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Composition and structural analysis of alpha-dextrins from potato amylopectin

Qin Zhu, Eric Bertoft *

Department of Biochemistry and Pharmacy, Åbo Akademi University, BioCity, P.O. Box 66, SF-20521 Turku, Finland

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Abstract

Potato amylopectin was hydrolyzed with the alpha-amylase of *Bacillus amyloliquefaciens* to produce intermediate α-dextrins with a degree of polymerization < 1000. The products were then fractionated by methanol precipitation into samples of different size distributions that were analyzed by gel-permeation chromatography. All samples contained mixtures of high and low molecular weight dextrins, but in different proportions. Selected fractions were treated successively with phosphorylase and beta-amylase to obtain ϕ,β-limit dextrins. A further alpha-amylolysis of the limit dextrins was performed to investigate their compositions and suggested that the amylopectin was build up of structurally different domains. The size of the units of clusters probably ranged from dp 30–70. Dextrins of this size were only slowly hydrolyzed further by the alpha-amylase and the long internal chains had been hydrolyzed into chains of intermediate lengths. The ratio of A:B-chains and of A:Ba-chains increased with decreasing size of the dextrins, whereas Ba:Bb-chains decreased. The changes in the ratios can be used to investigate the mode of interconnection of structural units in the amylopectin. © 1996 Elsevier Science Ltd.

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1. Introduction

Since first suggested by Nikuni [1] and independently by French [2], several modified cluster models of the structure of the amylopectin component of starch have been derived [3–5], and it is now generally accepted that clusters of chains constitute the basic units of the macromolecule. The distribution of the unit chains of amylopectins of

* Corresponding author.

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different origin have been extensively investigated. Gel-permeation chromatography generally reveals chain profiles with two peaks corresponding to short and long chains with lengths (c.l.) of 11–21 and 37–50 α-D-glucosyl residues, respectively [6–11]. Several amylopectins, however, possess polymodal distributions [5]. Two main groups of chains have been defined [12], namely A-chains (unsubstituted) and B-chains (substituted by other chains), and it is assumed that the short chain group contains A- and shorter B-chains (Bs), whereas the long chains are longer B-chains (BL) that interconnect individual clusters [5,13].

Limit dextrins that are produced with exo-acting enzymes, by which the external chains of the amylopectin molecule are hydrolyzed until the enzyme reaches a branch point, are used to study the inner structure and the ratio of A:B-chains in the amylopectin molecule. Whereas beta-amylolysis limit dextrins (β-LD) have been widely used [6,8,14–17], other limit dextrins have been used sparingly. Phosphorolysis limit dextrins (ϕ-LD) were obtained from waxy-maize [13,18] and waxy-sorghum [18] amylopectins, and the beta-amylolysis products of ϕ-LD (ϕ,β-LD) were used in a comparative study of several amylopectins [8].

Hizukuri and Maehara [19] sub-divided the B-chains into Ba- and Bb-chains and defined the Bb-chains as only substituted by other B-chains, whereas the Ba-chains carry at least one A-chain. They isolated the Ba-chains from β-LD of wheat amylopectin and showed [19] that the longer chains carry more A-chains than the short ones, the average ratio of A:Ba-chains being 2.1:1 and the ratio of Ba:Bb-chains 1.7:1.

Most of the investigations have so far been carried out on the entire macromolecule and little attention has been focused on the sizes and organization of the units of clusters within the amylopectin. The exact definition of a unit cluster is a difficult task and different conclusions seem to be obtained depending on the method of analysis. Bender et al. [17] investigated the non-cyclic dextrins obtained from potato and maize amylopectin by the action of cyclodextrin glucosyltransferase and suggested they contained at least three types of clusters. The degree of polymerization (dp) of the β-LD of the clusters ranged from 40–140 and were similar in both samples, but the clusters were suggested to be less tightly packed in the potato. From β-LD of wheat and potato amylopectin, Finch and Sebesta [20] produced limit dextrins with a maltotetraoseforming amylase from *P. stutzeri* and suggested that they represented clusters of uniform size but with different fine structures. The dp of the dextrin from the potato sample was ~ 140 (M_n ~ 23,000), or three times larger than the corresponding dextrin from wheat.

We have studied the compositions of dextrins obtained during the action of *Bacillus amyloliquefaciens* alpha-amylase on the β-LD of some starches and suggested models for the interconnection of the clusters in waxy-barley [21] and smooth pea starch [22] that possessed clusters in the dp-range of 65–85 and 32–55, respectively. Waxy-maize amylopectin was extensively investigated [13] and suggested to contain larger units of clusters with dp 150–200 that are build up of densely branched sub-units with 5–8 chains and dp 35–60.

We have now extended our studies to the amylopectin of the tubers of potato. The aim was to isolate α-dextrins of different size classes and to investigate their compositions to be able to identify the clusters. Potato amylopectin is known to contain a higher proportion of long chains [10] and more esterified phosphate groups [23] than the

amylopectins of cereals [24,25]. Recently, Jane and Shen [26] showed that the length of the long chains of potato amylopectin found at the periphery of the starch granules were shorter than in amylopectin at the core or in smaller granules. They also found a lower phosphorus content in the starch containing amylopectin with shorter long chains, and Bay-Smidt et al. [27] showed that the extent of phosphorylation greatly differs between cultivars and increases considerably from the cortex of the potato tubers toward the interior.

2. Experimental

Substrate and enzymes.—Starch granules (gift from Professor Zhang Wijie, Zhe Jiang University, China) from potato tubers (*Solanum tuberosum* L.) were defatted by extraction overnight with hot aq 85% MeOH in a Soxhlet apparatus. The amylopectin was isolated from the defatted granules by the method of Gilbert [28].

Alpha-amylase of *Bacillus amyloliquefaciens* [(1 → 4)-α-D-glucan glucanohydrolase; EC 3.2.1.1] with an activity [29] of 475 U/mg and amyloglucosidase of *Aspergillus niger* [(1 → 4)-α-D-glucan glucohydrolase; EC 3.2.1.3] were purchased from Boehringer–Mannheim, whereas phosphorylase *a* from rabbit muscle [(1 → 4)-α-D-glucan:orthophosphate α-D-glucosyltransferase; EC 2.4.1.1] and beta-amylase from sweet potato [(1 → 4)-α-D-glucan maltohydrolase; EC 3.2.1.2] were from Sigma. Isoamylase of *Pseudomonas amyloderamosa* (glycogen 6-glucanohydrolase EC 3.2.1.68) and pullulanase of *Klebsiella pneumoniae* (amylopectin 6-glucanohydrolase EC 3.2.1.41) were obtained from Hayashibara, Japan.

