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Structural changes during development in the amylose and amylopectin fractions (separated by precipitation with concanavalin A) of starches from maize genotypes*

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ABSTRACT

The starches from four maize genotypes (waxy, normal, amylose-extender, and *ae ae/wx wx*) have been sampled at three stages of development, and fractionated by precipitation of the amylopectin with concanavalin A. The resulting amylose and amylopectin fractions were examined by gel chromatography before and after treatment with debranching enzyme. Both mature and immature samples were efficiently fractionated. The proportion of amylose in the starches of the *n* and *ae* genotypes, measured by concanavalin A precipitation, increased on development. A small proportion of the *ae/wx* mutant starch was not precipitated by concanavalin A, and its properties were those of an "intermediate fraction" of low molecular weight with a degree of branching and average chain-length intermediate between amylose and amylopectin. The amylose of *n* starch increased in molecular size during development, whereas that of *ae* decreased. No differences in molecular size and chain-length distribution were detected within a genotype for the amylopectins of *wx*, *n*, and *ae/wx* starches during development, but the component from *ae* starch decreased in molecular size and increased in average chain-length. Comparison of the amylopectins from *wx*, *n*, and *ae* starches showed a decrease in molecular size and an increase in average chain-length, in that order.

INTRODUCTION

Whole starches, solubilised from granules isolated during the development of seeds[1–6], tubers[7], and leaves[8,9], characteristically show an increase in the apparent content of amylose, as measured by iodine-interacting processes, as well as by an increase in viscosity. Establishing precisely the contribution made to these changes by the individual components, amylose and amylopectin, and also the contribution of any intermediate fraction, has been difficult since such methods of fractionation as complexing with 1-butanol and gel chromatography give incomplete fractionation of starches from young organs and also of many from mature organs.

Some comparisons of fractions prepared by complexing with 1-butanol have been made[10]. In two normal (*n*) maize cultivars sampled at three stages of development, the complexing fractions (amylose) were chromatographed on a mixed Sephacryl (400S, 500S, 1000S) column, when an increase in average molecular size with time of development was found. The fractions (amylopectin) in the supernatant solution also showed

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an increase in molecular size. Debranching of the mature supernatant-solution fraction by pullulanase, followed by chromatography on Sephacryl S200/Biogel P6, gave a trimodal elution profile with a shoulder on the lowest molecular weight peak on the side of low molecular size. In other studies of potato starch fractions[11], increases in molecular size and in intrinsic viscosities[7] of the complexing fractions were found. Ultracentrifugation also indicated increasing M_w, and light-scattering of the supernatant-solution fractions was consistent with an increase in M_w. The intrinsic viscosities of amyloses, prepared by complexing with 1-butanol, increased with age in both *n* and high-amylose pea-seed starches[3]. Several studies of gel-chromatographic behaviour of whole starches and beta-limit dextrins of mature maize starches from different genotypes have been made[12–14].

Beta-limit dextrins from whole maize starch, prepared from samples at 3, 4, and 7 weeks after pollination, of different genotypes, and also from phytoglycogen, showed differences in their elution patterns on gel chromatography after debranching[15]. When whole rice starches [*n* and waxy (*wx*)][6] were debranched with isoamylase and chromatographed on Fractogels TSK 55 and 50(s), the ratios of the proportions of glucan in the two peaks of low M_w remained constant for each genotype; this result was interpreted as showing constant chain-length distribution for the amylopectin fraction.

The introduction of concanavalin A as a precipitant for amylopectin[16,17] has provided the possibility for examining the amylose and amylopectin fractions from starches at different stages of development of the accumulating organ.

We now report on the changes in amylose and amylopectin, separated with concanavalin A, of starches of four maize genotypes [*n*, *wx*, amylose extender (*ae*), and the double mutant *ae ae/wx wx* (*ae/wx*)].

RESULTS AND DISCUSSION

The amylose contents of the four genotypes (*n*, *wx*, *ae*, and *ae/wx*) were estimated at three times of sampling (14–20, 34, and 117 days after pollination). In the first samples, the endosperm was at the milky stage, and in the second at the dough stage. The third samples were mature and fully dried. The amylose content was measured by precipitating the amylopectin with concanavalin A and estimating the glucan content in the supernatant solution with the phenol–sulphuric acid reagent[18]. Total starch was also determined with the phenol–sulphuric acid reagent. As has been previously indicated by iodine interaction[1–9], the amylose content increased with time of sampling after anthesis (Table I) for those genotypes containing amylose (*n* and *ae*). The double mutant (*ae/wx*) also contained a small proportion of material not precipitated by concanavalin A at each time of sampling, but a definite trend related to development was not apparent. The structure of this material and its possible role in the fractionation of *ae* starch are discussed later.

