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SOME STRUCTURAL FEATURES OF AMYLOMAIZE STARCH

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Key Word Index—*Zea mays*; Gramineae; amylomaize starch; amylose; amylopectin; intermediate material.

Abstract—Amylomaize starch was sub-fractionated into two components, complexing (C-fraction) and non-complexing (S-fraction) fractions, and properties of the two fractions were examined. Further fractionation of these fractionated starches on Sepharose CL-2B revealed that C-fraction of the amylomaize starch was composed of 51.5% high MW material and 48.5% low MW material. S-fraction also contained 33.4% high MW material, but this fraction was characterized by the presence of 66.6% low MW material whose λ_{max} of the peak was different from that of the low MW material in the C-fraction. Elution patterns of S-fraction starch with and without debranching treatment on Sepharose CL-4B indicated that the MW of this low MW material was lower than that of amylose fraction in the original starch granule. Both high and low MW materials in the S-fraction were composed of branched molecules. Fractionation of debranched C-fraction starch on Sephadex G-200 indicated that this starch contained 47.5% of two tractions with average chain lengths of 54 and 19 which were longer than those of normal amylopectin. Although S-fraction starch after debranching was also composed of the two fractions with the same chain lengths, the ratio of Fr. III/Fr. II in the S-fraction was noticeably lower than that in the C-fraction. In addition, any peak which corresponded to short-chain amylose was not detectable from the elution patterns of all the debranched starches. These results reconfirmed the presence of amylopectin characteristic for amylomaize starch, and suggested that amylomaize starch might have a significant amount of an unknown material corresponding to neither amylose nor amylopectin fraction in the original starch granules. It was also suggested that the unknown material might be smaller than the amylopectin molecule and might be a branched chain molecule rather than a linear one.

INTRODUCTION

When compared with normal maize (*Zea mays*) starch, amylomaize starch is characterized either by an increased amylose component[1], or by some unique properties and structures[2–10]. The following suggestions have been proposed:

1. Amylomaize amylopectin has longer inner and outer branches than those of normal amylopectin[2, 3].
2. Amylomaize starch contains amylose of a lower degree of polymerization than normal amylose[4, 5].
3. Amylomaize starch contains an unusually high proportion of intermediate material fitting neither the definition of amylose nor amylopectin [5].

In spite of many investigations, however, the structural features of amylomaize starch have not been well characterized.

The use of gel filtration for debranched starches has enabled the analysis of unit chain profiles of amylopectin fraction and amylose content in the original starch granule[11–15]. Recent reports[13–15] indicated that the average chain lengths (CL) of unit chain fractions in amylomaize starch were longer than those in normal or waxy (wx) maize starch. These reports seemed to support the first suggestion. For confirmation of the second and third suggestions, further investigations are necessary.

In the present study, we have carried out a sub-fractionation by addition of *n*-BuOH to a DMSO-amylomaize starch dispersion, and have examined the properties of the sub-fractionated starches. The purpose of the present study was to investigate the structure of each component of amylomaize starch.

RESULTS

General properties

Properties of original starch, *n*-BuOH complexing (C-fraction), and *n*-BuOH non-complexing (S-fraction) starches are shown in Table 1. Values of fractionation yields for C- and S-fractions were 55.2% and 44.8%, respectively. The values were calculated from triplicated small-scale fractionations, each using 1 g of original amylomaize starch. As pointed out by Adkins and Greenwood[4], our larger scale fractionation also made a physical loss of *ca* 15% of the original starch. Amperometric iodine binding capacity, β-amylolysis limits, and λ_{max} of C-fraction starch were higher than those of S-fraction starch. The original starch showed intermediate values between the C- and S-fraction starches.