Production of α-dextrins.—Amylopectin (3.0 g) was dissolved in 90% Me_2SO (60 mL) by boiling on a water bath for 30 min, water (220 mL) was added, and the solution was then stirred overnight at 50 °C. The mixture was cooled to 25 °C and a solution (20 mL, 9 U) of alpha-amylase in 0.2 M NaOAc buffer (pH 6.5) was added to give a final concentration of 30 mU of enzyme and 10 mg substrate/mL. At intervals, 5 M KOH (60 μL) and water (0.4 mL) were added to small aliquots (0.2 mL) of the hydrolysate before gel-permeation chromatography. For the isolation of α-dextrins a large aliquot (298 mL) was treated with 5 M KOH (7.0 mL) after hydrolysis for 60 min and the mixture was fractionated with 5 vol MeOH into a precipitate (sample I) and a supernatant (sample II). Sample I was further sub-fractionated with series of increasing MeOH–H_2O ratios from 0.5:1 to 5:1 into precipitates of α-dextrins of different sizes as described by Bertoft and Spoof [30]. The precipitates were washed with methanol, then acetone, and air-dried.

Characterization of amylopectin and α-dextrins.—Amylopectin or α-dextrins (3.0 mg) were dissolved by heating in deionized water (0.5 mL) and 0.25 M NaOAc buffer (0.1 mL, pH 3.5) and isoamylase (0.25 mL, diluted 1:150 in water) were then added. The solution was boiled after incubation for 4 h at 35 °C to stop the reaction and the reducing value was determined [31]. Total carbohydrate content was measured with phenol–H_2SO_4 [32] and the average chain length (c.l.) was then calculated as total carbohydrate/reducing value. The c.l. of the amylopectin was estimated by gel-permeation chromatography of the debranched sample as described below. The average external

chain length (e.c.l.) and the average internal chain length (i.c.l.) were calculated from the phosphorolysis-beta-amylolysis limit value [33].

The procedure used for the analysis of the ϕ,β-limit was a slight modification of that described earlier [33]. The concentration of the stock solution of amylopectin or α-dextrins was 1.5 mg/mL and the total carbohydrate was measured with the phenol–H_2SO_4 method, whereas the amyloglucosidase reaction was used to measure the glucoamylolysis limit (γ-limit) [34].

The content of glucose 6-phosphate residues was analyzed in the amylopectin by hydrolysis of a sample (150 mg) in hot 0.7 M HCl (3.1 mL) as described by Hizukuri et al. [23]. Glucose 6-phosphate was then assayed enzymically [35] by the glucose 6-phosphate dehydrogenase catalyzed reduction of $NADP^+$.

Production of phosphorolysis-beta-amylolysis limit dextrins (ϕ,β-LD).—ϕ-LD were prepared as described earlier [13], but the phosphorolysis reaction was repeated once to reach the limit. The ϕ-LD were then dissolved in 90% Me_2SO (50 mg/mL) on a boiling water bath for 30 min, diluted (9 vol) with 0.1 M NaOAc buffer (pH 4.8), and beta-amylase was added to give a final concentration of 1 U/mg of carbohydrate. The mixture was incubated overnight at room temperature and then boiled. The maltose formed from the α-dextrin samples was removed on a PD10 column (Sephadex G-25, Pharmacia). The limit dextrins were then precipitated with methanol (5 vol) overnight at 4 °C, washed with acetone, and air-dried. The ϕ,β-LD of the amylopectin was precipitated with methanol, redissolved and dialyzed against water, and finally lyophilized.

Alpha-amylolysis of ϕ,β-LD.—The limit dextrins were dissolved in 90% Me_2SO (50 mg/mL) by heating on a boiling water bath and diluted with water (3 vol). A solution of alpha-amylase in sodium acetate buffer was added to give conditions identical with the treatment of the amylopectin described above. Aliquots taken at time intervals were analyzed by gel-permeation chromatography.

Partial debranching of ϕ,β-LD.—To a boiled solution (0.15 mL) of the ϕ,β-LD in 90% Me_2SO (20 mg/mL) was added water (0.9 mL), 0.1 M NaOAc buffer (0.45 mL, pH 3.5), and a diluted (100 ×) solution of isoamylase (1.5 μL, ~ 9 U). The mixture was incubated at 25 °C and aliquots (0.75 mL), taken after 4 and 5 h, were boiled for 5 min to stop the reaction. 5 M KOH (40 μL) was added to one part (0.4 mL) of the aliquot before gel-permeation chromatography.

Beta-amylolysis of partially debranched ϕ,β-LD.—To an other part (0.3 mL) of the partially debranched ϕ,β-LD was added 0.2 M NaOAc (20 μL) to adjust the pH to 4.8 and the volume was diluted to 0.39 mL with 0.1 M NaOAc buffer (pH 4.8) before beta-amylase (10 μL) was added. The mixture was incubated overnight at room temperature, treated with 5 M KOH (40 μL), and analyzed by gel-permeation chromatography.

Complete debranching of ϕ,β-LD.—Limit dextrins in 90% Me_2SO (0.15 mL, 20 mg/mL) were diluted with water (0.45 mL) and 0.1 M NaOAc buffer (0.385 mL, pH 5.5) before undiluted pullulanase (15 μL) was added. The mixture was incubated overnight at room temperature and boiled for 5 min. An aliquot (0.4 mL), treated with 5 M KOH (40 μL), was then analyzed by gel-permeation chromatography. The rest of the sample was treated with beta-amylase to confirm that the debranching was complete.

Gel-permeation chromatography.—Samples containing 0.5–0.8 mg of carbohydrates in ~0.5 M KOH (0.4 mL) were eluted from columns (1.0 × 90 cm) of Sepharose CL-6B or Superdex 75 (Phamacia) with 0.5 M KOH at 1 mL/min and 0.1 M KOH at 0.5 mL/min, respectively. Fractions (0.5 mL) were analyzed for carbohydrates, using the phenol–H_2SO_4 reagent [32]. The columns were calibrated with dextrins of known molecular weight as described [30,36].

3. Results

The gel-permeation chromatogram obtained from Superdex 75 of the debranched potato amylopectin preparation showed that it was free of amylose-like chains (Fig. 1). The amylopectin displayed a bimodal distribution with long chains (dp > 35) and short chains. The c.l. of these groups was 66 and 14, respectively, and the molar ratio of short to long chains was 6.6, which was similar to other reports [3,6,10]. The c.l. of the whole sample was 21.3 (Table 1) and was lower than those reported earlier [5,6,10].

