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Note

Chains of intermediate lengths in waxy-maize amylopectin

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According to the cluster model[1–3], amylopectin, the branched component of starch, comprises chains of (1→4)-linked α-D-glucopyranosyl residues inter-connected by (1→6) linkages in close groups. It has been shown by gel-permeation chromatography that debranching of amylopectins characteristically gives[4–11] short and long chains with average chain lengths (c.l.) of 11–25 and 40–60 residues, respectively. In several amylopectins[11–13], the short chains may be divided into two poorly separated groups with c.l. 11–13 and 18–19. The former group probably represents A-chains and the latter group short B-chains (B1-chains)[14]; A-chains do not carry other chains, whereas B-chains carry A- or B-chains[15]. Hizukuri[14] reported a polymodal distribution of the unit chains in many amylopectins in which groups of long B-chains exist. These so-called B3- and B4-chains have c.l. ~70 and ~110, respectively, and were suggested to extend into three or more clusters, whereas the major group of long chains (B2-chains) extends into two clusters. Small proportions of long chains have been reported by other workers[16,17]

When waxy-maize amylopectin was debranched with isoamylase, gel-permeation chromatography of the products on Sephadex G-50 (Fig. 1) gave two major peaks. The column was calibrated by the method of Mercier and Whelan[18] in which the average d.p. in each fraction was determined as the ratio between the total carbohydrate and the content of reducing-end residues. Determination of the latter values by the Nelson reagent for fractions of higher d.p. requires large amounts of the debranched sample[18]. However, even when the concentration of the sample was increased from 5 to 10 mg/mL and the volume from 0.5 to 2 mL, it was difficult to determine reproducibly d.p. values of >30.

The standard curve (Fig. 2) shows a linear relation between log d.p. and K_{av} 0.2 and 0.8. The calibration at higher d.p. could be estimated only approximately. Based on this calibration, the profiles of the unit chains (Fig. 1) were used to calculate the c.l. of the amylopectin [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]. Similar profiles of the unit chains and values of c.l. were obtained with two concentrations of the sample applied (Table I). However, the values of c.l. were lower than those determined from the concentration of reducing-end groups in the debranched sample prior to gel-permeation chromatography and those based on the

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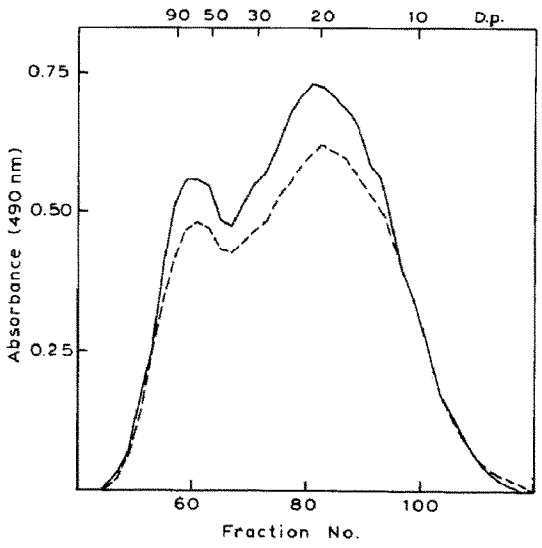

Fig. 1. Distribution of the unit chains after debranching of waxy-maize amylopectin with isoamylase by gel-permeation chromatography on Sephadex G-50; samples used were 2.5 (———) and 20 mg (— — —).

