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Investigation of the fine structure of alpha-dextrins derived from amylopectin and their relation to the structure of waxy-maize starch

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ABSTRACT

Alpha-dextrins, obtained by fractional precipitation with methanol of the products of the action of *Bacillus subtilis* alpha-amylase on waxy-maize amylopectin, were debranched with isoamylase and the distributions of the unit chains were analysed by gel-permeation chromatography. The large alpha-dextrins still contained long B-chains after hydrolysis for 60 min, but these were absent from the small dextrins with chain numbers of ~11 or less. The small dextrins contained increased amounts of chains with lengths intermediate of those of the long B-chains and the main part of the short chains. After hydrolysis for 210 min, almost all of the long B-chains had disappeared and the chains with intermediate lengths had been shortened further. The distributions of the unit chains of the internal chains, obtained by debranching of the phosphorolysis (ø)-limit dextrins, gave similar results and showed that the ratio of A- to B-chains was unchanged during the alpha-amylolysis. Models for the fine structure of the intermediate alpha-dextrins are proposed.

INTRODUCTION

A breakthrough in the structural analysis of the starch component amylopectin came with the introduction of debranching enzymes which selectively hydrolyse the (1→6) linkages to give (1→4)-linked chains of α-D-glucopyranose residues that can be fractionated by gel-permeation chromatography. Although the profile of unit chains is characteristic for amylopectins of different origins[1–5], there are important characteristics in common. Thus, the chains can be categorised as short and long chains, which, for waxy-maize amylopectin, have average lengths (c.l.) of 11–20 and 38–50, respectively[1,4–10]. For at least some amylopectins, these main groups can be divided into sub-groups[5,11,12], and reports[3,5,7,9,10] of the molar ratio of short to long chains have varied between 6.4 and 11. The source of the waxy-maize may be an important factor in this variation, since the temperature of the environment affects both the amylose content and the fine structure of the amylopectin in rice[13].

The cluster structure proposed for amylopectin[14–16] has been supported in several reports[12,17–22] and is now widely accepted. In the model, the branches are located in clusters from which short external chains extend as double helices that build up the crystalline regions of the starch granule[19–21,23]. Studies [24] of the trisaccharide panose

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[α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p*] have been extended to the branching points of amylopectin[25] and showed that a left-handed helical structure represents a conformation of low energy. This type of double helix is also found in crystalline amylose[26].

Little is known about the size of the clusters and how they are interlinked. In seeking to answer these questions, the initial stages of the hydrolysis of waxy-maize amylopectin by the alpha-amylase of *Bacillus subtilis* have been investigated[27,28]. The amylolysis involves two independent processes[29], namely, cleavage of the internal chains (endo process), which produces branched alpha-dextrins, and cleavage of the external chains, which yields maltohexaose (exo process). The branched products can be separated from the maltohexaose by precipitation with methanol and subdivided into different sizes by fractional precipitation[30]. The distributions of unit chains in such fractions have now been analysed and the results are discussed in relation to the fine structure of amylopectin.

EXPERIMENTAL

Waxy-maize starch granules (amylopectin, Sigma) were deproteinised and defatted as described[30]. The alpha-dextrins, produced by the action of alpha-amylase of *B. subtilis* [(1→4)-α-D-glucan glucanohydrolase, EC 3.2.1.1; Koch–Light] on waxy-maize starch and precipitated by methanol, were identical to those described[30]. Isoamylase from *Pseudomonas amyloderamosa* (glycogen 6-glucanohydrolase, EC 3.2.1.68) was obtained from Hayashibara Shoji Inc., purified[30] beta-amylase from sweet potato [(1→4)-α-D-glucan maltohydrolase, EC 3.2.1.2] from Sigma, and phosphorylase *a* from rabbit muscle [(1→4)-α-D-glucan:orthophosphate α-D-glucosyltransferase, EC 2.4.1.1] from Boehringer-Mannheim.

Production of phosphorolysis (ø)-limit dextrins. — To boiled samples of either amylopectin or alpha-dextrins (50 mg in 25 mL) were added 1.1M sodium phosphate buffer (2.5 mL, pH 6.8), 2.8mM EDTA (1.25 mL; disodium salt), and a freshly prepared aqueous solution (6.25 mL, 25 U) of phosphorylase. After incubation overnight at room temperature, the mixture was boiled, cooled to room temperature, and centrifuged. To the supernatant solution was added methanol (6 vol.); after 3 h, the precipitate was collected by centrifugation and dissolved in water (5 mL), and phosphate was removed using Sephadex G-25 (PD-10 column; Pharmacia) by elution with water. The limit dextrins were precipitated with ethanol (4 vol.) overnight at 4°, collected by centrifugation, washed with acetone, and air-dried. No α-D-glucose 1-phosphate, analysed as described[29], was produced on treatment of the limit dextrins with phosphorylase, which confirmed that the phosphorolysis reaction had reached the true limit.

Debranching experiments. — The sample (10 mg in 0.65 mL of water) was boiled for 15 min, cooled to 23°, and 0.1M sodium acetate buffer (0.1 mL, pH 3.5) together with an aliquot (0.25 mL, ~300 U) of freshly diluted (x 500) isoamylase were added. The mixture was stirred overnight, boiled, and centrifuged. To an aliquot (0.5 mL) of the supernatant solution was added water (0.5 mL) and 5M KOH (0.1 mL) before analysis by gel-permeation chromatography. The pH of another aliquot (0.3 mL) was adjusted

to 4.8 with 0.2M acetic acid and the volume to 0.565 mL with 0.1M sodium acetate buffer (pH 4.8) before the addition of beta-amylase (35 μL, 1 U/μL). The mixture was stirred for 3 h, 5M KOH (60 μL) was added, and an aliquot (0.5 mL) was analysed by gel-permeation chromatography. Only maltose was found in these control experiments, which confirmed that the debranching was complete.

