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Fine structure of wheat amylopectin: the mode of A to B chain binding*

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ABSTRACT

The fine structure of wheat amylopectin was analyzed by means of a new enzymic method, as well as by conventional methods. Its general properties were found to be similar to those of rice amylopectin with intermediate iodine affinity, but the chain-length distribution was clearly distinguishable from that of rice amylopectins. The B chain, as defined by Peat and co-workers [J. Chem. Soc. (1956) 3025], was classified into Ba and Bb chains on the basis of whether A chains were bound (Ba) or not bound (Bb), and the number of A chains bound to a Ba chain was determined from the ratio of non-reducing to reducing residues of the stepwise degradation products of the amylopectin with β-amylase, isoamylase, and β-amylase. A Ba chain was found to carry 2.1 A chains on average, but the longer Ba chains bound increased numbers of A chains (up to four). The A: B and the Ba: Bb chain ratios were found to be 1.26:1 and 1.5:1, respectively, indicating that ~40% of the B chains carried no A chains.

INTRODUCTION

Amylopectin molecules are constructed from hundreds or thousands of short amylose chains consisting mainly of 6 to ~100 glucosyl residues, along with some minor extra long chains (EL) of about 1000 glucosyl residues with (1→6)-α-D-glucosidic linkages. The chains of these building units, with are liberated by debranching enzymes, show characteristic distributions as to length according to their sources[1–5]. These distributions support the cluster structures proposed by Z. Nikuni[6], D. French[7], and others[8–10], but give little information on the mode of their assembly.

Peat and co-workers[11] classified the amylopectin chains into A, B, and C chains as a means of structural analysis. A chains are those involved in α-(1→6)-linkages by only the reducing-end glucose units, B chains are those linked at their reducing ends to another chain, while at the same time carrying one or more chains as branches, and C chains are those that only carry the reducing group. The A chains are trimmed to yield maltosyl or maltotriosyl stubs with β-amylase, depending on whether there is an even or odd number of glucosyl residues[12], and upon sebsequent debranching with pullulanase or R-enzyme, they give maltose and maltotriose. Therefore, the number of A chains can

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be calculated from the amounts of maltose and maltotriose. Peat *et al.*[11] debranched waxy maize β-limit dextrin (β-LD) with R-enzyme and found that the amounts of maltose and maltotriose supported Meyer's structure[13]. Later, Gunja-Smith *et al.*[14] proposed a revised Meyer structure in which half of the B chains carry two A chains and the rest only carry B chains. This structure was proposed on basis of the analysis of the isoamylase debranched products of φ,β-dextrin (the limit hydrolysis product from successive treatments with phosphorylase and β-amylase) of amylopectin and glycogen. This regular structure, of which the outermost B chains carry two A chains, is interesting.

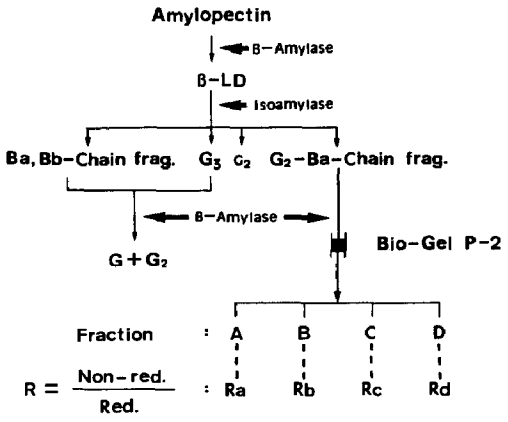

Fig. 1. Outline of the procedures for determination of the number of A chains bound to a B chain.

The main purpose of this study is to analyse the number of A chains binding to a B chain as the first step for elucidating the molecular architecture of amylopectin. Wheat amylopectin was used as a choice specimen because it links the least amount of phosphate[15], with interferes with analysis. The strategy used is shown in Fig. 1. Specifically, amylopectin is first processed to β-LD with sweet potato β-amylase, then with *Pseudomonas* isoamylase, and again with β-amylase. The resulting B chains with maltosyl stub(s) are divided into several fractions by size-exclusion chromatography. The number of A chains binding to a B chain of the fractionated maltosyl-Ba chains is determined by hydrolyzing the maltosyl stubs with pullulanase or by assaying the reducing and non-reducing residues. The hydrolysis of small amounts of maltosyl stubs with isoamylase are inevitable, but this effect is not detrimental as is described later. At this point, we classify the B chains into two kinds: Ba and Bb chains. The former carry at least one A chain, and the latter carry no A chains, but instead bear one or more B chain(s). The results of the present study revealed that longer Ba chains carried an increased number of A chains. This may imply random branching in the outermost layers of the amylopectin molecule. Conventional general analyses were also carried out, and the fine structure of the amylopectin is discussed.

