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Structures of sub-fractions of corn amylose

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ABSTRACT

Incubation of corn amylose in aqueous 1-butanol at 40° gave insoluble (F_{ppt}) and soluble (F_{sup}) fractions. F_{sup} had a lower affinity for iodine (i.a. 16.0 g.100 g^{-1}) and a higher weight-average d.p. ($\overline{d.p.}_w$ 5910) than F_{ppt} (i.a. 20.5, $\overline{d.p.}_w$ 2460). F_{ppt} and F_{sup} contained linear and slightly branched molecules in the molar ratios 60:40 and 84:16, respectively. Of the branched molecules in the amylose, ~ 89% was found in F_{ppt}. This main branched molecule had $\overline{d.p.}_w$ 5700, number-average d.p. ($\overline{d.p.}_n$) 2200, and 6 chains on average, and comprised the short ($\overline{d.p.}_n$ ~ 18) and extremely long ($\overline{d.p.}_n$ > 370, $\overline{d.p.}_w$ > 2760) chains. The branched molecules in F_{sup} had $\overline{d.p.}_w$ 7840, $\overline{d.p.}_n$ 1620, and 20 chains on average, and comprised short ($\overline{d.p.}_n$ ~ 18), long (d.p.$_w$ > 230), and extremely long (d.p.$_w$ > 2730) chains. Maltohexaose was the shortest side-chain of the branched molecules. The large branched molecule in F_{sup} seemed to comprise an immature cluster.

INTRODUCTION

Amyloses, isolated from various starches by the conventional method[1,2], contain linear and slightly branched molecules (6–19 chains on average) in various molar ratios 30–89:11–70[1,3–10]. The linear molecule is the true amylose, and the branched molecule is the so-called third (intermediate) component of starch[11], because it has three extremely long chains and three short chains having an average length similar to that of amylopectin[8]. However, the structures of the third components from various plants are not well characterised. The structure of corn amylose resembles that of rice amylose[5] and we now report on the sub-fractions of corn amylose.

EXPERIMENTAL

Materials. — Sweet-potato beta-amylase[12] was recrystallised from aqueous ammonium sulfate. Crystalline *Pseudomonas* isoamylase was a product of Hayashibara Biochemical Laboratories Inc. (Okayama).

Preparation of sub-fractions of amylose and their beta-limit dextrins. — Amylose[1,2] from corn starch was dissolved in aqueous 10% 1-butanol by heating under nitrogen and the solution was kept at 40° for 2 h. The precipitate was collected by centrifugation (~40°, 10 000*g*, 15 min) and dissolved in aqueous 10% 1-butanol, and the solution was

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kept under the same conditions as above. This procedure was repeated twice. The final precipitate (F_{ppt}) was washed with ethanol and ether, then dried *in vacuo* over $CaCl_2$. The combined supernatant solutions were filtered through a glass filter (G5), concentrated at 40–50°, and diluted with ethanol (2 vol.). The precipitate was collected, and dried to give F_{sup}. The yields of F_{ppt}, F_{sup}, and amylopectin were 10, 1.6, and 44 g, respectively, from 60 g of starch.

The beta-limit dextrin (β-LD) of each sub-fraction was prepared[4] by gel-filtration chromatography of the beta-amylolysate on Bio-Gel P-2.

Preparation of the fractions of short and long chains from the isoamylolysate of the amylose sub-fraction[8]. — The isoamylolysate of each sub-fraction was incubated for 2 h at 30° in aqueous 10% 1-butanol. The fractions with short (SCF) and long (LCF) chains were obtained in the supernatant solution and precipitate, respectively.

Analytical methods. — The iodine affinity (i.a.) was determined at 25° by a modified[13] amperometric titration[14], the blue value as described[15], and the number-average d.p. ($\overline{d.p._n}$) by the modified Park–Johnson method[3]. The weight-average d.p. ($\overline{d.p._w}$) and distribution of d.p. were determined[16] by gel-permeation h.p.l.c. on three columns (Tosoh, TSKgel, G6000PW, G4000PW, and G3000PW) in series, using a differential refractometer (Tosoh, RI-8011) and a low-angle laser-light-scattering photometer (Tosoh, LS-8) as detectors. The average chain-length ($\overline{c.l.}$) was determined by the rapid Smith-degradation method[3] with minor modifications[2]. The average number of chains per molecule ($\overline{n.c.}$) was calculated as $\overline{d.p._n}/\overline{c.l.}$ The beta-amylolysis limit ($\beta_{a.l.}$) was determined as described[2]. Carbohydrate was determined by the phenol–sulfuric acid method[17]. The treatment with isoamylase was carried out as described[3]. Malto-oligosaccharides were analysed at room temperature by column h.p.l.c. (Tosoh, TSKgel NH_2-60), using a differential refractometer (Tosoh, RI-8000) as detector. The solvent was aqueous 50% acetonitrile at 0.8 mL.min^{-1}. Amylose, partially hydrolysed by dilute acid, gave the malto-oligosaccharide standards.