Gel-permeation chromatography. — Solutions (0.5 mL) of debranched samples were eluted from a column (1.5 x 90 cm) of Sephadex G-50 (fine, Pharmacia) with 0.5M KOH at 1 mL/min. Fractions (1 mL) were analysed for carbohydrates, using the phenol–sulphuric acid reagent[31]. The column was calibrated with debranched amylopectin as described[32], and had a void volume of 48 mL and a total volume of 129 mL.

RESULTS

The alpha-dextrin fractions studied had been characterised partially[29] and some relevant data are listed in Table I. The individual alpha-dextrins in each fraction were detected[30] as peaks in gel-permeation chromatography, and designated dI–dV, cI–cVI, and bI–bV in order of increasing molecular weight. Mixture I contained alpha-dextrins produced from waxy-maize starch by the action of alpha-amylase of *B. subtilis* for 60 min. Fractional precipitation of this mixture with methanol[30] gave sub-fractions 7.1.1–9. Mixture II contained precipitable alpha-dextrins obtained after hydrolysis for 210 min, and gave sub-fractions 11.3–13. Oligosaccharides not precipitated by methanol were obtained as mixture III, and included maltohexaose together with small proportions of branched oligosaccharides of low molecular weight.

TABLE I

Some data for alpha-dextrin samples

Fraction[a]	*Hydrolysis time (min)*	*Composition*[b]	*D.p.*[a]	*Chain No.*[c]	*ø-Limit*[c] *(%)*
Amylopectin	0				47
I	60	bII–cIII			40
7.1.1	60	bV–bIII	1550	98.1	40
7.2.1	60	bI–cVI	625	41.3	37
7.2	60	cVI–cV	515	32.5	41
7.3	60	cV–cIV	325	20.5	40
8.2	60	cIII	191	13.6	39
8.3	60	cII–cI	127	9.1	41
9	60	cII–dV	95	7.1	40
II	210	cII–dV			22
11.3	210	cIII	165	14.8	16
12.1	210	cII	117	10.3	17
12.2	210	dV	75	6.6	23
13	210	dIV	67	6.3	26
III	210	dI	6	0.9	36

[a] See ref. 30. [b] Intermediate alpha-dextrins constituting the main part of the fraction (see ref. 30). [c] From ref. 29.

The distributions of the unit chains of the products obtained after debranching waxy-maize amylopectin with isoamylase are shown in Fig. 1a. Two maxima of c.l. were found in accord with the results of earlier investigations[1,5,6,8–10]. The profile for mixture I showed that the proportion of long chains had decreased after the initial action of the alpha-amylase. Only traces of these chains remained in mixture II and the lengths of the short chains had clearly decreased (Fig. 1a).

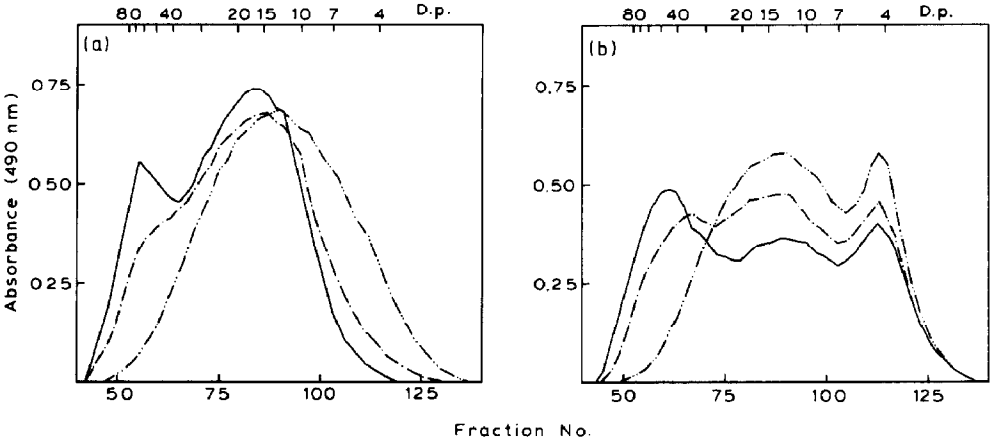

Fig. 1. Distribution of the unit chains of (*a*) waxy-maize amylopectin (———), mixture I (—·—), and mixture II (—··—); and (*b*) their ø-limit dextrins.

ø-Limit dextrins, prepared from amylopectin and mixtures I and II, were debranched with isoamylase. The profile of unit chains of the amylopectin ø-limit dextrin (Fig. 1b) had three major groups with c.l. values that corresponded to those found in beta-limit dextrins[1,6,7,9,10,33]. The maltotetraose peak represented the residual parts of the A-chains[34]*. The remainder of the profile represented the internal parts of the B-chains plus three D-glucosyl residues from their external stubs[29]. These chains could be divided into two heterogeneous main groups, namely, short chains (B1-chains[12]), with c.l. 5–27, and long chains with a peak c.l. of 40–50.

In mixture I, the three major groups of chains were still present, although the maximum c.l. of the long chains had decreased to ~35 (Fig. 1b). The ø-limit dextrins of mixture II contained only traces of long internal chains. The overall internal lengths of the heterogeneous group of short B-chains did not decrease during the alpha-amylolysis.