EXPERIMENTAL

Preparative methods and reagents. a. Preparation of amylopectin. — Starch was extracted with ice-cold water from wheat flour [semi-strong, which was provided by Nisshin Flour Milling Co. (Tokyo)] in the form of a dough, and the prime starch fraction was dried over calcium chloride under reduced pressure. Amylopectin was separated from the thin aqueous paste of the starch by precipitating the amylose as its 1-butanol and 3-methyl-1-butanol complexes, as previously described[16,17]. Before the extraction, the starch was defatted by dissolving it in dimethyl sulfoxide and precipitating it twice with alcohol[17]. This procedure is used because the lipid in starch prevents its molecular dispersion on pasting.

b. Preparation of β-LD. — A mixture of amylopectin (10 g) and crystalline sweet potato β-amylase[18] (10^5 IU) in 50mM acetate buffer (500 mL, pH 4.8) in cellulose tubing (Visking Co.) was incubated for 24 h at 37°, under dialysis against the same buffer, which was renewed three times during the first 12 h. After the reaction had been terminated by heating the mixture for 10 min at 100°, the coagulated protein was removed by centrifugation, and then ethanol (500 mL) and a 0.1% lithium bromide solution (0.1 mL) were added to the solution. The resulting precipitate was collected by centrifugation, dissolved in water (200 mL), and then reprecipitated by the addition of ethanol (200 mL) and 0.1% lithium bromide (0.1 mL). The precipitate recovered on centrifugation was washed by successive suspension and filtration in ethanol and ether, and it was then dried under reduced pressure in a desiccator over calcium chloride. The yield of β-LD was 4.0 g.

c. Preparation of maltosyl-Ba chain fragments (β, i, β-LD). — β-LD (500 mg) was dissolved in 5mM acetate buffer (100 mL, pH 3.5) and then hydrolyzed with isoamylase (150 IU) for 1 h at 45° (case I) or for 4 h (case II). The reaction was terminated by boiling for 15 min. After the pH of the mixture had been adjusted to 4.8 with M sodium hydroxide, β-amylase (5 IU·mg^{-1} β-LD′) was added to the mixture, followed by incubation for 2 h at 37°, and the reaction was stopped by boiling for 10 min. Through this β-amylolysis, linear dextrins, which were produced on splitting of the linkages between the B chains by the preceding isoamylolysis, were hydrolyzed into mainly maltose and small amounts of glucose and maltotriose, which were removed by gel-filtration on Bio-Gel P-2, and the rest of the products (β, *i*, β-LD′) were collected. To complete this β-amylolysis, β, *i*, β-LD′ was treated again with a large amount of β-amylase (50 IU·mg^{-1} β,*i*, β-LD′) for 24 h at 37°, and then the mixture was processed as for the first β-amylolysis. The yields of maltosyl-Ba chain fragments in cases I and II were 196 mg and 124 mg, respectively. The maltosyl-Ba chain fragments were separated into four fractions by Bio-Gel P-2 size-exclusion chromatography.

Preparation of Ba chain fragments. — Maltosyl-Ba chain fragments (1 mg.mL^{-1}) were debranched with pullulanase (0.5 IU.mg^{-1}) in 50 mM acetate buffer at pH 6.0 for 6 h at 40°. The pH of fraction D was adjusted with 0.01N sodium hydroxide without use of any buffer, because the buffer interfered with the h.p.l.c. analysis involving NH_2-bonded silica. The complete hydrolysis was confirmed by the 99% hydrolysis of the product with β-amylase.

Reagents and enzymes. — Isoamylase and pullulanase of crystalline grade were the products of Hayashibara Biochemical Lab., and sweet potato β-amylase was prepared by crystallization from three different solvents: water, acetone[18], and ammonium sulfate (0.36–0.40 saturation, pH 3.7). Other reagents were of the highest grade commercially available.

Analytical methods. — Total carbohydrate was determined by the phenol–sulfuric acid method[19]. The reducing residue was assayed colorimetrically using Somogyi's[20] and Nelson's[21] reagents, the heating time being extended to 30 min to give the same reducing power regardless of the chain length, or by the modified Park–Johnson method as previously described[22,23]. The number-average degree of polymerizations ($\overline{\text{d.p.n.}}$) was calculated from the reducing residue and total carbohydrate values. Non-reducing residues of oligosaccharides were determined by enzymic assaying of glycerol produced on rapid Smith degradation[24].

The average chain lengths ($\overline{\text{c.l.}}$) of amylopectin and maltosyl-Ba chain fragments were determined also by assaying of reducing and non-reducing residues. The $\overline{\text{c.l.}}$ of amylopectin was also calculated from the liberated reducing power after isoamylolysis, which was carried out with a 0.5% (w/v) solution for 12 h at 45° and pH 3.5 (50mM acetate buffer) with *Pseudomonas* isoamylase (0.3 $U.mg^{-1}$). The two values of $\overline{\text{c.l.}}$ coincided within experimental error.