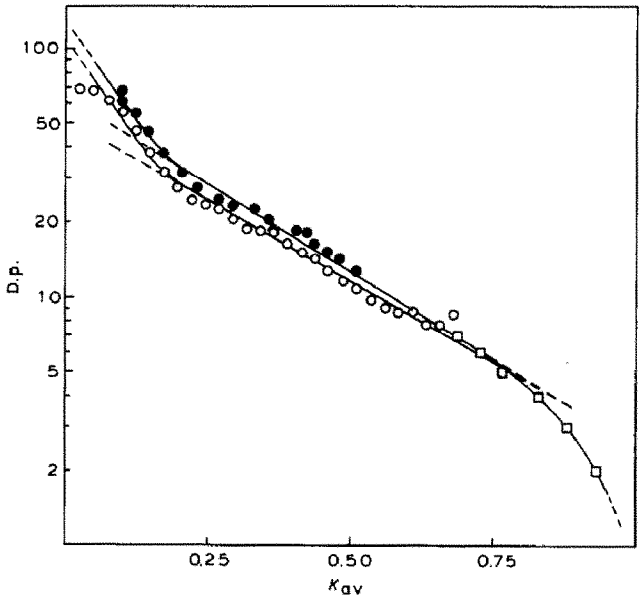

Fig. 2. Calibration curves for Sephadex G-50. The d.p. was determined by reducing-end group analysis of the fractions of a sample of debranched amylopectin. K_{av} is the partition coefficient defined as $V_e - V_o/V_t - V_o$, where V_e is the elution volume of the sample, V_o is the void volume, and V_t is the total volume: K_{av} was calculated from the fraction number (○, curve 1), or from the volume at which 50% of the dextrins in the fractions were eluted (●, curve 2); □, commercial dextrins.

proportion of non-reducing units. These findings indicated that the calibration of the gel was incorrect.

Every second fraction from an identically debranched and fractionated amylopectin sample (using the higher applied concentration) was then re-chromatographed on Sephadex G-50. As seen in Fig. 3, each fraction was eluted within a large range of volumes, which indicated heterogeneity with respect to c.l. Using the standard curve in Fig. 2, the molar distributions of the chains in the fractions were calculated and the volumes at which 50% of the chains had been eluted (examples given in Fig. 4) were used as a new value for the partition coefficient. The molar-based standard curve thus obtained (Fig. 2) was steeper than that based on the original fraction number and increased the value of c.l. by two D-glucosyl residues when estimated from the profile of the distribution of chains in Fig. 1. This value was in better agreement with those measured by the other methods (Table I).

The shape of the profiles of the distribution of chains of the re-chromatographed fractions that contained shorter chains indicated a nearly Gaussian distribution. How-

TABLE I

Comparison of the chain length (c.l.) of waxy-maize amylopectin obtained by different methods

Method[a]	*A*	*B*	*C*	*D*	*E*	*F*	*G*
c.l.	18.7	18.2	20.7	20.1	20.2	21.3	21.7

[a] *A*, Based on the profile of unit chains obtained by gel-permeation chromatography of a 2.5-mg sample and calculated with standard curve 1 (Fig. 2); *B*, as in *A* after chromatography of a 20-mg sample; *C*, as in *A* but calculated with standard curve 2 (Fig. 2); *D*, as in *C* after chromatography of a 20-mg sample; *E*, based on the proportion of reducing-end residues after debranching; *F*, based on the proportion of non-reducing end residues determined by Smith degradation[26]; *G*, based on enzymic analysis of non-reducing end residues[26].

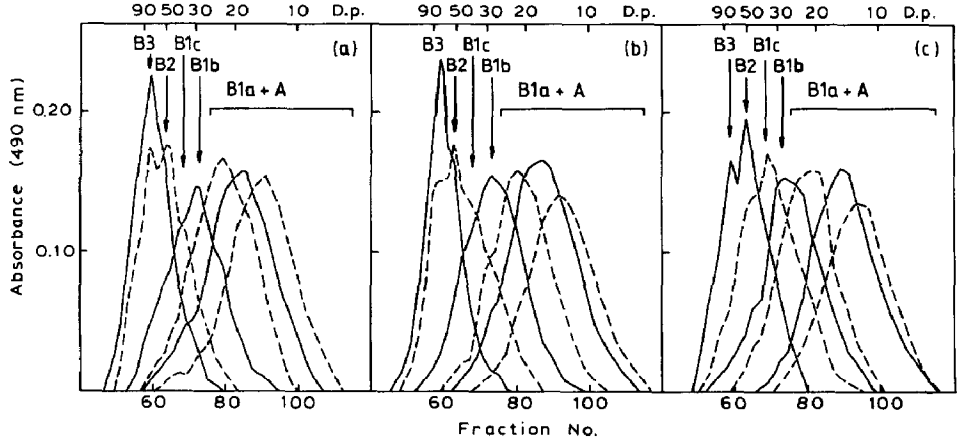

Fig. 3. Re-chromatography of fractions from Fig. 1 on Sephadex G-50. (a) fractions 57, 63, 69, 75, 81, and 87; (b) fractions 59, 65, 71, 77, 83, and 89; (c) fractions 61, 67, 73, 79, 85, and 91. A–B3 indicate the different types of chains.