The two samples (*n* and *ae*) known to contain amylose were then fractionated preparatively[17] and the fraction (amylose) not precipitated by concanavalin A was examined by gel chromatography on Sepharoses CL-2B and CL-4B prior to and after

TABLE I

Amylose content (%) of maize starch samples estimated by concanavalin A precipitation

Genotype	*First sampling*	*Second sampling*	*Third sampling*
n	14.6	21.1	22.4
ae	11.7	37.3	43.6
ae ae/wx wx	8.5	3	5

treatment with the debranching enzyme, isoamylase. The glucan contents and iodine spectral characteristics (λ_{max} and A_{max}) were determined for each column fraction. The ratios were calculated of the A_{max} to glucan concentration and the relative molecular sizes as a weight-average elution value (K_{WAV}):

$$-\log K_{WAV} = -\log (V_{EW} - V_o)/(V_t - V_o),$$

where V_t is the total volume of the column, V_{EW} is the weight-average elution volume, and V_o is the void volume, and also as a peak elution value (K_{PAV}):

$$-\log K_{PAV} = -\log (V_{EP} - V_o) / (V_t - V_o),$$

where V_{EP} is the elution volume of the peak. These results, together with the λ_{max} values of the fractions, are shown for the *n* samples in Table II and for the *ae* samples in Table III. Selected, illustrative elution profiles are shown in Fig. 1 for *n*, and in Fig. 2 for *ae* samples. The average 100 A_{max}/[glucan] values were ~ 2 at all sampling times and the λ_{max} values in the range 630–640 nm. Also, the elution profiles (Figs. 1 and 2) indicate that these values were maintained across all column fractions, showing that concanavalin A not only gave efficient fractionation of mature starches but also of the early samples.

With the *n* samples (Table II), the $-\log K_{WAV}$ values, particularly on Sepharose CL-4B (Fig. 1C and 1D), show that the molecular size increased with time of seed development. Treatment of all the *n* amyloses with debranching enzyme (isoamylase, Table II and Fig. 1B) caused a slight decrease in the molecular size ($-\log K_{WAV}$), indicating some branching in these molecules, as found previously for amylose fractionated from wheat starch by complexing with 1-butanol[19] and from *n* pea starch by precipitation with concanavalin A[17]. Material of low molecular size, similar to the α-(1→4) chains produced by debranching *wx* starch (Fig. 3A), did not appear, indicating that branching involved relatively long chains.

Examination of the *ae* samples showed a quite different pattern of development with respect to molecular size. At the first sampling (Table III, Fig. 2A), the value was high and then decreased at the second and third sampling times (Table III, Figs. 2C and 2D). The *ae* amylose had a higher relative weight-average molecular size than *n* amylose at the first sampling (Tables II and III), but, by the time of the third sampling, the *ae*

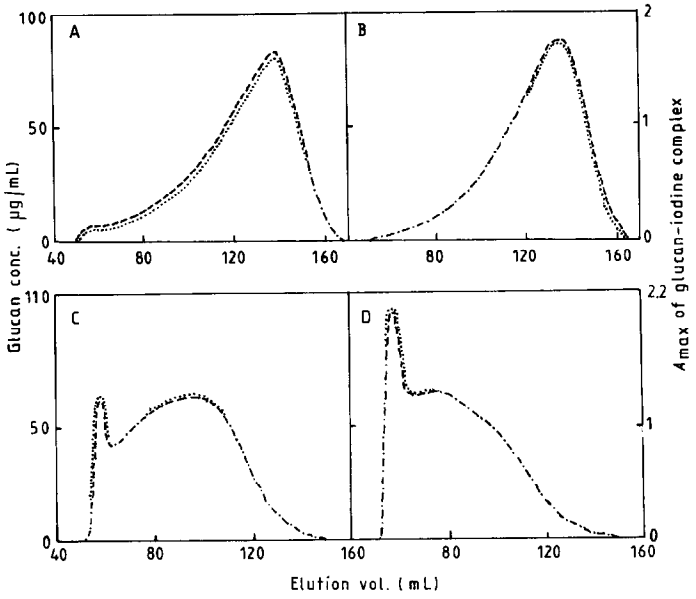

Fig. 1. Gel chromatography of *n* amylose fractions: *A*, 1st sampling on Sepharose CL-2B; *B*, 1st sampling, debranched, on Sepharose CL-2B; *C*, 1st sampling on Sepharose CL-4B; *D*, 3rd sampling on Sepharose CL-4B; ———, glucan; ·····, colour.

value was lower than that of *n*. Like the *n*-amylose fractions, when the *ae* samples were treated with isoamylase, there was a decrease in the weight-average molecular size (Table III), indicating that α-(1→6) linkages were present. The behaviour of debranched *ae* amylose at the first sampling time (Table III, Figs. 2A, 2B, and 2F) was similar to that of the *n* samples (Table II, Figs. 1A and 1B), in that there was depletion of material of high molecular size but no appearance of a fraction of low molecular size in the debranched material. However, the second and third *ae* debranched amyloses produced an inflexion point on Sepharose CL-2B and a peak on Sepharose CL-4B at a relatively lower molecular size (Table III, Fig. 2E). The elution volumes of these peaks on Sepharose CL-4B were earlier than debranched *wx* starch chains (Fig. 3A). The significance of these results is discussed later.

The characteristics of the elution profiles of the amylopectin fractions of the four genotypes at the three stages of development are given in Table IV, and selected profiles are illustrated in Figs. 4 and 5.

