B_{fp}	2.3	3.5
B_{fp}:(BS−B_{fp})	0.9	0.6

[a] Chain categories as defined in Table 5.
[b] Ratio of S:L in native amylopectin.
[c] Ratio of S:L in ϕ,β-LD.

a relative weight basis. The next question of interest is their connection to the cluster model of amylopectin. According to Hizukuri (1986), the long B-chains are involved in the interconnection of the clusters. This cannot, however, be the case for the long A-chains of PAPS, because A-chains do not carry other chains and cannot link clusters together. Recent results obtained by treatment of the granular starches of both WMS and PAPS in diluted acid (lintnerisation) suggested that the long chains are completely amorphous, because no traces of long chains remained in the granular residues (Bertoft, 2004). Most probably therefore, neither the long A-chains nor the long B-chains are part of the clusters (even though the BL-chains might link the clusters together).

Gidley and Bulpin (1987) noticed that very short chains of DP < 10 do not form double helices and, therefore, do not crystallise (only together with a longer chain this is possible). Indeed, the results from lintnerisation showed that the chains of DP 6–8 are not crystalline (Bertoft, 2004). Therefore, also the A_{fp}-chains might be found outside the clusters. Another result that indirectly suggested this came from the conclusion by Jane, Wong, and McPherson (1989) that A-crystalline starches possess branches that are scattered into the crystalline lamella, whereas B-type starches do not have scattered branches. Such branches should be found in clusters containing many short chains. Yet, WMS possessed only half the number of A_{fp}-chains compared to PAPS (Table 5). This chain category did, therefore, not correlate with the pattern of scattered branches.

Thus, it appears that the shortest and longest A-chains, together with the long B-chains, are all found within the amorphous areas of the starch granules and do not constitute part of the clusters. The ratio of these A-chains to the long B-chains was 0.6 in WMS and exactly 1.0 in PAPS. It was also noted that in PAPS the ratio of A_{fp}:B2 was 1.0, and further that the ratio of long A:B3 also was 1.0 (Table 6). Though this might be a coincidence, it was interesting to find that in WMS, which possessed practically no B3-chains, there were also no long A-chains present. Possibly, therefore, long A-chains are only found connected to B3-chains, whereas A_{fp}-chains are found together with B2-chains.

The rest of the A- and B-chains (DP 9–34, Figs. 7b and 8b) are probably clustered with their external part largely involved in the crystalline lamellae (Imberty et al., 1991). The lengths of the clustered A-chains corresponded to the distribution of crystalline chains remaining after acid treatment (Bertoft, 2004), and represented 90 and 80% of all A-chains in WMS and PAPS, respectively (Table 6). The ratio of clustered A:clustered B (= BS) chains was 1.1 in WMS and slightly higher in PAPS. As noted above, the B_{fp}-chains were probably found as parts of the building blocks inside clusters together with the clustered A-chains. The ratio of these A-chains to B_{fp} was notably higher in PAPS (3.5) than in WMS (2.3). The rest of the clustered B-chains (with DP ~20–34) should represent the chains involved in the interconnection of the building blocks inside the clusters (Bertoft & Koch, 2000). The ratio of B_{fp} to these latter chains (equal to BS−B_{fp}) was lower in PAPS than in WMS. The ratios between the three types of clustered chains are important, because they reflect the principal architecture of the clusters of WMS and PAPS. Two (simple) cluster models are shown in Fig. 9 and show that comparatively small differences in the organisation could explain the differences in chain ratios.

Table 7 summarises the average lengths of the reconstructed chain categories. The chains involved in the clusters were remarkable similar in WMS and PAPS. The clustered A-chains possessed the same average length as the external segments of the B-chains. Apart from the A_{fp}-chains, the different categories in PAPS were constantly slightly longer than in WMS. ECL of the B-chains was one residue longer in PAPS, but the major difference was found

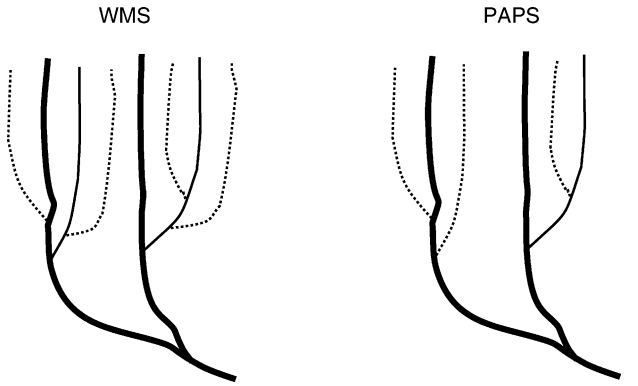

Fig. 9. Model of a cluster in WMS and PAPS, respectively, showing the clustered chains organised into two branched building blocks. Clustered A-chains (···), B_{fp}-chains (—) and the rest of the short B-chains (BS − B_{fp}) that are involved in interconnecting building blocks inside the clusters (—). The ratio of clustered A:BS is the same in both types of clusters, but the ratio of clustered A:B_{fp} is higher in PAPS, whereas B_{fp}:(BS−B_{fp}) is higher in WMS.

