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Composition of building blocks in clusters from potato amylopectin

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Abstract

The cluster structure of amylose-free potato starch was recently analysed with respect to the size and unit chain composition of the clusters [Bertoft, E. (2007). Composition of clusters and their arrangement in potato amylopectin. *Carbohydrate Polymers*, *68*, 433–446]. The present work describes a further investigation of the cluster structure in terms of the internal organisation of the chains inside the clusters. This structural level represents the molecular organisation of the amorphous lamellae in starch granules. It was found that the chains were organised into very densely branched areas, characterised as building blocks, by which the clusters were constructed. The building blocks were isolated by extensive α-amylolysis of samples containing clusters. Doubly and triply branched blocks were identified in HPAEC-PAD, but larger dextrins with a degree of polymerisation (DP) $\geqslant 20$ were not resolved in the chromatograms. The majority of the branched blocks were, however, small, singly branched dextrins with DP 5–10. The overall composition of building blocks in different clusters was fairly similar, but based on the density of the blocks inside the clusters two major groups of clusters were distinguished. The average internal chain length between branches in the building blocks was only ~2 glucosyl residues, whereas the inter-block chain length inside the clusters was estimated to 7–8 residues. A structural role of sub-groups of the short chains that form the clusters is suggested on the basis of the inter-block chain length. A minor part of the building blocks appeared to possess a strong tendency to form molecular aggregates that were detected by gel-permeation chromatography, but the structural background for this phenomenon remained unknown.
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Keywords: Potato amylopectin starch; Amylopectin structure; Cluster structure; Building blocks

1. Introduction

Amylopectin, the major, branched component in starch, is built up from clusters of short chains of 6–35 (1 → 4)-linked α-D-glucosyl residues (Manners, 1989). In native starch granules the external chain segments of the clusters are found as double helices inside thin (4–6 nm) crystalline lamellae (Waigh et al., 2000), though it was suggested that the smallest external chains with a length (CL) of 6–8 are found outside these structures (Bertoft, 2004b). The internal part of the clusters contains α-(1 → 6)-branches between the chains and forms amorphous lamellae. The total thickness of an amorphous and crystalline lamella is 9–10 nm and stacks of the lamellae form larger, semi-crystalline rings (Jenkins, Cameron, & Donald, 1993). The molecular structure of the double helices and the crystallite structures they form (distinguished as A- and B-types) have been characterised in great detail (Buléon, Colonna, Planchot, & Ball, 1998; Imberty, Buléon, Tran, & Pérez, 1991; Kiseleva et al., 2005; Vermeylen, Goderis, Reynaers, & Delcour, 2004). In contrast, the molecular structure of the amorphous lamellae, and thus the organisation of chains in the internal part of the units of clusters, is largely unknown.

Units of clusters have been isolated from amylopectins by a limited degree of hydrolysis with the endoacting α-amylase of *Bacillus amyloliquefaciens* (Bertoft, 2004a). The external chains of the clusters were largely removed by the exoacting enzymes phosphorylase and β-amylase and the resulting limit dextrins (named ϕ,β-limit dextrin, ϕ,β-LD) possess only the internal structure, including all

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branches of the clusters (Bertoft, 1989). Structural analyses of the limit dextrins thus enables one to study the internal organisation of chains in the clusters and thereby the molecular structure of the amorphous lamellae of the starch granules.

In the form of limit dextrins the size of the clusters varies widely in different plants from a degree of polymerisation (DP) approximately 30–200 (Bertoft, 2004a). The average internal chain length (ICL) between the branches in the clusters is known from only few examples to be approximately 4.2–5.4 (Bertoft, 1989; Zhu & Bertoft, 1996). Waxy rice possessed highly branched clusters as judged from the very low ICL of 2.4–3.0, and it was found that the branches were heterogeneously outspread within the ϕ,β-LD of the clusters (Bertoft, Zhu, Andtfolk, & Jungner, 1999). Units of small, branched building blocks were isolated from the rice amylopectin by an extensive hydrolysis with the α-amylase of *B. amyloliquefaciens* (Bertoft et al., 1999). The blocks were of DP 5–50 and contained at average 2–3 branches (Bertoft & Koch, 2000). A cluster was found to contain from 2.3 to 5.8 blocks and two structurally different types of clusters were distinguished on the basis of the size-distribution of their building blocks (Bertoft et al., 1999). Later, building blocks were analysed in two double mutants of maize (Gérard, Planchot, Colonna, & Bertoft, 2000). The blocks were of similar sizes as in rice, but with different size-distributions. Overall, *wxdu* maize possessed similar building blocks as waxy rice, but in *aewx* maize, which contained smaller clusters, the building blocks were also smaller.

In a recent study, the cluster structure of amylose-free potato starch (PAPS) was investigated in terms of the size and unit chain composition of the clusters and their mode of interconnection in the amylopectin macromolecule (Bertoft, 2007). By the use of a diluted solution of *B. amyloliquefaciens* α-amylase (0.03 U/mL) and a short hydrolysis time of 1 h, intermediate α-dextrins were produced and size-fractionated by precipitation in methanol. Large dextrins contained groups of 2–4 clusters (fractions named 2.I–4.I), whereas small dextrins were found to be more or less single clusters (fractions 5.I–6.2.I). All fractions were further treated with the enzyme a second (designated II) or third (III) time in order to isolate the single clusters from each size-fraction. Finally, each fraction was treated with phosphorylase and β-amylase to obtain the ϕ,β-LD (i.e., the internal structure of the dextrins). In this form the majority of the clusters possessed DP 50–75, but a minor group of clusters was of DP ~ 25 (sample 6.3.I). A single cluster contained between 3 and 11 chains depending on the size, and the degree of branching was estimated to between 7.6% and 14.1%. The unit chain composition of the clusters showed that long chains with CL > 35, which interconnect clusters (Hizukuri, 1986), had been hydrolysed by the α-amylase in order to release the clusters. A detailed description of the samples is found in Bertoft (2007).

In the present work, the same limit dextrin preparations obtained from PAPS (Bertoft, 2007) were further analysed with the aim to investigate the organisation of the unit chains within the clusters. The samples were therefore treated further with a more concentrated α-amylase solution to isolate small, branched building blocks. The blocks were then characterised with respect to their size and composition of chains.

2. Materials and methods

2.1. Samples and enzymes

Lyophilised samples of ϕ,β-LD produced from PAPS (Bertoft, 2004c) and α-dextrins at different stages of hydrolysis (Bertoft, 2007) were identical to those described previously. α-Amylase of *B. amyloliquefaciens* [(1 → 4)-α-D-glucan glucanohydrolase; EC 3.2.1.1], with an activity (Bertoft, Manelius, & Qin, 1993) of 600 U/mg, was purchased from Boehringer-Mannheim (now Roche), Germany. Pullulanase from *Klebsiella pneumoniae* (amylopectin 6-glucanohydrolase; EC 3.2.1.41), with an activity of 404 U/mL given by the supplier, was from Hayashibara Shoji Inc., Japan.

2.2. Characterisation of size-categories of building blocks

ϕ,β-LD of PAPS (20 mg) was dissolved in 90% dimethylsulphoxide (DMSO, 0.36 mL) on a boiling water bath and gently stirred at room temperature for 3 days. The sample was then diluted in hot water (1.44 mL) and traces of undissolved material (estimated to <1% of the dry weight) were removed by centrifugation at 1800*g* for 30 min. The ϕ,β-LD in the supernatant (1.2 mL) was then subjected to extensive α-amylolysis by the addition of a solution (0.133 mL) of α-amylase (30 U/mL) in 0.01 M NaOAc buffer, pH 6.5, to give a final enzyme concentration of 3 U/mL. The incubation took place at 25 °C for 5 h and was stopped by boiling for 10 min. The fresh mixture of building blocks (0.5 mL) was applied to a column (1 × 90 cm) of Superdex 30 (Pharmacia, Sweden), which was calibrated as described previously (Bertoft & Spoof, 1989), and eluted with water at 0.5 mL/min. The experiment was repeated once to obtain larger quantities of size-fractionated building blocks. Aliquots (50 μL) of collected fractions (2 × 0.5 mL) were analysed for carbohydrates with the phenol–sulphuric acid reagent (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The fractions were then pooled to give six different size-categories of building blocks. Each pooled fraction was divided into three parts and analysed as follows: (a) The sample was concentrated by rotary evaporation to 1 mg/mL and the molecular weight distribution was obtained by re-chromatography of an aliquot (0.2 mL) on the column of Superdex 30. (b) The composition of building blocks were analysed by HPAEC (after dilution or concentration to obtain suitable PAD response) as described below. (c) The carbohydrate concentration was adjusted to 0.15 mg/mL. To an aliquot (0.9 mL) were added 0.01 M NaOAc buffer, pH

5.5 (0.1 mL), and pullulanase (1 μL). After incubation overnight at room temperature, the debranched sample was boiled and analysed by HPAEC.