The composition of the hydrolysis mixture after treatment of the amylopectin with alpha-amylase is shown in Fig. 2a. The endo-attack performed by the enzyme resulted in an effective initial depolymerization. Whereas originally the amylopectin eluted at the void volume of Sepharose CL-6B, the molecular weight distribution of the 60 min hydrolysate (the sample used for further study) possessed α-dextrins within a broad dp-range from 30–1000, and only trace amounts of larger material remained. The dextrins obtained at this stage were intermediate products as shown by their subsequent hydrolysis by the enzyme. Simultaneously with the larger dextrins, a heterogeneous group of small dextrins with dp < 20 appeared and represented the main products after 5 h of hydrolysis.

Sample I was obtained by precipitation with 5 vol MeOH of the 60 min hydrolysis mixture. It contained dextrins with a dp between 20–1000 (Fig. 2b) and the recovery (73%) was similar to the amount (82%) of carbohydrates with dp > 20 in the original mixture. The carbohydrates that remained in the supernatant (sample II) had a narrow

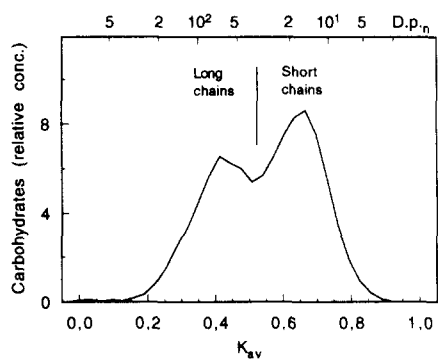

Fig. 1. Gel-permeation chromatography on Superdex 75 of the unit chain profile of debranched potato amylopectin.

Table 1
Characterization of amylopectin and fractions of α-dextrins and their ϕ,β-limit dextrins

Parameter	Amylopectin	2	3.3	4.2	6	Sample II
α-Dextrins						
Yield (%) [a]	–	6.6	5.7	4.4	3.3	27.1
Dp-distribution [b]	> 5000	70–1500	40–800	25–800	20–800	2–35
γ-limit (%)	97	86	94	96	99	–
ϕ,β-limit (%)	60	54	54	55	53	90
C.l.	21.3	15.0	14.9	14.8	14.9	–
E.c.l. [c]	14.3	9.6	9.5	9.6	9.4	–
I.c.l. [c]	6.0	4.4	4.4	4.2	4.5	–
ϕ,β-Limit dextrins						
Yield (%) [d]	79	91	90	84	59	–
Dp [e]	–	140	68	49	40	–

[a] The yield obtained from the mixture of dextrins after 60 min of alpha-amylolysis.
[b] Distribution of dextrins comprising 90% of the total carbohydrate material.
[c] E.c.l = c.l. × (% ϕ,β-limit/100) + 1.5, and i.c.l = c.l. − e.c.l − 1.
[d] Percentage of the theoretical yield expected from the ϕ,β-limit value.
[e] From gel-permeation chromatography on Superdex 75 (Fig. 3a).

molecular weight distribution and included mainly the group of small dextrins. Sample I was fractionated with a series of increasing MeOH–H_2O ratios from 0.5:1 to 5:1 into 6 fractions. Some fractions (3, 4, and 5) were further sub-fractionated into 12 fractions. Four fractions, namely samples 2 and 6 (obtained from sample I) and samples 3.3 and 4.2 (sub-fractions of samples 3 and 4, respectively), that represented different size-distributions of the dextrins of sample I, were chosen for further studies. The yields of the samples ranged from 3.3–6.6% (Table 1). Their molecular weight distributions (and the distributions of all the other fractions that are not shown) were surprisingly broad (Fig. 2c). A separation of the dextrins at the low molecular weight side was obtained, but at the high molecular weight side only a poor separation was achieved. Thus, samples 3.3, 4.2, and 6 all contained dextrins up to dp ~ 800 (Table 1).

The gel-permeation chromatograms possessed peaks and shoulders that indicated the presence of groups of intermediate α-dextrin products similar to those obtained for the amylopectin of waxy-maize [30], waxy-barley [21], and smooth pea starches [22], and they have been named in Fig. 2c according to size as dIII–cV in analogy to the nomenclature used in the earlier studies. The estimated mean dp of the dextrins are given in Table 2. Beside these groups, both larger and smaller dextrins existed, though any specific groups were not identified among them.

More than half of the phosphate groups in potato amylopectin are esterified at the 6-position [23] and the amount of glucose 6-phosphate was shown to be a reliable measure of the total phosphorus in potato starch [27]. The relative molar content of glucose 6-phosphate in the amylopectin was 0.11%, which suggested a low extent of phosphorylation in comparison with other potato starches [27], though it was possible that some phosphate was removed by the acid hydrolysis prior to measurement. Because of a limit amount of the samples, the phosphate content was not examined in more

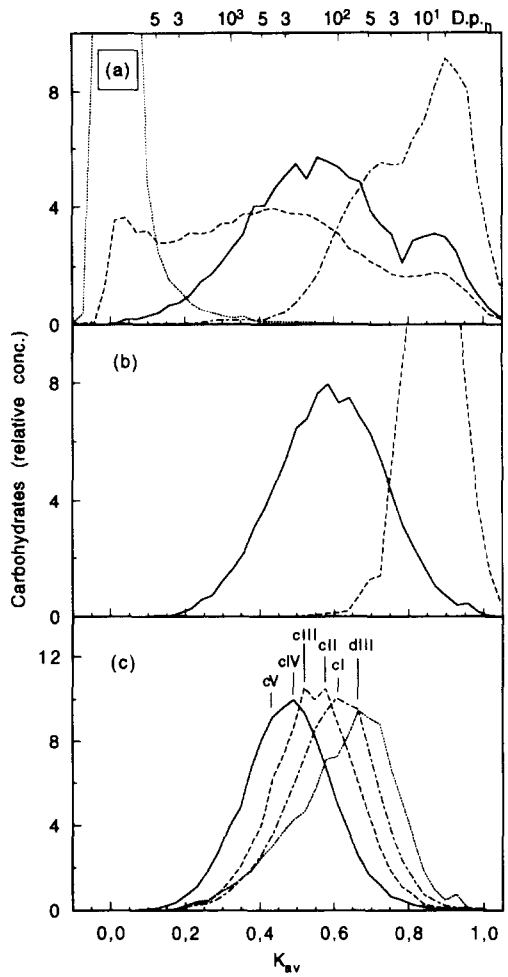

Fig. 2. Molecular weight distribution on Sepharose CL-6B of (a) the hydrolysates of potato amylopectin obtained after the action of alpha-amylase before (· · · · ·) and after hydrolysis for 30 (– – –), 60 (————), and 300 min (– – –), (b) the α-dextrins fractionated after precipitation with 5 vol MeOH from the 60 min hydrolysate (sample I, ————) and the dextrins remaining in the supernatant (sample II, – – –), and (c) samples 2 (————), 3.3 (– – –), 4.2 (– – –), and 6 (· · · · ·) obtained from sample I by fractional precipitation; cV–dIII indicates the positions of groups of intermediate α-dextrins.