Table 7
Average chain lengths of reconstructed chain categories in WMS and PAPS

Chain category[a]	WMS	PAPS
A_{fp}	7.1	6.7
Clustered A	12.1	13.2
Long A	–	63.8
BS	20.6	21.9
BL	48.1	52.6
ϕ,β-BS[b]	9.5	9.8
ϕ,β-BL[b]	37.1	40.7
BS-ECL[c]	12.1	13.0
BL-ECL[c]	12.0	12.9
All ECL[d]	11.8	13.8
B-TICL[e]	12.4	16.7
BS-TICL[e]	8.5	8.8
BL-TICL[e]	36.1	39.7
B-ICL[f]	5.4	6.5

[a] Chain categories as defined in Table 5.
[b] Experimental values from ϕ,β-LD.
[c] External chain length = CL − (CL of ϕ,β-LD) + 1, of the BS or BL-chains.
[d] Includes all A- and B-chains.
[e] Total internal chain length = CL − ECL of different B-chain categories.
[f] The average internal chain length of B-chains = (NC:B) − 1, in which NC:B is the number of chains per B-chain from Table 2.

in the length of the inner part (TICL) of the long B-chains and the existence of long A-chains in PAPS.

3.5. A two-directional backbone model of amylopectin fine structure

A cluster model based on Hizukuri (1986) is shown in Fig. 10a, in which the major feature is the interconnection of the clusters by the long B-chains. The original model has been updated with some later findings, which relates the model to the semicrystalline structure as it appears inside the starch granules. Thus, the external chains form double helices (Imberty et al., 1991) that crystallise into thin (5–7 nm) lamellae (Jenkins et al., 1993). Some branches are scattered into the crystallites, but the majority are located in the amorphous lamellae (Jane et al., 1997), inside which the chains are comparatively flexible and bend into directions even perpendicular to that of the double helices (O'Sullivan & Pérez, 1999). The universal thickness of one crystalline plus one amorphous lamella is ~9 nm (Jenkins et al., 1993). If the semicrystalline structure is treated with diluted acid, the amorphous lamellae are removed (Biliaderis, Grant, & Vose, 1981). The long B-chains are thereby cleaved and shortened into lengths that correspond to the length of the resistant double helices. Therefore, the former long B-chains cannot be distinguished from other chains in acid treated granular residues.

A slight modification of the Hizukuri model, in which the long B-chains have been removed from their positions as integrated parts of the clusters, is shown in Fig. 10b. The B-chains in this model form a backbone to which the clusters

