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Note

Polymodal distribution of the chain lengths of amylopectins, and its significance

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Amylopectin is a branched polysaccharide composed of thousands of short (1→4)-α-D-glucan chains linked to each other by α-(1→6) linkages. Several models have been proposed for its molecular structure[1,2], the cluster models presented by Nikuni[3] and French[4] appearing to be accepted as the most probable[1,2,5–8].

Hizukuri[9] has characterised the distributions of the chain lengths (c.l.) of amylopectins from 20 species by gel chromatography with monitoring with a low-angle laser-light-scattering photometer and a differential refractometer. This technique allows the weight-average molecular weights of any particular part, or at any point, of the elution to be determined directly[9–11]. The distribution profiles showed two peaks (corresponding to c.l. values of 13–21 and 37–50) for most species, as observed by others[1,2,7,12,13], but three peaks for amylopectins from wheat, tapioca, and tulip. However, a trimodal or a more polymodal distribution was thought to be normal for amylopectins, because the chain lengths were expected to have a periodicity corresponding to the distance between adjacent linked clusters. To find the periodicity, the distributions of chain lengths of some common amylopectins were re-investigated by h.p.l.c.

The resolution on h.p.l.c. was improved by using Toyo Soda SW-series gel-columns (Fig. 1). Tapioca amylopectin, which showed a trimodal distribution curve with PW-series gel-columns[9], showed a tetramodal distribution profile. In addition, potato and kuzu[14] amylopectins also showed similar tetramodal, instead of bimodal, distribution profiles (Figs. 2 and 3). The distribution curve for waxy-rice amylopectin (Fig. 4) showed three distinct peaks with a slight shoulder on the shorter-retention-time side of the major peak, suggesting a tetramodal distribution similar to that in the other species. These elution curves could be divided into four peaks and one tailing fraction (first eluted) as shown in Figs. 1–4, although the boundaries are somewhat arbitrary due to overlapping of the peaks, and the fractions were designated as A, B1, B2, B3, and B4 in reverse order of elution. These distribution profiles indicate that the previous[9] fraction F1 was composed of fractions B2, B3, and B4, and that fraction F2 contained fractions A and B1. The chain lengths at the

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maxima of these peaks were similar or identical to the weight-average chain lengths of the fractions, probably due to the narrow distribution of molecular sizes in each fraction. These values, together with weight and molar fractions, are listed in Table I. These distribution properties are distinctive for different species and, in general, the amounts rather than the c.l. of these fractions seem to be significant factors for discrimination of the species. Several specimens from different varieties of rice were found to have similar distributions with large amounts of fraction A. Studies of these species characteristics are continuing.

The distribution characteristics of the chain lengths (Figs. 1–4, and Table I) are inconsistent with the Haworth, Staudinger, or Meyer models, but consistent with the cluster models of Nikuni[3] and French[4] which have been supported by other investigators[5–8]. The general features of these models are that amylopectin is composed of compact parts of oriented chains (clusters) which are randomly or somewhat regularly branched, and that the clusters are linked by long chains which

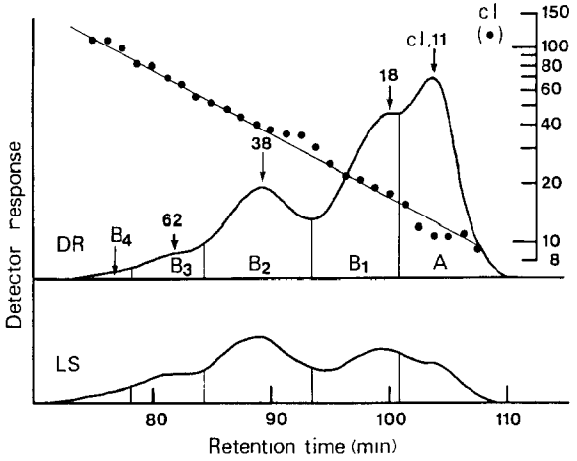

Fig. 1. Gel-permeation h.p.l.c. of tapioca amylopectin: LS, low-angle laser-light-scattering photometer; DR, differential refractometer.