RESULTS AND DISCUSSION

Amylose, isolated[1] from corn starch, was fractionated into insoluble (F_{ppt}) and soluble (F_{sup}) fractions by incubation in aqueous 10% 1-butanol at 40° (*cf.* 8° in the conventional preparation of amylose). The yields of F_{ppt}, F_{sup}, and amylopectin from the starch were 16.7, 2.7, and 73.3%, respectively. The sum (19.4%) of the yields of F_{ppt} and F_{sup} was similar to the amylose content (20–21%)[18] of corn starch, and the ratio of F_{ppt} and F_{sup} by weight was 86:14. Table I shows the properties of the amylose sub-fractions. The $\overline{d.p._n}$ and $\overline{d.p._w}$ values of F_{sup} were 580 and 5910, respectively, which were about half and twice those of F_{ppt}, respectively. The molar ratio of F_{ppt} and F_{sup} was calculated to be 77:23, using the ratio $(W_r)_{Fppt}/(\overline{d.p._n})_{Fppt}:(W_r)_{Fsup}/(\overline{d.p._n})_{Fsup}$ where W_r is the ratio of the sub-fractions by weight.

F_{sup} had i.a. 16.0, $\overline{c.l.}$ 140, and $\beta_{a.l.}$ 72%; these values were lower than those (i.a. 20.5, $\overline{c.l.}$ 360, $\beta_{a.l.}$ 85%) of F_{ppt}. The n.c. of F_{sup} was 4.1, which was higher than that (3.0) of F_{ppt}. The branch linkages were α-(1→6) because of the complete degradation on

TABLE I

Properties of F_{sup} and F_{ppt}, of the corn-amylose fraction and their beta-limit dextrins (β-LD_{Fsup} and β-LD_{Fppt})

	F_{sup}	β-LD_{Fsup}	F_{ppt}	β-LD_{Fppt}
Iodine affinity (i.a., g/100 g)	16.0	16.0	20.5	20.2
Blue value	1.21	1.11	1.38	1.36
λ_{max} (nm)	633	628	644	643
Number-average d.p. ($\overline{d.p._n}$)	580	800	1050	1100
Weight-average d.p. ($\overline{d.p._w}$)	5910	3880	2460	2820
Apparent d.p. distribution[a]	270–28 000	260–21 000	500–9800	240–11 000
$\overline{D.p._w}/\overline{d.p._n}$	10.2	4.85	2.34	2.56
Average chain-length ($\overline{c.l.}$)	140	40	360	180
Average number of chains ($\overline{n.c.}$)	4.1	20.0	3.0	6.0
Beta-amylolysis limit ($\beta_{a.l.}$, %)	72		85	
$\beta_{a.l.}$ with pullulanase (%)	100		100	
Molar fraction[b]				
Branched molecule	0.16		0.40	
Linear molecule	0.84		0.60	

[a] $\overline{D.p._w}$ values of the sub-fractions (10% by weight) having the lowest and highest molecular weights.
[b] Calculated[2] from $\overline{n.c.}$ of the sub-fraction and its β-LD.

simultaneous incubation with pullulanase and beta-amylase. These results imply that F_{sup} comprised a branched molecule with a slightly higher degree of branching or a higher amount of a branched molecule than F_{ppt}. The former alternative applied (see below).