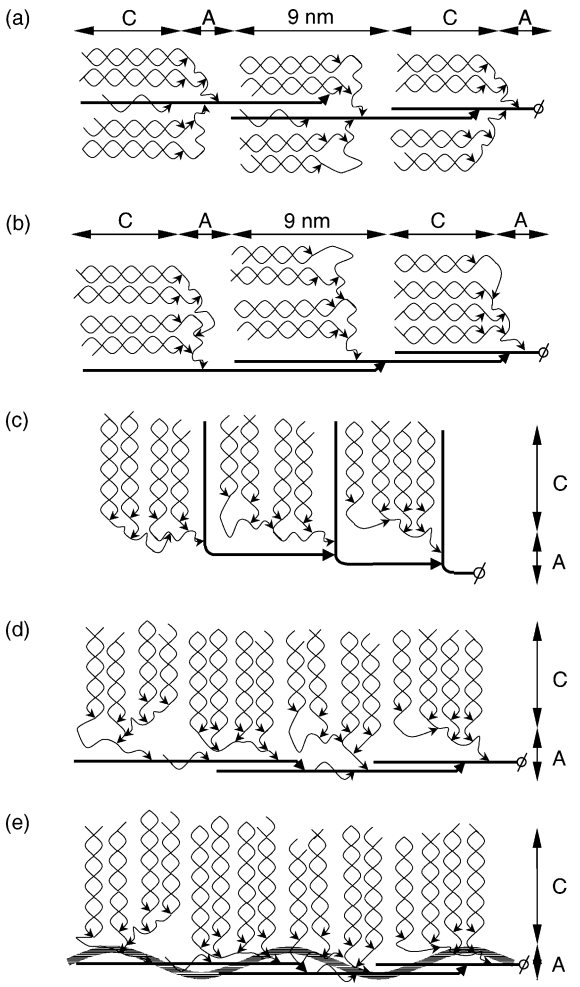

Fig. 10. Possible cluster models that cannot be distinguished on the basis of the unit chain distribution of native or acid treated (lintnerised) starches. The crystalline (C) and amorphous lamellae (A) found inside starch granules are indicated. External segments of clustered chains form double helices. Long B-chains (drawn as B2-chains) are shown as bold lines and the reducing end is indicated (Ø). (a) The cluster model by Hizukuri (1986) redrawn to highlight some findings from later years. The B2-chains are part of the clusters and partly crystalline. (b) One-directional backbone model. The B2-chains form a backbone to which the clusters are anchored. (c) A partial Robin et al. (1975) structure, in which the internal parts of the B2-chains are amorphous. (d) Two-directional backbone model. The entire B2-chains are amorphous and extend in perpendicular direction to the clustered chains. Some A_{fp}-chains are attached to the B2-chains. B3- and long A-chains will also be found in the amorphous lamella when present. (e) The two-directional backbone model in a normal starch, where the amylose (thick wave-line) is found together with the amorphous chains of amylopectin.

are attached. As in the original model all chains extend in one direction, and it would be impossible to distinguish between the two alternatives on the basis of their unit chain compositions. A backbone, and cluster-like, model was in fact suggested by M. Richter more than three decades ago, but published in a paper by Babor, Kalac, and Tihlarik (1968). It is also notable that the architecture with a backbone chain, to which side chains are attached, is commonly found among branched polysaccharides.

In Fig. 10c, the backbone model has been further rearranged, so that the direction of all external chains is perpendicular to that of the internal segments of the long B-chains. The major effect on the semicrystalline structure is that the direction of the crystalline and amorphous lamellae now is changed. Another important difference is that only the external segment of the long B-chains is found inside the crystalline lamella, whereas all of the interior parts are placed in the amorphous lamella. The model is a 'partial Robin et al.' structure, because it was suggested by Robin, Mercier, Duprat, Charbonnière, and Guilbot (1975) as a part of their amylopectin model. In fact, it represented one out of three alternatives to interconnect the clusters, as recently discussed by Thompson (2000).

A further, slight modification is shown in Fig. 10d, in which the external parts of the long B-chains are placed into the amorphous lamella. The structure is a two-directional backbone model, in which the clustered chains are standing in one direction, with their external parts forming the crystalline lamella, and the long B-chains are found in a perpendicular direction. It should be noted that the unit chain composition is (principally) identical to that of the other models in Fig. 10. They all represent, therefore, structural alternatives that have not been discussed previously. A major feature of the two-directional model is that the entire long B-chains are amorphous. It was shown by Hizukuri et al. (1983) that variations in the lengths within the group of short chains determine the type of crystallinity (A, B, or C), and thus the long chains might not be involved in the crystalline areas. As suggested above, the short fingerprint A-chains are attached to the B2-chains and are also found in the amorphous lamella. The long A-chains of PAPS will also be found there together with the B3-chains (not shown in Fig. 10d). The model is therefore also based on the results from acid treatments that suggested that all long chains (and the entire length of the chains) and the A_{fp}-chains are amorphous (Bertoft, 2004).

In normal starch granules amylose molecules are present. It was shown by cross-linking the components of granular starch that amylose is found next to amylopectin molecules, rather than forming bundles with other amylose molecules (Jane, Xu, Radosavljevic, & Seib, 1992). Except in high-amylose starches (Tester, Debon, & Sommerville, 2000), the amylose is generally believed to be amorphous inside the granules. The two-directional backbone model offers a room for amylose, where it could be found completely inside the amorphous lamella together with the amorphous chains of amylopectin (Fig. 10e). (Other amylose molecules, e.g. those involved in lipid complexation (Morrison, 1995), are allocated to amorphous 'growth rings'.) It was shown by Jenkins and Donald (1995) that the thickness of the amorphous lamella decreases and the crystalline lamella increases (retaining the 9 nm repeat distance) in the presence of amylose. In the discussion to their results, they referred to a personal communication with M.J. Gidley, who suggested that the amylose might penetrate the amorphous lamella and be oriented transverse to the chains in the crystalline lamella. (The other possibility ('mechanism 1') was that the amylose itself takes part in the crystalline area (Jenkins & Donald, 1995)).

In 1993, Oostergetel and van Bruggen proposed that the crystalline lamellae form a left-handed super-helical structure inside starch granules. The super-helix has a diameter of 18 nm and a central cavity with a diameter ~8 nm. Waigh et al. (1999) described the turns of the super-helix as being build up of nine 'pie shaped lamellar motifs'. The amorphous lamellae between these motifs form a backbone for the double helices. A simplified super-helix model based on Hizukuri's structure (1986) is presented in Fig. 11a.