For the *wx* samples (Table IV, Figs. 4A and 4B), the ratio of $100A_{max}$/[glucan] and the λ_{max} values were 0.8 and 530 nm, respectively, and these were constant over the whole range of column fractions. The profiles were similar for the three sampling times. Where so much of the total material is eluted at, or close to, the void volume as was found with both column matrices [Sepharose CL-2B and Fractogel TSK 75(s)], the reproducibility of the $-\log K_{WAV}$ values is poor, but the elution profiles indicated that there was little variation apparent in the molecular size with time of development, unless it occurred at

TABLE II

Characteristics of elution profiles of *n* amylose fractions

Amylose state	*Sampling time*	*Chromatography matrix*	$100A_{max}/[glucan]^a$	λ_{max} *(nm)*	$-\log K_{WAV}$	$-\log K_{PAV}$
Original	First	Sepharose CL-2B	1.9	630 (640)	0.21	0.12
	Second		1.9	(620) 630 (640)	0.24	0.13
	Third		1.9	(620) 630	0.24	0.12
Debranched	First	Sepharose CL-2B	2.0	630 (640)	0.22	0.14
	Second		2.0	630	0.22	0.14
	Third		2.0	(620) 630 (640)	0.22	0.16
Original	First	Sepharose CL-4B	2.0	630, 640	0.49	V_o, 0.45
	Second		2.0	630, 640	0.50	V_o, 0.50
	Third		2,1	630, 640	0.59	V_o, 0.90
Debranched	First	Sepharose CL-4B	2.1	630, 640	0.50	V_o, 0.43
	Second		2.0	630 (640)	0.46	V_o, 0.51
	Third		2.1	640 (630)	0.58	V_o, 0.79

[a] Conc. in μg/mL.

TABLE III

Characteristics of elution profiles of *ae* amylose fractions

Amylose state	*Sampling time*	*Chromatography matrix*	$100A_{max}/[glucan]$	λ_{max} *(nm)*	$-\log K_{WAV}$	$-\log K_{PAV}$
Original	First	Sepharose CL-2B	2.0	630 (640)	0.34	0.34
	Second		1.9	(620) 630, 640	0.18	0.13
	Third		1.9	(620) 630, 640	0.18	0.11
Debranched	First	Sepharose CL-2B	1.9	630, 640	0.28	0.31
	Second		2.0	(620) 630, 640	0.18	0.13 + inflexion
	Third		2.0	630, 640	0.16	0.13 + inflexion
Original	First	Sepharose CL-4B	2.1	630 (640)	0.97	V_o
	Second		2.0	630 (640)	0.47	V_o, 0.55
	Third		2.0	630 (640)	0.48	V_o, 0.54
Debranched	First	Sepharose CL-4B	2.0	630, 640	0.68	V_o
	Second		2.1	630 (640)	0.45	V_o, 0.55, 0.16
	Third		2.1	630, 640	0.43	V_o, 0.55, 0.14

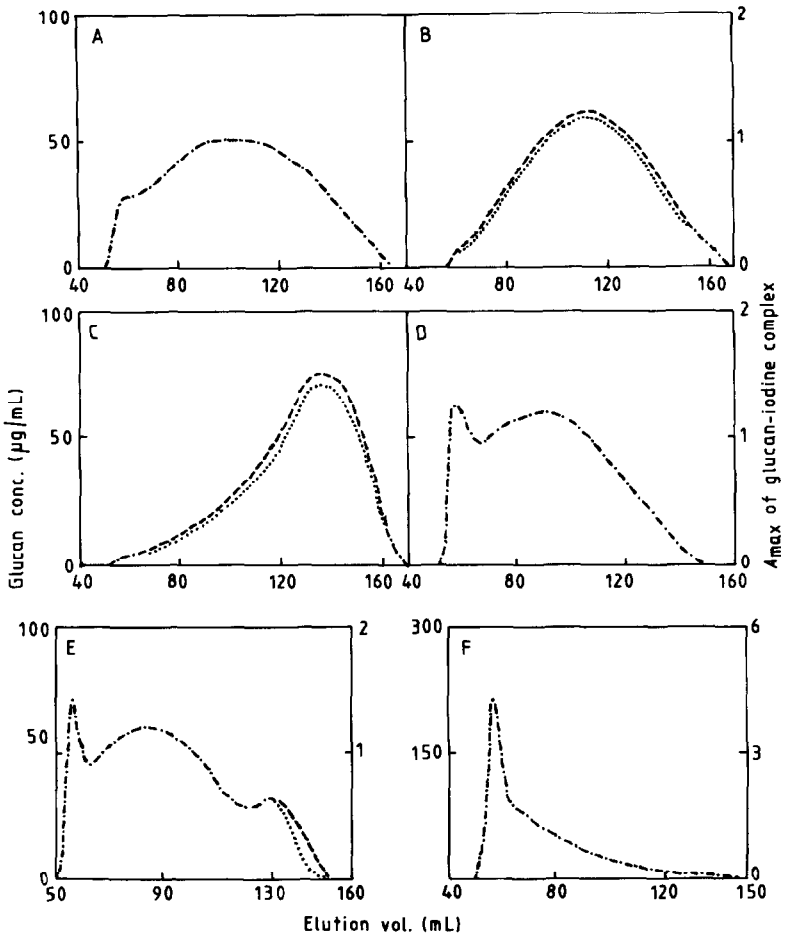

Fig. 2. Gel chromatography of *ae* amylose fractions: *A*, 1st sampling on Sepharose CL-2B; *B*, 1st sampling, debranched, on Sepharose CL-2B; *C*, 2nd sampling on Sepharose CL-2B; *D*, 2nd sampling on Sepharose CL-4B; *E*, 2nd sampling, debranched, on Sepharose CL-4B; *F*, 1st sampling, debranched, on Sepharose CL-4B; - - - -, glucan; ·····, colour.