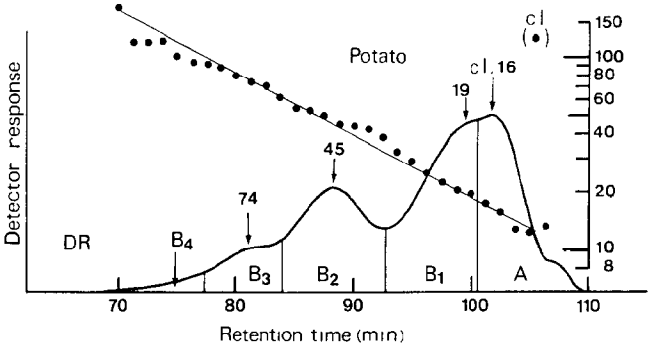

Fig. 2. Gel-permeation h.p.l.c. of potato amylopectin.

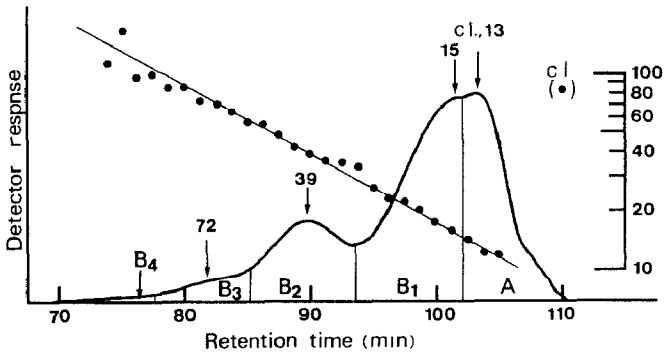

Fig. 3. Gel-permeation h.p.l.c. of kuzu amylopectin.

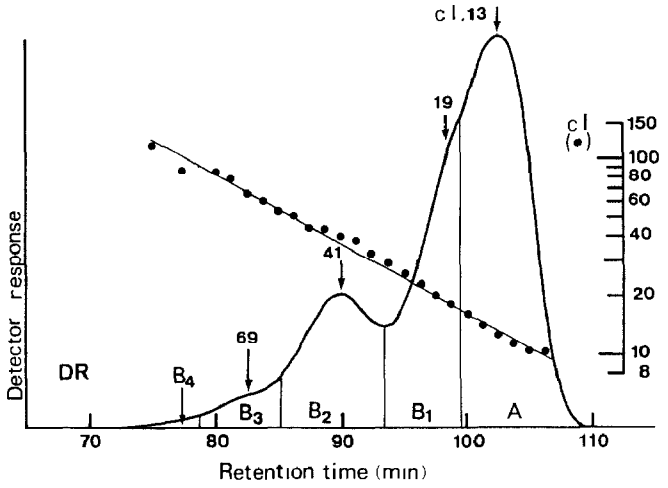

Fig. 4. Gel-permeation h.p.l.c. of waxy-rice amylopectin.

extend into two or more clusters. Consequently, the chain lengths are distributed in a polymodal manner with periodic peaks at multiple lengths between two adjacent clusters. The profiles shown in Figs. 1–4 reveal such periodical distributions and can be interpreted according to the cluster structure models with the following assumptions: (*a*) fractions A and B1–B4 are the A- and B-chains, respectively, which bind at C-6 of the other chains through their reducing residues (the A-chains carry no chains and the B-chains carry the A- or other B-chains[15]); (*b*) the chains in fractions A and B1 make a single cluster; (*c*) the chains in fractions B2 and B3 extend into 2 and 3 clusters, respectively, and the chains in fraction B4 stretch across more than 4 clusters.

The average chain-lengths of fractions B1, B2, and B3 of the four specimens are in the ranges 20–24, 42–48, and 69–75, respectively, the relative lengths being ~1:2:3, and, thus, the length between adjacent linked clusters can be inferred to