Fractionation on Sepharose CL-2B

Original, C-fraction, and S-fraction starches were fractionated on a column of Sepharose CL-2B, and

the elution patterns are shown in Fig. 1. All starches were composed of two peaks in high and low MW regions. The elution pattern of C-fraction was similar to that of the original. A peak in the low MW region of the S-fraction shifted to a lower MW than those peaks of the original or of the C-fraction. Table 2 shows percentages of high and low MW materials in each starch which were calculated from the carbohydrate content of the elution patterns. The content of the high MW material in the C-fraction was somewhat higher than that of the low MW material. The proportion was reversed in the S-fraction starch which contained a considerable amount of low MW material. In a further comparison, values of the fractionation percentages were converted to percentage of original starch by calculations from *n*-BuOH fractionation yields. The percentage of high MW material in the original amylomaize starch (43.6%) agreed well with the sum of the percentage value of high MW materials of C- and S-fractions (43.4%). The same tendency was observed for low MW materials (56.4 and 56.6%), indicating that unfavorable degradation did not occur during the analyses. Although the λ_{max} of each peak of high MW material was the same in all starches, those of the low MW material differed a little in original, C-fraction, and S-fraction, the λ_{max} being 630, 645, and 605 nm, respectively.

Fractionation on Sepharose CL-4B

As shown in Fig. 2, starches of original, C-fraction, and S-fraction were debranched by isoamylase (EC 3.2.1.68), and the starches were then fractionated on a column of Sepharose CL-4B. By debranching of C-fraction starch, the peak of high MW material in this fraction disappeared, and a new peak appeared in the much lower MW position than the peak of low MW

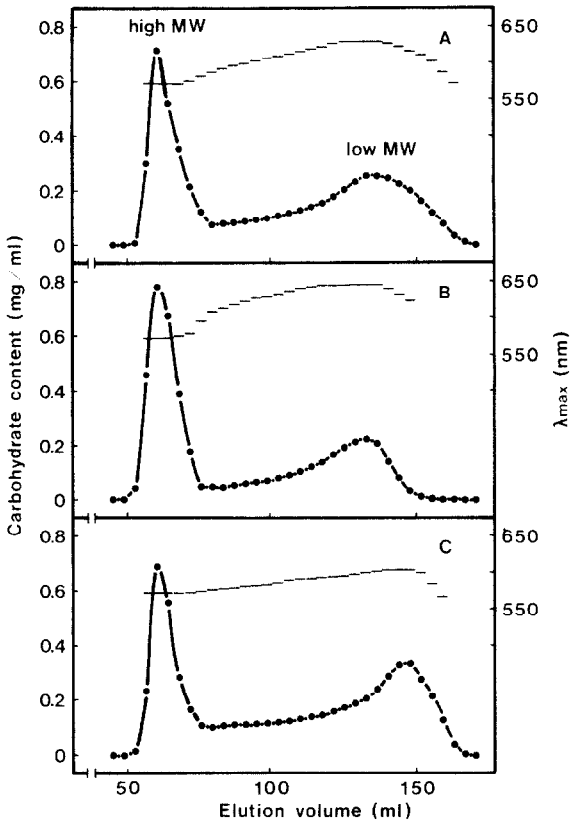

Fig. 1. Elution patterns of starches without debranching on Sepharose CL-2B. Carbohydrate content (●—●) and λ_{max} of the starch–iodine complex (lateral bar) in each tube were measured as described in Experimental. Starches examined were original (A), C-fraction (B), and S-fraction (C).

Table 1. Properties of original, C-fraction, and S-fraction starches

Starch	Fractionation yield (% original starch)	I_2-binding capacity (mg I_2/100 mg sample)	β-Amylolysis limits (%)	λ_{max} (nm)
Original	(100.0)	9.4	68.1	602
C-fraction	55.2 ± 0.9	11.0	81.6	628
S-fraction	44.8 ± 0.9	5.7	58.4	586

Table 2. Fractionation percentages of high and low MW materials, and λ_{max} of each peak

Starch	High MW side			Low MW side		
	(%)*	(% original)†	λ_{max} (nm)	(%)*	(% original)†	λ_{max} (nm)
Original	43.6	—	572	56.4	—	630
C-fraction	51.5	28.4	572	48.5	26.8	645
S-fraction	33.4	15.0	572	66.6	29.8	605

*Estimated from fractionations of starches without debranching on Sepharose CL-2B.