The c.l. distributions of amylopectin and Ba chain fragments were analyzed by gel-exclusion or amino-bonded silica h.p.l.c. as described below. Amylopectin (500 mg) was dissolved in water (200 mL) by heating at 100°. After the solution had been cooled to 45° and the pH was adjusted to 3.5 with 0.1M hydrochloric acid, isoamylase (150 IU) was added, followed by incubation for 12 h. The reaction was terminated by heating for 10 min at 100°, and the product was lyophilized. The debranched amylopectin (30 mg) was dissolved in M sodium hydroxide (0.15 mL), the resultant solution was diluted with water (0.8 mL) and 0.5M sodium phosphate buffer (0.4 mL, pH 6.1) containing 0.1% sodium azide, the pH of the solution was adjusted to 6.1 with M hydrochloric acid, and then the volume was made up to 2 mL with water. The solution (0.3 mL), which had been filtered through a membrane filter (0.22 μm Millex GS, Millipore Corp.), was subject to h.p.l.c. analysis (Toso 803D) on two columns and two TSK guard columns SW(GC) connected in the following order: Asahipak GS-320 (7.6 × 500 mm), GC (7.5 × 7.5 mm), TSK-gel G2000SW (7.5 × 600 mm) and GC, instead of the three TSK-gel SW columns in the previous experiment[2], because the two columns under the present operation conditions exhibited similar resolution to that of the three TSK-gel SW columns previously used[2]. Elution was carried at 35° with 0.1M sodium phosphate buffer (pH 6.1) containing 0.02% sodium azide at 0.4 $mL \cdot min^{-1}$. The eluent was monitored with a differential refractometer (r.i., Toso 1011) and a low-angle laser-light-scattering photometer (l.a.l.l.s., Toso LS-8000), as described[2]. The molecular weight or degree of polymerization was calculated using a standard pullulan specimen (P-10, 10200 daltons; Hayashibara Biochemical Lab.). In the case of maltosyl-Ba chain fragments, appropriate amounts (2–3.3 mg per 400–800 μL) were injected into the system. The c.l. distributions of Ba chain fragments of fraction D were also examined by h.p.l.c. on an

NH_2-bonded silica column (TSK gel NH_2-60, 4.6 × 250 mm), with a mixture of 62:38 acetonitrile–water as the eluent at flow rate of 0.8 mL·min^{-1}.

The A:B chain ratio was determined as follows. Isoamylase (3 IU) was added to an aqueous solution of β-LD (10 mg·0.5 mL^{-1}), of which the pH was adjusted to pH 3.5–4.0 with M hydrochloric acid, and then the mixture was incubated for 12 h at 45°. After the reaction had been terminated by boiling, the pH of the mixture was readjusted to pH 6.0 with M sodium hydroxide. Pullulanase (0.5 IU·mg^{-1} of β-LD) was added to the solution, and then the mixture was incubated for 4 h at 40°. The total amount, in moles, of the hydrolyzate was determined by the Somogyi–Nelson method[20,21]. The hydrolyzate was frozen at ~ −10° and then thawed at room temperature. The resulting insoluble materials, which consisted of long chains of Ba chain fragments, were removed by filtration with a membrane filter (0.22 μm, Millipore). The combined amount, in moles, of maltose and maltotriose was determined by h.p.l.c. on NH_2-bonded silica with the solvent system of 65:35 acetonitrile–water. The A:B chain ratio was calculated with equation *1*.

$$\frac{\text{A chain}}{\text{B chain}} = \frac{[\text{maltose} + \text{maltotriose}]}{[\text{total molecule}] - [\text{maltose} + \text{maltotriose}]} \qquad (1)$$

The iodine affinity, blue value, λ_{max} of an iodine-stained solution, β-amylolysis limit and [η] (M KOH, 22.5°) were determined by the procedures described alsewhere[25,26].

RESULTS

General properties. — The physicochemical properties examined by conventional methods are listed in Table I. The properties are similar to those of rice amylopectins with intermediate iodine affinities, *i.e.*, IR48, IR64 (ref. 27) and a japonica variety[17], except that the $\overline{\text{d.p.n}}$ was a little lower than those (9000–15 000) of these rices, and the phosphorus content was also lower.

