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**Note**

# Amylose percentage and distribution of unit chain-length of maize starches having a specific genetic background*†

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In this study, starch granules prepared from kernels of the single mutants, *amylose-extender (ae)*, *waxy (wx)*, and *opaque*-2 ($o_2$), and the double-mutant combinations *ae* $o_2$ and *wx* $o_2$ from four maize (*Zea mays* L.) inbred lines, Oh43, B37, C103, and W64A were fractionated by gel filtration after debranching by isoamylase. The contents of each fraction and distribution of the linear α-D-(1 → 4)-linked unit-chains were investigated. No noteworthy differences were observed in the aforementioned criteria between single mutants and their respective double-mutant combinations with $o_2$, and among four inbred lines. It was confirmed that starch granules of the *ae* mutants have intermediate fraction(s) different from typical amylose and amylopectin.

Several of the endosperm mutants of maize (*Zea mays* L.) display differences in pasting temperature, BEPT**, viscosity, gel-stability, starch-granule digestibility, and the production of amylose, amylopectin, water-soluble polysaccharides, and sugars (see Fuwa *et al.*[1] for references). Greenwood and his co-workers[2] found that iodine-binding curves for starches of *amylose-extender (ae)* mutants were different from that of normal maize-starch, and amylose contents estimated by potentiometric iodine-titration were higher (by about 15%) than those of complexes with 1-butanol. Furthermore, they[2] separated from the *ae* starches an intermediate fraction different from typical amylose and amylopectin. It is remarkable that the *ae* starches had large portions of the intermediate fraction, whereas normal maize-starches did not. Recently, Boyer *et al.*[3] and Yamada *et al.*[4] reported that maize starches of the *amylose-extender waxy (ae wx)* double-mutant consists merely of an atypical amylopectin. They suggested the abnormal amylopectin was similar to the intermediate fraction

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**BEPT denotes Birefringence Endopoint Temperature.

of the *ae* starches. Thus, in investigating components of maize starches, it is necessary to account not only for typical amylose and amylopectin, but also for these intermediate fraction(s).

Our investigations on susceptibility of various starch granules to amylases required fuller knowledge about starch components. To help elucidate the details, we prepared starch granules from kernels of several single mutants and their double-mutant combinations having $o_2$ of four, inbred lines of maize, and measured their content of amylose and the distribution of linear, α-D-(1 → 4)-linked, unit-chains by the use of gel filtration after debranching by isoamylase.

## RESULTS AND DISCUSSION

Figs. 1A and 1B show typical elution patterns on a Sephadex G-75 column of maize starch after debranching by isoamylase. A peak appeared near the void volume [fraction I (Fr. I)], a peak or shoulder near the average chain-length ($\overline{c.l.}$) of 50 [fraction II (Fr. II)], and a peak at $\overline{c.l.}$ 20 [fraction III (Fr. III)]. Fr. I comprised material of higher molecular weight, regarded as amylose in the original granules. Frs. II and III constituted the linear unit-chains of amylopectin, because they displayed peaks at about $\overline{c.l.}$ 50 and 20, respectively.

Table I shows that there are no significant differences apparent in the contents of each of Frs. I, II, and III in the percentage and chain length of each of the apices from Frs. II and III, whether the high-amylose maize starches are debranched by isoamylase or by a combination of isoamylase and pullulanase. In addition, there were no differences between their elution patterns, and thus we used isoamylase for debranching all other starches.

Fig. 1D shows that the commercial, waxy-maize starch contains a trace of Fr. I. However, in the *wx* and *wx* $o_2$ starches of Oh43 and B37, there is no Fr. I (Table II). The elution patterns of *wx* and *wx* $o_2$ starches of Oh43 and B37 resemble that of the commercial *wx* starches very closely, except Fr. I. As these starches of the *wx* mutants consist essentially of amylopectin, the contents and elution patterns of Frs. II and III characterize typical amylopectin. As compared with the elution-

TABLE I

PERCENTAGE OF FRACTIONS I, II, AND III, AND AVERAGE CHAIN-LENGTH ($\overline{c.l.}$) OF HIGH-AMYLOSE MAIZE STARCH, DEBRANCHED BY ISOAMYLASE OR ISOAMYLASE PLUS PULLULANASE

| *Debranching enzymes* | *Fr. I* (%) | *Fr. II* (%) | *Fr. III* (%) | *Frs. II+III* (%) | *Peak of Fr. II* ($\overline{c.l.}$) | *Peak of Fr. III* ($\overline{c.l.}$) |
|---|---|---|---|---|---|---|
| Isoamylase | 44.0 | 36.8 | 19.2 | 56.0 | 52 | 18 |
| Isoamylase plus pullulanase | 42.8 | 36.0 | 21.2 | 57.2 | 45 | 18 |