Some representative chain-distribution curves of the sub-fractions obtained[30] from mixture I are shown in Fig. 2a. Fraction 7.1.1, which contained large alpha-dextrins (Table I), had a distribution of chains that resembled that of the original amylopectin with a peak of long chains at c.l. 60–70. This peak decreased in fractions 7.2.1–8.2, and long chains with c.l. ~45 preponderated in fraction 8.2. Only traces of

* A- and B-chains are (1→6)-linked to B-chains or to the C-chain which carries the sole reducing-end group. A-chains are unsubstituted and B-chains are substituted by other A- or B-chains[35].

long chains were detected in fractions 8.3 and 9. The position of the peak of the short chains at c.l. ~ 15 was unchanged in all sub-fractions, but, in the upper part of the range of these chains, an increase was seen in fractions 8.3 and 9.

The profiles of the unit chains of the ø-limit dextrins of the sub-fractions of mixture I showed a similar trend (Fig. 2b). The position of the peak of the long internal B-chains changed from c.l. 45–50 in fraction 7.1.1 to 30–40 in fraction 8.2. Therefore, these chains were divided into two groups (B2- and B3-chains). At the upper part of the range of short internal B-chains, there was an increase when the size of the ø-limit dextrins decreased, so that, in fractions 8.3 and 9, a new peak maximum at c.l. 24 appeared. These chains are designated B1c-chains. A slight increase of somewhat shorter chains in the range c.l. 15–21 indicated the existence of another group of short

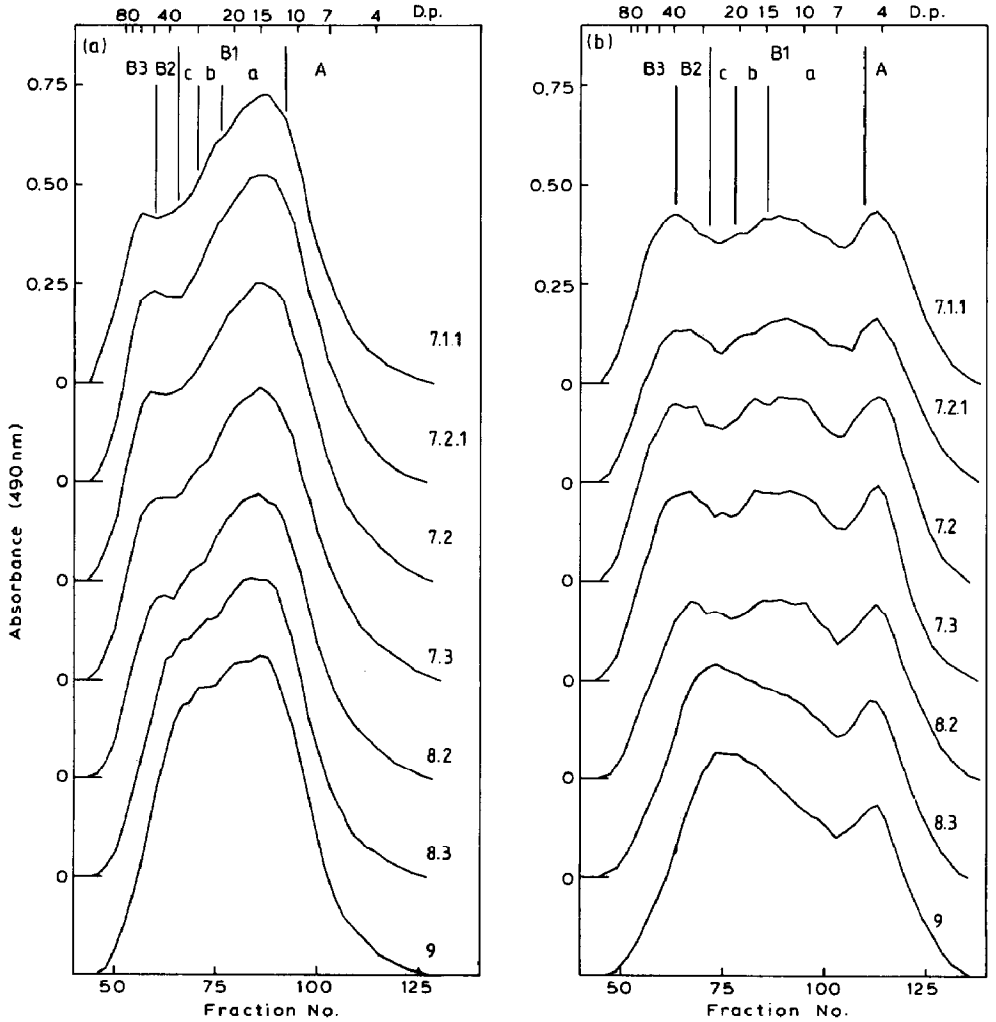

Fig. 2. Distribution of the unit chains of (*a*) samples fractionated from mixture I (see Table I), and (*b*) their ø-limit dextrins. The ranges of different chain types (A–B3) are indicated.

internal B-chains (B1b-chains). Within the third group of these chains (B1a-chains), no differences were detected between the sub-fractions. The concentrations of the A-chains were similar in all of the sub-fractions.

In the sub-fractions of mixture II (Fig. 3a), the larger alpha-dextrins (fraction 11.3) had a relatively broad distribution of c.l., whereas small alpha-dextrins (fractions 12.2 and 13) showed a narrower distribution. The B-chains of the ø-limit dextrins (Fig. 3b) showed distributions similar to those in Fig. 3a, with only a slight decrease in c.l. The smaller ø-limit dextrins in fractions 12.2 and 13 had an increased content of B1b-chains.