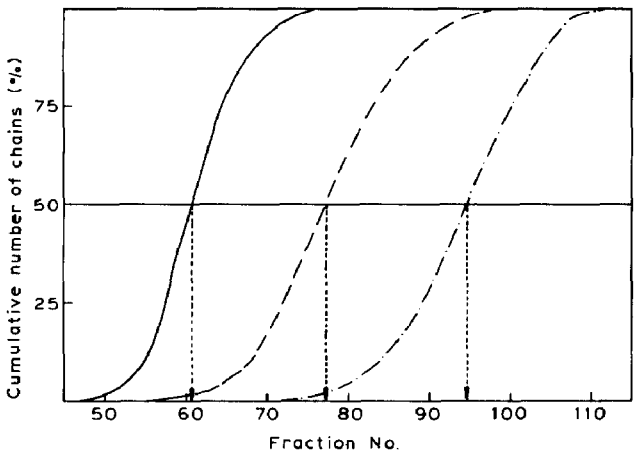

Fig. 4. Molar distribution of the unit chains in re-chromatographed fractions 59 (——), 73 (— — —), and 91 (—·—) (see Fig. 3). The volume at which 50% of the chains had eluted is indicated.

ever, the profiles of the fractions that contained longer chains possessed shoulders and peaks which indicated that groups of chains with more specific lengths were included (Fig. 3). Fractions 57–65, which contained the long chains of the amylopectin, comprised two distinct groups. In Fig. 1, these groups were not separated and were represented by a single, comparatively broad peak. One group had c.l. 70–75, although the exact value was difficult to estimate because of the uncertain shape of the standard curve close to the void volume. Presumably, these chains were B3-chains[14]. Akai *et al.*[4], who used Sephadex G-75, also found small proportions of chains of this length in waxy-maize starch. The other group of long chains (B2-chains) had c.l. ~46 which corresponded to the values 38–50 normally reported for waxy-maize starch[4,5,11,19–21].

The profiles of the unit chains of fractions 67–73 (Fig. 3) were also irregular. These fractions contained chains that corresponded to the upper part of the main group of short chains with c.l. 27–40. Two groups of chains with average c.l. of 36 and 29 were indicated within this range. Thus, the short B-chains (B1-chains) seemed to be composed of sub-groups, although, probably, there was no sharp distinction. The longer of these chains (B1c-chains), which could be seen as shoulders in fractions 63 and 65, was not detected in the profile of the unit chains of amylopectin (Fig. 1). In fact, they corresponded to the trough between the two main peaks. The length of B1b-chains corresponded to the position of a small shoulder on the chromatograms.

The third group of short B-chains (B1a-chains) had c.l. < 27. A small shoulder at c.l. ~13 (Fig. 1) may indicate the fraction of A-chains, but the re-chromatographed fractions that contained these small chains did not show any distinct groups. This finding indicated that the distinction between the lengths of A- and B1a-chains was diffuse.

When the linear chains from debranched amylopectin were fractionated by precipitation with methanol, the yields and d.p. of the fractions shown in Table II were obtained. Gel-permeation chromatography of the fractions showed a heterogeneous

distribution of chains (Fig. 5). Fraction 0.5:1 (methanol–water) was considered to contain retrograded material because it was eluted almost quantitatively in the void volume (not shown). Fraction 1:1 contained mostly long chains with a peak at c.l. 70–75, which corresponded to the long B3-chains. Fraction 2.5:1 possessed a peak at c.l. 45–55, which corresponded to the length of the B2-chains. However, a large part of the material in these fractions had short chains which explained the low d.p. of only 47 and 35, respectively (Table II).

The fraction of chains precipitated with 4:1 methanol–water was eluted in a volume intermediate of those of the long chains and the major part of the short chains (Fig. 5). The major part of the material corresponded to the length of B1c-chains, whereas a broad shoulder indicated the group of B1b-chains.

TABLE II

Data for methanol-precipitated dextrins

Methanol–water ratio	*Yield (%)*	*Carbohydrate content (%)*	*D.p.*
0.5:1	2.3	n.d.[a]	n.d.
1:1	11.4	88.1	47
2.5:1	8.7	87.0	35
4:1	9.4	87.1	31
6:1	33.5	88.6	19
12:1	1.0	n.d.	n.d.

[a] Not determined.