2.3. Analytical characterisation of the composition of building blocks in ϕ,β-LD of PAPS

ϕ,β-LD of PAPS (4 mg) was dissolved in hot DMSO (80 μL) and stirred 3 days before dilution in hot water (640 μL). A solution (80 μL) of α-amylase (60 U/mL) in 0.01 M NaOAc, pH 6.5, was then added to give a final concentration of 6 U/mL. The mixture was incubated at 35 °C for 3 h and then boiled. One part of the sample (80 μL) was diluted to 180 μL, treated with 5 M KOH (20 μL), and then applied to a column (1 × 90 cm) of Superdex 75 (Pharmacia, Sweden) that was calibrated with dextrins of known DP (Bertoft & Spoof, 1989). The sample was eluted at 0.5 mL/min with 0.01 M KOH. Fractions (0.5 mL) were collected and analysed for carbohydrates with the phenol–sulphuric acid reagent (Dubois et al., 1956). Another part of the building block mixture (20 μL) was diluted in water (475 μL) and 5 M NaOH (5 μL) and analysed by HPAEC as described below. A third part of the mixture (15 μL) was diluted in water (885 μL) and 0.01 M NaOAc buffer, pH 5.5 (100 μL), before addition of pullulanase (1 μL). After reaction overnight at room temperature, the mixture was boiled and analysed by HPAEC. Additional samples of building blocks from the ϕ,β-LD of PAPS were made similarly, but with higher concentrations of α-amylase (up to 12 U/mL), and analysed with HPAEC.

2.4. Characterisation of building blocks in ϕ,β-LD of dextrins obtained by α-amylolysis

ϕ,β-LDs (4 mg) of α-dextrin fractions containing single clusters or groups of clusters (Bertoft, 2007) were dissolved in hot water (720 μL). A solution of α-amylase (80 μL, 60 U/mL) in 0.01 M NaOAc buffer, pH 6.5, was added and incubated at 35 °C for 3 h. The sample was then boiled, lyophilised, re-dissolved in hot DMSO (0.2 mL), and finally stirred at room temperature overnight. One part of the sample (20 μL) was diluted in water (160 μL) and 5 M KOH (20 μL) and analysed on the column of Superdex 75 as described above. Another part (10 μL) was diluted in water (690 μL) and analysed with HPAEC, whereas a third part (15 μL) was debranched with pullulanase as described above.

2.5. High-performance anion-exchange chromatography (HPAEC)

The HPLC system (series 4500i, Dionex, USA) was equipped with a BioLC gradient pump and pulsed amperometric detection (PAD). The column (250 × 4 mm, CarboPac PA-100 combined with a guard column) was eluted at 1 mL/min with a mixture of eluent A (150 mM NaOH, 85%) and eluent B (150 mM NaOH containing 500 mM NaOAc, 15%) from 0 to 1 min, whereafter the sample (25 μL) was injected. A gradient was made by increasing the proportion of eluent B as follows: 1–16 min from 15% to 34%; 16–27 min from 34% to 40%; 27–53 min from 40% to 49%; 53–55 min from 49% to 100%; and 59% to 61 min from 100% to 15% (return to the start mixture). The PAD response was corrected to quantitative values by the method of Koch, Andersson, and Åman (1998). The isolated size-categories of building blocks (above) were used to correct the response for branched dextrins, and it was found that the same correction factors as for linear dextrins were valid.

3. Results

3.1. Characterisation of size-categories of building blocks

The ϕ,β-LD of amylose-free potato starch (PAPS) was identical to the sample used for preparation of clusters (Bertoft, 2007). In a semi-preparative scale, building blocks were prepared by treating the sample with a solution of the α-amylase of *B. amyloliquefaciens* (3 U/mL) for 5 h. The size-distribution obtained by gel-permeation chromatography (GPC) on Superdex 30, using water as the elution medium, is shown in Fig. 1. The sample was extensively degraded into dextrins with DP up to the order of 80. The average DP was only 5.7, however, because a majority of the dextrins constituted a group with a peak at DP 6–7. Another, minor peak at DP 45 was an artefact, as discussed below.

Fractions were pooled into groups I–VI, as indicated in Fig. 1, to represent different size-categories of building blocks of PAPS (Table 1). The pooled fractions were then analysed again by GPC. In Fig. 1, the distribution of dextrins is shown when drawn on the basis of the yield of each fraction, together with a reconstruction of the building

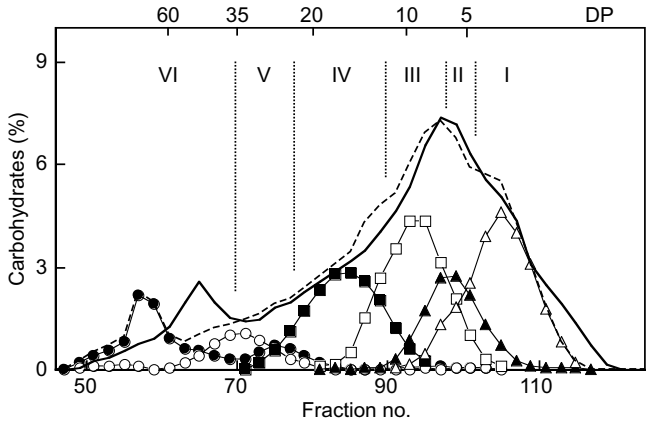

Fig. 1. Gel-permeation chromatography on Superdex 30 of the hydrolysate obtained from ϕ,β-LD of PAPS by treatment of α-amylase (3 U/mL) for 5 h (————). The column was eluted with water. Fractions were pooled as indicated and re-chromatographed as I (△), II (▲), III (□), IV (■), V (○), and VI (●). A reconstruction of the hydrolysate was made on the basis of fractions I–VI (--------).

Table 1
Characterisation of size-categories of building blocks from ϕ,β-LD of PAPS

Fraction[a]	GPC			HPAEC							
	Yield (%)	Collected DP range[b]	DP[c]	DP[d]	Linear[e]		Branched[f]				
					Mole (%)	DP	DP	CL	ICL[g]	NC[h]	DB[i]
I	24.4	1–4	2.8	2.8	96.0	2.7	6.4	3.2	1.5	2.0	17.8
II	13.5	5–6	5.2	6.1	55.7	5.3	7.1	3.7	2.5	1.9	15.2
III	24.0	7–11	8.4	9.0	10.1	5.9	9.4	4.1	2.7	2.3	13.1
IV	18.5	12–22	15.2	16.2	1.6	2.8	16.4	4.6	2.5	3.6	15.8
V[j]	6.7	23–35	27.8	22.4	5.8	2.5	23.6	5.0	2.8	4.7	15.7
VI[k]	12.4	>35	40.3	–	–	4.7	–	7.1	–	–	–

[a] Fractions collected by preparative GPC on Superdex 30.
[b] Estimated DP range of pooled fractions.
[c] DP obtained by re-chromatography on Superdex 30 of pooled fractions.
[d] DP obtained by HPAEC of pooled fractions.
[e] Linear building blocks of DP 1–6.
[f] Branched building blocks of DP ⩾ 5.
[g] Average internal chain length = (CL − ECL) × NC/(NC − 1) − 1, in which ECL is estimated to be 2 in the building blocks.
[h] Number of chains = DP/CL.
[i] Density of branches = (NC − 1)/DP × 100.
[j] The average DP of dextrins at DP > 19 was estimated from GPC.
[k] The fraction contained aggregates of building blocks (see Section 3).

block profile of the ϕ,β-LD of PAPS. Samples I–IV possessed size-distributions as expected, but samples V–VI possessed distributions at higher DP than originally collected. Especially in sample VI, the peak at DP 45 shifted position to ~80 and, as a consequence, the reconstructed curve of PAPS did not correspond to the original chromatogram. In addition sample VI possessed a minor group between DP 20 and 35. When samples of building blocks from PAPS were allowed to stand longer times (over night) at room temperature before chromatography, the peak at DP 45 also shifted position (not shown), which suggested that parts of the building blocks tended to aggregate, despite their small size.