The division of the long and short B-chains into sub-groups was also used for the phosphorylase-treated samples in Fig. 1b, and the ranges and average chain lengths are

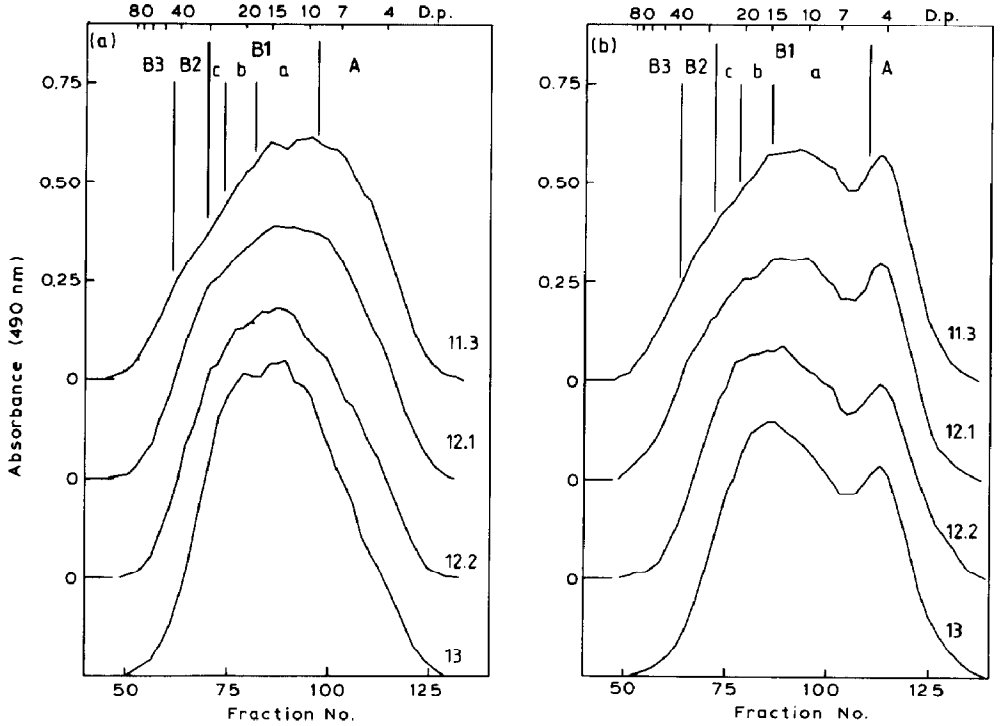

Fig. 3. Distribution of unit chains of (*a*) samples fractionated from mixture II (see Table I), and (*b*) their ø-limit dextrins. The ranges of different chain types (A–B3) are indicated.

given in Table II. The total internal chain length (t.i.c.l.) is defined as the length of a B-chain without its external chain*. The short B1a-chains had, on average, a t.i.c.l. of only 6, whereas the B1b- and B1c-chains were, on average, 9 and 15 residues longer, respectively. B2-chains had a t.i.c.l. of 30 and B3-chains as much as 55. The latter value had decreased in mixtures I and II, whereas the internal lengths of the other chains were the same in the amylopectin and its products of amylolysis.

The relative molar concentrations of individual types of chains had changed

* The t.i.c.l. includes all of the branch-point residues, whereas the average internal chain length (i.c.l.) refers to the segment between two branches and excludes the branch-point residues.

during the alpha-amylolysis (Table II). Originally, the long B2- and B3-chains constituted ~8% of the chains in the amylopectin. However, the proportion of B3-chains may have been overestimated due to overlap of the peak of B2-chains. Only 3% of the long chains remained in mixture II, which had a markedly increased ratio of short to long chains. A moderate increase of the proportions of B1a- and B1b-chains was deteced, whereas those of the A-chains were unchanged. Small differences also occurred within each series of sub-fractions (Table III). Noteworthy was the decrease of the proportions of B3-chains and the increase of those of the B1c- and B1b-chains within the sub-fractions of mixture I (fractions 7.1.1–9).

In addition to larger intermediate alpha-dextrins, the alpha-amylase of *B. subtilis*

TABLE II

Distribution of chain lengths in the ø-limit dextrins

Fraction	*Whole*	*A*	*B1a*	*B1b*	*B1c*	*B2*	*B3*	*A:B*	*S:L*[a]
Amylopectin									
C.l.-range		<5	5–14	15–21	22–27	28–40	>40		
C.l.[b]	11	4	9	18	24	33	58		
T.i.c.l.[c]			6	15	21	30	55		
Mole (%)	100	51	32	6	3	4	4	1.0:1	11:1
Mixture I									
C.l.-range		<5	5–14	15–21	22–27	28–40	>40		
C.l.[b]	10	4	9	18	24	33	55		
T.i.c.l.[c]			6	15	21	30	52		
Mole (%)	100	49	34	7	4	3	3	1.0:1	16:1
Mixture II									
C.l.-range		<5	5–14	15–21	22–27	28–40	>40		
C.l.[b]	9	4	9	18	24	33	48		
T.i.c.l.[c]			6	15	21	30	45		
Mole (%)	100	50	37	7	3	2	1	1.0:1	32:1

[a] Ratio of short (A–B1c) to long (B2–B3) chains. [b] Average chain length calculated from gel chromatograms as $\Sigma A_i/\Sigma(A_i/\text{d.p.}_i)$, in which A_i is the absorbance and d.p._i is the d.p. of fraction i. [c] Total internal chain length defined as c.l. − 3.

TABLE III

Percentage molar distribution of chains in ø-limit dextrins.

Sample	*A*	*B1a*	*B1b*	*B1c*	*B2*	*B3*
7.1.1	53	32	6	3	3	3
7.2.1	52	33	6	3	3	3
7.2	51	34	6	3	3	3
7.3	50	34	6	4	3	3
8.2	51	34	6	4	3	2
8.3	48	34	8	5	3	2
9	48	34	9	5	3	1
11.3	51	36	7	3	2	1
12.1	51	36	7	3	2	1
12.2	51	37	7	3	2	-
13	51	37	8	3	1	-

also produced maltohexaose and a small proportion of branched low-molecular-weight material that was not precipitated by methanol[30]. This material (mixture III), when analysed on Sephadex G-50 (Fig. 4), was found to contain maltohexaose and a range of dextrins up to d.p. ~30. Debranching caused an increase of the peak for maltohexaose and an accumulation of chains with c.l. ~ 10 together with small proportions of chains with c.l. up to 20.