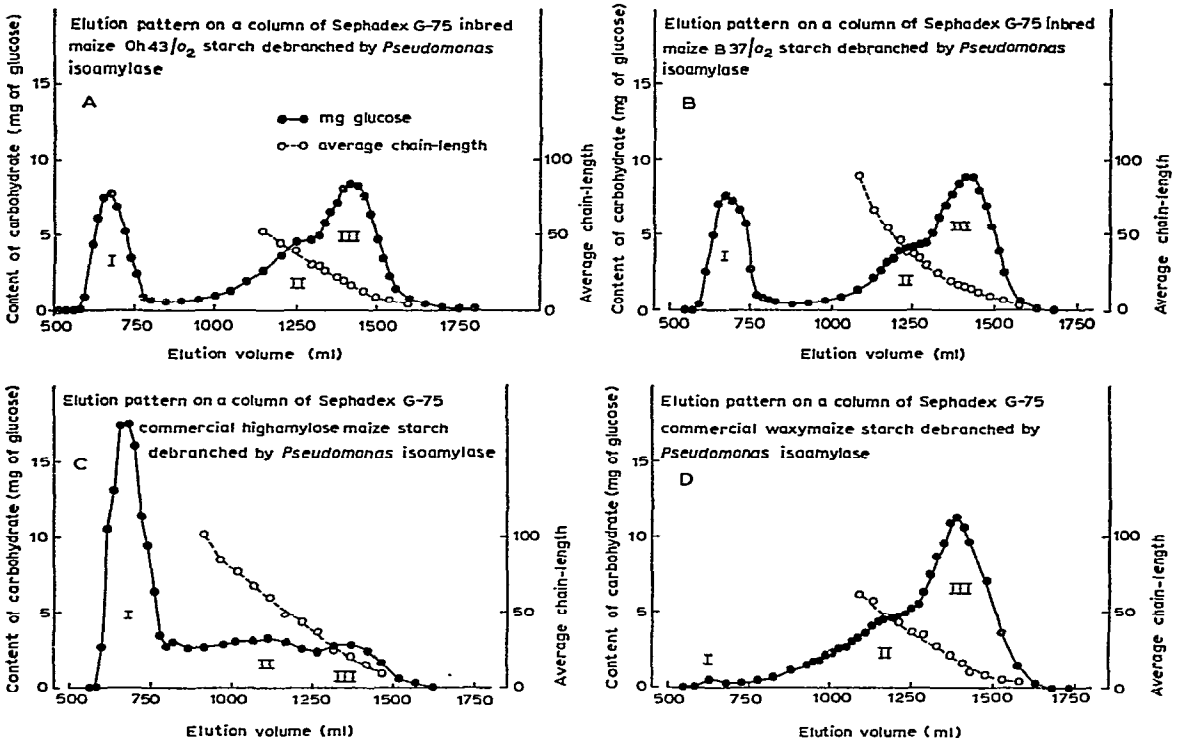


Fig. 1. Elution patterns on a column of Sephadex G-75 of four maize starches debranched by isoamylase: (*A*) *opaque-2* ($O_2$) maize starch of Oh43, (*B*) $O_2$ maize starch of B37, (*C*) commercial high-amylose VII maize starch, (*D*) commercial waxy maize starch (—●—) content of carbohydrate determined by the phenol-sulfuric acid method[5] and expressed as mg of glucose; average length of unit chain (—○—) calculated from the carbohydrate content and the number of reducing ends, as determined by the chromotropic acid method[6,7]).

patterns of the $o_2$ and *wx* or *wx* $o_2$ (see Fig. 1), Frs. II and III displayed similar elution patterns. Apparently, the $o_2$ starches contained typical amylopectin.

Fig. 1C shows that the high-amylose VII starches had higher contents of carbohydrate between Fr. I and Fr. II than those of the $o_2$ mutants. Starches of the *ae* and *ae* $o_2$ mutants of the Oh43, B37, C103, and W64A maize inbred-strains were similar in their elution profiles to that of the high-amylose VII starch. This indicates that the high-amylose maize starches contained intermediate fraction(s) different from typical amylose and amylopectin. The elution patterns of Frs. II and III of the high-amylose maize starches were also different from those of the $o_2$ and *wx* mutants. Apparently, the high-amylose maize starches contained an anomalous amylopectin and/or an amylose of short chain-length.

Table II shows the percentage contents of Frs. I, II, and III, and the chain lengths of apices in Frs. II and III. The contents of Fr. I were about 28% of the $o_2$, 0% of the *wx* and *wx* $o_2$, and 1% of the commercial waxy-maize starch, respectively,