Amylose comprises branched and linear molecules, and no method is available at present for their quantitative isolation. Therefore, the structure of the branched molecule is analysed on the basis of the derived beta-limit dextrin[4] (β-LD). β-LD_{Fsup} and β-LD_{Fppt} were prepared from F_{sup} and F_{ppt}, respectively, and their properties are listed in Table I. The iodine-binding and staining properties of each β-LD were similar to those of the parent sub-fraction as reported for whole amylose[5]. β-LD_{Fsup} had $\overline{d.p._n}$ 800 and $\overline{d.p._w}$ 3880 (*cf.* 1100 and 2820 for β-LD_{Fppt}). The $\overline{d.p._w}$ and $\overline{d.p._n}$ of the branched molecule of F_{sup} were calculated to be 7840 and 1620, respectively, assuming $\beta_{a.l.}$ to be 50.5% and using the equation $\overline{d.p._w}$ or $\overline{d.p._n}$ = ($\overline{d.p._w}$ or $\overline{d.p._n}$ of β-LD)/(100 − $\beta_{a.l.}$)/100. The value of $\beta_{a.l.}$ was the average for branched molecules of amyloses from various plants[11]. The $\overline{d.p._w}$ and $\overline{d.p._n}$ of the branched molecule of F_{ppt} were calculated to be 5700 and 2220, respectively.

β-LD_{Fsup} and β-LD_{Fppt} had $\overline{c.l.}$ 40 and 180, respectively (*cf.* ~10 for amylopectin β-LD). The c.l. of the branched molecules of F_{sup} and F_{ppt} appeared to be 80 and 370, respectively, which were calculated as ($\overline{d.p._n}$ of branched molecule)/$\overline{n.c.}$ These results, and the high i.a. of β-LD_{Fsup} (16.0) and β-LD_{Fppt} (20.2), implied that the structure of the branched molecule could be distinguished clearly from that of amylopectin. The $\overline{n.c.}$ of each β-LD indicated that the branched molecules of F_{sup} and F_{ppt} had 20 and 6 chains on average, respectively. Thus, the branched molecule of F_{sup} had a higher degree of branching and a lower $\overline{c.l.}$ than that of F_{ppt}.

The branched molecule was a minor component in corn amylose[5] and was present in both of the amylose sub-fractions, since its molar fraction was calculated[4] to be 0.16 for F_{sup} and 0.40 for F_{ppt} from the n.c. of the amylose sub-fractions and their β-LDs (Table I). From the molar fraction (M_{fb}) of the branched molecule and the molar ratio (M_r) of F_{sup} and F_{ppt} (23:77), the molar ratio of the F_{sup} and F_{ppt} branched molecules was calculated to be 11:89 [using the equation $(M_r)_{Fppt} \times (M_{fb})_{Fppt}:(M_r)_{Fsup} \times (M_{fb})_{Fsup}$], which indicated that F_{sup} involved a minor portion of the corn branched molecules.

The isoamylolysates of F_{sup} (i-F_{sup}) and F_{ppt} (i-F_{ppt}) had $\overline{d.p.}_w$ 1730 and 2360, respectively (*cf.* 5910 and 2460 for F_{sup} and F_{ppt}, respectively). Each isoamylolysate was fractionated by incubating with aqueous 1-butanol into soluble (short-chain, SCF) and insoluble-precipitate (long-chain, LCF) fractions (see Table II). The yield-ratio of SCF and LCF was 12:88 for F_{sup} and 1:99 for F_{ppt}. The SCFs had similar blue values (0.15–0.22), $\overline{d.p.}_n$ (16–18), and c.l. (17–19), and were linear since they were hydrolysed completely by beta-amylase. The chromatogram of SCF (Fig. 1) showed that the d.p. at the maximum was 11, and that maltohexaose was the smallest chain although there was an unidentified, asymmetrical, small peak at $T \sim 8$ min. The smallest chain differed from that (maltotetraose) of a potato amylose[3]. LCF from F_{sup} had a higher i.a. (18.4) and blue value than the parent, implying that the relatively large amount of the short chains of F_{sup} decreased the i.a. of the branched molecules. On the other hand, LCF from F_{ppt} had the same i.a. as the parent, probably due to a small proportion of short chains. LCF from F_{sup} had $\overline{d.p.}_n$ 510 and $\overline{d.p.}_w$ 1880 (*cf.* 950 and 2360 for LCF from F_{ppt}). Each LCF had a higher c.l. and $\beta_{a.l.}$ than the parent sub-fraction. LCFs had n.c. >1 and were incompletely hydrolysed by beta-amylase, indicating that isoamylase had not cleaved all of the branch linkages[3], perhaps due to the extremely long side-chains as suggested[8] for the branched molecule from rice starch. These results indicate that the branched molecules of F_{sup} and F_{ppt} comprised short side-chains with $\overline{d.p.}_n \sim 18$ and may have had extremely long side-chains, and that the branched molecule of F_{sup} had a higher proportion of short chains than that of F_{ppt}.