TABLE I

General properties of wheat amylopectin

Property	
Iodine affinity, g·100 g^{-1}	0.76
Blue value	0.14
λ_{max}, nm	552
[η], mL·g^{-1}	146
$\overline{\text{D.p.n}}$	4800
$\overline{\text{C.l.n}}$	19
β-Amylolysis, %	57
Po, ppm	< 1

C.l. distribution. — The h.p.l.c. size-exclusion chromatogram of isoamylase-debranched wheat amylopectin showed a polymodal distribution with peak apices at d.p. 11.4, 17.7, 40, and 1600, and peak shoulders at d.p. 13.5 and 80. The chains were fractionated into six fractions, as indicated in Fig. 2, although the cuts were somewhat

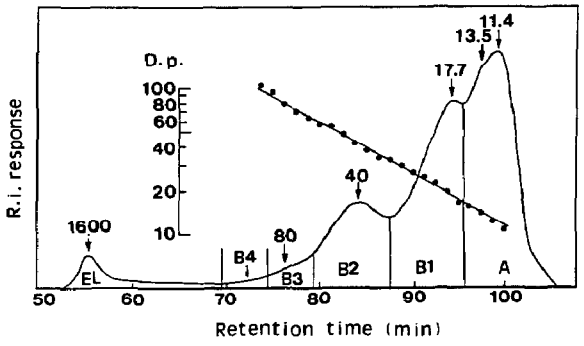

Fig. 2. C.l. distribution of wheat amylopectin. Numbers indicate d.p.

arbitrary. The weight-average c.l. (c.l.w.) and amounts by weight and by moles of the fractions are summarized in Table II. The smallest chain fraction (A-fraction), which is considered to comprise mainly A chains, amounted to over 60 mole-percent, suggesting A chains predominate over B chains. The cluster length was inferred to be 36 from the difference between the B_3 and B_2 fractions in c.l.[2]. This value, which is larger than those (27–28) in the cases of waxy rice, tapioca, kuzu, and potato amylopectins[2], seems to be characteristic. The line profile of the distribution, except for the extra-long chain fraction (EL), is similar to that of tapioca amylopectin, but the slight shoulder at d.p. 13.5 is characteristic for this polymer, having not been observed previously for any other amylopectins.

The amount of EL was 4.5%, which is similar to those of the corresponding fractions (Fla) of rice amylopectins[4] with intermediate iodine affinities such as IR48, IR64 (ref. 25), and a japonica variety (product of Hokkaido)[3]. The iodine affinity of the wheat amylopectin was also similar to those of the above amylopectins with intermediate iodine affinities. The positive relationship between iodine affinity and the amount of

TABLE II

C.l. Distribution of wheat amylopectin

Fraction	*Whole*	*A*	*B1*	*B2*	*B3*	*B4*	*EL*	*A/B1-4*
C.l.(Max)		11	18	40	80			
C.l.w	25[a]	13	22	43	79	140		
Weight(%)	100	42.0	32.7	16.7	3.2	0.9	4.5	
Mol(%)	100	63.2	28.4	7.5	0.8	0.1	0	1.7

[a] Other than EL

the EL fraction observed for rice amylopectins[4], that is, Fla (%) = 5.4.(i.a.) + 0.33, was true for the wheat amylopectin EL fraction also.

Hydrolysis of β-LD with isoamylase. — The segments connecting the A chains to the Ba chains of amylopectin were isolated, after stepwise degradation of the amylopectin with β-amylase, isoamylase, and β-amylase (second β-amylolysis), as maltosyl-Ba chain fragments, as shown in Fig. 1. The structural changes which occured during the process are shown in Fig. 3. Amylopectin is first degraded to the limit with β-amylase,

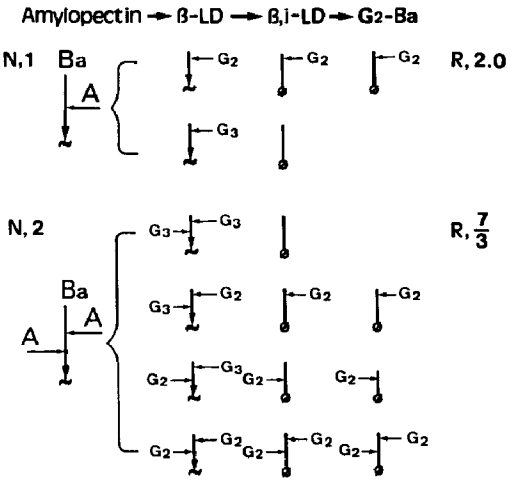

Fig. 3. Structural changes in the A and Ba chains of amylopectin on stepwise sequential hydrolysis with β-amylase, isoamylase, and β-amylase. *N*, the A:Ba chain ratio of amylopectin: *R*, the ratio of non-reducing:reducing residues of the maltosyl Ba chain fragments (G_2 – Ba). G_2 and G_3, maltosyl and maltotriosyl stubs of A chains; ~, amylopectin residue; φ, reducing residue; —, α-(1→4)-glucan; ↓, α-(1→6)-linkage.