TABLE II

PERCENTAGE OF FRACTIONS I, II, AND III, AND AVERAGE CHAIN LENGTH ($\overline{C.L.}$) OF APICES OF PEAKS II AND III

| *Inbred line* | *Genotype* | *Fr. I* (%) | *Fr. II* (%) | *Fr. III* (%) | *Frs. II+III* (%) | *Peak of Fr. II* ($\overline{c.l.}$) | *Peak of Fr. III* ($\overline{c.l.}$) |
|---|---|---|---|---|---|---|---|
| | $o_2$ | 28.3 | 26.4 | 45.3 | 71.7 | 40 | 17 |
| | *wx* | 0 | 32.1 | 67.9 | 100.0 | 45 | 17 |
| OH43 | *wx* $o_2$ | 0 | 36.5 | 63.5 | 100.0 | 46 | 18 |
| | *ae* | 39.6 | 40.7 | 19.7 | 60.4 | 53 | 18 |
| | *ae* $o_2$ | 39.0 | 36.8 | 24.2 | 61.0 | 55 | 17 |
| | $o_2$ | 28.2 | 21.7 | 50.1 | 71.8 | 40 | 17 |
| | *wx* | 0 | 33.9 | 66.1 | 100.0 | 44 | 17 |
| B37 | *wx* $o_2$ | 0 | 34.9 | 65.4 | 100.0 | 48 | 18 |
| | *ae* | 38.0 | 34.7 | 27.3 | 62.0 | 45 | 18 |
| | *ae* $o_2$ | 37.0 | 36.0 | 27.0 | 63.0 | 45 | 19 |
| C103 | *ae* | 34.6 | 38.7 | 26.7 | 65.4 | 40 | 17 |
| | *ae* $o_2$ | 35.3 | 33.2 | 31.5 | 64.7 | 43 | 17 |
| W64A | *ae* | 36.7 | 36.1 | 27.2 | 63.3 | 44 | 18 |
| | *ae* $o_2$ | 39.3 | 34.0 | 26.8 | 60.8 | 43 | 18 |
| Commercial | HA* | 55.0 | 31.5 | 13.5 | 45.0 | 60 | 20 |
| | waxy | 1.1 | 36.3 | 63.7 | 98.9 | 48 | 19 |

*Different lots of commercial, high-amylose maize starch from those used in Table I.

and agreed with the amylose percentage as measured by potentiometric iodine-titration[8]. In the high-amylose maize starches, the contents of Fr. I were 55% of the high-amylose VII, and 35–40% of the *ae* and *ae* $o_2$ starches in the four inbred lines. These values were 10–15% lower than the amylose percentage measured by the other methods[8,9]. This fact, as pointed out by Greenwood and his co-workers[2] and by Boyer *et al.*[3], related to the aforementioned intermediate fraction(s), and this is a striking characteristic of the high-amylose maize starches. However, the elucidation of the relationship between the results obtained and the digestibility of starch granules needs further study. There were no significant differences in the chain length of apices of Frs. II and III among the four inbred lines of maize, and between starches of the double-mutant combinations having $o_2$ and those of their respective, nonopaque single mutants.

## EXPERIMENTAL

*Materials.* — Samples of starch granules were prepared from mature maize (*Zea mays* L.) kernels of the *ae* and *ae* $o_2$ mutants of inbreds Oh43, B37, C103, and W64A, and the $o_2$, *wx* and *wx* $o_2$ mutants of Oh43 and B37. Starches were prepared according to Schoch's method[10]. As controls, commercial high-amylose VII maize

starch (Shikishima Denpun Co., Japan) and waxy-maize starch (Clifford Lobe Co., Ltd., Australia) were used.

*Methods.* — Starches were debranched by the method of Mercier and Kainuma[11], and debranched starches were fractionated according to the method of Akai *et al.*[6]. Starch granules (a definite wet-weight between 450 and 455 mg) were dissolved in 10 ml of dimethyl sulfoxide, incubated for 1 h at 40°, and 20 ml of 0.01M acetate buffer, pH 3.5, and 8850 U* of crystalline *Pseudomonas* isoamylase (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) were added. The mixture was incubated for 24 h at 40° for debranching. When both isoamylase and pullulanase (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) were used for debranching, further incubation under similar conditions was performed with 10 ml of 0.5M acetate buffer, pH 5.5, and 40 IU† of crystalline pullulanase. Five-fold volumes of ethanol were added to the mixture and starches were precipitated. The precipitates were washed twice with ethanol and then twice with ether, and dried *in vacuo*. The dried samples were suspended in 5 ml of water, and gelatinized with 1 ml of 6M sodium hydroxide under nitrogen. After 1 h, ungelatinized materials were centrifuged off (1,200*g* 10 min). The supernatant was diluted to 10 ml with water and crystalline sodium chloride added to a concentration of 5% (w/v). These samples (10 ml each) were charged onto a column (5.0 × 85 cm) of Sephadex G-75 and eluted with 0.05M sodium hydroxide containing 5% sodium chloride. Fractions (10 ml) were collected, and neutralized with 0.5M hydrochloric acid. The amount of carbohydrate in each tube was measured by the phenol–sulfuric acid method[5]. Reducing end-groups were determined colorimetrically by the chromotropic acid method, according to Akai *et al.*[6,7]. The average chain length (c.l.) was calculated from the amount of carbohydrate and number of reducing ends. The contents of each fraction (Frs. I, II, and III, see Results and Discussion) were calculated from the elution areas.

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*One unit is expressed according to ref. 12.
†One unit is the amount of pullulanase that releases 1 μmol of maltotriose from pullulan in 1 min at pH 5.0 and 30°.

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