†Calculated from fractionation yield described in Table 1.

material before debranching. Although the peak of low MW material was still observed in the same position, the λ_{max} of this peak after debranching was higher than that before debranching. In the case of S-fraction starch, two peaks of both high and low MW materials were eliminated by debranching, and only a new single peak with a slight shoulder was observed in the lower MW position than the peak of low MW material in S-fraction.

Fractionation on Sephadex G-200

Debranched starches were fractionated on a column of Sephadex G-200, and elution patterns are shown in Fig. 3. Each elution pattern was composed of three peaks; the first peak (Fr. I) appeared near the void volume, the second peak (Fr. II) around the $\overline{CL}$ of 55, and the third peak (Fr. III) around the $\overline{CL}$ of 20. The elution pattern of the C-fraction was similar to that of the original. The first peak of S-fraction was significantly lower than those of the other Frs. I. Percentages of Frs. I–III of the debranched starches are shown in Table 3. Proportions of Frs. I–III were 52.5, 24.6, and 22.9% in C-fraction, respectively, and the $\overline{CL}$ of each peak of the Frs. II and III was 54 and 19. In contrast to the C-fraction, debranched S-fraction starch contained a much smaller amount of Fr. I (6.6%) than the original or C-fraction, while an increment in Fr. II (65.3%) was observed. However, $\overline{CL}$ of each peak of Frs. II and III agreed with those of the C-fraction. The ratio of Fr. III/Fr. II in C-fraction was *ca* twice as high as that in the S-fraction. Percentages of debranched C- and S-fraction starches were converted into percentage of original starch according to their fractionation yields. Each sum of Frs. I–III in both C- and S-fractions respectively agreed approximately with the corresponding percentage of Frs. I–III in the original amylomaize starch. When wx and normal maize starches were debranched, the elution patterns were almost the same as previously reported [13–15], and each $\overline{CL}$ of the peaks of Frs. II and III was shorter than those in original amylomaize, C-fraction, and S-fraction.

Fractionation on Sephadex G-75

In order to confirm the results of fractionation on Sephadex G-200, fractionation of Frs. II and III of debranched starches were analysed on a column of Sephadex G-75. The elution patterns are shown in Fig. 4. Like the fractionation on Sephadex G-200, the elution patterns of debranched starches on Sephadex G-75 were also composed of three peaks, which cor-

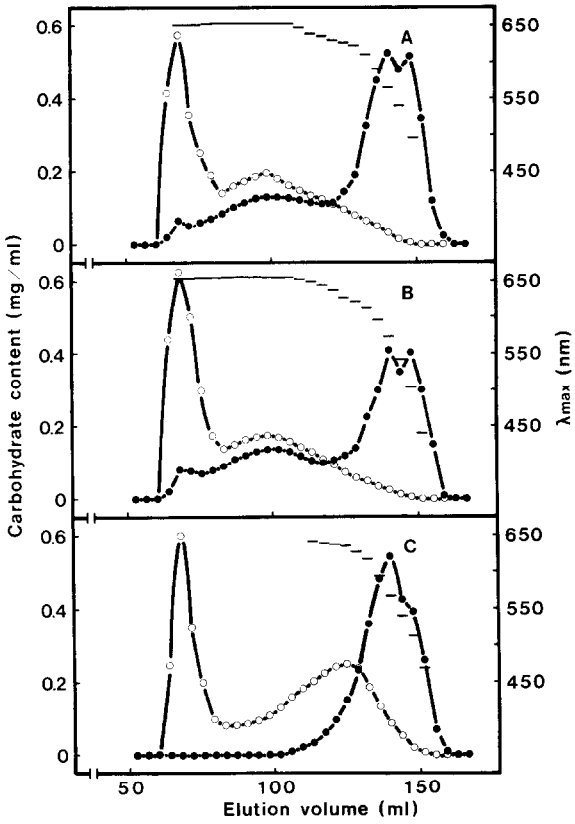

Fig. 2. Elution patterns of starches with (●—●) and without (○—○) debranching on Sepharose CL-4B. Starches examined were original (A), C-fraction (B), and S-fraction (C). Lateral bar indicates λ_{max} of the starch–iodine complex in each tube after debranching.