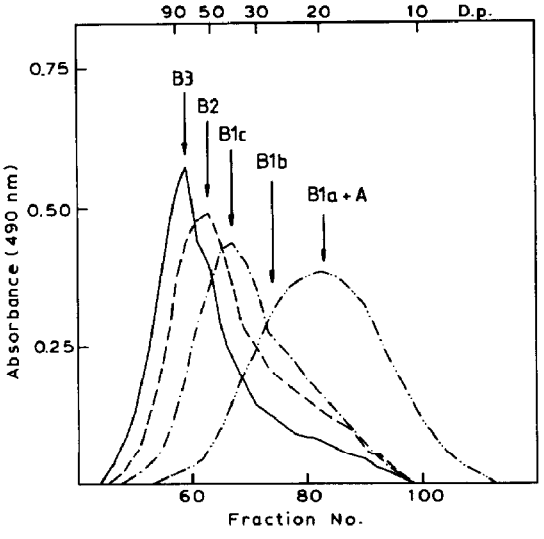

Fig. 5. Profiles on Sephadex G-50 of the distribution of chains of dextrins precipitated from debranched amylopectin at methanol–water ratios of 1:1 (——), 2.5:1 (— — —), 4:1 (—·—), and 6:1 (—···—). A–B3 indicate the different types of chains.

Fraction 6:1 possessed a nearly Gaussian distribution of chains with no sign of individual peaks. With 12:1 methanol–water, only traces of carbohydrates precipitated and the total yield of precipitated material was ~66%. Thus, a large part of the short chains could not be precipitated and, presumably, these included most of the A-chains.

It is concluded that the short B-chains of waxy-maize amylopectin are composed of sub-groups which can be divided into a major group of B1a-chains with c.l. < 27 and two minor groups (B1b- and B1c-chains) with c.l. ~29 and ~36, respectively. Chains with intermediate lengths (B1b- and B1c-chains) were also found in branched dextrins obtained by the action of the alpha-amylase of *B. subtilis* on waxy-maize starch, and the significance of these chains to the fine structure of amylopectin is discussed elsewhere[22]

EXPERIMENTAL

Debranching of amylopectin. — Waxy-maize starch granules (amylopectin, Sigma) were deproteinised and defatted as described[23]. An aqueous suspension of the granules (5 mg in 0.65 mL) was gelatinised by boiling 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 (× 500) isoamylase from *Pseudomonas amyloderamosa* (glycogen 6-glucanohydrolase, EC 3.2.1.68; Hayashibara Shoji Inc.) were added. The mixture was stirred overnight and then treated with 5M KOH (0.1 mL) to stop the reaction. In some experiments, the amount of starch granules was increased to 20 mg and all volumes were doubled. The completeness of the debranching was confirmed by using beta-amylase as described[22].

Linear dextrins. — (*a*) *Quantitative production.* Amylopectin was debranched as described above, with the following modifications. The starch granules (500 mg in 90 mL) were gelatinised for 1 h, the buffer volume was 10 mL, and the isoamylase (50 μL, ~30 000 U) was added undiluted. After 24 h, the mixture was heated at 100° for 15 min, then cooled slowly to room temperature, before the addition of 5M KOH (10 mL). The solution was stirred gently for 2 h, traces of undissolved material were removed by centrifugation, and the pH was adjusted to ~11 with M HCl.

(*b*) *Fractional precipitation.* The details of the method have been described[23]. To an aqueous solution of the dextrins prepared in (*a*) was added methanol to a methanol–water ratio of 0.5:1. The mixture was stored for 45 min, and the precipitate was collected by centrifugation, washed twice with acetone, and air-dried. The methanol–water ratio of the supernatant solution was adjusted to 1:1, and the procedure was repeated with ratios of 2.5:1, 4:1, 6:1, and 12:1.

Prior to gel-permeation chromatography, the dextrin samples were dissolved in 0.5M KOH (2 mg/mL). The d.p. was calculated as the molar concentration of total carbohydrate/reducing-end residues. After neutralisation with 0.5M HCl, the total carbohydrate concentration was determined by use of the phenol–sulphuric acid reagent[24] and reducing-end residues by the Nelson method[25].

(*c*) *Gel-permeation chromatography.* Solutions (0.5 mL) of debranched amylopectin and of methanol-precipitated dextrins were eluted from a column (1.5 × 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[24]. The column was calibrated by applying an increased volume (2 mL) that contained ~20 mg of debranched amylopectin, and the fractions were neutralised with 0.5M sulphuric acid prior to analysis of total carbohydrate and reducing-end residues. The void volume (fraction 53) and the total volume (fraction 135) were determined as described[23].

When fractions were re-chromatographed, an aliquot (0.1 mL) was taken for carbohydrate analysis and the remainder (~0.9 mL) was applied to the column.

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