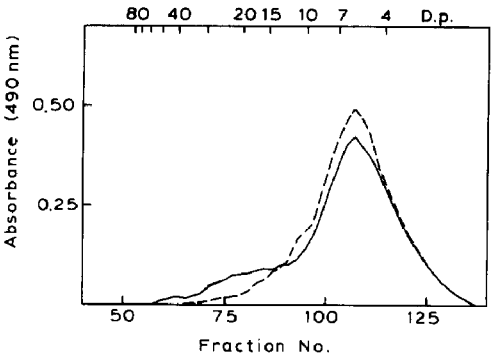

Fig. 4. Gel-permeation chromatography on Sephadex G-50 of mixture III before (———) and after (— — —) debranching.

DISCUSSION

Gel-permeation chromatography on Sephadex G-50 gave only two distinct groups of chains for waxy-maize amylopectin and the products of alpha-amylolysis (Figs. 1a–3a). However, for the series of debranched ø-limit dextrins (Figs. 1b–3b), there were several sub-groups of chains. Although there was probably no sharp distinction between the groups, their c.l. could be estimated approximately (Table II). The short chains (A- and B1-chains) had c.l. $\leqslant 27$, which is comparable to the division of unit chains for β-limit dextrins[6], and the ratio of short to long chains in the amylopectin agreed with those reported[3,5].

Usually, the ratio of A- to B-chains is measured with β-limit dextrins in which the A-chains have been reduced to maltosyl and maltotriosyl stubs[36]. It was concluded for rice amylopectin[37] that the closest branching involved adjacent D-glucosyl residues. As this is probably true for other amylopectins, it follows that, in a β-limit dextrin in which ~ 50% of the B-chains have only one external D-glucosyl residue[36], the shortest possible B-chain is maltotriose which can increase slightly the estimated proportion of A-chains. In a ø-limit dextrin, the external length of the B-chains is 3 D-glucosyl residues[29] and the shortest possible B-chain will be maltopentaose. Therefore, the maltotetraose in Figs. 1b–3b represents solely the A-chains and the ratio of A- to B-chains was estimated to be 1.0:1 (Table II), which accords with reported ratios[10,16].

The large peak of short chains in several amylopectins is composed of two poorly separated peaks[11,12]. Hizukuri[12] assumed that the shorter of these chains were A-chains and termed the somewhat longer chains as B1-chains. Except for an indistinct shoulder

at c.l. 13, there was no indication of two peaks in the profile of unit chains of the waxy-maize amylopectin (Fig. 1a). However, the proportions of A-chains were the same in amylopectin and its ø-limit dextrin, and, if it is assumed that most were shorter than the other chains, then the molar concentration in Table II can be used to calculate the approximate range of c.l. In this way, chains corresponding to c.l. < 14 were found to constitute $\sim 50\%$, and the average c.l. was 9 (Table IV).

Two populations of short B-chains were reported by Mercier[6] in the β-limit dextrin of waxy-maize starch. Three populations are now reported in addition to two populations of long chains. If an assumption is made that is analogous to that for the A-chains, then the approximate ranges for the B-chains before the phosphorolysis can be calculated. The result is shown in Table IV and the ranges are also indicated in Figs. 2a and 3a. The exact value of c.l. for the B3-chains is uncertain because of the difficulty in estimating the shape of the standard curve close to the void volume of the gel[32]. All types of chains were attacked by the alpha-amylase, as shown by the decrease of the values of c.l. in mixtures I and II.

The estimated average c.l. for the different sub-groups of B-chains in the amylopectin is in good agreement with the results obtained by re-chromatography of the fractions from gel-permeation chromatography of debranched waxy-maize starch[32]. The B1b- and B1c-chains of intermediate size were produced during the action of the enzyme and had increased proportions in the smaller dextrins (Figs. 2 and 3). However, as these types of chains also exist in the amylopectin[32], they seem to be characteristic structural components.

When the total length of the internal segment of each type of chain is known from the debranching of the ø-limit dextrins (Table II), the external chain lengths in the amylopectin and the alpha-dextrins can be estimated (Table IV). The values of e.c.l. were remarkably similar for the B1b–B3-chains in the amylopectin and had an average

TABLE IV

Chain lengths in amylopectin and alpha-dextrins

Fraction	*Whole*	*A*	*B1a*	*B1b*	*B1c*	*B2*	*B3*
Amylopectin							
C.l.-range		<14	14–26	27–32	33–39	40–54	>54
C.l.[a]	19	9	18	29	36	46	70
E.c.l.[b]		9	12	14	15	16	15
Mixture I							
C.l.-range		<12	12–22	23–29	30–36	37–49	>49
C.l.[a]	15	7	15	25	32	42	63
E.c.l.[b]		7	9	10	11	12	11
Mixture II							
C.l.-range		<9	9–18	19–24	25–30	31–44	>44
C.l.[a]	11	6	11	20	27	35	50
E.c.l.[b]		6	5	5	6	5	5

[a] Average chain length calculated from gel chromatograms as $\Sigma A_i/\Sigma(A_i/\text{d.p.}_i)$, in which A_i is the absorbance and d.p._i is the d.p. of fraction i. [b] External chain length calculated as c.l. − t.i.c.l., in which t.i.c.l. is the total internal chain in Table II.

of 14–16. B1a-chains had an e.c.l. that was intermediate of those of the other B- and A-chains (the whole chain is external in the latter). Overall, the results support the suggestion[10] that all of the chains in amylopectin have similar external segments. In mixture I, all of the external chains had been reduced by 2–4 residues. As maltohexaose is produced by fission of external chains[29,38], this result indicated that ~50% of these chains had been attacked after alpha-amylolysis for 60 min. After further alpha-amylolysis (mixture II, 210 min), all of the external chains had been reduced to an average of 5–6 residues.