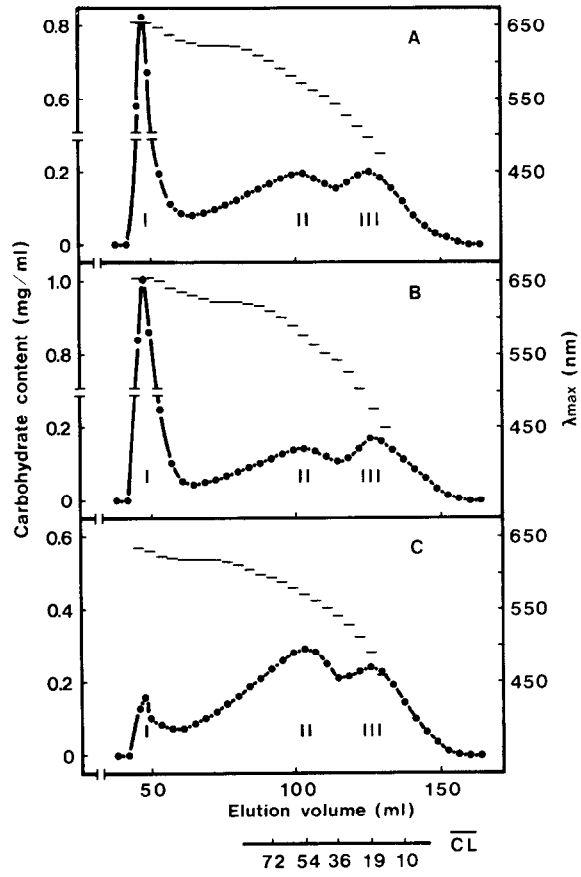

Fig. 3. Elution patterns of debranched starches of original (A), C-fraction (B), and S-fraction (C) on Sephadex G-200. Amounts of carbohydrate and reducing end-group in each tube were measured as described in Experimental, and average chain length ($\overline{CL}$) was calculated from the ratio of the carbohydrate and the reducing ends. Lateral bar indicates λ_{max} of the debranched starch–iodine complex in each tube.

Table 3. Results from fractionations of debranched starches on Sephadex G-200

Starch	Fr. I (%)	Fr. II (%)	Fr. III (%)	Fr. II + III (%)	Ratio of Fr. III/Fr. II	Peak of Fr. II ($\overline{CL}$)	Peak of Fr. III ($\overline{CL}$)
Original	34.0	40.6	25.4	66.0	0.63	54	19
C-fraction	52.5	24.6	22.9	47.5	0.93	54	19
	(29.0)*	(13.6)	(12.6)	(26.2)			
S-fraction	6.6	65.3	28.1	93.4	0.43	54	19
	(3.0)	(29.3)	(12.6)	(41.8)			
wx	0.3	27.3	72.4	99.7	2.65	38	16
Normal	23.5	20.3	56.2	76.5	2.77	39	16

*Percentage of original amylomaize starch: values were calculated from fractionation yield described in Table 1.

responded to those on Sephadex G-200. $\overline{CL}$s of the peaks in original, C-fraction and S-fraction starches were 54, 54 and 56 for Fr. II, and 19, 19 and 19 for Fr. III, respectively. These values approximately agreed with those obtained on Sephadex G-200.