which trims A chains to yield maltosyl or maltotriosyl stubs, depending on whether there is an even or odd number of glucose residues, and then B chains, until one or two glucosyl residue(s) remain at the branch linkages for the non-reducing residues[12]. This β-LD was disjointed completely into chains with isoamylase, except at α-(1→6)-maltosyl linkages, which are highly resistant to the enzyme. Most of the latter remain unhydrolysed. The ideal situation is to hydrolyze all the α-(1→6)-linkages except those involving maltosyl stubs of A chains. Figure 4 shows the time course of the hydrolysis of wheat amylopectin β-LD. Under these conditions, hydrolysis proceeded rapidly within the first 30 min and then slowly during the rest of the period. However, liberation of maltotriose attained a limit at 30 min hydrolysis, while the splitting of maltose increased gradually. This result suggests that the branch linkages between maltotriosyl stubs of A chains and Ba chain fragments, and probably those between the mutual Bb chain stubs, are hydrolyzed completely within 30 min. Based on these results, the isoamylolysis was carried out for 1 h (case I) and 4 h (case II) in the following experiments. Case II was carried out to ensure the hydrolysis of linkages other than 6-*O*-α-maltosyl linkages and to examine the effect of splitting the maltosyl residue on analysis.

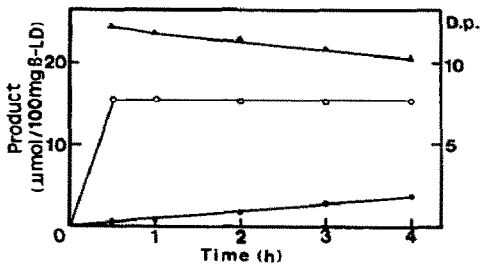

Fig. 4. Time courses of the debranching of β-LD with *Pseudomonas* isoamylase. ▲, d.p.; o, maltotriose; ●, maltose.

Properties of maltosyl Ba chain fragments. — After the second β-amylolysis, maltosyl Ba chain fragments were fractionated into four fractions by Bio-Gel P-2 size-exclusion chromatography, as shown in Figs. 5 and 6. The fractionations were

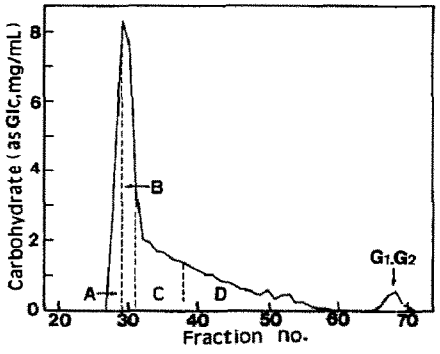

Fig. 5. Fractionation of maltosyl-Ba chain fragments (case 1) on a Bio-Gel P-2 column (2.6 × 90 cm). Sample size, 5 mL (235 mg as D-glucose); solvent, water; flow rate, 30 mL·h^{-1}; fraction size, 5 mL.

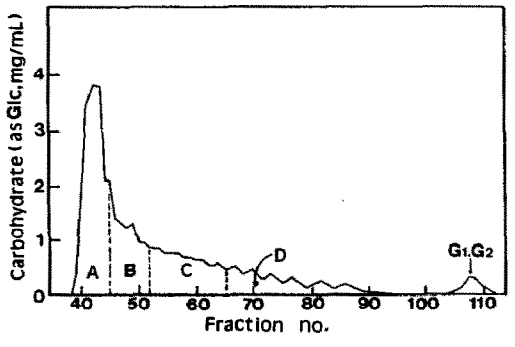

Fig. 6. Fractionation of maltosyl-Ba chain fragments (case 2) on a Bio-Gel P-2 column (3.2 × 90 cm). Sample size, 5 mL (177 mg as D-glucose); solvent, water; flow rate, 27 mL·h^{-1}; fraction size, 4.5 mL.

arbitrary, but they were fractionated into nearly equal amounts by weight in case I. These chromatograms suggest similar, wide molecular-weight distributions. The d.p., c.l., number of branch linkages, etc., of the maltosyl-Ba chain fragments and their subfractions are summarized in Tables III and IV. The branch linkages increased with an increase in molecular weight. The average branch numbers of the maltosyl-Ba chain fragments were 1.4 and 1.3 for specimens hydrolyzed with isoamylase for 1 and 4 h, respectively. The fragments produced maltose equivalent to the increased reducing power on hydrolysis with pullulanase, and thus all side chains of those branched fragments were confirmed to be maltose. Therefore, fractions A, B, C, and D in Table III had 1.8, 1.7, 1.4, and 1.1 maltosyl stubs per Ba-chain fragment, respectively. However, this does not imply that a Ba chain of amylopectin carries 1.1–1.8 A chains, because half of the A chains, which were trimmed to yield maltotriosyl stubs with β-amylase, were liberated on the subsequent hydrolysis with isoamylase. Therefore a correction is necessary as to the real number of A chains bound to a Ba chain.