very high values. Similarity in the molecular architecture of the *wx* amylopectin molecules during development was shown by gel chromatography on Fractogel TSK 50(s) of the α-(1→4) glucan chains produced by debranching with isoamylase (Table V and Fig. 6A). The weight-average molecular size of the total of the chains and the proportions of chains in the two size ranges (medium and short), and their peak elution volumes, were similar at each time of sampling.

The details of the *n* amylopectin fractions are also listed in Table IV and in two profiles shown in Figs. 5A and 5B. The ratios of $100A_{max}$/[glucan] and the λ_{max} values were similar over the whole of the elution profiles, consistent with efficient fractionation at all sampling times, indicating that concanavalin A effectively fractionates immature starches containing amylose as well as mature starches. The characteristics of the

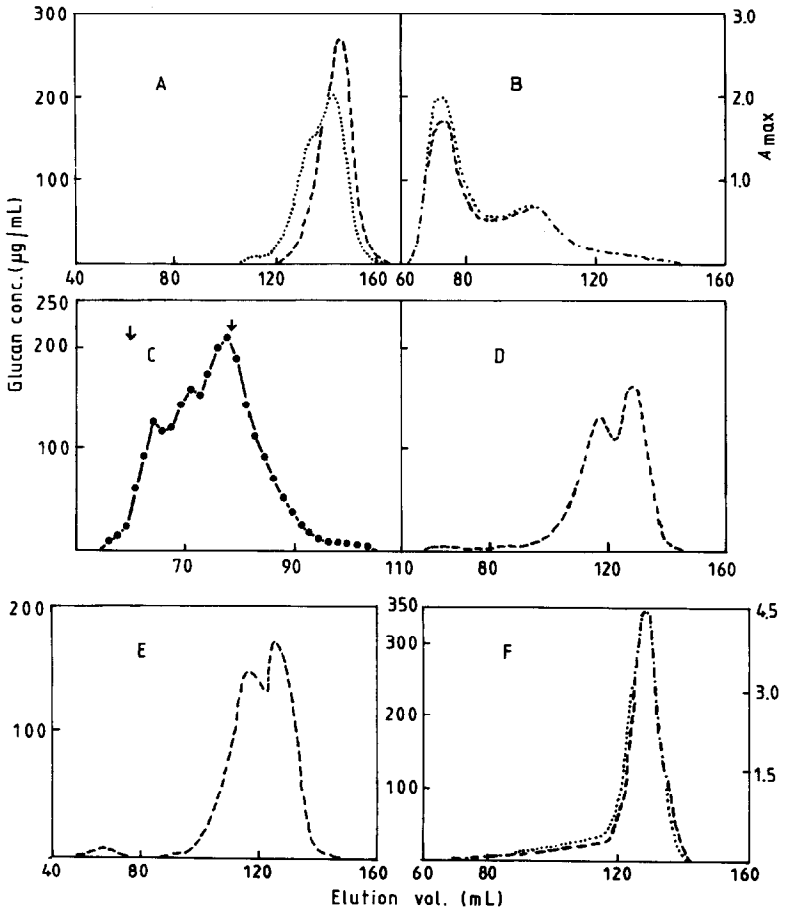

Fig. 3. Gel chromatography of *ae* and *ae/wx* debranched starches: *A*, *wx* (- - - -) and *ae/wx* (·····) (3rd samplings) on Sepharose CL-4B; *B*, *ae/wx*, precipitated by concanavalin A, on Fractogel TSK 75(s); *C*, *ae/wx*, soluble in concanavalin A, on Fractogel TSK 50(s); *D*, *ae*, precipitated by concanavalin A (3rd sampling), on Superose 6B; *E*, *ae/wx* (3rd sampling) on Superose 6B; *F*, *ae/wx*, soluble in concanavalin A (1st sampling), on Fractogel TSK 75(s); - - - -, glucan; ·····, colour.

profiles showed little variation for the three times of sampling, with the proviso that, where so much of the total material is eluted at or close to the void volume, the reproducibility of $-\log K_{WAV}$ is low and there is the potential for variation in the higher range of molecular weights of molecules that show no separation, eluting at the void volume. The similarity of architecture of these molecules at the different sampling times was confirmed by debranching followed by gel chromatography on Fractogel TSK 50(s) (Table V and Fig. 6B). The $-\log K_{WAV}$ values of the whole elution profiles, the percentages of the three types of chains (long, medium, and short), and the elution volumes of the peaks of these were similar at each time of sampling.