TABLE I

DISTRIBUTION OF THE CHAIN LENGTHS OF AMYLOPECTINS

Fraction	*Whole*	*A*	*B1*	*B2*	*B3*	*B4*	*A/B1–4*
Waxy rice							
C.l. (max.)		13	19	41	69		
C.l. (average)	24	13	22	42	69	101	
Weight (%)	100	50.0	26.2	18.9	4.1	0.8	
Mole (%)	100	69.2	21.7	8.0	1.0	0.1	2.2
Tapioca							
C.l. (max.)		11	18	38	62		
C.l. (average)	26	12	21	42	69	115	
Weight (%)	100	38.5	32.5	23.0	5.1	0.9	
Mole (%)	100	47.0	41.9	9.4	1.5	0.2	0.89
Kuzu							
C.l. (max.)		13	16	39	72		
C.l. (average)	26	13	20	42	70	119	
Weight (%)	100	30.7	42.7	20.2	5.4	1.0	
Mole (%)	100	47.0	41.9	9.4	1.5	0.2	0.89
Potato							
C.l. (max.)		16	19	45	74		
C.l. (average)	35	16	24	48	75	104	
Weight (%)	100	27.8	34.9	26.0	9.1	2.3	
Mole (%)	100	44.2	38.1	14.0	3.1	0.6	0.79

be in the range of c.l. 20–24. However, the difference in c.l. between fractions B2 and B1 and that between fractions B3 and B2 do not agree (Table II). The latter values are a little greater than the former. This discrepancy is probably due to the possibility that the B2 chains do not extend over the whole of two adjacent clusters, whereas the B3 chains go through three clusters, including a single whole cluster. Thus, the c.l. between the nearest two linked clusters would be B3–B2 (27–28) rather than B2–B1. Fig. 5 depicts a cluster model for amylopectin showing these relationships. The sums of the chains of fractions A and B1 are 89–91/100 chains for waxy-rice, tapioca, and kuzu amylopectins, and 82/100 chains for potato amylopectin, suggesting that 80–90% of the chains constitute a single cluster and the remaining 10–20% form inter-cluster connections, most of which connect two adjacent clusters. These connecting chains seem to be relatively abundant in potato starch and are probably characteristic of B-type starches because they have higher amounts[9] of fractions B2–B4.

The A,B-chain ratios are 0.8–0.9 for tapioca, kuzu, and potato amylopectins, and 2.2 for waxy-rice amylopectin. These values may be a little lower or higher than the values obtained on enzymic analyses[2]. However, this does not imply that assumption (*a*) is invalid, since these values are approximate because of the overlapping of the fractions (A and B1). In addition, the A,B-ratio is not yet agreed generally. MacGregor and Morgan[8] found a trimodal distribution of the c.l. of barley amylopectin and inferred that their fractions 3 and 4, which correspond to

TABLE II

DIFFERENCES IN THE CHAIN LENGTHS BETWEEN FRACTIONS

Amylopectin	*B1–A*	*B2–B1*	*B3–B2*
Waxy rice	9	22	27
Tapioca	9	21	27
Kuzu	7	22	28
Potato	8	24	27

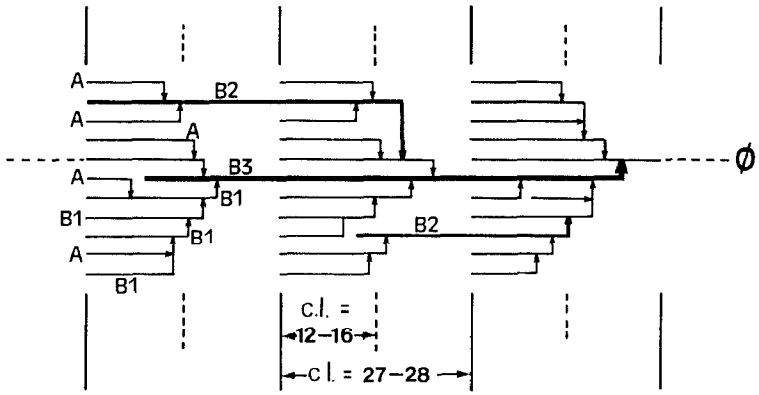

Fig. 5. A cluster model for amylopectin. For definition of A and B1–B3, see text: ϕ, reducing chain-end; —, (1→4)-α-D-glucan chain; →, α-(1→6) linkage.

fractions B1 and A, respectively, were composed mainly of A chains. However, this is less probable because the A,B-chain ratios are calculated to be 4–10, which are considerably higher than those reported[2].