Table 2
Dp of groups of dextrins produced from amylopectin by alpha-amylolysis and their ϕ,β-limit dextrins

	dIIa	dIIb	dIII	cI	cII	cIII	cIV	cV
α-dp [a]	—	—	70	110	150	210	270	450
ϕ,β-dp [b]	≤ 12	13–29	33	50	70	120	160	220
ϕ,β-Limit (%) [c]	—	—	53	55	53	43	41	51

[a] Estimated from Fig. 2.
[b] Estimated from Fig. 3.
[c] The limit value of the dextrin suggested from the difference in dp.

detail. However, the existence of phosphorylated α-D-glucosyl residues can also be shown by the production of γ-limit dextrins through the action of the amyloglucosidase of *Aspergillus niger* that cannot by-pass the phosphate groups [34]. The γ-limit of the amylopectin was 97% (Table 1), which also suggested a low degree of phosphorylation. Sample 2 possessed a lower limit value (86%) and possibly, therefore, the phosphorylated residues were enriched in this fraction. The other samples were similar to the amylopectin.

The ϕ,β-limit value can be used as an alternative to the β-limit for the characterization of amylopectin [33]. Sample II possessed a high limit value showing that it mostly contained linear dextrins, whereas the limit values of samples 2–6 were lower than that for amylopectin (Table 1). The c.l. had been reduced by the alpha-amylase by ~ 6 residues at average and was similar in all the α-dextrin samples. The reduction was mostly due to the attack by the alpha-amylase at the external chains.

ϕ,β-Limit dextrins were isolated on a preparative scale from the amylopectin and samples 2–6. The yields of these preparations ranged from 79–91% of that theoretically expected based on the ϕ,β-limit values, except for sample 6 that was obtained in a lower yield (Table 1). Gel-permeation chromatograms were obtained from the column of Superdex 75 (Fig. 3a). The amylopectin ϕ,β-LD eluted mostly at the void volume and the preparation contained a small amount of maltose that had remained after dialysis. Samples 2–6 were partly included into the gel and their average dp-values were calculated to range from 40–140 (Table 1). The distribution of sample 6 possessed two major groups of ϕ,β-LD of sizes that corresponded to the original α-dextrins, but they were now found in different proportions. The low yield of this sample indicated that the smaller dextrins largely had remained in the supernatant when the sample was precipitated with methanol.

Groups of ϕ,β-LD that originated from the α-dextrins were weakly indicated in the chromatograms, suggesting broad distribution of individual dextrins within each group and large overlapping between the groups (Fig. 3a). Their average dp could therefore only be roughly estimated. The difference in the dp-values of the α-dextrins and ϕ,β-LD agreed mostly with the ϕ,β-limit values, though the dp of dextrins cIII and cIV suggested a lower ϕ,β-limit (Table 2). Whether this indicated that some groups of dextrins possessed shorter external chains or simply was due to uncertain dp-positions is not known.

The samples were treated a second time with the alpha-amylase to obtain additional data of their compositions. Because ϕ,β-LD contain very short external chains [33], initially only endo-attack at internal chains will occur [37]. The amylopectin ϕ,β-LD was initially hydrolyzed fast and when the average dp of the sample was ~ 30 the rate of the depolymerization became low (Fig. 4). At this stage (2 h of hydrolysis) the dextrin groups cIV–cII predominated in the hydrolysis mixture (Fig. 3b) and after 5 h, when the reaction was stopped, cII–dIII remained (Fig. 3c). A range of very small dextrins with dp < 30 was also found (dII), probably including glucose, maltose, and maltotriose that are produced from the new external chains formed by the cleavage of internal chains [38].

When samples 2–6 were treated with the alpha-amylase, the hydrolysis rate became low and similar in all samples after 2 h (Fig. 4), and dextrins cII–dIII predominated at

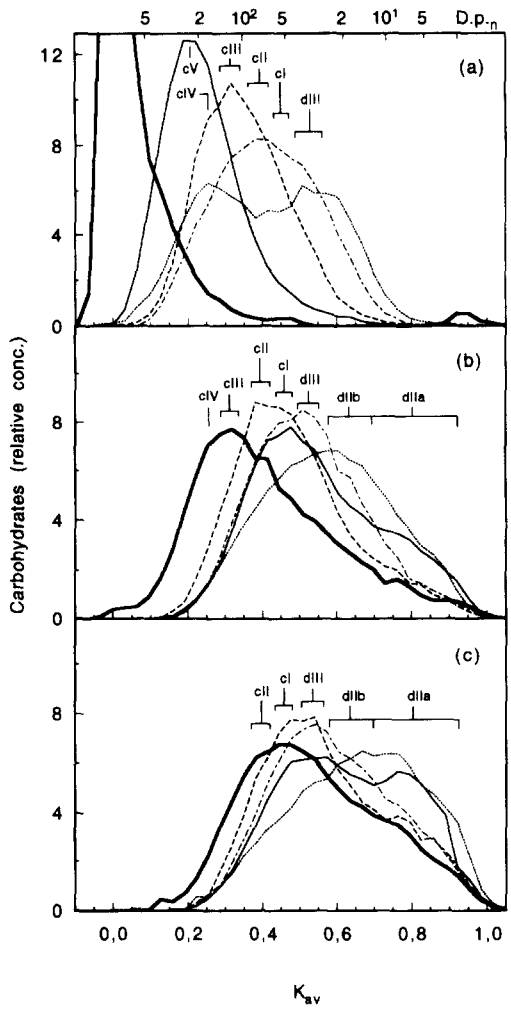

Fig. 3. Molecular weight distribution on Superdex 75 of ϕ,β-LD: (a) the samples before treatment with alpha-amylase, and the hydrolysis mixtures after (b) 2 h and (c) 5 h; amylopectin (bold line), samples 2 (————), 3.3 (– – –), 4.2 (– · –), and 6 (· · · · ·). cV–dIIa traces groups of ϕ,β-LD.

this stage (Fig. 3b). After 5 h, dextrins cI and dIII still constituted the major fractions (Fig. 3c), which showed that they were comparatively resistant to the alpha-amylase attack. The ϕ,β-LD cIV and cIII of sample 6 were also degraded into cI and dIII, whereas the original dextrins of these types gave rise to the heterogeneous group dII, which was sub-divided into dIIa and dIIb (Fig. 3c and Table 2).