The gel-permeation chromatograms (Fig. 2) and the data in Table I showed that F_{sup} had a wider distribution of d.p. than F_{ppt}, and that F_{sup} was composed of two fractions (F1 and F2 in order of elution) whereas F_{ppt} gave a single fraction. F1 and F2 had $\overline{d.p.}_w$ 7230 and 380, respectively, and their ratio by weight was 81:19 (Table III). Figure 2A shows that a plot of d.p. against retention time gave a steeper slope for the large molecule of F1 than for the small molecules, probably due to a slightly higher degree of branching (*cf.* the data for polyethylene[19]). This conclusion was supported by the results of debranching with isoamylase (see below).

β-LD_{Fsup} gave fractions, F1 and F2, similar to F_{sup} (Fig. 3A), which indicated that F_{sup} comprised large and small branched molecules. The ratio of F1 and F2 by weight was 87:13, and they had $\overline{d.p.}_w$ 4420 and 260, respectively (Table III). From these values, $\overline{d.p.}_w$ of the large and small branched molecules were calculated to be 8930 and 530, respectively, assuming the $\beta_{a.l.}$ of the branched molecules to be 50.5%[11]. The large molecule of F1 seemed to be more abundantly branched than the small molecule, because of the steeper slope of the plots of d.p. of the large molecules (Fig. 3A). A single

TABLE II

Properties of the fractions with short (SCF) and long (LCF) chains of isoamylase-debranched sub-fractions (F_{sup} and F_{ppt})

	F_{sup}		F_{ppt}	
	SCF	*LCF*	*SCF*	*LCF*
Yield (%)	12	88	1	99
I.a. (g/100 g)		18.4		20.2
Blue value	0.22	1.29	0.15	1.44
λ_{max} (nm)	544	634	534	642
$\overline{D.p._n}$	18	510	16	950
$\overline{D.p._w}$		1880		2360
Apparent d.p. distribution		200–9000		200–10 000
$\overline{C.l.}$	19	345	17	540
$\overline{N.c.}$	1.0	1.5	1.0	1.8
$\beta_{a.l.}$ (%)	99	77	100	92

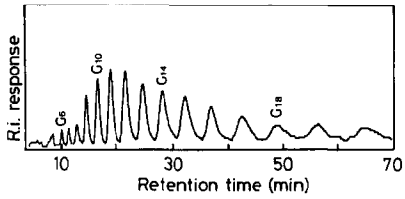

Fig. 1. Analysis of the short-chain component of the isoamylase-debranched sub-fraction by h.p.l.c. on a TSKgel NH_2-60 column (number: d.p.). The conditions were as given in the text.

TABLE III

Weight proportions and $\overline{d.p._w}$ of fractions[a] F_{sup} and β-LD_{Fsup}, and their isoamylolysates (i-F_{sup} and i,β-LD_{Fsup})

	Weight proportion (%)			$\overline{D.p._w}$		
	F1	*F2*	*F3*	*Whole*	*F1*	*F2*
F_{sup}	81	19	0	5910	7230	380
i-F_{sup}	72	18	10	1730	2370	210
β-LD_{Fsup}	87	13	0	3880	4420	260
i,β-LD_{Fsup}	67	16	17	1860	2730	230

[a] See Figs. 2A and 3A.

elution profile of β-LD_{Fppt} (Fig. 3B) indicated that the branched molecule of F_{ppt} had a similar branched structure. The $\overline{d.p._w}$ of the branched molecule of F_{ppt} was estimated as 5700, from the $\overline{d.p._w}$ (2820) of β-LD_{Fppt}. Therefore, corn amylose appears to comprise three branched components with high ($\overline{d.p._w}$ 8930), medium (5700), and low (530)