It was suggested[29,30] that the intermediate alpha-dextrins are characterised by a constant number of chains. If this is true, then the distribution of c.l. for the internal chains should be identical for an alpha-dextrin regardless of the duration of the alpha-amylolysis. Fraction 8.2, obtained after alpha-amylolysis for 60 min, contained mostly the intermediate product cIII (Table I), and the same dextrin was obtained in fraction 11.3 after 210 min. Therefore, the ø-limit products of these two fractions would be expected to have similar or identical distributions of c.l. However, a comparison of the profiles in Figs. 2b and 3b shows that they are different, the proportion of long B-chains being much lower in fraction 11.3. A similar result was obtained for fractions 8.3 and 9, which contained alpha-dextrins cII–dV, and fractions 12.1 and 12.2 in which the proportion of B1c-chains had been reduced during the alpha-amylolysis. The sub-fractions were heterogeneous and contained alpha-dextrins of different sizes[30]. In Table I, only those dextrins that constituted the main part of the fractions are listed. The minor components will influence the profile, but it is unlikely that this could cause the large differences between the sub-fractions. Therefore, the alpha-dextrins in fractions 8.2, 8.3, and 9 were not identical to those in fractions 11.2, 12.1, and 12.2. The question arises as to whether alpha-dextrins produced early in the alpha-amylolysis have structures that are completely different from those obtained at later stages, or whether a minor change, including a loss of one or a few branches, results in a relatively large change in the profile of the internal chains. On the basis of the pattern of hydrolysis of amylopectin[27,28,30], the latter alternative seems more probable despite a slight decrease in the chain number.

In Fig. 5a a structure for the waxy-maize amylopectin is suggested that contains clusters composed of A- and B1a-chains, of which the external parts participate in the crystalline regions of the starch granules[19–20,23] in accordance with other cluster models[12,15]. The total length of a cluster equals the length of the B1a-chains of ~18 D-glucosyl residues. A clue to the density and arrangement of the branchings within the clusters comes from studies of small oligosaccharides[37,39,40]. The clusters are inter-linked by B1b-chains and longer chains. B3-chains extend into 4 clusters that correspond to a length of ~70 residues. These chains are linked to the non-reducing end segments of the B2-chains. The position of this branching may correspond to the small proportion of branching points near to the non-reducing ends in Nägeli amylodextrins reported by Umeki and Kainuma[23]. The B2-chains, in turn, are linked to a cluster of short chains near the ends of the B3-chains and extend into ~2.5 clusters. The B1c-chains are linked to the inner segments of the long B2- and/or B3-chains and extend into 2 clusters.

Alternatively, the B1b-chains are linked to B1a-chains in clusters in these regions and extend into 1.5 clusters, and the overall concentration of B1b- and B1c-chains will equal that in Table II.

The structure in Fig. 5a is regular and may be considered as built up of larger units. One such (marked) unit has the size and chain number that corresponds to alpha-dextrin bII (Tables I and V), characterised[28] as a "super-cluster". This large unit is built up of smaller units that contain 19 (dextrin cIV) or 26 chains (dextrin cV). In order to show the regularity of the proposed structure, a more schematic overview is drawn in Fig. 5b. In the large unit, dextrin cV is linked to two cIV units, which is a slight modification of the structure proposed[28] in which each cIV unit binds two other identical units (inset).