It is accepted that the crystalline domains of starch granules appear to be composed of A-chains and the exterior parts of B-chains[4,16], and that this length seems to be in the range of c.l. 12–16 (length of fraction A) on average. This value agrees with the exterior chain-length of amylopectins[2]. Similar values for the lengths of crystalline domains have been estimated by different approaches. Robin *et al.*[5] and Hood and Mercier[17] have proposed c.l. 15 for the cluster of tapioca amylopectin, and French[4] suggested c.l. 12 for that of lintnerised potato starch. The properties of a single cluster are supposed to be dependent mainly on the sizes and amounts of fractions A and B1, and the species characteristics. Some 22–25 chains may be included in a single cluster, assuming that periods (99–110 Å)[18,19] seen on X-ray small-angle diffraction correspond to the distance between clusters and are based on the packing of the double helical chains in crystalline cells[20].

EXPERIMENTAL

Materials. — Starch specimens and crystalline isoamylase were the same as those used previously[9].

Methods. — Amylopectin was debranched as described elsewhere[21]. H.p.l.c. was performed as reported[9], but gel columns of TSK G3000SW and G2000SW (×2) connected in sequence were used instead of a series of TSK PW columns, and eluted with 0.1M sodium phosphate buffer (pH 6.2) containing 0.02% of sodium azide at 0.61 mL/min. Other conditions were as described[9].

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REFERENCES

1 R. L. WHISTLER AND J. R. DANIEL, in R. L. WHISTLER, J. N. BEMILLER, AND E. F. PASCHALL (Eds.), *Starch: Chemistry and Technology*, 2nd edn., Academic Press, New York, 1984, pp. 153–182.
2 D. J. MANNERS, *Cereal Foods World*, 30 (1985) 461–467.
3 Z. NIKUNI, *Chori Kagaku*, 2 (1969) 6–14; *Denpun Kagaku*, 22 (1975) 78–92.
4 D. FRENCH, *Denpun Kagaku*, 19 (1972) 8–25.
5 J. P. ROBIN, C. MERCIER, R. CHARBONNIER, AND A GUILBOT, *Cereal Chem.*, 51 (1974) 389–406.
6 D. J. MANNERS AND N. K. MATHESON, *Carbohydr. Res.*, 90 (1981) 99–110.
7 N. PALMER, L. E. MACASKIE, AND K. K. GREWELL, *Carbohydr. Res.*, 114 (1983) 338–342.
8 A. W. MACGREGOR AND J. E. MORGAN, *Cereal Chem.*, 61 (1984) 222–228.
9 S. HIZUKURI, *Carbohydr. Res.*, 141 (1985) 295–306.
10 T. TAKAGI AND S. HIZUKURI, *J. Biochem. (Tokyo)*, 95 (1984) 1459–1467.
11 S. HIZUKURI AND T. TAKAGI, *Carbohydr. Res.*, 134 (1984) 1–10.
12 Z. GUNJA-SMITH, J. J. MARSHALL, C. MERCIER, E. E. SMITH, AND W. J. WHELAN, *FEBS Lett.*, 12 (1970) 101.
13 H. AKAI, K. YOKOBAYASHI, A. MISAKI, AND T. HARADA, *Biochim. Biophys. Acta*, 252 (1971) 427–431.
14 A. SUZUKI, S. HIZUKURI, AND Y. TAKEDA, *Cereal Chem.*, 58 (1980) 286–290.
15 S. PEAT, W. J. WHELAN, AND G. J. THOMAS, *J. Chem. Soc.*, (1952) 4546–4548.
16 Z. NIKUNI AND S. HIZUKURI, *Mem. Inst. Sci. Ind. Res., Osaka Univ.*, 14 (1957) 173–181.
17 L. F. HOOD AND C. MERCIER, *Carbohydr. Res.*, 61 (1978) 53–66.
18 C. STERLING, *J. Polym. Sci.*, 56 (1962) S10–S12.
19 S. HIZUKURI, M. KAKUDO, AND Z. NIKUNI, *Nippon Nogei Kagaku Kaishi*, 38 (1964) 520–524.
20 H.-C. HAROLD AND A. SARKO, *Carbohydr. Res.*, 61 (1978) 7–40.
21 S. HIZUKURI, T. KANEKO, AND Y. TAKEDA, *Biochim. Biophys. Acta*, 760 (1983) 188–191.