The *ae* genotype showed a different pattern on development (Table IV, Figs. 5C, 5D, 5E, and 5F) than the *n* and *wx* amylopectins. At the first sampling, the ratio of $100A_{max}$/[glucan] was similar to *n* and *wx*, and the λ_{max} was low (530 nm). The molecular

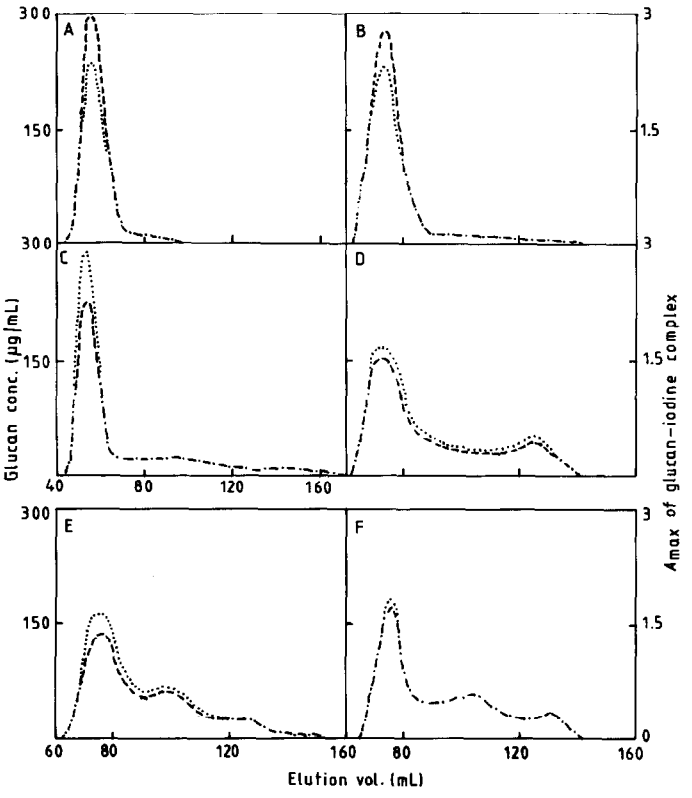

Fig. 4. Gel chromatography of *wx* and *ae/wx* starches: *A*, 3rd sampling of *wx* on Sepharose CL-2B; *B*, 3rd sampling of *wx* on Fractogel TSK 75(s); *C*, 3rd sampling of *ae/wx* on Sepharose CL-2B; *D*, 1st sampling of *ae/wx* on Fractogel TSK 75(s); *E*, 2nd sampling of *ae/wx* (whole starch) on Fractogel TSK 75(s); *F*, 3rd sampling of *ae/wx* (whole starch) on Fractogel TSK 75(s); - - - -, glucan; ·····, colour.

size, as indicated by $-\log K_{WAV}$ (Table IV, Figs. 5C and 5D), was high and similar to *n*. At the second and third samplings, the elution profiles of *ae* indicated a lower molecular size (Table IV, Figs. 5E and 5F), the ratios of $100A_{max}$/[glucan] were higher (>1), and the λ_{max} values were higher (560 nm). These properties were found over the whole of the elution profiles, showing that the results are not a consequence of inefficient fractionation by concanavalin A. The elution profiles of the chains after debranching also differed between the first sampling and the two later samplings (Table V, Figs. 6E and 6C). Initially, the $-\log K_{WAV}$ value was lower, the percentages of medium and long chains were lower, and that of the short chains was higher, indicating a structure similar to that of *n* amylopectin for the first sampling but different at the two later times. These observations were repeated in another growing season. The result complemented the findings on the structure of the amylose fraction at the first sampling, which indicated it was more like *n* amylose in molecular size. The data for the second and third samples are consistent with the production of amylopectin fractions with longer average chain-lengths, as found previously with mature high-amylose pea starch[17]. The structural difference between *n* and *ae* amylopectins is reflected in the levels and number of

TABLE IV

Behaviour of amylopectin fractions on gel chromatography

Source of amylopectin	*Time of sampling*	*Column matrix*[a]	$100A_{max}/[glucan]$	λ_{max} *(nm)*	$-\log K_{WAV}$	$-\log K_{PAV}$
wx	1	CL-2B	0.8	530 (540)	1.4	V_o
		TSK 75(s)	0.8	530 (540)	0.98	V_o
	2	CL-2B	0.8	530	1.3	V_o
		TSK 75(s)	0.8	530	1.1	V_o
	3	CL-2B	0.8	530	1.5	V_o
		TSK 75(s)	0.8	530 (520)	1.0	V_o
n	1	CL-2B	0.9	540, 550	0.76	V_o
		TSK 75(s)	0.9	540 (550)	0.71	V_o
	2	CL-2B	0.9	540, 550	0.78	V_o
		TSK 75(s)	0.9	540 (550)	0.74	V_o
	3	CL-2B	0.9	540, 550	0.71	V_o
		TSK 75(s)	0.9	540 (550)	0.66	V_o
ae	1	CL-2B	0.8	530	0.85	V_o
		TSK 75(s)	0.9	530	0.6	V_o
	2	CL-2B	1.1	560	0.56	V_o
		TSK 75(s)	1.1	560	0.53	V_o
	3	CL-2B	1.2	560	0.54	V_o
		TSK 75(s)	1.2	560, 570	0.52	V_o
ae/wx whole starch	1	CL-2B	1.2	560	0.70	V_o, 0.07
		TSK 75(s)	1.1	560	0.56	V_o, 0.09
	2	CL-2B	1.2	560	0.79	V_o, 0.48
		TSK 75(s)	1.1	560	0.53	V_o, 0.41, 0.08
	3	CL-2B	1.2	560	0.72	V_o, 0.56
		TSK 75(s)	1.1	560	0.55	V_o, 0.40, 0.09