The number of A chains bound to a Ba chain. — Figure 3 illustrates the relationship between the number (N) of A chains bound to a Ba chain of amylopectin, and the ratio (R) of non-reducing to reducing residues of the maltosyl Ba chain fragments. In the

TABLE III

Properties of maltosyl-Ba chain fragments (case I) of wheat amylopectin

Fraction	*A*	*B*	*C*	*D*	*Whole*
$\overline{\text{D.p.w}}$	45.7	33.3	18.2	9.0	19.5
$\overline{\text{C.l.w}}$	16.5	12.4	7.7	4.3	8.3
R[a]	2.8	2.7	2.4	2.1	2.4
N[a]	3.2	2.9	2.1	1.2	2.1
Amount					
% in moles	9.1	12.6	26.1	52.2	(100)
% by weight	23.4	23.5	26.7	26.4	(100)

[a] See Fig. 3.

TABLE IV

Properties of maltosyl-Ba chain fragments (case II) of wheat amylopectin

Fraction	*A*	*B*	*C*	*D*	*Whole*
$\overline{\text{D.p.w}}$	43.1	24.2	13.5	7.9	18.9
$\overline{\text{C.l.w}}$	15.0	9.7	6.1	3.8	8.2
R[a]	2.9	2.5	2.2	2.1	2.3
N[a]	3.4	2.4	1.6	1.2	2.0
Amount					
% in moles	16.6	17.4	29.8	36.2	(100)
% by weight	39.2	23.1	22.2	15.5	(100)

[a] See Fig. 3.

cases of N-values of 1, 2, and 3, R values are 2, 7/3, and 19/7, respectively, as illustrated, in part, in Fig. 3. This relationship between N and R is expressed by equation *2* where C means combinations, and as shown in Fig. 7.

$$R = \frac{\sum_{i=1}^{N} {}_N\mathrm{C}_i(1+i)}{2^N - 1} \tag{2}$$

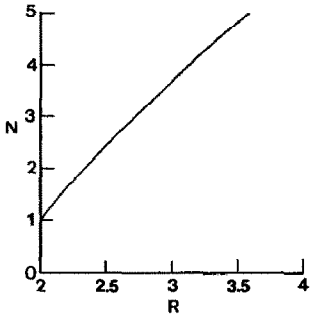

Fig. 7. Relationship between R and N with Eq. *2*. R and N, (see text).

It is advantageous that the R-values are affected only slightly by the extent of hydrolysis of maltosyl-Ba chain fragments, as judged from the R-values of whole fractions obtained on hydrolysis for 1 and 4 h with isoamylase. This is rationalized by that when $N = 1$, $R = 2$, regardless of the extent of hydrolysis. In case where $N = 2$, the R-value changes

$$R = \frac{7}{3} \cdot \frac{(300 - 2A)}{(700 - 6A)} \cdot \mathrm{Ra} = \mathrm{K} \cdot \mathrm{Ra} \tag{3}$$

according to equation *3*, where Ra is the value obtained at A(%) hydrolysis of the maltosyl Ba chain fragment, and K is the correction factor for R due to the liberation of maltosyl stubs. As shown in Table V, when A is $<20\%$, K is <1.05, and the error is negligible.

The R-values of the largest molecular fractions (fraction A) of the maltosyl-Ba chains in cases I and II were 2.8 and 2.9, respectively (Tables III and IV), which imply that Ba chains in the fractions carry 3.2 and 3.4 A chains on average, and that some Ba chains bind at least as many as four A chains. More precisely, if fraction A is assumed to be a mixture of Ba chains carrying three and four A chains, 60–80% and 20–40% of the Ba chains carry three and four A chains, respectively. Similarly, the smallest molecular fraction D was calculated to be a mixture of Ba chains carrying one and two A chains, in the ratio of 4:1. The R-value of unfractionated, whole maltosyl Ba chain fragments was

TABLE V

Change in *R* due to the hydrolysis of maltosyl-Ba chain fragments with isoamylase

A[a] (%)	5	10	15	20	30	40
K[a]	1.01	1.02	1.03	1.05	1.08	1.12

[a] K, correction factor for A (%) hydrolysis of the maltosyl-Ba chains.

2.4 (case II), which corresponds to $N = 2.1$; therefore, a Ba chain carries 2.1 A chains on average. This is in accord with Whelan's revised Meyer model[14], in which a Ba chain carries two A chains, but the data for the subfractions revealed that a Ba chain bound variable numbers, 1–4, of A chains, depending on the length of the Ba chain. Therefore, the mode of A chain binding to B chains appears to be random and does not fit Whelan's regular model, but it does support the cluster structure.

