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Note

Structural features of Nägeli amylodextrins from waxy-maize, sweet-potato, and potato starches. Presence of a linear polysaccharide in the purified Fraction II from sweet-potato and potato amylodextrins

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Previously, we reported[1] the structural features and properties of Nägeli amylodextrins from waxy-maize, sweet-potato, and potato starches. The amylodextrins were separated, by gel filtration on a column of Bio-Gel P-6, into two fractions, namely, Fraction II (d.p. 30–33, singly branched fraction) and Fraction III (d.p. 15–18, linear fraction), which were purified by repeated gel-filtration on the same column. The results of limit beta-amylolysis, the λ_{max} value of the iodine complex, and the elution profiles of the beta-amylolyzate of the amylodextrins from three starch types suggested that the structural features of Fraction II and Fraction III differ from each other.

The present Note deals with the presence of a linear polysaccharide in Fraction II from sweet-potato or potato amylodextrin.

RESULTS AND DISCUSSION

Waxy-maize, sweet-potato, and potato amylodextrins (5 mg each) were chromatographed on a column of Bio-Gel P-6. The elution profiles are shown in Figs. 1–3.

The elution profiles of Fraction II from three starch types on Toyopearl HW-40 F, before and after simultaneous treatment with pullulanase and isoamylase, are shown in Figs. 4–6.

After debranching of Fraction II from waxy-maize, sweet-potato, and potato amylodextrins with pullulanase and isoamylase, the elution profiles were different from each other. The debranched Fraction II from waxy-maize amylodextrin showed a small peak corresponding to the original Fraction II, and a peak having the same elution volume as that of G_{15} (G = Glc). These results suggest that the lengths of the A-chain and C-chain of waxy-maize Fraction II are almost the same (d.p. 15).

After debranching, Fraction II of sweet-potato amylodextrin was separated into three peaks, one corresponding to the original Fraction II, and two corresponding,

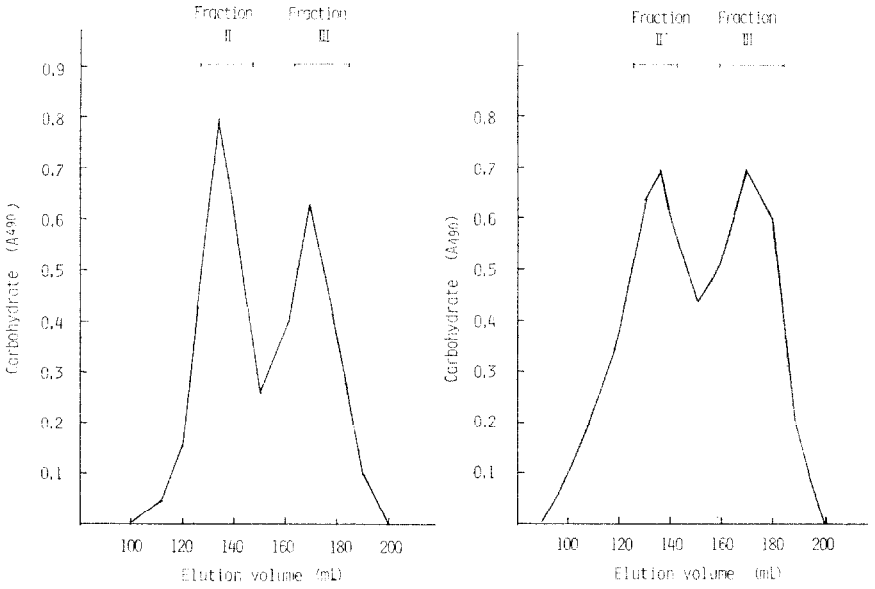

Fig. 1. Elution profile of waxy-maize amylodextrin on Bio-Gel P-6 (1.5 × 140 cm) at 50°.

Fig. 2. Elution profile of sweet-potato amylodextrin on Bio-Gel P-6 (1.5 × 140 cm) at 50°.