The rate of the hydrolysis of samples 3.3–6 was comparable to the amylopectin ϕ,β-LD, though the initial rates already were lower because of the low dp-values at the start of the reaction. The depolymerization of sample 2 proceeded, however, unexpectedly at a much faster rate than of the other samples (Fig. 4). The reason for this was a

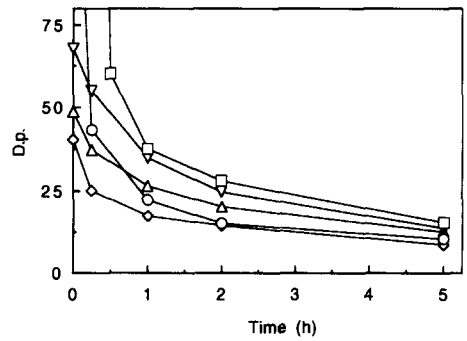

Fig. 4. Dp as a function of time during alpha-amylolysis of the ϕ,β-LD of amylopectin (□) and samples 2 (○), 3.3 (▽), 4.2 (△), and 6 (◇).

much larger production of the group of dII-dextrins, in particular dIIa (Fig. 3b, c), which were obtained by a rate similar as the corresponding group in sample 6.

The ϕ,β-LD of the amylopectin and the α-dextrins were debranched with pullulanase and their unit chain compositions are shown in Fig. 5. The maltose formed in this

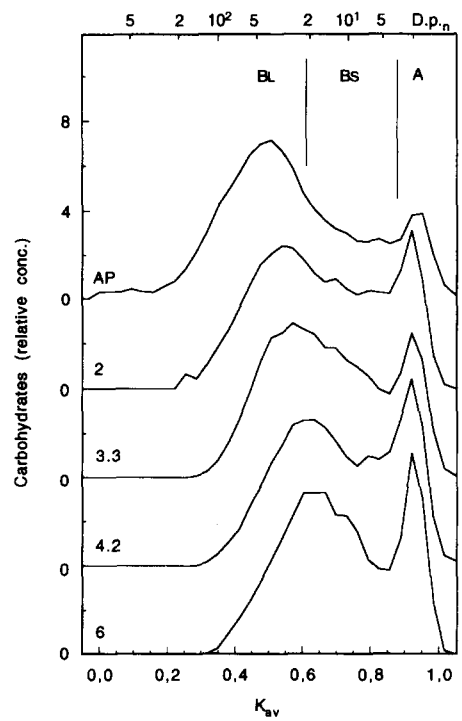

Fig. 5. Distribution on Superdex 75 of the unit chains of completely debranched ϕ,β-LD of amylopectin (AP) and samples 2–6. BL, BS, and A are long B-chains, short B-chains, and A-chains, respectively.

Table 3
Chain ratios in ϕ,β-limit dextrins

Sample	(A + Bs):BL	Bs:BL	A:B	A:Ba	Ba:Bb
Amylopectin	6.6:1	2.1:1	1.45:1	2.12:1	2.16:1
2	10.9:1	3.8:1	1.51:1	2.21:1	2.14:1
3.3	11.2:1	4.0:1	1.47:1	1.95:1	3.04:1
4.2	16.2:1	5.2:1	1.76:1	2.53:1	2.29:1
6	15.9:1	5.0:1	1.82:1	2.74:1	1.99:1

reaction represented the A-chains [33], whereas the rest of the chromatogram showed the distribution profile of the internal parts of the B-chains. The molar ratio of A:B-chains in the amylopectin was 1.45:1 (Table 3). Robin [15] estimated the ratio to 0.70:1 from the molar amount of maltose and maltotriose obtained after debranching of the β-LD. Hizukuri [5] obtained a ratio of 0.79:1 from debranched intact amylopectin, when assuming that the shortest sub-group of the short chains represented the A-chains. The group of short chains should then constitute all A-chains together with the short B-chains [5] (Bs-chains) and we suggested [13] that the latter chains also possess the shortest internal segments. The molar amount of the A- and Bs-chains should therefore be the same in the ϕ,β-LD and in the original molecule. The ratio of short to long chains in the amylopectin was 6.6:1 (Fig. 1), which suggested a division of the B-chains of the amylopectin ϕ,β-LD into short and long at dp 20. There was, however, no obvious indication of these sub-groups in the chromatogram (Fig. 5). The long B-chains (BL-chains) possessed a peak at dp ~ 40 and their average length was 46.3, whereas the c.l. of the Bs-chains was 8.5 (Table 4). The molar ratio of Bs:BL in the amylopectin was 2.1 (Table 3).

The length of the BL-chains decreased after the action of alpha-amylase (Fig. 5). The peak position changed with the size of the dextrins and in sample 6 the position was at

Table 4
Average length of B-chains in ϕ,β-limit dextrins

Sample	Bb + Ba − A [a]		Ba − A [b]		Bb + Ba [c]	
	Bs [d]	BL [e]	Bs	BL	Bs	BL
Amylopectin	9.7	50.4	9.2	49.1	8.5	46.3
2	9.5	46.7	9.6	46.0	8.2	37.7
3.3	9.7	39.0	9.7	37.7	8.6	34.2
4.2	10.4	34.1	10.6	35.3	8.3	32.4
6	10.1	34.2	10.3	34.5	8.9	31.5

[a] The dp of the mixture of linear Bb-chains and Ba-chains branched with maltosyl-stubs obtained after partial debranching.
[b] The dp of the internal core of Ba-chains branched with maltosyl-stubs obtained after successive beta-amylolysis of partially debranched samples.
[c] The dp of the mixture of linear Bb- and Ba-chains after complete debranching.
[d] Short B-chains with dp ≤ 20.
[e] Long B-chains with dp > 20.

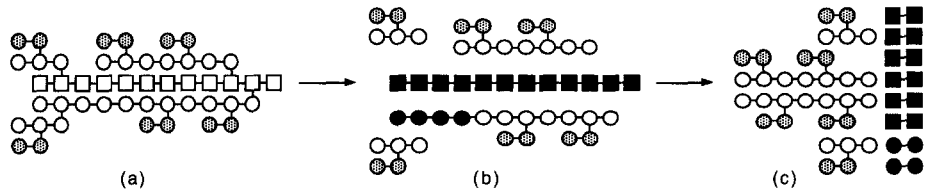

Fig. 6. Principle of partial debranching of ϕ,β-LD with isoamylase; (a) before debranching: gray circles represent the maltosyl-stubs of A-chains, white circles Ba-chains, and white squares Bb-chains; (b) after partial debranching: black squares represent the Bb-chains and black circles the newly formed external parts of Ba-chains that are susceptible to successive beta-amylolysis, whereas the white circles show the 'internal core' of the Ba-chains; (c) the products of the successive beta-amylolysis: black squares and circles represent the maltose formed.

dp ~ 18. The c.l. decreased from 37.7 in sample 2 to 31.5 in sample 6 (Table 4). The length of the Bs-chains, however, remained almost unaffected. The ratio of Bs:BL and of all the short chains (A + Bs) to long (BL) chains both increased with decreasing dp (Table 3). The alpha-amylolysis affected also the distribution of chain-types, so that samples 3.3–6 constituted a series of increasing A:B ratio. However, sample 2 possessed a higher ratio than sample 3.3.