DISCUSSION

Properties of C- and S-fraction starches were analysed before and after debranching treatment with isoamylase, by fractionation on Sepharose CL-2B and CL-4B. The fractionation of the starches without debranching indicated that each starch was composed of two different materials (Figs. 1 and 2). From the results of the λ_{max} of each peak of high MW materials before debranching and elution pattern after debranching, it was suggested that each of the high MW material in the C- and S-fractions corresponded to the amylopectin fraction in the original starch. C-fraction starch contained a large amount of amylopectin fraction (*ca* 50%, Table 2). This result indicated that either amylose or amylopectin in amylomaize starch was complexing by an addition of *n*-BuOH. Boyer *et al.*[16] reported that amylose-extender waxy (ae wx) maize starch consisted only of an altered amylopectin, and the fine structure of the ae wx starch was similar to that of amylomaize amylopectin. In addition, Yamada and Taki[17] reported that ae wx starch was partially complexing with *n*-BuOH. Therefore, in the present experiment, contamination of amylopectin in *n*-BuOH complexing material (C-fraction starch) might be due to the altered structure of amylomaize amylopectin. When wx maize starch, which was composed entirely of normal amylopectin[18], was fractionated by *n*-BuOH, only very small amount of the complexing material could be obtained.

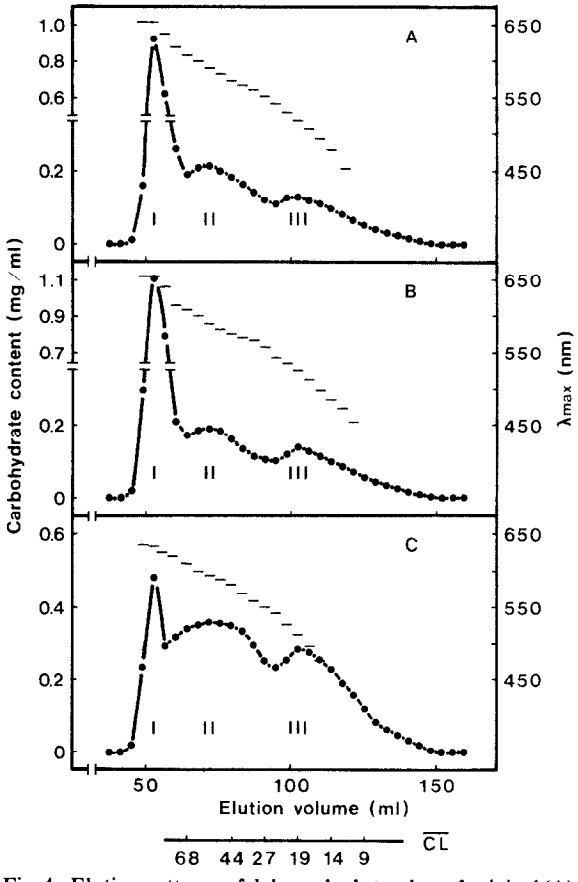

Fig. 4. Elution patterns of debranched starches of original (A), C-fraction (B), and S-fraction (C) on Sephadex G-75. Details are the same as in Fig. 3.

Elution patterns of C-fraction starch on two kinds of Sepharose indicated that the low MW material in the C-fraction mainly corresponded to the amylose fraction in the original starch (Figs. 1 and 2). It was noted that the λ_{max} of the peak of this low MW material after debranching shifted to a higher wavelength than before debranching (645 to 655 nm). However, there might be some difficulties to elucidate that this low MW material, which had been considered to be amylose fraction, was actually lightly branched amylose, or contaminating low MW material which should have been in the S-fraction.