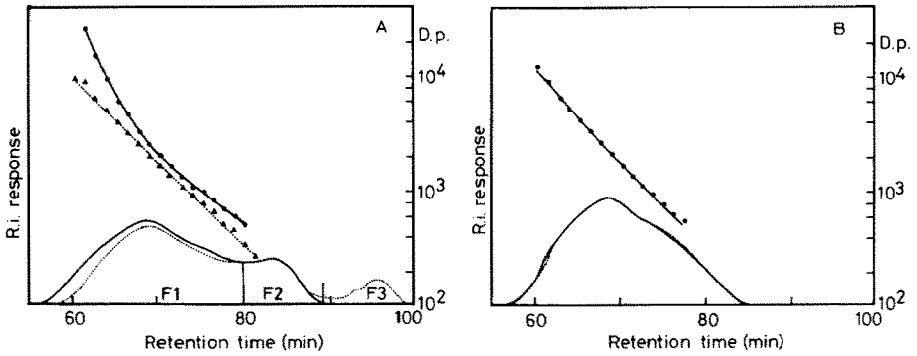

Fig. 2. Gel-permeation h.p.l.c.[16] of the sub-fractions (—: A, F_{sup}; B, F_{ppt}) and their isoamylolysates (...: A, i-F_{sup}; B, i-F_{ppt}) on connected columns of TSKgel G6000PW, G4000PW, and G3000PW; and the d.p. (●, sub-fraction; ▲, isoamylolysate).

molecular weights, of which the medium-branched molecule comprises a major proportion.

The isoamylolysate (i-F_{sup}) of F_{sup} gave three fractions, F1–F3, in order of elution (Fig. 2A). The ratios of the F1–F3 by weight were 72:18:10, and the $\overline{d.p.}_w$ of the F1 and F2 were 2370 and 210, respectively (Table III). F1 showed a linear and shallow slope of the plot of d.p. against retention time (Fig. 2A) compared with F_{sup} F1, indicating that the latter was more abundantly branched. i-F_{sup} F1 with the same retention time as F_{sup} F1 had a lower d.p., suggesting that the former had a higher limiting viscosity number $[\eta]$ since the hydrodynamic volumes were the same and proportional to $[\eta]\cdot(d.p.)$. i-F_{sup} F1, which had the higher $[\eta]$, appeared to be more slender in shape than F_{sup} F1. i-F_{sup} F1 was lower in proportion and $\overline{d.p.}_w$ than F_{sup} F1. i-F_{sup} F2 had a lower $\overline{d.p.}_w$ than the F_{sup} F2, but a similar proportion. i-F_{sup} F3 was a short side-chain fraction since F_{sup} gave no F3, and the amount of i-F_{sup} F3 was similar to that (12%) of SCF from F_{sup}. These results

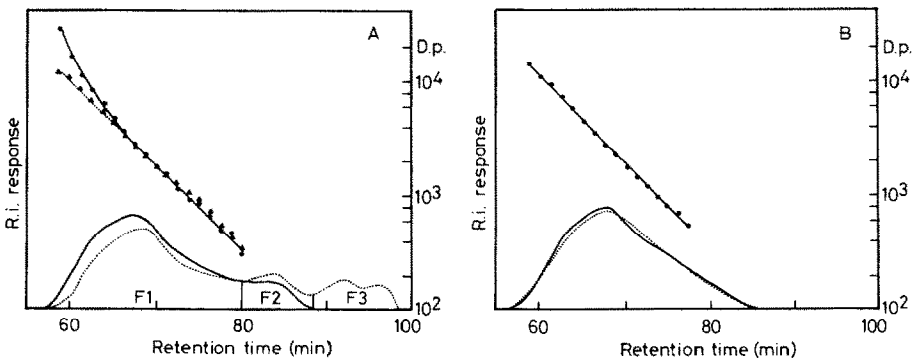

Fig. 3. Gel-permeation h.p.l.c.[16] of the β-LDs (—: A, β-LD_{Fsup}; B, β-LD_{Fppt}) and their isoamylolysates (...: A, i,β-LD_{Fsup}; B, i,β-LD_{Fppt}) on connected columns of TSKgel G6000PW, G4000PW, and G3000PW; and the d.p. (●, β-LD; and ▲, isoamylolysate).

imply that the F_{sup}-F1 branched molecules, especially the large molecules, were abundantly branched and became slender on isoamylolysis after release of the short chains (*i*-F_{sup} F3) and, perhaps, long chains having a d.p. similar to that of *i*-F_{sup} F2. These conclusions were supported as follows.