Samples I–VI were analysed in more detail by HPAEC (Fig. 2). Sample I contained almost only linear dextrins, mostly at DP 1–3 and smaller amounts at DP 4–6. The average DP was 2.8, which corresponded to that estimated by GPC (Table 1). Sample II contained, in addition to linear dextrins at DP 4–6, branched dextrins at DP 5–9 known to elute in front of the linear dextrins of corresponding DP (Ammeraal, Delgado, Tenbarge, & Friedman, 1991). A single peak, suggesting that they all contained a single branch, represented each size of branched dextrin. The average DP of sample II was 6.1 and was somewhat higher than estimated by GPC (5.2). When by HPAEC a separation of the linear and branched dextrins was achieved, it was possible to estimate the average DP of the two types to 5.3 and 7.1, respectively (Table 1). Sample II was further debranched with pullulanase to give a series of linear dextrins from DP 2 to 6 and traces at DP 7 (Fig. 2). Because the amounts of the original linear dextrins in the sample were known, they were subtracted from the chains of the debranched sample to obtain the unit chains of the branched dextrins. From the values obtained, the average chain length (CL) of the branched dextrins was estimated to 3.7, and the average number of chains (NC = DP/CL) was 1.9, which confirmed the singly branched nature (Table 1). The branching density was 15.2%. The external chain length (ECL) of the dextrins was not exactly known and might vary somewhat. However, from the work of Umeki and Yamamoto (1972a, 1972b) describing the attack of α-amylases at the vicinity of a branch point, it can be estimated that ECL was close to 2. With this assumption, the average internal chain length (ICL, which in a singly branched dextrin corresponds to the length of the segment from the branch to the reducing end) was estimated to 2.5.

Sample III contained mostly (90%) branched dextrins from DP 6–13 (Fig. 2). Single peaks, with the exception of DP 10, of which there were two peaks, represented all sizes. Because NC was slightly higher than 2 (Table 1), the dextrins at DP 11–13 were probably doubly branched, whereas dextrins of DP 10 were found as both doubly and singly branched types. In sample IV branched dextrins up to DP 19 were distinguished as peaks. From DP 14 there were two or three peaks at each DP, making the estimation of the correct DP-value for each peak somewhat hazardous. At DP > 19 the number of dextrin types at each DP-value was apparently even higher and the NC-value was 3.6. The average DP of the dextrins at DP > 19 was estimated from GPC (Fig. 1), and used to correct the PAD-response in the HPAE chromatogram in this area to obtain an average value for the whole sample analysed by HPAEC. Despite the somewhat uncertain calculations, the average DP estimate (16.2) was in satisfying agreement with that (15.2) from GPC (Table 1). Sample V contained mostly dextrins at DP > 19, as expected from the GPC-result, but in addition there were sharp peaks irregularly outspread at lower DP. The same result was obtained for sample VI and it seemed that the aggregated material, that

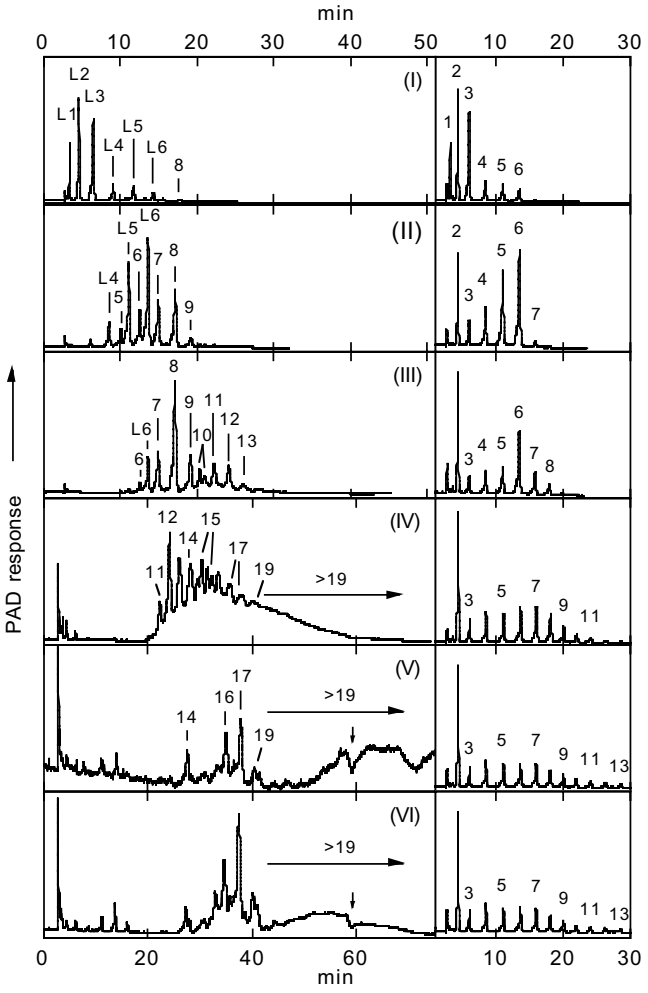

Fig. 2. Distribution of dextrins in size-fractions of building blocks (I–VI) obtained by HPAEC-PAD before (left) and after debranching (right). Numbers indicate DP and in the chromatograms to the left linear dextrins are denoted "L". Note the different time-scale in I–III compared to IV–VI. (Small arrows in V and VI show a distortion in the baseline.)

these samples apparently contained, to some extent was dissolved in the rather harsh conditions in HPAEC.

Samples III–VI were also debranched and the chain profiles are shown in Fig. 2. In larger dextrins a slight increase of somewhat longer chains were found up to DP ~ 13. The average CL increased therefore from 4.1 to 7.1 (Table 1). The ICL-value remained, however, more or less constant at 2.5–2.8. The NC-value increased in direct proportion to DP up to 4.7 in sample V, whereas the degree of branching only slightly increased from 13.1% to 15.7%.

3.2. Composition of building blocks in ϕ,β-LD of PAPS

Because the building blocks tended to form aggregates as a function of time, the protocol for the further experiments in analytical scale was changed in order to minimize their formation. Thus, the α-amylase concentration was increased to the double (6 U/mL), the incubation time shortened to 3 h, and the elution medium for GPC changed to 0.01 M KOH. At this pH (12) the conformation of the dextrins is more extended than in pure water and a column of Superdex 75 was therefore used instead of Superdex 30. In Fig. 3 the composition of dextrins obtained from the ϕ, β-LD of PAPS is shown. The resolution of the chromatogram was higher than in Fig. 1 and indicated four major groups of dextrins (designated 1, 2, 3, and 5) with peak positions at DP ~ 3, 6–7, 11–12, and 20–25, respectively. The latter peak apparently replaced the peak of aggregated dextrins at DP 45 in Fig. 1, of which there was no indication left.

A higher resolution of the dextrins up to DP 19 was obtained by HPAEC (Fig. 4a). The chromatogram possessed large peaks for glucose to maltotriose corresponding to group 1 in Fig. 3. Group 2 was resolved as peaks for maltotetraose to maltohexaose and singly branched dextrins from DP 5 to 8. The rest of the chromatogram possessed comparatively small peaks, partly because of smaller amounts, but also because of the fact that the PAD response decreases for larger dextrins. A group of peaks at DP 9–13 corresponded to group 3 obtained by GPC. As discussed above, there was two peaks at DP 10, which suggested that both singly and doubly branched dextrins existed of this size. The peaks resolved at DP 14–19 were assigned group 4. This group was not distinguished by GPC. The peaks were very small and their exact DP-values could only be roughly estimated. At DP > 19 the PAD response was very low and no peaks were resolved that corresponded to peak 5 at DP 20–25 obtained by GPC. Dextrins remaining in the column were finally washed out by a sharp increase of the sodium acetate gradient and were thereby detected as an apparent peak at the end of the chromatogram.

The dextrins obtained by the concentration of enzyme used (6 U/mL) were not α-limit dextrins. When the substrate was treated with a two times more concentrated

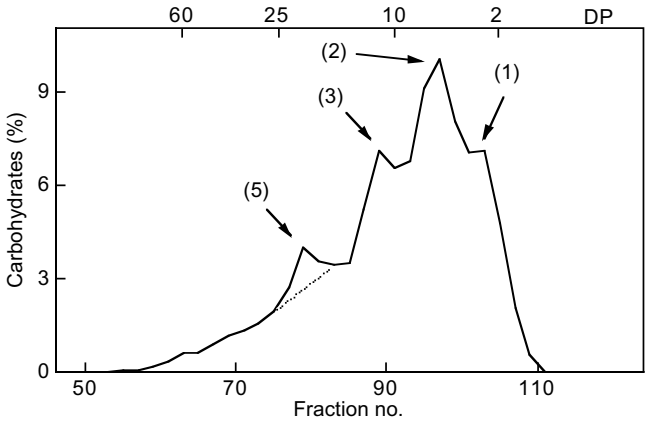

Fig. 3. Gel-permeation chromatography on Superdex 75 of the hydrolysate obtained from ϕ,β-LD of PAPS by treatment of α-amylase (6 U/mL) for 3 h. The column was eluted with 0.01 M KOH. Groups of building blocks are indicated. Group 5 possibly contained aggregated dextrins as indicated by the dotted line.