The low MW material in the S-fraction was evidently different from the other materials. This low MW material amounted to *ca* 65% of the S-fraction starch (*ca* 30% of original amylomaize starch, cf. Table 2), and its MW was lower than the amylose fraction (Fig. 2). Adkins and Greenwood[4] reported that *n*-BuOH non-complexing material in amylomaize

starch was a mixture of amylopectin of normal properties and short chain amylose. Recently, Boyer *et al.*[10] reported a fractionation of *n*-BuOH non-complexing material in amylomaize starch on Bio-gel A-50 m and proposed the existence of short-chain amylose (*ca* 100-glc units). It was very interesting that in our experiments, the low MW material in S-fraction also seemed to have some characteristics of the short-chain amylose. However, elution patterns of S-fraction starch with and without debranching on Sepharose CL-4B indicated that a peak of the low MW material disappeared by debranching treatment, and a new peak was detected in the lower MW region (Fig. 2). Furthermore, the peak corresponding to short-chain amylose with $\overline{DP}$ of *ca* 100 could not be observed from the elution pattern in debranched S-fraction starch on Sephadex G-200 (Fig. 3). These results suggested that the low MW material in S-fraction was not a linear molecule, but a branched one.

It has been reported on an existence of material having properties differing from those of both amylose and amylopectin, and the material is called 'intermediate material'[19–21]. Whistler[19] reported that intermediate material in normal maize starch had a lower MW than amylose and gave a deep-blue color with I_2 and λ_{max} of 588 to 600 nm for I_2 solution. Banks *et al.*[5] proposed that amylomaize starch contained an unusually high proportion of intermediate material which fits neither the definition of amylose nor amylopectin. Accordingly, it was very likely that the low MW material in the S-fraction might correspond to the so-called 'intermediate material', although the fine structure of the intermediate material has been scarcely elucidated in both normal and amylomaize starch.

Frs. I–III of debranched C-fraction starch on Sephadex G-200 corresponded to amylose fraction, longer chain, and shorter chain fractions of amylopectin in the original starch, respectively. C-fraction starch contained 52.5% amylose fraction (29.0% of original starch, cf. Table 3), and the value was somewhat higher than that of the low MW material in the C-fraction (Table 2). This might be due to a slight contamination of amylose fraction into the high MW material. The $\overline{CL}$ of each peak of Frs. II and III in C-fraction was longer than those of normal or wx maize starch (Table 3), as previously reported[13–15]. The results reconfirmed that amylomaize amylopectin had longer branches than those of normal amylopectin[2, 3].

As shown in Fig. 2, a peak corresponding to the amylose fraction was not detectable from the elution pattern of debranched S-fraction starch on Sepharose CL-4B. The result would indicate that S-fraction starch might not contain any amylose fraction in the original starch granules. Therefore, Fr. I of the S-fraction seemed to correspond, not to amylose, but to a part of Fr. II. Coincidences of $\overline{CL}$ of each peak of Frs. II and III, and of λ_{max} of the high MW material peak, between C- and S-fractions, supported the suggestion that each amylopectin of the C- and S-fractions was the same one (Tables 2 and 3). In addition, the ratio of Fr. III/Fr. II in the S-fraction was noticeably lower than that in the C-fraction. These results suggested that low MW material in S-fraction was mainly composed of Fr. II having $\overline{CL}$ close to 54.

In ordinary amylose measurements, properties of starch against iodine were used to estimate the amylose content. The amylose content of amylomaize starch used in the present study was estimated to be *ca* 50% by the iodine binding capacity of the starch and corresponding amylose[22]. Although no amylose was expected in S-fraction starch, a noticeable amount of iodine binding capacity was observed in this fraction starch (Table 1). This fact indicated that a true amylose content in the amylomaize starch was significantly lower than that estimated from the iodine method. From the present study, we concluded that 50% amylomaize starch was composed of *ca.* 30% amylose, 40% amylomaize-specific amylopectin, and 30% unknown of a material fitting neither the definition of amylose nor amylopectin, and that the amylomaize starch might contain only negligible amount of amylopectin of normal properties, and short-chain amylose, if they existed.