ae/wx precipitated by Con A	1	TSK 75(s)	1.2	560	0.67	V_o, 0.12
	2	TSK 75(s)	1.2	560	0.61	V_o, 0.35
ae/wx **not** precipitated by Con A	1	TSK 75(s)	1.4	620–550	0.08	0.05

[a] Sepharose CL-2B and Fractogel TSK 75(s).

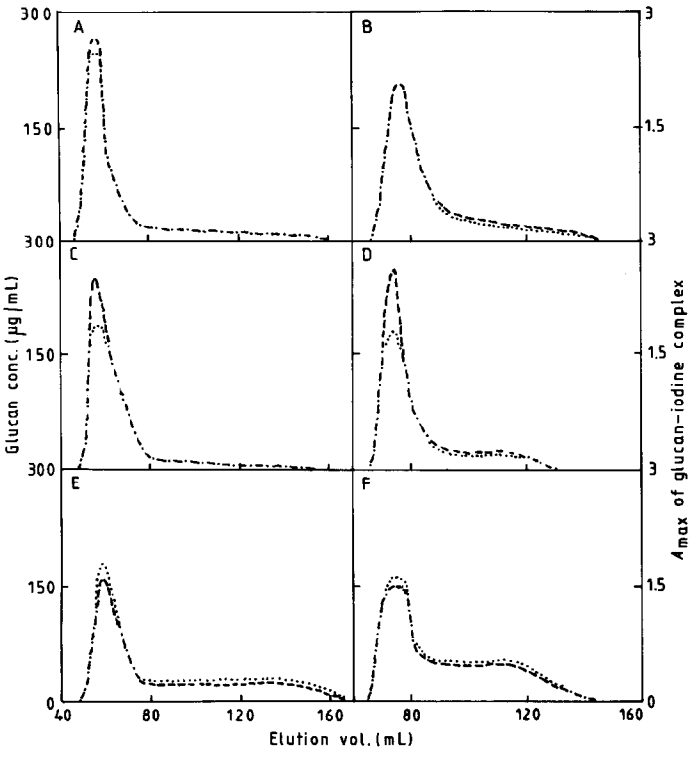

Fig. 5. Gel chromatography of *n* and *ae* amylopectin fractions: *A*, 1st sampling of *n* on Sepharose CL-2B; *B*, 1st sampling of *n* on Fractogel TSK 75(s); *C*, 1st sampling of *ae* on Sepharose CL-2B; *D*, 1st sampling of *ae* on Fractogel TSK 75(s); *E*, 2nd sampling of *ae* on Sepharose CL-2B; *F*, 2nd sampling of *ae* on Fractogel TSK 75(s); - - - -, glucan; ·····, colour.

branching enzymes that have been found in maize[20] and pea seeds[21–23] and pea leaves[24].

On comparison of the three amylopectins from the *wx*, *n*, and *ae* genotypes, significant patterns in structural differences can be detected. The molecular size decreases as the amylose content increases, and the λ_{max} and average $100A_{max}$/[glucan] both increase from 530 nm to 560 (570) nm and from 0.8 to 1.2, respectively. The elution profiles of the debranched amylopectins show an increase in $-\log K_{WAV}$ which is associated with an increase in medium and long chains at the expense of short chains. All of these results are consistent with an increase in average chain-length of the amylopectins in the sequence *wx* to *n* to *ae*.

The data obtained for the double mutant *ae/wx* provide further information about the fractions of *ae* starch. As described above, when this starch was treated with concanavalin A, some of the glucan remained in solution. The amount was greater at the first sampling than later. The whole and the debranched starches from the three developmental stages were examined by gel chromatography and the results are shown in Table IV, with some elution profiles illustrated in Figs. 4C, 4D, 4E, and 4F. Like *ae* amylopectin, the ratio $100A_{max}$/[glucan] and the λ_{max} values were higher than for *n* amylopectin and there was a considerable proportion of material of lower molecular

TABLE V

Characteristics of elution profiles of debranched amylopectins on Fractogel TSK 50(s)