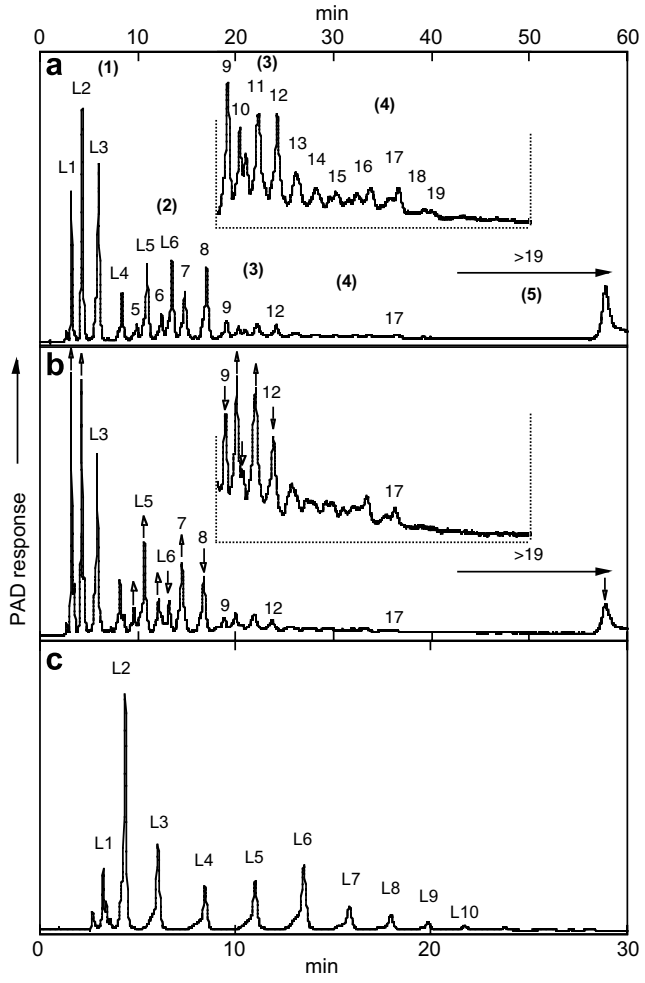

Fig. 4. Distribution by HPAEC-PAD of building blocks in PAPS obtained by treatment with (a) 6 U/mL and (b) 12 U/mL of α-amylase for 3 h, and (c) the debranched sample from (a). Insets in (a) and (b) are magnifications of the chromatograms from 18–50 min. Numbers indicate DP and linear dextrins are denoted "L". In (a) bold numbers in parenthesis show different groups of building blocks comparable with those in Fig. 3. In (b) small arrows indicate increase or decrease of peak size compared to the peaks in (a). Note the different time-scale in (c).

enzyme solution it was possible to detect small changes that showed that a further, extremely slow degradation of the branched dextrins continued (Fig. 4b). Of the linear dextrins, maltohexaose was hydrolysed, whereas maltopentaose, maltose, and glucose were formed. This result was in agreement with the results of Robyt and French (1963) who showed that, in comparison to maltoheptaose, maltohexaose is only slowly degraded into preferentially maltopentaose and glucose. Thus, the building blocks under study could be characterised as near-limit dextrins, rather than absolute limit dextrins.

The mixture of building blocks from PAPS (in Fig. 4a) were also debranched and analysed by HPAEC (Fig. 4c). The amount of maltose increased very much compared to maltotriose. Maltohexaose predominated among the longer chains. Only trace amounts (less than one mole%) represented chains of DP $\geqslant$ 10.

3.3. Composition of building blocks in clusters

Groups of clusters from PAPS, and the clusters from each of the groups, were isolated in a previous work (Bertoft, 2007). From the ϕ,β-LD of each sample, building blocks were produced and analysed by GPC on Superdex 75 (Fig. 5). The same traits as described above for PAPS in Fig. 3 were found. Each of the samples also possessed the characteristic peak of group 5, which in most cases resembled a distortion in the otherwise rather smooth part of the chromatogram as indicated by dotted lines (Fig. 5). This distortion tended to increase with decreasing size of the clusters from 1.5 wt% in sample 2.III to 6.2% in 6.3.I. In PAPS the distortion represented ~2% (Fig. 3). All samples were also analysed by HPAEC (chromatograms not shown). The general trends and all peaks that was described for PAPS (Fig. 4a) were also found in each sample, though in slightly variable amounts. In each case also, there was no indication of peaks (or elevated amounts of dextrins) at DP 20–25 that would correspond to the peak of group 5 in GPC. It was therefore possible that the apparent distortion in the chromatograms in Fig. 5 was due to aggregated dextrins that remained in the samples despite the efforts to inhibit their formation. The GPC was performed at pH 12, whereas HPAEC at pH > 13, which might have been enough to dissolve possible aggregates completely.

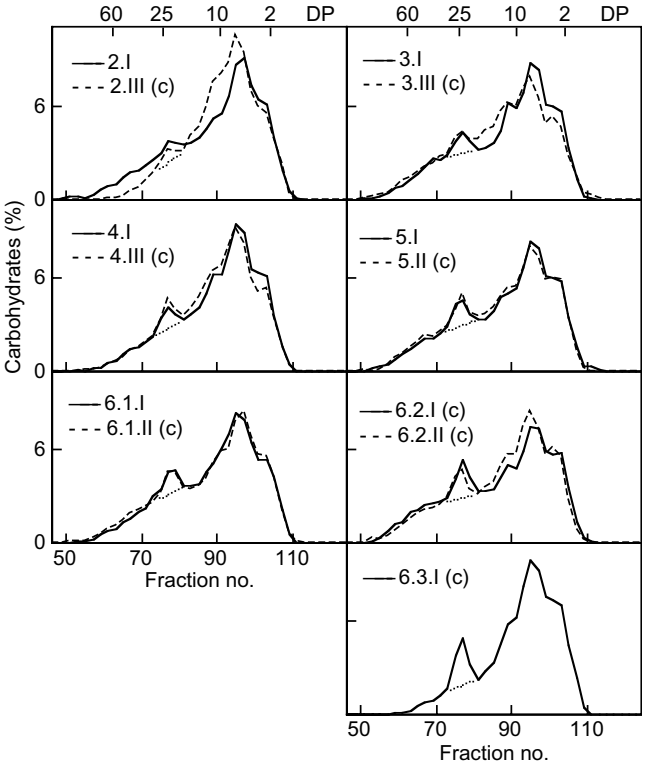

Fig. 5. Gel-permeation chromatography on Superdex 75 of the building blocks obtained from samples 2–6.3. Samples 2.I–4.I consisted of groups of clusters. Samples marked (c) contained free clusters. Small, dotted lines indicate peaks of possibly aggregated dextrins at DP 20–25.

In Table 2 the amount of dextrins with DP ⩾ 20 (i.e., building blocks of group 5), as represented by the area under the curves in Fig. 5, is given for all samples. The amount ranged from 13% in 2.III to 32% in 6.2.I. The average DP of this material ranged from 27 to 33. As discussed above, the PAD-response in HPAEC decreases with the DP of the dextrins and must be corrected in order to achieve a quantitative estimation (Koch et al., 1998). The DP-values estimated by GPC for the dextrins of DP ⩾ 20 were therefore used to correct the PAD-response of the HPAE-chromatograms in the corresponding area. As shown in Table 2 the corrected relative weight in HPAEC was generally lower than suggested by GPC (with the exception of sample 2.I). At average GPC suggested an amount of 24%, whereas HPAEC only showed 18%. However, when the areas corresponding to dextrins of both groups 4 and 5 (i.e., DP ⩾ 14) were compared, the values from GPC and HPAEC largely agreed. The average values were 31% with both methods. Thus, this suggested that aggregates of DP ~ 25 detected by GPC were dissolved, at least partly, into dextrins of DP 14–19 when analysed by HPAEC. As shown in Fig. 2, peaks in this area were obtained in the chromatogram of fraction VI, which was rich in aggregates. Results of the analyses of the structure of building blocks by HPAEC are presented in Table 3. The average DP of the whole α-amylolysis mixture of PAPS was 5.5 and was slightly lower than in the majority of the fractions isolated from PAPS. On a molar basis, two out of three molecules were linear with DP ⩽ 6, whereas one third was branched with an average DP of 10.8. In all the other samples the molar proportion of linear dextrins was lower. With the exception of samples 2.III and 6.3.I, the DP of the branched blocks was slightly higher than in PAPS in all fractions. All the other data were very similar in all the samples. Thus, CL of the branched building blocks was 3.7–4.2, ICL 1.7–2.6, average number of chains per block (NC) 2.6–3.3, and the degree of branching (DB) was in the order of 14.9–17.1%. These average values suggested that all samples contained rather similar compositions of building blocks possessing a common type of internal structure.