EXPERIMENTAL

Materials. Amylomaize seeds in the dent inbred M-14 background were obtained from Dr. E. Amano in the Department of Induced Mutation, National Institute of Genetics (Misima, Japan). They were field-grown in 1980 at The Tsukuba Agricultural Technical Center and harvested at 36 days after self-pollination. The ears were immediately placed on dry ice in the field and stored at −20° until used. Endosperm tissues were prepared by removing embryo and pericarp from kernels, and their starches were isolated, and then defatted by MeOH as previously described[9, 15]. Crystalline *Pseudomonas* isoamylase and sweet potato β-amylase (EC 3.2.1.2) were purchased from Hayashibara (Okayama, Japan) and Sigma. All other reagents were analytical grades.

Analytical procedures. Amperometric iodine binding capacities of starches were measured at 10° according to the method of ref.[15, 22]. Absorption spectra of starch–iodine complex were measured by the procedure of ref.[23] with some modifications. Starch soln, containing 2–3 mg of starch/100 ml, was added to iodine reagent soln which was diluted by H_2O instead of satd $CaCl_2$. The spectra of the soln were recorded over the range 400–700 nm. β-Amylolysis limits were determined in a mixture containing 2 mg of starch, 50 mM NaOAc buffer (pH 5), 1 M *meso*-erythritol, and 100 IU of β-amylase [24]. *meso*-Erythritol was added in order to prevent α-glucosidase (EC 3.2.1.20) activity[25]. After complete hydrolysis (30°, 24 hr), liberated maltose and total carbohydrates were measured by copper reduction[26] and phenol–H_2SO_4 method[27].

Sub-fractionation of starches by an addition of *n*-BuOH was performed according to the procedure of ref. [4]. Defatted starch granules (4 g) were dispersed with 90% (v/v) DMSO to obtain a final concn of 2% (w/v). To the starch–DMSO dispersion, 7 vols of 6% (v/v) *n*-BuOH containing 0.1% NaCl were added, and the mixture was allowed to stand at room temp. for 1 hr. *n*-BuOH complexing material was collected by centrifugation at 10 000 *g* for 10 min, and washed with Me_2CO and Et_2O, and finally dried *in vacuo*. *n*-BuOH non-complexing fractions were immediately concentrated *ca* 8-fold by evapn at 35°, precipitated with 5-fold vols of MeOH, and allowed to stand overnight at room temp. The precipitated material was collected, washed with

Me_2CO and Et_2O, and then dried *in vacuo*. Both materials dried were used as *n*-BuOH complexing and non-complexing material.

Debranching of starches with isoamylase was according to the method of ref. [28]. Starch (40 mg) was dispersed in 90% DMSO at a concn of 2% (w/v), and the dispersed starch was incubated at 40° for 24 hr in a mixture containing 20 mM NaOAc–HCl buffer (pH 3.5) and 4720 U [29] of isoamylase. After debranching, 10-fold vol. of EtOH was added to the digest, and starches precipitated were collected by centrifugation at 10 000 g for 10 min. Since values of β-amylolysis limits of debranched products under these conditions were >98% in all starches, the debranching of the starches was almost complete.

Fractionation of starches with or without debranching. Starches with or without debranching were suspended with 1 ml of H_2O and dissolved with 0.5 ml of 1 M NaOH. The soln was made up to 5 ml with H_2O. Starch soln (3 ml) was applied on each column of Sepharose CL-2B, CL-4B, Sephadex G-200, and G-75 (1.6 × 76 cm each) previously equilibrated with 0.05 M NaOH containing 0.02% NaN_3, and eluted with the same soln at room temp. Each fraction (1.9 ml) was collected at the rate of 10 ml/hr, and neutralized with 1 M HCl. The amounts of carbohydrates and reducing end-groups in each fraction were determined by the phenol–H_2SO_4 method [27] and copper reduction [26]. The $\overline{CL}$ was calculated from the ratio of carbohydrates and reducing ends. Recoveries of carbohydrates charged on a column were >95% under these conditions.

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