Ba- and Bb-chains can be analyzed by a partial debranching with *Pseudomonas* isoamylase [19]. This enzyme attacks very slowly maltosyl-chain stubs compared to longer chains [39]. In a β-limit dextrin half of the A-chains have been reduced to maltosyl-stubs and if the enzyme is allowed to attack partially the branches, so that all branches are hydrolyzed except the maltosyl-chain stubs, half of the A-chains remain bound to the Ba-chains [19]. If a ϕ,β-LD, in which all A-chains are represented as maltosyl-stubs [33], is used as the substrate, they will all remain attached to the Ba-chains and only the Bb-chains become linear. This principle is illustrated in Fig. 6. If the products of the partial hydrolysis are attacked by beta-amylase, all Bb-chains will be further converted into maltose and the remaining branched dextrins represent the Ba-chains with their A-chain stubs still attached (here designated Ba–A-chains). Two types of Ba-chains can exist in the ϕ,β-LD. One type carries an A-chain next to the non-reducing end of the chain and will not be attacked further by beta-amylase. The other type has a B-chain at that position and will possess a newly formed external chain after the partial debranching, which is hydrolyzed into maltose by the successive beta-amylolysis. The remaining chains represent, therefore, the internal core-chains, which will be defined as the part of a Ba-chain extending from the first A-chain at the non-reducing side (including the external glucosyl- or maltosyl-chain stub) to the reducing end of the chain (Fig. 6).

The extent of the partial debranching reaction is critical, because ideally all branches except maltosyl branches should be attacked [19]. Fig. 7 shows an example of the reaction with sample 3.3. The molecular weight distribution continued to decrease as the reaction proceeded up to 4 h, after which almost identical curves were obtained and practically no carbohydrates were seen at the position for maltose. The partial debranching reaction had thus reached the extent required and 5 h was taken as the incubation

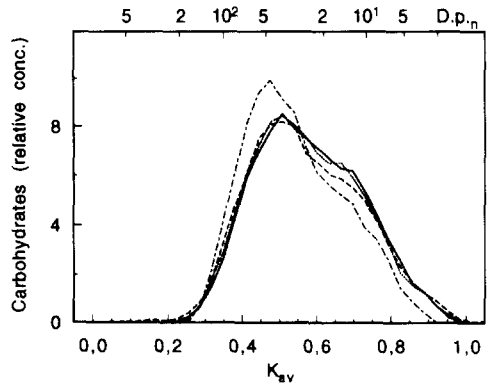

Fig. 7. Molecular weight distribution on Superdex 75 of partially debranched ϕ,β-LD (sample 3.3) with isoamylase after 2 (– – –), 3 (– – –), 4 (· · · · ·), and 5 h (————).

time for the reaction, whereas 4 h served as a control sample to confirm that the reaction rate was negligible at this stage.

The molecular weight distributions of the partially debranched ϕ,β-LD of the

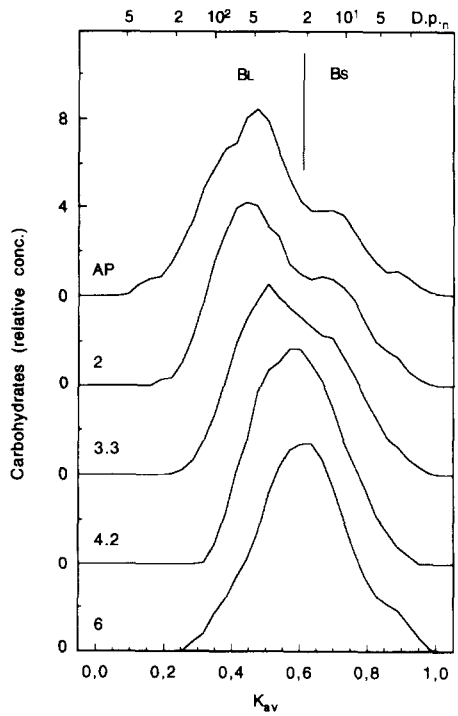

Fig. 8. Distribution on Superdex 75 of the Ba–A- and Bb-chains after partial debranching of the ϕ,β-LD of amylopectin and samples 2–6.

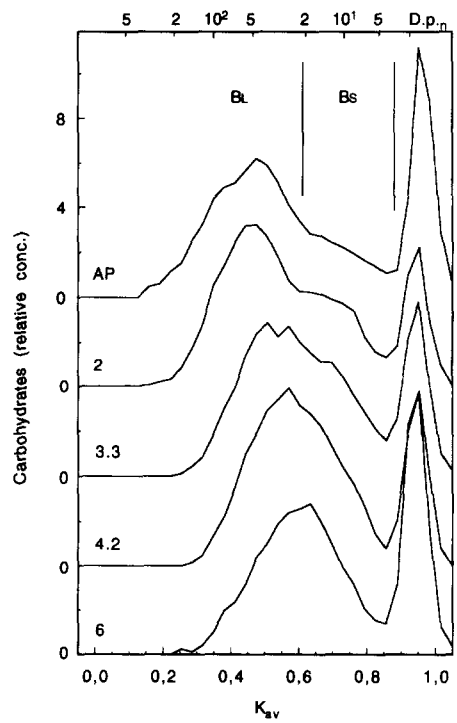

Fig. 9. Distribution on Superdex 75 of the 'internal core' of the Ba–A-chains obtained after successive beta-amylolysis of partially debranched ϕ,β-LD. The peak at $K_{av} \sim 0.94$ is the maltose.

amylopectin and its α-dextrins are shown in Fig. 8. The two groups of short (dp ≤ 20) and long (dp > 20) chains were better resolved than after the complete debranching and their average dp in the amylopectin were 9.7 and 50.4, respectively (Table 4). The long chains were composed of two overlapping sub-groups with peak dp at ~ 50 and ~ 80 (Fig. 8). A shoulder at dp ~ 35 in sample 2 indicated the new sub-group of long chains that had been formed after alpha-amylolysis. This group predominated in sample 3.3, and in samples 4.2 and 6 the dp had been reduced to ~ 20 and the chromatogram possessed a single broad distribution of chains. The calculated dp of the Bs- and BL-chains was higher than after the complete debranching because the maltosyl-stubs were attached to the Ba-chains (Table 4).