Table 2
Comparison of the amounts of building blocks of groups 4 and 5 obtained by GPC and HPAEC[a]

Sample[b]	DP	wt%	wt%	wt%	wt%
	Group 5 GPC[c]	Group 5 GPC	Group 5 HPAEC	Group 4 + 5 GPC	Group 4 + 5 HPAEC
PAPS	28.8	15	13	22	23
2.I	31.9	23	24	30	36
2.II	30.2	20	16	27	30
2.III (c)	27.0	13	10	20	22
3.I	32.0	27	20	33	32
3.II	32.1	23	19	30	32
3.III (c)	32.3	28	20	36	32
4.I	29.9	21	13	28	27
4.II	30.2	23	16	30	31
4.III (c)	29.3	22	10	29	25
5.I	32.2	28	22	35	36
5.II (c)	32.1	29	23	36	36
6.1.I	30.4	26	14	33	28
6.1.II (c)	31.4	26	21	36	36
6.1.III (c)	31.7	31	28	38	43
6.2.I (c)	33.0	32	24	39	37
6.2.II (c)	32.7	29	20	35	31
6.3.I (c)	27.7	18	7	23	24
Average	–	24	18	31	31

[a] Group 4 corresponds to dextrins with DP 14–19, group 5 to dextrins of DP ⩾ 20. The ranges estimated in GPC are not exact because each fraction represented itself a certain range of DP-values.
[b] (c) indicates fraction containing clusters and roman numbers indicate successive stages of α-amylolysis (from Bertoft, 2007).
[c] Average DP of group 5 estimated by GPC and used for correction of PAD-response in HPAEC.

Table 4 summarizes the composition of clusters and building blocks in the fractions isolated from PAPS. The average DP and the peak-DP of the fractions, as well as the number of clusters in the dextrins, were analysed previously (Bertoft, 2007). From the molar amount of building blocks contained in the samples and the number of dextrins that they contained originally, which was known (Bertoft, 2007), the number of branched blocks in the dextrins was calculated. The number decreased with the size of the dextrins, and single clusters were composed of 2.1–3.7 building blocks. In analogy to the density of branches, a value characterising the density of building blocks in the dextrins was obtained as

$$(\text{density of blocks}) = (\text{number of building blocks}) / (\text{DP of the fraction}) \times 100. \quad (1)$$

The density of blocks increased when the clusters were produced from the groups of clusters (series 2, 3, and 4) from roughly 6 to 7.

The linear dextrins were fragments derived from the segments between building blocks. Because every branched building block is connected to another block through a linear segment, the number of such segments equals the number of building blocks. It was therefore possible to estimate the average DP of the inter-block segments as

$$\text{IB} - \text{DP} = (\text{mole\% linear dextrins}) \times (\text{DP of linear dextrins}) / (\text{mole\% branched blocks}). \quad (2)$$

IB-DP ranged from 2.9 to 4.9, and in all series the value was lower in the fractions representing clusters than in those being groups of clusters. The difference was, however, not large and the clusters of series 2–4 possessed lower IB-DP than clusters of the other series (Table 4).

4. Discussion

The small dextrins that were analysed in this work represented units of building blocks from which the clusters of PAPS were built up. The size-range of the blocks was comparable with that found for the amylopectin from waxy rice starch (Bertoft et al., 1999). However, in rice the

Table 3
Characterisation of the structure of building blocks in PAPS and fractions containing clusters and groups of clusters

Sample[a]	Whole[b]	Linear[c]		Branched[d]					
	DP	Mole (%)	DP	Mole (%)	DP	CL[e]	ICL[f]	NC[g]	DB[h]
PAPS	5.5	67.6	3.0	32.4	10.8	4.0	2.2	2.7	15.7
2.I	7.0	59.4	3.0	40.6	12.7	4.1	2.1	3.1	16.5
2.II	6.3	59.2	2.9	40.8	11.4	4.1	2.2	2.8	15.8
2.III (c)	5.7	57.1	2.5	42.9	9.9	3.8	2.0	2.6	16.2
3.I	6.6	61.4	3.1	38.6	12.2	4.2	2.4	2.9	15.6
3.II	6.5	59.9	2.9	40.1	11.8	4.1	2.3	2.9	16.1
3.III (c)	6.7	54.5	2.6	45.5	11.6	4.0	2.0	2.9	16.4
4.I	6.1	60.8	2.9	39.2	11.0	4.0	2.1	2.8	16.4
4.II	6.4	58.4	2.8	41.6	11.4	4.2	2.6	2.7	14.9
4.III (c)	6.0	54.3	2.4	45.7	11.3	3.7	1.7	2.8	15.9
5.I	6.8	60.4	3.0	39.4	12.7	4.1	2.1	3.1	16.5
5.II (c)	6.5	62.9	2.9	37.1	12.6	4.1	2.2	3.1	16.7
6.1.I	5.5	65.4	2.5	34.6	11.1	3.9	2.0	2.8	16.2
6.1.II (c)	6.6	60.5	2.8	39.5	12.3	4.0	2.0	3.1	17.1
6.1.III (c)	7.4	57.5	2.9	42.5	13.5	4.1	2.0	3.3	17.0
6.2.I (c)	7.1	59.7	3.1	40.3	13.2	4.1	2.1	3.2	16.7
6.2.II (c)	6.1	63.6	2.8	36.4	11.8	4.0	2.1	2.9	16.1
6.3.I (c)	5.1	66.1	2.5	33.9	10.3	3.7	1.7	2.7	16.5

[a] (c) indicates fraction containing clusters and roman numbers indicate successive stages of α-amylolysis (from Bertoft, 2007).
[b] Average values for the whole sample.
[c] Linear dextrins of DP 1–6.
[d] Branched building blocks of DP ⩾ 5.
[e] Chain length obtained by debranching and subtraction of linear dextrins.
[f] Average internal chain length = (CL − ECL) × NC/(NC − 1) − 1, in which ECL is estimated to be 2 in the building blocks.
[g] Number of chains = DP/CL.
[h] Density of branches = (NC − 1)/DP × 100.

Table 4
Composition of building blocks in fractions isolated from PAPS

Fraction[a]	DP[b]	Peak-DP[b]	Number of clusters[c]	Number of blocks[d]	Density of blocks[e]	IB-DP[f]
2.I	246	380	3.9	14.4	5.8	4.4
2.II	99	170	1.9	6.3	6.3	4.2
2.III (c)	51	70	1	3.7	7.3	3.3
3.I	118	217	3.4	6.9	5.9	4.9
3.II	84	130	2.3	5.2	6.2	4.3
3.III (c)	40	50	1	2.7	6.7	3.2
4.I	87	135	1.9	5.6	6.4	4.5
4.II	59	72	1.1	3.9	6.6	3.9
4.III (c)	42	54	1	3.2	7.7	2.9
5.I	64	72	1.3	3.7	5.7	4.6
5.II (c)	46	54	1	2.7	5.8	4.8
6.1.I	78	115	1.3	4.9	6.3	4.8
6.1.II (c)	55	75	1	3.3	6.0	4.3
6.1.III (c)	53	70	1	3.0	5.7	3.9
6.2.I (c)	54	55	1	3.1	5.7	4.5
6.2.II (c)	48	55	1	2.9	5.9	4.9
6.3.I (c)	31	25	1	2.1	6.7	4.9

[a] (c) indicates fraction containing clusters and roman numbers indicate successive stages of α-amylolysis (from Bertoft, 2007).
[b] Obtained by chromatography on Sepharose CL 6B (from Bertoft, 2007).
[c] Estimated average number of clusters found per dextrin molecule (from Bertoft, 2007).
[d] Average number of branched building blocks in the dextrins.
[e] (Number of blocks)/(DP of fraction) × 100.
[f] Inter-block DP = (mole% linear dextrins) × (DP of linear dextrins)/(mole% of branched blocks).

proportion of large blocks with DP > 30 was considerably higher than in PAPS. Another major difference was the heterogeneous composition of building blocks in waxy rice, which indicated domains of clearly different structural composition, whereas PAPS apparently was homogeneous as shown by the similar composition of building blocks throughout all isolated fractions. The structural characterisation of the size-fractionated building blocks from PAPS

(Table 1 and Figs. 1 and 2) should therefore be generally applicable on all the fractions subsequently analysed.