Starch source	*Sampling time*	$-\log K_{WAV}$	$-\log K_{PAV}$	*Percentage of chains*		
				Long	*Medium*	*Short*
wx	First	0.32	0.47, 0.28	0	26	74
	Second	0.31	0.44, 0.29	0	25	75
	Third	0.33	0.47, 0.28	0	27	73
n	First	0.45	V_o, 0.64, 0.37	8	27	66
	Second	0.43	V_o, 0.60, 0.59	7	22	70
	Third	0.47	V_o, 0.61, 0.37	10	24	66
ae	First	0.41	V_o, 0.60, 0.39	3	29	69
	Second	0.48	V_o, 0.60, 0.39	7	40	53
	Third	0.50	V_o, 0.65, 0.41	5	48	47
ae/wx	First	0.44	V_o, 0.54, 0.36	2	52	46
	Second	0.41	V_o, 0.50, 0.34	2	54	44
	Third	0.40	V_o, 0.50, 0.32	1	55	45
ae/wx not precipitated by Con. A	First	0.56	1.0, 0.68, 0.49	4	72	24
	Third	0.56	1.0, 0.63, 0.53	2	75	23

weight, leading to a smaller molecular size as indicated by the $-\log K_{WAV}$ values on Fractogel TSK 75(s). The weight-average molecular size, unlike that for the *ae* genotype, showed no change. Debranching (Table V, Fig. 6D) gave a distinctive elution profile on Fractogel TSK 50(s) with a high proportion of chains of medium length combined with a low proportion of long chains, and this pattern was similar at all stages.

The nature of the material not complexing with concanavalin A was then investigated. Firstly, the whole starch (from the second sampling) was debranched and chromatographed on Sepharose CL-4B to detect the presence of any long amylose-like molecules. The elution profile (Fig. 3A) showed that no significant peak due to material of very high molecular size (at the void volume) was present. Debranching of *ae* amylopectin followed by chromatography on Superose 6B[25] gave the profile shown in Fig. 3D — there was a small amount of material that was eluted early in the profile (55–90 mL). Three possible sources of this fraction are a few extra long α-(1→4) chains in the amylopectin structure, incomplete debranching, or a small proportion of amylose in the amylopectin fraction. The λ_{max} was high (610–630 nm) and a similar result was found with *n* amylopectin. Debranching of *ae/wx* starch (Fig. 3E) also produced some glucan in this elution range. The presence of extra long chains in amylopectin is in accord with the "cluster model"[26,27] for its structure. These chains have been detected in rice amylopectins[28,29] prepared by complexing with 1-butanol and in potato amylopectin prepared by concanavalin A precipitation[16]. When fractionation with 1-butanol is applied to *n* starches, although the amylose fraction contains amylopectin, the amylopectin fraction appears to be free of amylose[5].

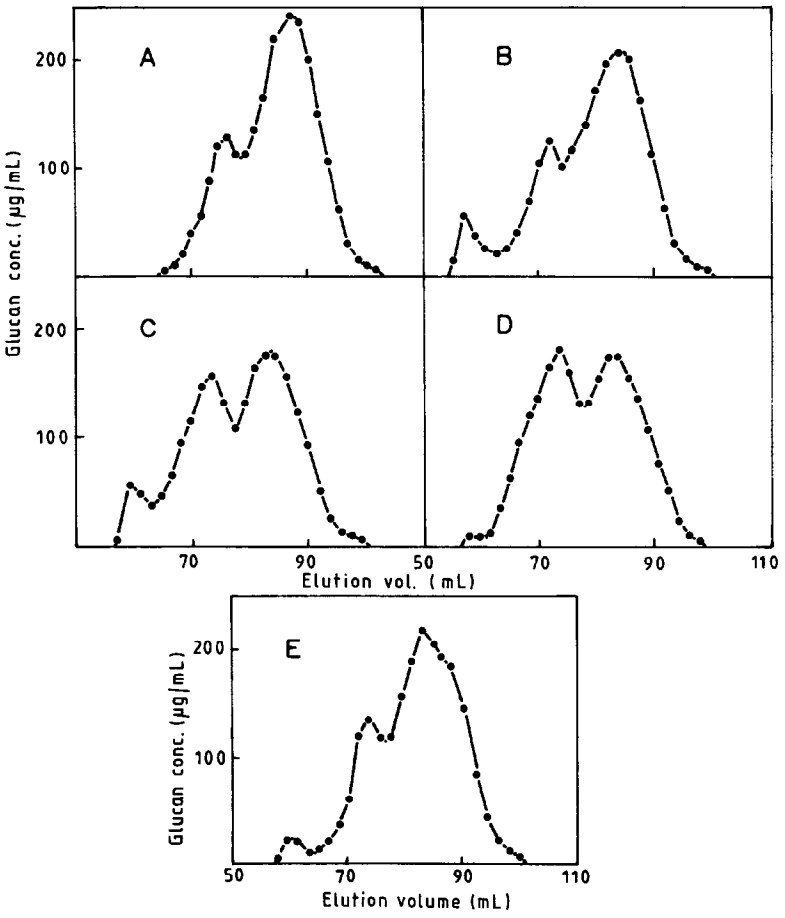

Fig. 6. Chromatography on Fractogel TSK 50(s) of debranched maize amylopectins: *A*, *wx*, 2nd sampling; *B*, *n*, 2nd sampling; *C*, *ae*, 2nd sampling; *D*, *ae/wx*, 2nd sampling; *E*, *ae*, 1st sampling.