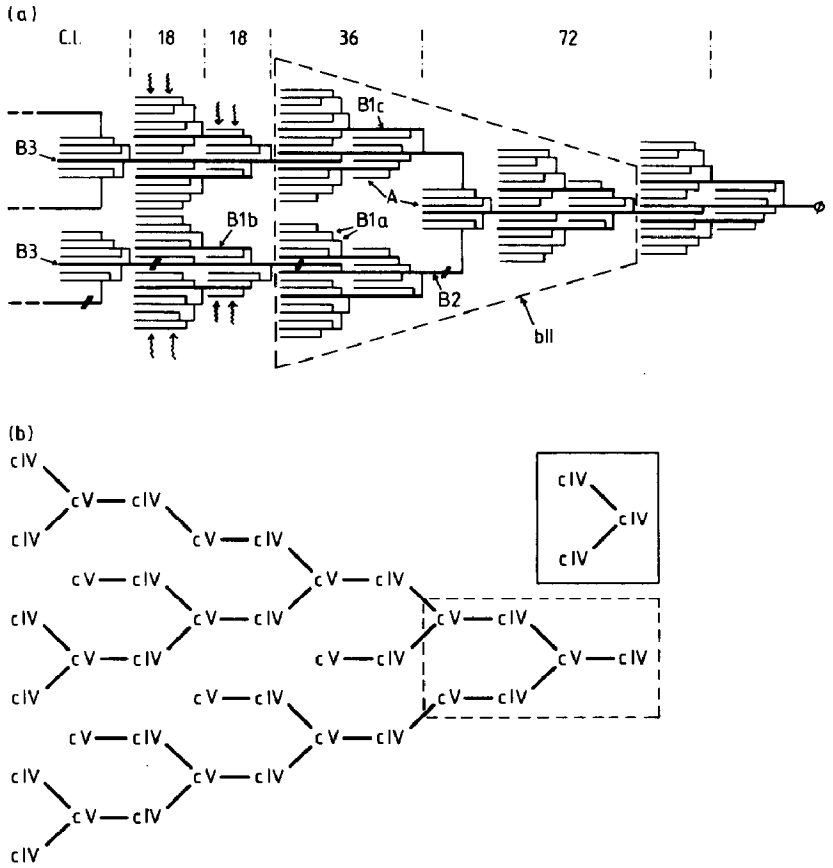

Fig. 5. Proposed structure for waxy-maize amylopectin. (*a*) Units of clusters built up of A- and B1a-chains (———) are interconnected regularily by longer B-chains (▬▬). A larger structural unit of the size of alpha-dextrin bII is indicated. Endo-attack (↙) by the *B. subtilis* alpha-amylase occurs preferentially on long internal-chain segments. Exo-attack (↓) occurs simultaneously on external chains: |, (1→6) linkage; Ø, D-glucosyl residue at the reducing end. (*b*) The same structure drawn with the symbols for intermediate alpha-dextrins, and a comparison with the structure proposed earlier[28] (inset). The part of the structure detailed in (*a*) is indicated.

The alpha-amylase of *B. subtilis* contains 9 subsites that are distributed on both sides of the active site[38,41]. Preferential endo-attack probably involves longer internal chains where all of the subsites will be occupied. Some of these sites are marked in Fig. 5a, and complex mixtures of alpha-dextrins will be produced during the hydrolysis. Simultaneously with the endo-attack, maltohexaose is produced from the external chains[29]. After hydrolysis for 60 min, the mixture of products contained mostly alpha-dextrins cIV–bI[30] of intermediate size, the structures of which are proposed in Fig. 6 and which are the main products. Attack at other internal chains can result in the formation of dextrins of intermediate compositions and sizes that are not detected readily in gel-permeation chromatography, in addition to the smaller alpha-dextrins that are produced simultaneously[27,28]. The characteristics of the structures are shown in Table V. The number of individual types of chains is based on the concentrations given in Table III and on the chain number proposed for each dextrin[28]. Other important parameters are compared with those found by enzymic analysis[29]. The estimated values of c.l. are somewhat lower than those reported earlier and are caused probably by the calibration of the column used in gel-permeation chromatography[32].

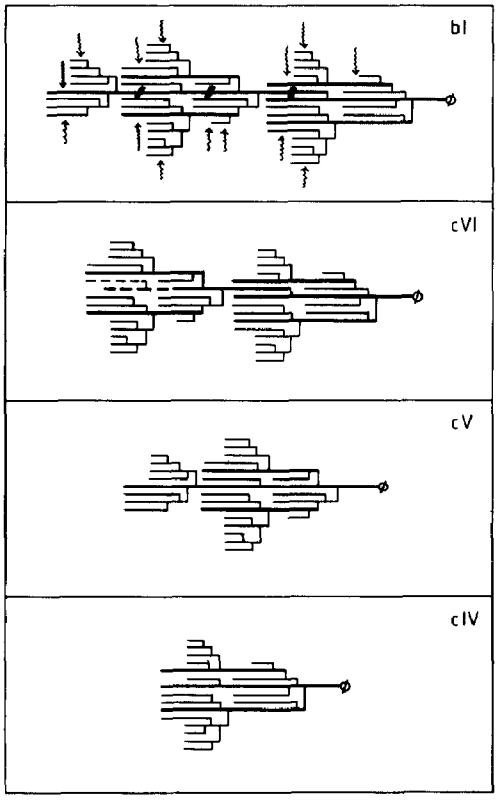

Fig. 6. Proposed structures for alpha-dextrins obtained by the action of *B. subtilis* alpha-amylase on amylopectin for 60 min. Endo-attack at the sites indicated on the internal chains in dextrin bI gives the smaller dextrins. Dotted chain segments are alternative structures. The symbols are as in Fig. 5.

TABLE V

Characteristics[a] of proposed alpha-dextrin structures obtained after 60 min of hydrolysis

Parameter	*bII*	*bI*	*cVI*	*cV*	*cIV*
No. of B3	1	1	0	1	0
B2	2	1	1–2	0	1
B1c	2	1	1–3	0–1	0–1
B1b	4	3	2–3	1–2	1–2
B1a	21	15	12	9	6
A	32	23	18–19	14	10
Chain No.	62	44	36.5	26	19
	(58)	(45)	(36)	(26)	(19)
A:B	1.1:1	1.1:1	1.0:1	1.2:1	1.1:1
S:L[b]	20:1	21:1	23:1	25:1	18:1
D.p.[c]	855	603	499	348	256
β-D.p.[c,d]	468	330	270	189	138
	(475)	(370)	(295)	(211)	(151)
β-Limit (%)	45	45	46	46	46
	(47)	(47)	(48)	(48)	(47)
C.l.[e]	13.8	13.7	13.7	13.4	13.5
	(15.7)	(15.7)	(15.8)	(15.8)	(15.8)
E.c.l.[e]	8.3	8.2	8.3	8.2	8.2
	(9.9)	(9.4)	(9.5)	(9.5)	(9.5)
I.c.l.[e]	4.6	4.9	4.6	4.4	4.6
	(5.4)	(5.4)	(5.5)	(5.5)	(5.6)

[a] Numbers in paranthesis are the experimental values reported[28,29]. [b] Ratio of short (A–B1c) to long (B2–B3) chains. [c] Based on the length of individual chain types in mixture I (Tables II and IV). [d] Degree of polymerisation of the theoretical β-limit dextrin in which the length of a B-chain is t.i.c.l. + 1.5 and the length of an A-chain is t.i.c.l. + 2.5. [e] The average length of a chain segment between two branches calculated as in ref. 29.