The molecular weight distributions obtained by the successive beta-amylolysis of the partially debranched ϕ,β-LD became only slightly different in all samples (Fig. 9 and Table 4) and probably, therefore, the majority of the Ba-chains possessed A-chains rather than B-chains close to the non-reducing end. The sub-groups of chains were also seen, which showed that the Ba–A-chains were represented in all length-classes. The molar amount of Ba-chains was estimated from the chromatograms and the amount of Bb-chains was the difference between all B-chains and the Ba-chains, which gave molar ratios of A:Ba and Ba:Bb in the amylopectin of 2.12:1 and 2.16:1, respectively (Table 3). The effect of the alpha-amylolysis on the A:Ba-chain ratio was complicated. Sample

2 had a slightly higher ratio than the original amylopectin, whereas sample 3.3 had a lower ratio, which then increased in the smaller dextrins. Interestingly, the changes in the ratio of Ba:Bb-chains were the opposite.

4. Discussion

The alpha-amylolysis pattern of the potato amylopectin (Fig. 2a) was similar to that obtained for waxy-maize [30] and waxy-barley amylopectin [21]. The reduction of the length of the external chains (Table 1) showed that the extent of the exo-attack proceeded equally regardless of the size of the dextrins in the hydrolysis mixture. However, whereas maltohexaose preferentially was produced from the other amylopectins, the group of low molecular weight dextrins isolated in sample II obviously contained a range of mostly linear dextrins. This is in agreement with the result of Robyt and French [38].

When sample I was fractionated with methanol into the series of precipitates, the recoveries of samples containing larger dextrins were generally good (~ 90%). Smaller dextrins were obtained in lower yields and much with a distribution (not shown) similar to that of sample 6 (Fig. 2c) remained in the supernatant. It was also difficult to obtain a good separation of the α-dextrins. Obviously, some high molecular weight dextrins did not precipitate at lower methanol concentrations and were therefore recovered together with smaller dextrins when more methanol was added. Thus, the fractionation was apparently not only based on the size of the dextrins, but also on other properties, e.g., their structure. This behavior was different from the dextrins of waxy-maize amylopectin [30] that easily were obtained in high yields and comparatively narrow size-distributions. Interestingly [17], fractionated dextrins obtained from potato amylopectin after the action of cyclodextrin glycosyltransferase, also obtained co-precipitating high molecular weight material among smaller dextrins, though in these fractions the groups of large and small dextrins were completely separated by gel-permeation chromatography on Sepharose CL-6B.

The presence of certain groups of α-dextrins in the hydrolysate suggested that the potato amylopectin was built up of blocks of clusters by a similar principle as in other starches [13,21]. The smooth distribution profiles (Fig. 2) indicated, however, comparatively broad size-distributions within the groups, which could depend on several factors, e.g., variations in the length of individual internal chains or of external chains both before and after the attack by the alpha-amylase. Unfortunately, there is no method available to measure the distributions of these chain segments.

If ϕ,β-LD are used as substrate, the latter cause to variation is excluded. The groups of dextrins obtained from the ϕ,β-LD of the amylopectin were, however, also poorly resolved (Fig. 3). Besides variations in internal chain length segments, clusters of small sizes also could cause a broad diversity of intermediate dextrins, because such units can be combined into more size-alternatives than large ones. The ratio of short to long chains is lower in potato amylopectin than in cereal starches [10,40], which suggests that it is build up of smaller clusters.

The hydrolysis rate decreases when only densely branched substrates remain avail-

able to the alpha-amylase [41]. The dextrin-groups cII–dIII were attacked with a very slow rate and cI and dIII still constituted major products after 5 h of hydrolysis (Fig. 3). This suggested that the size of the limit dextrins of the clusters were found in the dp-range 30–70 (Table 2), which corresponded to the smaller sizes suggested by Bender et al. [17] but was smaller than the dp of ~ 140 suggested by Finch and Sebesta [20].

The high rate of alpha-amylolysis of the limit dextrins in sample 2 (Fig. 4) showed that they contained more of easily accessible internal chain segments than the other samples, despite the similar average value for i.c.l. (Table 1). The large production of the smallest dextrins dIIa (Fig. 3) suggested a composition of very small structural units different from the dextrins of samples 3.3 and 4.2. Sample 2 had also a lower γ-limit value than the other samples (Table 1), indicating a higher degree of phosphorylation. Therefore, it seems possible that not only a concentration of phosphate groups to certain parts of either the starch granules or within the macromolecule could exist [26,34,42], but also that the structural composition of the carbohydrate chains was different locally.

The partially debranched sample of amylopectin (Fig. 8) showed that short and long internal chains existed in the potato amylopectin, though they were not resolved in the unit chain profile of the completely debranched ϕ,β-LD (Fig. 5). A similar single broad distribution of internal B-chains was obtained by Robin [15] from the β-LD of potato amylopectin and suggested a different type of internal structure compared to cereal starches, in which the distinction between the groups is more obvious [6,13–15].

The unit chain profiles of samples 2–6 (Fig. 5) showed that the alpha-amylolysis of the ϕ,β-LD reduced the length of the internal BL-chains. The similarity in the pattern of the profiles after the partial debranching and the successive beta-amylolysis (Figs. 8 and 9, Table 4) indicated that both the Ba- and the Bb-chains had been attacked. Sample 2 possessed the longest chains, which was consistent with the high rate of alpha-amylolysis of that sample.

To produce individual unit clusters the interconnecting long chains must be cut and an isolated unit should therefore not possess BL-chains. Samples 2–6 were heterogeneous mixtures of dextrins and BL-chains were found in all of them. However, in sample 4.2, that mostly contained dextrins cIII–dIII (Fig. 3a), chains of intermediate lengths preponderated (Fig. 5, Fig. 8, and Fig. 9) and the ratio of short to long chains (A + Bs:BL) was clearly higher than in samples 2 and 3.3 (Table 3). Apparently, the alpha-amylase attack occurred at certain positions along the internal chain segments so that new internal chains appeared with specific lengths intermediate to the original short and long chains. These new chains were attacked further at a lower rate, showing that the remaining linear chain segments were too short to interact effectively with all 9 sub-sites of the enzyme [38,43]. The formation of B-chains with c.l. 30–35 was also reported by Bender et al. [17] after hydrolysis with cyclodextrin glycosyltransferase. The low resolution of the BL- and Bs-chains in the gel-permeation chromatogram of the amylopectin (Fig. 5) strongly suggested that these types of chains already pre-existed in the macromolecule and the formation of more such chains during the alpha-amylolysis was directly connected with the fine structure of the amylopectin. Similar types of chains (called B1b and B1c) were shown to exist in native waxy-maize starch [36] and were enriched in the α-dextrins [13]. In a structure model of that amylopectin [13], smaller sub-units were interconnected by these chains into units of dextrins cIV and cV.