Each size-fraction of building blocks was a complex mixture of dextrins. By HPAEC it was possible to distinguish linear dextrins from branched dextrins (Fig. 2). The smaller sized dextrins of the latter type were singly branched and eluted as single peaks. The smallest branched dextrin produced by the α-amylase of *B. amyloliquefaciens* has DP 5 and is 6^2-α-maltosylmaltotriose (French, Smith, & Whelan, 1972). Dextrins at DP 6–8 were also singly branched, but it is known that a single peak in fact hides several types of singly branched molecular species (Jodelet, Rigby, & Colquhoun, 1998). Umeki and Yamamoto (1972a) showed that dextrins at DP 6 obtained by extensive hydrolysis of waxy rice starch consisted of 42% 6^3-α-maltosylmaltotetraose, 15% 6^2-α-maltosylmaltotetraose, and 42% 6^2-α-maltotriosylmaltotriose. A similar result was reported by Jodelet et al. (1998) who used the α-amylase of *B. licheniformis* and corn amylopectin as the substrate. Therefore, it is clear that the larger branched dextrins were even more complicated mixtures and only average values were possible to present in this work. Nevertheless, these average estimations gave a good characterisation of the structural nature of the building blocks. Thus, the blocks possessed from 2 to 5 chains and the average CL tended to slightly increase with the number of chains. However, the internal structure of the blocks was indifferent, as indicated by the similar ICL value and branching density (Table 1).

As already discussed above, a part of the dextrins that by GPC eluted at DP > 20 tended to form aggregates. The structural nature that could describe this unexpected behaviour remained uncertain. From the values presented in Table 2, it appears that this material was at least partly dissolved during HPAEC into the dextrins detected as group 4 at DP 14–19. All fractions possessed aggregates. Though it was not possible to accurately quantify the aggregates, the amount seemed to increase in fractions representing dextrins of smaller sizes (samples 5–6 in Fig. 5). These fractions (especially sample 6.1–6.3) were found to be difficult to size-fractionate by methanol and possibly contained a minor component of different structure (Bertoft, 2007). The major part of the building blocks with DP > 20 (group 5) seemed, however, to behave ''normally'' in GPC, and the overall characterisation of blocks of this size was most probably correct (Table 1).

The overview of the structure of the building blocks in the different fractions of groups of clusters and free clusters suggested only small apparent differences (Table 3). The average DP of the branched blocks tended to be slightly larger in clusters isolated from samples 5 to 6.2 (12–13) in comparison with those from samples 2 to 4 (10–11). However, other structural parameters (CL, ICL, NC, and DB) were similar in all samples, including sample 6.3.I that contained clusters of very small size (DP ~ 25). Besides a common structural feature of the building blocks, this also reflected the action pattern of the α-amylase towards the α-(1 → 4)-linkages between the blocks inside the clusters.

It was possible to estimate the average number of blocks contained in the clusters of PAPS to between 2.7 and 3.7 (Table 4). In the smallest cluster type (sample 6.3.I) the number was only 2.1. Because there were four groups of branched blocks identified (groups 2–5, Fig. 4a), it is clear that an individual cluster was generally built up from building blocks of only some of these groups. The estimated density of the building blocks was lower in fractions containing groups of clusters than in single clusters. This was because the inter-cluster regions contained only little branches (Bertoft, 2007).

A closer look suggested that the samples could be divided into groups on the basis of the density of the building blocks in the clusters (Table 4). Thus, the density of blocks was somewhat higher in the clusters of samples 2–4 (6.7–7.7) compared to the clusters in samples 5–6.2 (5.7–6.0). This was reflected in smaller inter-block segments in the former group. The very small clusters in sample 6.3.I possessed a high density of blocks similar to samples 2–4, but IB-DP was similar to the samples with low block density. The structure of the small clusters was therefore not comparable to the others. On this basis the clusters of PAPS were divided into three different types (A–C) with the average characteristic composition of blocks shown in Table 5. Peak-DP was the peak position obtained by

Table 5
Composition of building blocks in different types of clusters in PAPS

Type of cluster[a]	Peak-DP range[b]	Number of blocks[c]	Density of blocks[d]	IB-DP[e]	Block DP[f]	Molar distribution of groups of blocks (%)[g]			
						2	3	4	5
A	50–70	3.4–5.1	7.2	3.1	10.9	52	31	11	6
B	55–75	3.1–4.1	5.8	4.5	12.7	44	31	15	10
C	25	1.7	6.7	4.9	10.3	53	27	16	4

[a] The type of cluster is primarily based on the density of branched building blocks: Type A is represented by samples 2.III, 3.III, and 4.III, type B by 5.II, 6.1.II, and 6.2.I, and type C by sample 6.3.I.
[b] DP was obtained by chromatography on Sepharose CL 6B (from Bertoft, 2007).
[c] Estimated number at peak-DP.
[d] (Number of blocks)/(DP of fraction) × 100.
[e] Inter-block DP = (mole% linear blocks) × (DP of linear blocks)/(mole% of branched blocks).
[f] Average DP of the the branched building blocks obtained by HPAEC.
[g] The DP-range of group 2 = 5–8, group 3 = 9–13, group 4 = 14–19, and group 5 = ⩾ 20.

GPC in the previous investigation and represented the sizes of the majority of the typical clusters of PAPS (Bertoft, 2007). Whereas the density of blocks was not related to any specific size-category of the clusters, the average size of the blocks was higher (DP 12.7) in the clusters with low block density compared to those with high density (DP 10.9). The smallest clusters of DP 25 possessed the smallest building blocks (DP 10.3) and the average number of blocks was only 1.7 at peak-DP.

The size-distributions of the branched building blocks in the three types of clusters, based on the average values of the individual samples obtained by HPAEC-PAD, are compared in Fig. 6. For clarity, all peaks of identical DP were collected into a single peak. On a weight basis, all samples possessed nearly equal amounts of dextrins at DP 8, which was the most common of all building blocks, and at DP 9. In all other cases there were clear differences in the compostions. Thus, each structural type of cluster possessed a characteristic average distribution of blocks, which is summarised in Table 5.

Besides the general composition of the groups of building blocks in the different types of clusters (Table 5), an important question is how the building blocks are organised within the clusters. As clusters are formed from the short chains of amylopectin, it is of highest interest to understand in what way these chains are related to the structures of the building blocks. The short, clustered chains of amylopectin are sub-divided into A- and B1-chains (Hizukuri, 1986), defined as not carrying other chains and carrying other chains, respectively (Peat, Whelan, & Thomas, 1952). B-chains were further sub-divided into very short "fingerprint" B-chains (B_{fp}), also named short B1a(s)-chains, that in the ϕ,β-LD of PAPS have CL 3–7, long B1a(l)-chains (CL 8–17), and B1b-chains (CL 18–24) (Bertoft, 2004c). In the building blocks only fragments of these original chain categories remained.

On the basis of the HPAEC-PAD results (Fig. 2) the molar distributions of branched building blocks in fractions I–IV of the size-fractionated dextrins are presented as block diagrams in Fig. 7. Reconstructions of the quantitative unit chain distributions of the branched blocks are also shown. The amount of chains at DP 2 was in all samples approximately 40 mole%. In the original ϕ,β-LD of PAPS all A-chains were in the form of maltosyl stubs, whereas the B-chains were longer (Bertoft, 2004c). Thus, all of the original A-chains were found in the peak of maltose. However, when the α-amylase released building blocks from the interior of the clusters, new A-chains were possibly formed by cleavage of the B-chains between the blocks. As shown in Fig. 8, new A-chains are formed when building blocks are inter-connected through a Haworth-type of structure (Haworth, Hirst, & Isherwood, 1937). The DP of the new A-chain stub produced by the α-amylase is known to be 2 or 3 in limit dextrins (Umeki & Yamamoto, 1972a). One can expect that this mainly also was the case with the near-limit dextrins analysed in this investigation and, thus, approximately one half of the new A-chains were involved in the maltose peak, and the other half was found in the maltotriose peak. Another mode of building block interconnection is through the Staudinger-type of structure (Fig. 8) (Staudinger & Husemann, 1937). The fission of the inter-block chain will in this case result in a new short B-chain. The length of the B-chain varies much depending on the type of building block and maltotriose is the shortest possible length. Some new B-chains were therefore also involved in the peak for maltotriose.