After hydrolysis for 210 min, the alpha-dextrins in mixture II possess the structures shown in Fig. 7. In dextrins cIV, cIII, and cII, the continous shortening of the external B2-chain by-passes the branching point and the remainder of the chain is identical to a B1b-chain. This explains the difference in the profiles of the unit chains of the dextrins at different times of alpha-amylolysis (Figs. 2 and 3). Dextrin cIV is built from the units dIV, dV, and cI as suggested[28]. Combination of the dextrins dIV and dV will give cII, whereas cIII is composed of dV and cI. The characteristics of the structures are given in Table VI.

The structures proposed are intended to represent an average of closely related alpha-dextrins. Small differences in the chain numbers are probable, as indicated in Figs. 6 and 7. Because of the exo-attack of the enzyme, there will also be a range of dextrins of different sizes, depending on the lengths of the external chains at a particular time during the hydrolysis. For example, fraction 13, which contained mostly dextrin dIV, included a range of chains with different lengths (Fig. 3a) although this dextrin contained only ~ 5 chains.

Small alpha-dextrins may be produced slowly from the densely branched areas in the clusters. Probably, these include the dextrins not precipitated by methanol and obtained in mixture III together with maltohexaose (Fig. 4). The smallest single-

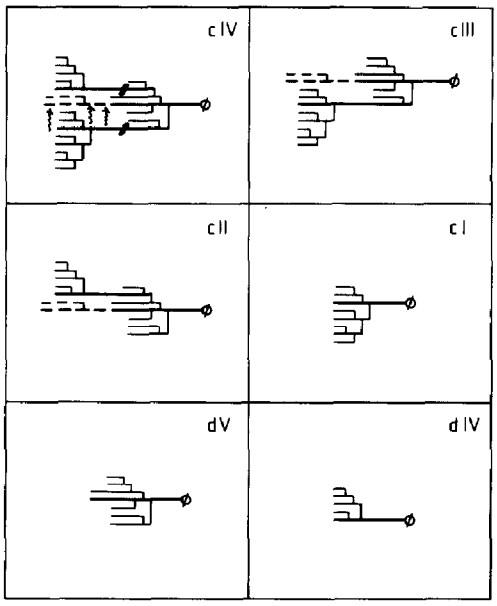

Fig. 7. Proposed structures for alpha-dextrins obtained by the action of *B. subtilis* alpha-amylase on amylopectin for 210 min. Endo-attack at the sites indicated on the internal chains in dextrin cIV gives the smaller dextrins. Dotted chain segments are alternative structures. The symbols are as in Fig. 5.

branched dextrin produced by the alpha-amylase is 6^2-α-maltosyl-maltotriose[42,43], which was isolated[43] in a yield of ~1.3% after extensive hydrolysis of waxy-maize starch. It is tempting to speculate that this oligosaccharide was produced mainly from the single (1→6) linkages on the B2- and B3-chains (Fig. 5a) which, from the structure proposed, would result in yields of that order.

The most important features of the structure proposed are that (*a*) the amylopectin contains 6 types of chains of characteristic lengths, (*b*) the A-chains have not more than ~15 D-glucosyl residues, (*c*) the external segments of the B-chains contain 12–16 residues (short external chains do not exist), (*d*) internal segments longer than ~18 residues are not found (which may indicate that the elongation of a chain will stop at this length; if not, a branch is introduced), and (*e*) units of clusters with 5–8 chains are regularly interconnected by longer chains.

As the profiles of the chains of most amylopectins are similar, the main features of the structure are probably general. Differences between amylopectins could then be found mainly in the size of the unit clusters and in the mode of their interconnection.

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TABLE VI

Characteristics[a] of proposed alpha-dextrin structures obtained after 210 min of hydrolysis

Parameter	*cIV*	*cIII*	*cII*	*cI*	*dV*	*dIV*
No. of B3	0	0	0	0	0	0
B2	0–1	0–1	0–1	0	0	0
B1c	1	1	0	0–1	0	0
B1b	1–2	0–1	1–2	0–1	1	1
B1a	6	5	4	3	3	2
A	9–10	7–8	5–6	4–5	3	2
Chain No.	18.5	14.5	11.5	8.5	7	5
	(19)	(14)	(11)	(8)	(7)	(5)
A:B	1.1:1	1.1:1	0.9:1	1.1:1	0.8:1	0.7:1
S:L[b]	36:1	28:1	22:1	-	-	-
D.p.[c]	198	155	125	84	71	54
β-D.p.[c,d]	132	103	84	53	47	37
	(151)	(106)	(82)	(58)	(45)	(35)
β-Limit (%)	33	34	33	37	35	32
	(30)	(30)	(31)	(33)	(37)	(40)
C.l.[c]	10.7	10.7	10.8	9.9	10.1	10.8
	(11.0)	(11.2)	(11.4)	(11.3)	(11.4)	(10.6)
E.c.l.[c]	5.5	5.6	5.6	5.7	5.5	5.5
	(5.3)	(5.4)	(5.6)	(5.7)	(6.3)	(6.2)
I.c.l.[c]	4.5	4.5	4.7	3.8	4.4	5.6
	(5.0)	(5.2)	(5.4)	(5.4)	(5.0)	(4.2)

[a] Numbers in paranthesis are the experimental values reported[28,29]. [b] Ratio of short (A–B1c) to long (B2–B3) chains. [c] Based on the length of individual chain types in mixture II (Tables II and IV). [d] Degree of polymerisation of the theoretical β-limit dextrin in which the length of a B-chain is t.i.c.l. + 1.5 and the length of an A-chain is t.i.c.l. + 2.5. [e] The average length of a segment between two branches calculated as in ref. 29.

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