Probably, the structure of the potato amylopectin was analogous, though the units were of smaller sizes.

The molar ratio of A:B-chains increased after the alpha-amylase attack (Table 3). This was different from the α-dextrins of waxy-maize starch that possessed a constant ratio of 1.0:1 regardless the size of the dextrins or the time of amylolysis [13], which pointed to differences in the mode of interconnection of the unit chains. In this investigation it was also shown that the ratios of A:Ba and Ba:Bb were different both between the dextrins and when compared to the original amylopectin. Sample 2 showed a slightly higher degree of substitution of A-chains on the Ba-chains compared with the average degree in the original macromolecule or in sample 3.3 (Table 3). This was also seen as comparatively large core segments of the Ba–A-chains (BL 46.0, Table 4), whereas after the complete debranching the mixture of Ba- and Bb-chains was more similar to the other samples (BL 37.7).

Samples 3.3, 4.2, and 6 possessed changes in their ratios that constituted increasing or decreasing series with decreasing molecular weight (Table 3). These differences can be used to trace the mode of interconnection of the structural units, because they are reflections of the type of attack performed by the alpha-amylase at the internal chains. A certain type of chain can thereby change to another type, and the effect on the ratios will be more obvious the smaller the dextrins are. In Fig. 10 the possible positions for the attack at the internal segments of Ba- and Bb-chains in some hypothetical dextrins are illustrated. For simplification the structures are drawn as ϕ,β-LD. In case I the attack occurs at a Ba-chain that is attached to a Bb-chain. The products from this reaction contain newly formed A-chains but the net amount of B-chains remains constant and thus the A:B ratio increases. The reaction, however, gives also rise to a secondary effect so that the Bb-chain, to which the new A-chain is bound, is transferred into a Ba-chain. This decreases the A:Ba-ratio and increases Ba:Bb. A similar type of attack is presented in case II, but because the Ba-chain now is attached to another Ba-chain, the secondary effect is absent and only an increase of A-chains is obtained and reflected as an increase in the ratios of A:B and A:Ba. Similar discussions can be made for the other cases.

Sample 3.3 contained mostly dextrins cIV and cIII that are considered to be built up by two or a few unit clusters. To produce these dextrins apparently most of the longest chains of the BL-group were attacked (Figs. 5, 8 and 9). When compared with the original macromolecule, sample 3.3 possessed a slight increase of A:B, a decrease of A:Ba and a clear increase of Ba:Bb (Table 3). This was consistent with cases I and V (Fig. 10) in which the attack occurs at either a Ba- or a Bb-chain. In both cases the chain is attached to a Bb-chain that is transformed into a Ba-chain. Thus, it was possible that the very long B-chains (that probably extended over several clusters [5]) were connected to Bb-chains at their reducing end, which presumably was found within a cluster, and that they were not branched before they extended into the next cluster.

The further degradation involved the hydrolysis of the rest of the BL-chains into the chains of intermediate lengths that interconnected smaller sub-units within the clusters (dextrins cII–dIII). This was characterized by a further increase of the ratio of A:B, but also by an increase of A:Ba and decrease of Ba:Bb, which was opposite to the initial stages (Table 3). No single case of attack pattern in Fig. 10 explained this, but certain combinations could produce such results. Thus, any combination of cases II or VI, in

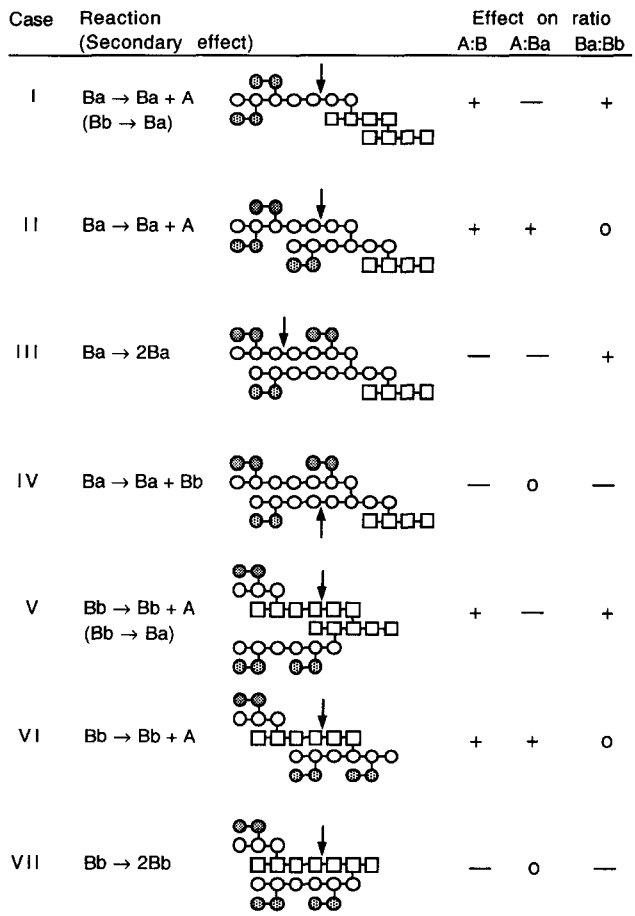

Fig. 10. Possible endo-attack positions (arrows) of alpha-amylolysis of ϕ,β-LD and the effect on the ratios of A:B-, A:Ba-, and Ba:Bb-chains: gray circles represent the maltosyl-stubs of A-chains, white circles Ba-chains, and white squares Bb-chains.

which an A-chain is produced without any secondary effect, with cases IV and VII, that gives a net increase in Bb-chains, was possible. (To give a more clear increasing effect on the A:B ratio, cases II and VI should occur more frequently than cases IV and VII). It was not possible to distinguish between these alternatives, but because the larger dextrins should have an increased amount of Ba-chains as a result of the previous attack, cases II and IV may be more frequent. Thus, several types of interconnection of the smaller dextrins existed. Whether certain types of dextrins possessed specific modes of interconnection was not distinguished experimentally, because all samples were mixtures of dextrins.

In conclusion, the alpha-amylolysis pattern suggested that potato amylopectin contained structurally different domains. The rate of the hydrolysis and the structural characteristics of the dextrins obtained indicated that the ϕ,β-LD of the units of clusters

had a dp of 30–70 and were build up of sub-units interconnected by chains with lengths intermediate to the short and long chains. The characteristic ratios of A- and B-chains obtained by a partial and complete debranching of ϕ,β-LD of the intermediate products of the alpha-amylolysis provided a tool for the investigation of the mode of interconnection of the structural units. Because the constitution of dextrins within the isolated samples was complex, a closer analysis of the details in the structure was not achieved. Future work should therefore focus on the techniques for the isolation of either different potato amylopectins or different domains within the macromolecule.

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