As shown above, the building blocks in fraction I were singly branched. Each molecule was therefore composed of one A- and one B-chain. Because A-chains in the form of maltose constituted ~41% in fraction I (Fig. 7), ~9% of the A-chains were maltotriose and theoretically they represented one half of the Haworth-type of building

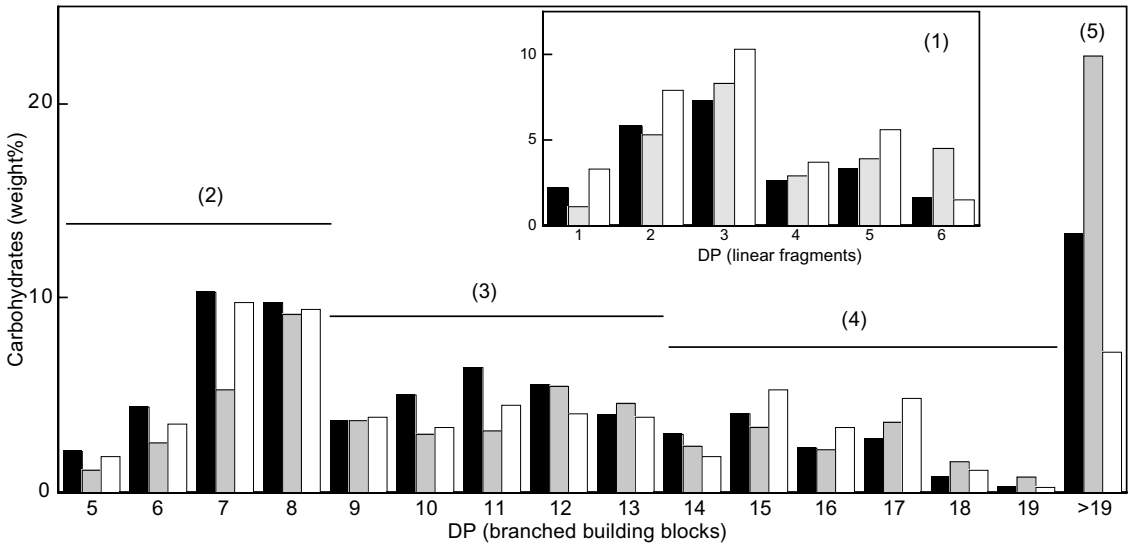

Fig. 6. Average size-distribution of branched building blocks in clusters of type A (black bars), B (grey bars), and C (white bars). Inset shows the average distribution of linear dextrins produced by α-amylase attack at inter-block chain segments. Numbers in parentheses are groups of blocks.

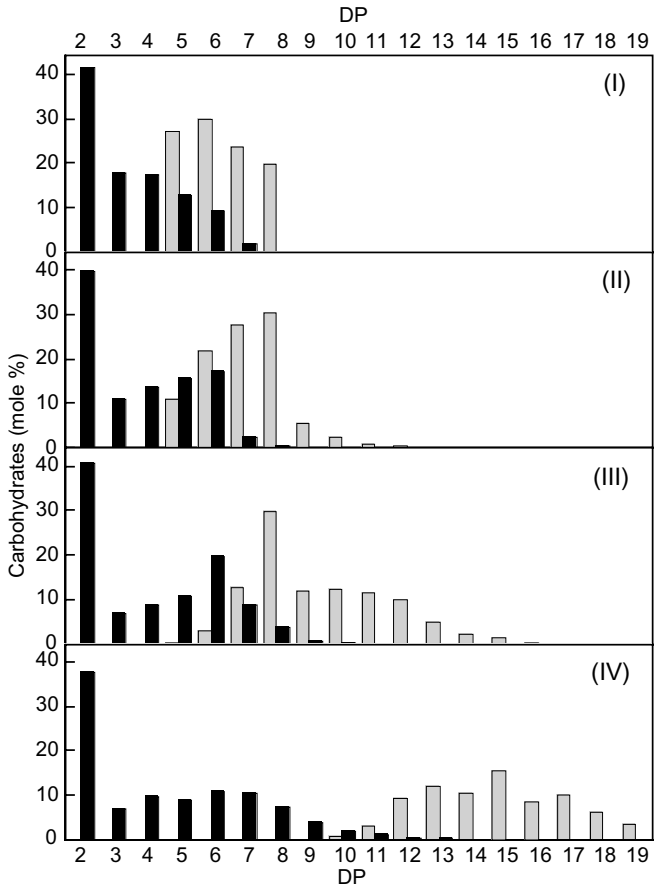

Fig. 7. Molar distribution of branched building blocks (grey bars) and their unit chain distribution (black bars) reconstructed from HPAEC-PAD of size-fractions I–IV.

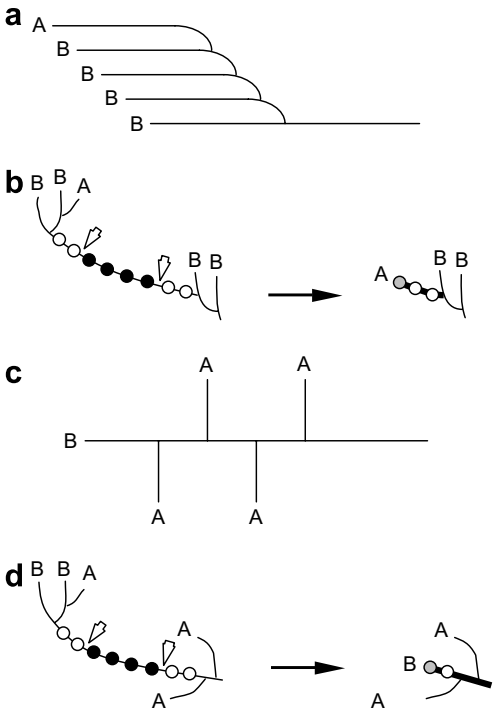

Fig. 8. The principles of interconnection of two building blocks inside clusters. (a) The Haworth-type of interconnection of unit chains and (b) two building blocks interconnected by the Haworth-type of structure. (c) The Staudinger-type of interconnection of unit chains and (d) two building blocks interconnected by the Staudinger-type of structure. Lines symbolises the chains of amylopectin and letters denote the types of chains (A or B). Black circles are residues that are removed by the attack of α-amylase (open arrows) and show the part included in the value of IB-DP. Open circles are residues not removed but participating in the inter-block segment. The structures to the right show the released blocks with internal Haworth (b) and Staudinger-type of branching (d), and in which the actual length of the new chain stubs slightly varies (grey circles).

block interconnections (Fig. 8). The rest of the maltotriose (~9 mole%) was B-chains and, when it was known that the original amount of such short B-chains in the ϕ,β-LD of the entire PAPS was only 1.68% (Bertoft, 2004c), a major part was formed by α-amylolysis at Staudinger-type interconnections. Because fraction II also contained singly branched dextrins, a similar discussion as for fraction I could me made. In this case a majority of the branched dextrins were of DP 7–8 and the unit chain distribution possessed a maximum at CL 6, showing that the B-chains were generally longer than in fraction I. The amount of chains as maltose and maltotriose was together only slightly more than 50%, suggesting that only very few, if any, B-chains were found as maltotriose in these blocks. For the larger dextrins in fractions III and IV the number of branches was more than one and the structures more complicated. In fraction III chains of CL 6 predominated, but in fraction IV (mainly containing blocks of group 4), in which the number of chains was 3.6, chains from CL 3 to 8 were found in rather similar amounts. The amount of maltose and maltotriose tended to slightly decrease to <50%, which showed that the ratio of A:B-chains was <1:1. This suggested that larger building blocks proportionally possessed more of the Haworth-type of structures than Staudinger-type. (In a pure Haworth structure the ratio of A:B-chains approaches zero, whereas in the Staudinger structure it approaches infinity, Fig. 8) On the other hand, the larger the building blocks became, the longer B-chains they contained, which was indicative of a Staudinger-type of structure along these longer chains. A range of structural organisations of areas of tight branching involving 4–6 chains was recently suggested for amylopectins from maize (Xia & Thompson, 2006). The structures were mixtures of the Haworth and Staudinger types with short chains largely corresponding to those found in fractions II–IV.

The ICL in building blocks was very short, only ~2 glucosyl residues. The ICL of the clusters was, however, considerably longer. In clusters of type A the ICL-value was 4.3–5.3 and in type B 5.9–6.4 (Bertoft, 2007). The latter value was in fact only slightly lower than in the original PAPS samples, in which it was 6.5 (Bertoft, 2004c). It was therefore interesting to examine the inter-block segment. The exact action pattern on internal chains possessed by the α-amylase is not known, but studies of the structure of the limit dextrins produced gives a good clue (Umeki & Yamamoto, 1972a, 1972b). As illustrated in Fig. 8, the

α-amylase most probably attacks these segments at points leaving 2–3 residues in the new A-chains and 1–2 external residues in new B-chains. The block at the other end of the segment retains also a similar number of residues at its reducing-end segment (and is included in the ICL-value). The inter-block segment is removed by the enzyme as the linear dextrins detected by HPAEC. Longer dextrins may be fragmented further, but under the conditions in this investigation maltopentaose and smaller dextrins were in practice resistant to attack. Thus, from the amount of linear dextrins the inter-block DP was obtained (Table 4). However, the actual length of the whole inter-block segment included also the chain stubs at both sides of the removed segment (Fig. 8). Because the expected average length of these segments is close to 2, it was possible to estimate an approximate average length between building blocks inside the clusters as IB-DP + 4. For type A, B, and C-clusters (Table 5), the length was 7.1, 8.5, and 8.9, respectively. This value showed that surprisingly long internal chains separated the building blocks, and it explained the high ICL-values of the isolated clusters of PAPS (Bertoft, 2007).