A:B chain ratio. — This ratio may be calculated from the reducing sugar liberated from β-LD at each step on successive degradation with isoamylase and pullulanase. This calculation is based on the assumption that the isoamylase does not hydrolyze the maltosyl stubs of A chains, but the stubs are hydrolyzed slowly as described, which leads to erroneous results[10]. Therefore, we specifically determined maltose and maltotriose by h.p.l.c. on a NH_2-bonded silica column after complete hydrolysis of β-LD with isoamylase and pullulanase. With this method, a tiny amount of maltotriose, which is derived from the B chains, as will be discussed later, may lead to an erroneous result. But the amount of maltotriose seemed to be negligible because the ratio of the yields of maltose and maltotriose was 1:1.02, and the A:B chain ratio was calculated to be 1.26:1 from the yields (Table VI). This value is a little lower than those reported by other workers[28–30], but it is similar to those reported for potato[31], waxy sorghum[31], and waxy rice[32].

TABLE VI

Chain ratios of wheat amylopectin

Chain chain	*Ratio*
A:B	1.26:1
A:Ba	2.1:1
Ba:Bb	1.7:1

Ba to Bb chain ratio. — From the fact that a Ba chain binds 2.1 A chains, and from the fact that the A:B chain ratio is 1.26:1, the Ba:Bb chain ratio was calculated to be 1.5:1. This implies that 60% of the B chains are Ba chains, and the rest are (40%) Bb chains. Gunja-Smith *et al.*[14] first suggested the presence of Bb chains in amylopectin and glycogen from the high accessibility of φ,β-LD for β-amylase after debranching with isoamylase.

Chain length distribution of Ba chain fragments. — Debranched fractions A–D of maltosyl-Ba chain fragments with pullulanase were subjected to h.p.l.c. on gel-exclusion columns or on an NH_2-bonded silica column. The main portion of Ba chain fragments of fraction A, the largest chain fragments, was distributed approximately in the range of d.p. 20–100, the peak apex falling at d.p. 37, with a slight shoulder at d.p. 60. A small number of long chains of over 1000 d.p. were observed (Fig. 8), but this may partly be

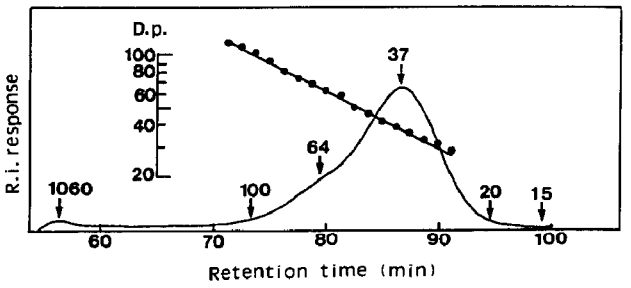

Fig. 8. C.l. distribution of the Ba chains of the fragments of fraction A determined by h.p.l.c.–r.i.–l.a.l.l.s.

due to incomplete hydrolysis with isoamylase, because a similar chromatogram with a longest chain of d.p. 450 was obtained for the specimen in case II. This fraction is possibly mainly derived from B_3–B_5 fractions of debranched amylopectin (Fig. 1). The Ba chain fragment of fraction B (Fig. 9) is distributed between d.p. 17–50, the peak apex being at d.p. 24. This fragment is supposed to be mainly derived from fractions B_2 and B_3 of debranched amylopectin (Fig. 1) The Ba chain fragments of fraction C (Fig. 10), which are distributed in the sharp d.p. range of 10–20, the peak apex being at d.p. 12.5, are considered to carry two A chains in amylopectin. The smallest and largest of the Ba chain fragments of fraction D were found to be maltotriose and G_{11}, respectively, on h.p.l.c. with NH_2-bonded silica (Fig. 11). This implies the presence of such heavily branched parts as an α-(1→4)-linkage intervening between two α-(1→6)-linkages (Fig. 12) in amylopectin, and is consistent with the finding of oligosaccharides with such structures in a hydrolyzate of waxy rice amylopectin with α-amylase by Umeki and Yamamoto[33].

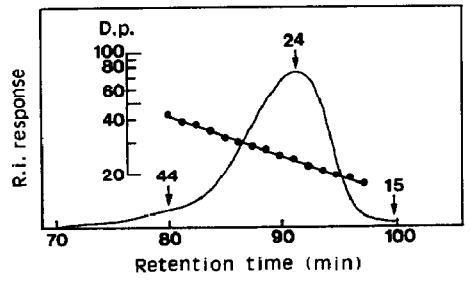

Fig. 9. C.l. distribution of the Ba chains of the fragments of fraction B determined by h.p.l.c.–r.i.–l.a.l.l.s.