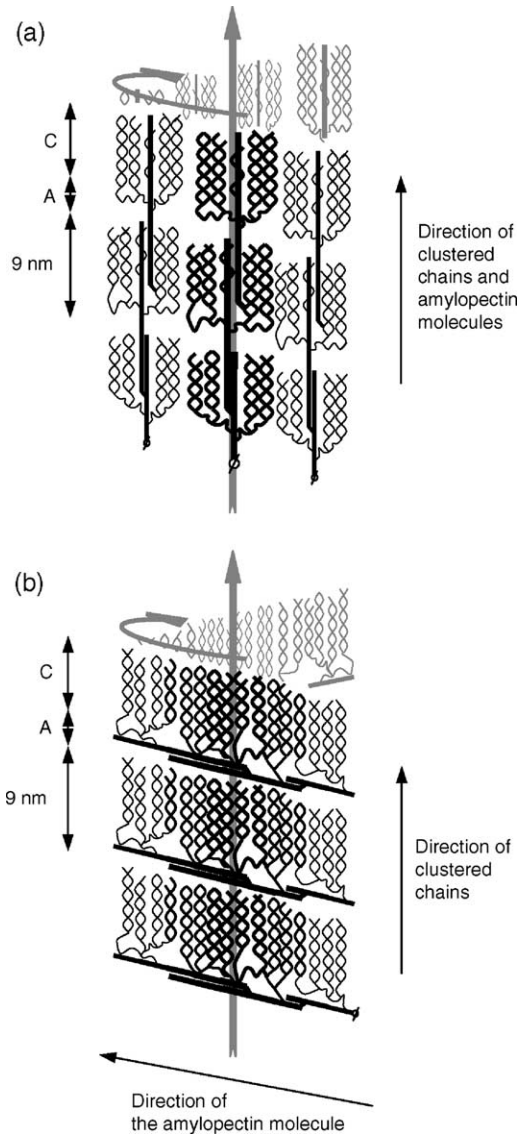

Fig. 11. The super-helix model of amylopectin schematically redrawn on the basis of Oostergetel and Bruggen (1993), Waigh et al. (1999). The axis and the turns of the super-helix are indicated by grey arrows. (a) The super-helix based on the cluster model by Hizukuri (1986) is a cooperative structure formed by several individual amylopectin molecules. (b) The super-helix based on the two-directional backbone model is formed by a single amylopectin molecule.

The super-helix is a cooperative structure build up of several individual amylopectin molecules. The double helices are lined up close together to form the left-handed crystalline lamella (Waigh et al., 1999). The direction of the clustered chains and of the individual amylopectin molecules follows that of the super-helix axis.

The two-directional backbone model (Fig. 10d) can also be adapted to a super-helix by simply twisting it into the helix (Fig. 11b). With the backbone structure the entire super-helix is build of a single amylopectin molecule. The crystalline lamella is structurally similar to the cooperative alternative, but the amorphous lamella is build up of a true backbone formed by the long chains of the amylopectin. The direction of the clustered chains is still similar to the super-helix axis, but the direction of the amylopectin molecule follows the turns of the super-helix.

It is tempting to suggest that a single macromolecular super-helix is identical to the units of blocklets observed by atomic force microscopy (Baldwin, Adler, Davies, & Melia, 1998; Gallant, Bouchet, & Baldwin, 1997). It might also be identical to the small ellipsoidal particles being formed during gelatinisation of starch granules (Atkin, Abeysekera, Cheng, & Robards, 1998). The possibility that a blocklet represents a single amylopectin molecule was discussed by Blennow, Bay-Smidt, and Bauer (2001) and Takeda, Shibahara, and Hanashiro (2003). One would expect that the super-helix have important influences on the properties of amylopectin. The amorphous backbone could allow much flexibility, permitting movements active during phenomenon like annealing (Tester & Debon, 2000) and gelatinisation (Waigh, Gidley, Komanshek, & Donald, 2000). Another interesting area is starch synthesis; a process that still is not understood and several theories exist (Smith, 1999). Among several problems, it has been difficult to understand how only one chain, out of several possible chains in a cluster, is selected to continue to grow into a long, intercluster chain. The two-directional model offers a solution, because it suggests that the synthesis of the short chains is separated in space from that of the long chains. Finally, it should be noticed that the two-directional backbone model only highlights some possible general principles for the arrangement of chain categories in amylopectin and hopefully contributes to a discussion of the structure. The details of the fine structure of amylopectins from different origin might be unique and require further investigation.

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