It is a fact that an actual definition of a unit cluster of amylopectin based on structural properties does not exist. The length of the inter-block segments suggested, however, that clusters could be defined as structures that possess internal chains shorter than nine glucosyl residues. As a consequence, this suggested that the interaction between internal chains and the α-amylase became weak at ICL < 9, which coincided with the number of subsites in the enzyme (Robyt & French, 1963; Thoma, Brothers, & Spradlin, 1970). In 1985, Thurn and Burchard published statistical model calculations for the angular dependence of scattered light from amylopectin and found that clusters are very densely branched (ICL 2) and contain only 4.22 chains. Interestingly, the size and ICL of their clusters was in the order of the building blocks in this investigation. However, Thurn and Burchard also found that very long chains of 22 residues separated the clusters, which is far more than in the inter-block segments estimated here. O'Sullivan and Pérez (1999) studied the mode of interconnection of two adjacent double helices using molecular modelling techniques. They found that several combinations of lengths of the two internal chain segments involved were possible, of which 4 and 6 residues were energetically most favourable in B-crystalline starch. These values are clearly longer than the ICL of the building blocks (Table 3), but correspond to the average ICL of the clusters (4.3–6.4). The authors also found that double helices connected through internal segments of 7 residues resulted in a larger separation in space of the two helices and suggested that it possibly represented a model for branching between two amylopectin crystals or unit clusters. Again, it is very interesting to notice that this ICL closely corresponds to the inter-block distance of 7–8 residues.

To summarise the results, an assembly of building blocks was drawn to form a hypothetical cluster of PAPS (Fig. 9). The cluster contains 10 chains, which resembles the clusters of sample 2.III (Bertoft, 2007). This cluster was of type A that, after removing the external chains to form the limit dextrin (structure [b]), possessed a peak-DP of 70 and five building blocks (Table 5). IB-DP of type-A clusters was 3.1 and thus the average length of the five inter-block chains was ~7 residues. ICL of the building blocks was 2.0 (Table 3). There are altogether four internal chains in the blocks that are not participating in the inter-block segments. From these figures it is possible to calculate the ICL of the cluster to be 4.8, which agree with that of the clusters of the A-type that can be estimated from the data given earlier (Bertoft, 2007).

Several different types of building blocks are found in Fig. 9. Two singly branched blocks of the types found in group 2 are shown in structures [c]. The external chains in these dextrins were formed by exo-acting enzymes during the preparation of ϕ,β-limit dextrins and are resistant

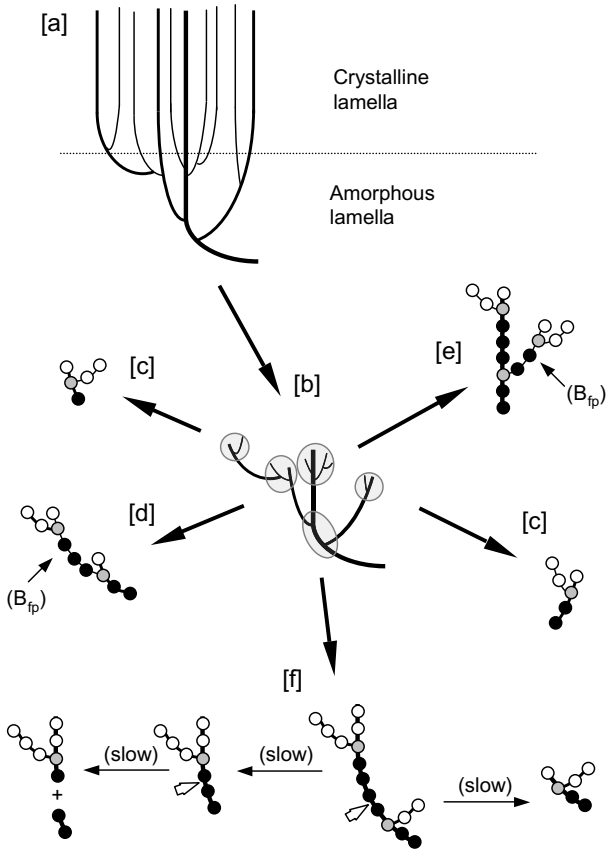

Fig. 9. A hypothetical cluster of potato amylopectin built up by units of building blocks (structure [a]). The external chains form actually double helices inside crystalline lamellae in starch granules, and the internal part contains most of the branches inside amorphous lamellae. The external chains are removed by exo-acting enzymes to obtain a ϕ,β-LD [b], in which the blocks are encircled and the inter-block chains are of CL 7–8. Structures [c] are small, singly branched blocks, [d] is a block in Haworth formation and [e] possesses both Haworth (involving a "fingerprint" B_{fp}-chain) and Staudinger formation around a comparatively long B-chain. In [f] a near-limit dextrin is extremely slowly attacked further by α-amylase (open arrows) to give limit dextrins. White circles are external residues, black circles internal residues, and grey circles residues in branch points.

to the α-amylase. In [d], a doubly branched block with DP 11 is shown. This block is typical of group 3. The A-chain is formed by cleavage of the inter-block chain, which connects the singly branched block through the Haworth structure. The B-chains in the block are also in Haworth formation. One of these was resistant to the α-amylase and is a B_{fp}-chain. In structure [e] there is also a B_{fp}-chain, again connected in Haworth conformation. Thus, B_{fp}-chains are typically involved in building blocks with three or more chains possessing the Haworth-type of structure and (most probably) found in blocks participating in the outer branches (closer to the non-reducing side of the clusters). PAPS possessed comparatively little B_{fp}-chains (12.45 mole%), whereas this type of chain was more common in waxy maize and waxy rice (Bertoft, 2004c; Bertoft & Koch, 2000), suggesting that the Haworth structure is more common in the latter amylopectins and may result in clusters of large sizes, such as those in waxy rice (Bertoft et al., 1999). Structure [e] is a triply branched dextrin of the size found in group 4. The central B-chain is comparatively long (CL 8), which was more common in larger blocks (Fig. 7). Along this chain the other chains are bound in the Staudinger formation.

Structure [f] in Fig. 9 is derived from the internal part of the cluster. The two A-chains are formed by α-amylolysis and therefore found as maltosyl and maltotriosyl stubs in approximately equal amounts. The internal chain in the dextrin is 4 residues long, but could possibly be slightly longer. It is here proposed that ICL of the order of 4–6 residues were found in the near-limit dextrins and were extremely slowly attacked by the α-amylase upon prolonged incubation (or by using more concentrated enzyme, Fig. 4b). In this case near-limit dextrins of group 4 (or 5) would be further hydrolysed into DP 6 and 9. The latter contains three residues at the reducing-end segment. As it is known that the α-amylase is capable to reduce this part into only one or two residues (Umeki & Yamamoto, 1972a), a very slow hydrolysis may occur to give a final limit-type of structure with DP 7.

Finally, the structural function of the different subgroups of the short B-chains emerge in Fig. 9. As already mentioned, B_{fp}-chains are short chains found in Haworth formation inside building blocks. All other types of B-chains found in the blocks, although of short and similar length as the B_{fp}-chains, were originally longer. A majority of these chains are involved in the interconnection of two building blocks through an inter-block chain length of 7–8 residues. As the part of the chain that remains in the building block, reduced by 1–2 residues at the reducing end, is 2–9, the original length was 8–17. This length corresponds to the B1a(l)-chains. A minor part of the B-chains are involved in the interconnection of building blocks through the Staudinger-type structure ([f]). This may involve two blocks, as in Fig. 9, or eventually three blocks in some cases. In any case, the length includes two inter-block segments and 2–3 segments from the internal parts of the blocks. The total length could therefore vary, but the order of 18–24 seems very reasonable and corresponds to the B1b-chains.

5. Conclusions

On the basis of the length of the internal chain segments of isolated clusters, it was possible to structurally define a cluster as a group of chains, in which the branches are found closer than ~9 residues from each other. The chains of the clusters of potato amylopectin were organised into densely branched building blocks interconnected by internal segments of 7–8 glucosyl residues. A single cluster was built up from 3 to 5 blocks. The average constitution of blocks in different clusters was almost similar, but on the basis of the density of branched building blocks the clusters were of two major types. In addition a small, minor type of clusters constituted a third group containing ~2 blocks. The blocks constituted four size-groups covering the approximate DP-range 5–20, of which a minor category of the larger dextrins at DP $\geqslant$ 14 tended to form aggregates. The blocks contained 2–5 chains, of which singly branched blocks constituted roughly half of all blocks. The average ICL within building blocks was only 2 glucosyl residues. Different sub-categories of the short B-chains of amylopectin possessed different structural roles in the amorphous, internal organisation of the clusters. Thus, "fingerprint" B-chains (B_{fp} or B1a(s)) participated within building blocks with $\geqslant$ 3 chains in Haworth-type formation. B1a(l)-chains were involved in the interconnection of two building blocks through one inter-block chain, whereas B1b-chains interconnected 2–3 blocks through two inter-block chains.

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