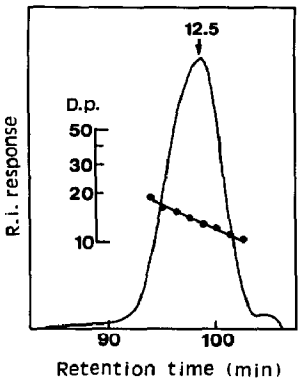

Fig. 10. C.l. distribution of the Ba chains of the fragments of fraction C determined by h.p.l.c.–r.i.–l.a.l.l.s.

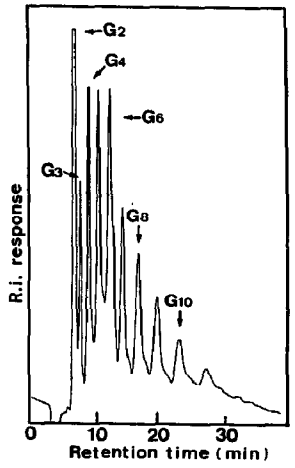

Fig. 11. C.l. distribution of the Ba chains fragments of fraction D determined by h.p.l.c. on NH_2-bonded silica. Gn, malto-oligosaccharides with d.p.n.

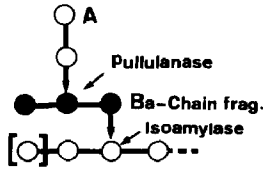

Fig. 12. Production of maltotriose from maltosyl Ba-chain fragments. –, α-(1→4)-glucosidic bond; ↓, α-(1→6)-glucosidic bond.

DISCUSSION

The general properties of wheat amylopectins are similar to those of rice amylopectins with intermediate iodine affinities[17,33], but the c.l. distribution (Fig. 2) is clearly distinguisable from those of several rice species and others[2] as well. Rice amylopectins do not show a clear boundary between the A and B_1 fractions. The A and B_1 peak heights of potato[2], sago[34], and water chestnut[35] amylopectins are nearly the same, but the A peak height of wheat amylopectin is considerably greater than the B_1 peak height. This profile of wheat amylopectin resembles that of tapioca amylopectin[2], but the latter has no characteristic shoulder on the A peak.

The Ba chain fragments of fraction A contained chains of hundreds or 1000s d.p., and longer chains carried more A chains, suggesting that the EL fraction is a constituent of the amylopectin molecule and not an amylose contaminant. The EL component has not been found in japonica type waxy rice amylopectin, but it has been found in varying amounts in other kinds of amylopectins. The c.l. and c.l. distribution of the A fraction of Ba chain fragments indicate that some Ba chains are involved in the formation of more than five clusters and carry several A chains, though there are only a small number of them. The amount and size of this EL component are considered to be very important factors in determining the molecular architecture and functional properties as well. Probably such renders the molecule an elongated species and results in an increase in viscosity.

The shortest Ba chain fragments in fraction D are supposed to be derived from the frequently branched, non-crystalline domain of a cluster. The c.l. distribution of 3–11 suggests that the inner c.l. distribution of the Ba-chain of this fraction is 1.5–9.5, with 1.5–4.5 apparently being predominant (Fig. 11), because one or two glucosyl residues of the Ba-chain fragments belong to the outer chains. Maltotriose was the smallest Ba chain fragment because it was confirmed by h.p.l.c. using an NH_2-bonded silica column that the smallest molecule of maltosyl-Ba chain fragments was maltopentaose (data not shown). The Ba chain fragment in case I fraction C could be involved in the formation of

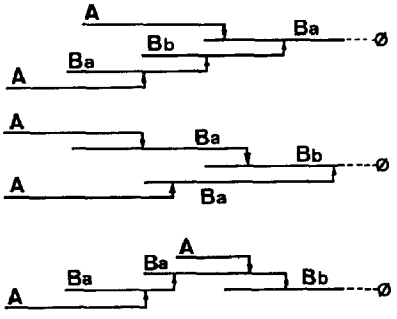

Fig. 13. The possible modes of linking of two Ba chains to a Bb chain. For simplification, a single A chain is linked to a Ba chain.

a crystalline domain because the peak apex of the c.l. distribution (Fig. 10) is 12.5, which is consistent with the finding of Pfannemüller[36] that c.l. 10 is the minimum for A type crystallization. The Ba:Bb chain ratio of 1.5:1 suggests that some Bb chains are linked to at least two Ba chains, as shown in Fig. 13.

In conclusion, the frequency of A chain binding to a Ba chain of amylopectin was first analyzed by means of a new enzymic method. This method, together with relevant h.p.l.c. techniques, provides important information for the elucidation and characterization of intricate branching structures of amylopectin and glycogen.

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