The fraction precipitated by concanavalin A was then chromatographed on Fractogel TSK 75(s) (Table IV and Fig. 3B). Compared to the whole starch, there was a slight increase in weight-average molecular size, accompanied by a reduction in the amount of material of low molecular weight. No change occurred in the $100A_{max}$/[glucan] and λ_{max} values. When the fraction not precipitated by concanavalin A was debranched and chromatographed on Fractogel TSK 50(s) (Table V and Fig. 3C), the weight-average molecular size was higher than for the whole starch (Fig. 6D) and there was a large increase in the proportion of medium-size chains, accompanied by a decrease in the proportion of short chains. The elution volumes for division of chains into long, medium, and short were the same as those used for the whole starches, and these are indicated by the arrows in Fig. 3C. This fraction (not precipitated by concanavalin A) from the first sampling was chromatographed on Fractogel TSK 75(s) (Table IV, Fig. 3F). It had a low molecular size and the average $100A_{max}$/[glucan] at 1.4 was intermediate in value between those for whole starch (at 1.2) and amylose (at 2.0). There

was a considerable range of values (1.9–1.3) over the elution profile. The λ_{max} values varied from 620 nm in the initial fractions (up to 115 mL) to 610–580 nm up to 120 mL, decreasing to 550 nm at 140 mL. The data indicate that it is probably best defined as "intermediate fraction", *i.e.*, of low molecular weight and intermediate between amylose and amylopectin in degree of branching and average chain-length. It has insufficient branching and too low a molecular size to complex with concanavalin A.

A possible consequence of this result is that, in the fractionation of *ae* starch, the fraction not precipitated by concanavalin A consists not only of amylose (defined as long unbranched chains plus those with limited branching by long chains) but also for a minor intermediate fraction. The appearance of an additional peak in the amylose fractions of the second and third samplings[8] on Sepharose CL-4B after debranching (Table III, Fig. 2E) at an elution volume of ~130 mL supports this possibility. Debranching of *ae/wx* starch produced a peak at a similar elution volume on Sepharose CL-4B (Fig. 3A), whereas the debranched chains of *wx* starch were eluted at a higher volume.

With high-amylose pea starch, the amylose content estimated by iodine titration was significantly higher than that by concanavalin A precipitation[16]. It was proposed that the difference was due to the greater interaction with iodine of the longer chains from the amylopectin of the high-amylose starch than of those from amylopectin of *n* starch. The results with *ae/wx* starch open the possibility that the amount of amylose in *ae* starch, when defined as long unbranched chains plus those lightly branched with long chains, is even lower than that estimated by concanavalin A precipitation, since the soluble fraction may contain a small proportion of an intermediate fraction. Since starch probably consists of a continuum of structures, varying in molecular weight, average chain-length, and degree of branching, which occur with differing frequency, it can be expected that the fractions produced from the same starch by the various methods (1-butanol complexing, gel chromatography, ultracentrifugation, and precipitation with concanavalin A) will effect separation at different points in the spectrum of structures.

EXPERIMENTAL

Plant sampling. — Normal (*n*, single cross B37/Mo17), waxy (*wx/wx CV.*DK 84A), amylose extender (*ae/ae, CV.*CP5577), and a double mutant (*ae ae/wx wx*, 3-way hybrid OH43/B37/W64A) were field-grown and hand-pollinated. The first samplings were made at 20 (*n* and *ae ae/wx wx*), 19 (*wx/wx*), and 14 days (*ae/ae*) after pollination. The grains were cut from the cob with a sharp knife prior to maceration. The second sampling was made 34 days after pollination and the grains were again cut from the cob. The third sampling was made 117 days after pollination, when the cobs had fully dried and whole grains could be readily removed.

Preparation of starch — The first and second samplings were macerated in 0.01M mercuric chloride, 0.1M sodium chloride, and toluene immediately after removal from the cob. The third sampling was soaked in 0.2M ammonium hydroxide to swell the grain

without concurrent enzymic degradation, and, after washing with water, macerated in 0.01M mercuric chloride–0.1M sodium chloride–toluene. After filtering the macerate through cheesecloth, the starch was collected by centrifugation, shaken with a toluene–sodium chloride mixture several times, centrifuged, washed with water, ethanol, acetone, and ether, and dried. Granules (1 g) were suspended in dimethyl sulphoxide (50 mL) in a centrifuge tube with a drying tube attached, and stirred overnight. The mixture was centrifuged, the supernatant solution was collected, and the residue was stirred with dimethyl sulphoxide (25 mL) overnight and centrifuged, when, for all samples, only a small residue of fibrous material remained. The supernatant solutions were combined, the starch was precipitated in three volumes of ethanol and centrifuged, and the precipitate was stored overnight in ethanol. The starch was centrifuged, washed with acetone and ether, and dried.

Estimation of amylose content. — Amylose contents of starches (*n*, *ae*, and *ae/wx*) were estimated at the three stages of sampling by measurement with the phenol–sulphuric acid reagent[18] of the glucan content of the total starch solution and of the supernatant solution after treatment with concanavalin A.

Preparative fractionation of starches with concanavalin A. — This fractionation was performed as described[16,17].

Chromatography of amylose and amylopectin. — These procedures were carried out as described[16,17].

Debranching and chromatography of fractions. — The previously described procedures[16] were used.

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