The isoamylolysate of β-LD_{Fsup} (*i*, β-LD_{Fsup}) also comprised three fractions, F1–F3 (Fig. 3A). The ratios of F1–F3 by weight were 67:16:17, and the $\overline{d.p.}_w$ of F1 and F2 were 2730 and 230, respectively (Table III). F1 was lower in proportion and $\overline{d.p.}_w$ than β-LD_{Fsup} F1. F1 gave a linear plot of d.p. against retention time, which had a steeper slope than that for the parent β-LD_{Fsup}. These results suggest that F1 is a more slender molecule than β-LD_{Fsup} F1, due to debranching. F2 comprised a slightly higher proportion than β-LD_{Fsup} F2, which implied that, on isoamylolysis, β-LD_{Fsup} F1 gave a long-stub chain with a $\overline{d.p.}_w$ (230), similar to that of *i*,β-LD_{Fsup} F2. F3 (Fig. 3A) was a short-stub chain fraction. Thus, the branched molecule of F_{sup} seemed to comprise short ($\overline{d.p.}_n \sim 18$) and long side-chains with a d.p. higher than that ($\overline{d.p.}_w$ 230) of *i*,β-LD_{Fsup} F2.

The isoamylolysates of F_{ppt} and β-LD_{Fppt} (*i*-F_{ppt} and *i*, β-LD_{Fppt}, respectively) had $\overline{d.p.}_w$ 2360 and 2760, respectively (*cf.* 2460 and 2820 for F_{ppt} and β-LD_{Fppt}, respectively). *i*-F_{ppt} and *i*,β-LD_{Fppt} had elution profiles that were similar to those of the respective parents (Figs. 2B and 3B), and there was no detectable increase in the proportion of the fraction corresponding to *i*,β-LD_{Fsup} F2 (Fig. 3A). This result may indicate that the branched molecule in F_{ppt} comprised no side chain corresponding to the long side-chain of the F_{sup} branched molecule. SCF from F_{ppt} was isolated but was not detectable on the chromatogram, due to its small amount.

The results for the amylose sub-fractions F_{sup} and F_{ppt} indicate that the corn amylose comprises branched molecules that differ in size, c.l., and degree of branching. F_{ppt} contained 89% of the branched molecules in the amylose, which had $\overline{d.p.}_w$ 5700, $\overline{d.p.}_n$ 2220, 6 chains on average, short side-chains ($\overline{d.p.}_n \sim 18$), and extremely long chains judging from the $\overline{c.l.}$ (540) of LCF and the $\overline{d.p.}_w$ (2760) of *i*,β-LD_{Fppt}. The latter are the main chains, and the side chains are resistant to isoamylase. The $\overline{d.p.}_n$ of the long chains could not be determined since the branched molecule cannot be isolated at present, but it seems to be higher than the $\overline{c.l.}$ [360 calculated as ($\overline{c.l.}$ of β-LD_{Fppt})/(100 − $\beta_{a.l.}$, 50.5%)/100] of the F_{ppt} branched molecule. The minor branched molecule (11%) was involved in F_{sup}, had $\overline{d.p.}_w$ 7840, $\overline{d.p.}_n$ 1640, and 20 chains on average, and seemed to comprise short ($\overline{d.p.}_n \sim 18$), long, and extremely long chains. The sizes of the long and extremely long chains could not be determined, but are presumed to be higher than those of *i*,β-LD_{Fsup} F2 ($\overline{d.p.}_w$ 230) and F1 ($\overline{d.p.}_w$) 2730), respectively. The F_{sup} branched

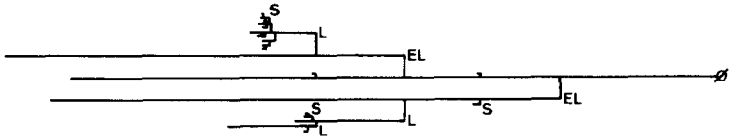

Fig. 4. Proposed structure of the branched molecule comprising immature clusters. EL, extremely long; L, long; and S, short chains; ∅, reducing terminal.

molecule is composed of the large ($\overline{d.p.}_w$ 8930) and small ($\overline{d.p.}_w$ 530) molecules. The elution patterns of the *i*-F_{sup} and *i*,*β*-LD_{Fsup} F3 on gel-permeation chromatography (Figs. 2A and 3A) showed a bimodal distribution of d.p. similar to those of the isoamylolysates of amylopectin and its *β*-LD (chromatograms not shown), respectively. Therefore, an abundantly branched amylose molecule, such as the large molecule in F_{sup}, seems to have a structure which resembles locally that of amylopectin, that is, an immature cluster[20] (Fig. 4).

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