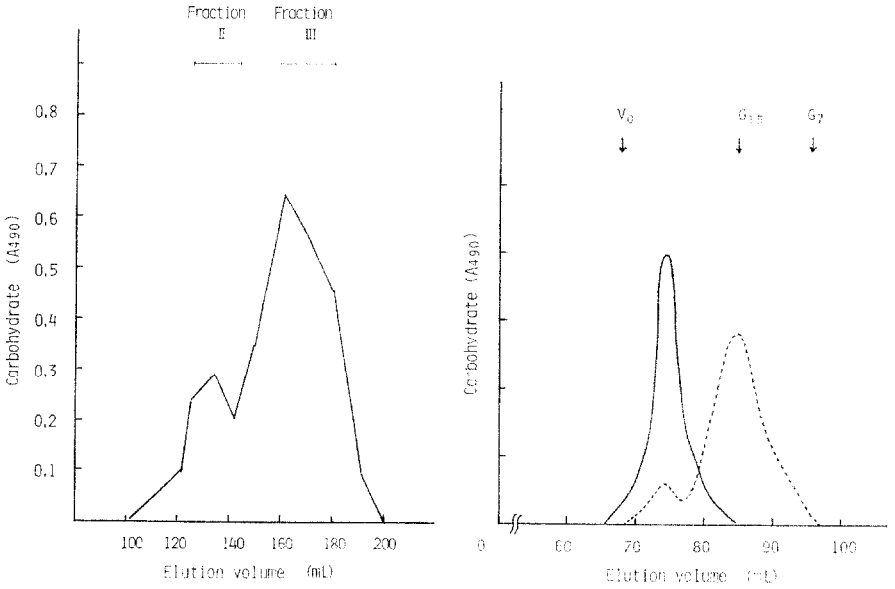

Fig. 3. Elution profile of potato amylodextrin on Bio-Gel P-6 (1.5 × 140 cm) at 50°.

Fig. 4. Elution profile of Fraction II from waxy-maize amylodextrin, before and after simultaneous treatment with pullulanase and isoamylase on Toyopearl HW-40 F. (——, Before treatment; ----, after treatment.)

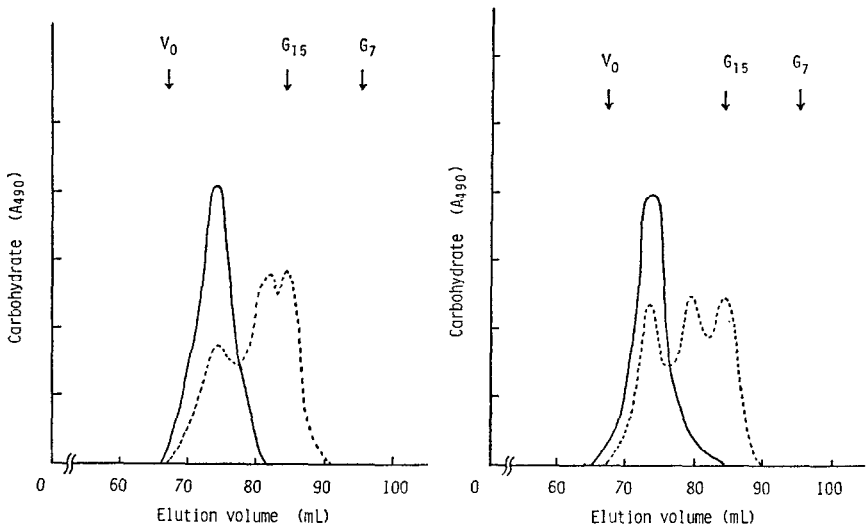

Fig. 5. Elution profile of Fraction II from sweet-potato amylodextrin, before and after simultaneous treatment with pullulanase and isoamylase on Toyopearl HW-40 F. (——, Before treatment; ----, after treatment.)

Fig. 6. Elution profile of Fraction II from potato amylodextrin, before and after simultaneous treatment with pullulanase and isoamylase on Toyopearl HW-40 F. (——, Before treatment; ---- after treatment.)

respectively, to the elution volumes of G_{16-17} and G_{15}. The debranched Fraction II of potato amylodextrin also showed three peaks, one corresponding to the original Fraction II and two corresponding, respectively, to the elution volumes of G_{18} and G_{15}. These results suggest that the chain lengths of the A-chain and C-chain of Fraction II, either from sweet-potato or potato amylodextrin, are different from each other: d.p. 16–17 and 15 (sweet potato), and d.p. 18 and 15 (potato). The proportion of the part resistant to debranching enzymes was larger in Fraction II from potato or sweet-potato amylodextrin than in that from waxy-maize amylodextrin.

The elution profiles of Fraction III of the three starch types, before and after simultaneous treatment with pullulanase and isoamylase, are shown in Figs. 7–9. In each case, after debranching, the elution profile of Fraction III showed the same elution profile as that of the original Fraction III. Fraction III was not cleaved by simultaneous treatment with pullulanase and isoamylase, but gave glucose and maltose on treatment with beta amylase. The portion of Fraction II, either from sweet-potato or potato amylodextrin, that was resistant to debranching enzymes was also completely hydrolyzed by beta amylase.

The resistant portions of Fraction II from sweet-potato and potato amylodextrins were re-treated with isoamylase, and the digests were applied to a column of Toyopearl HW-40 F. In each instance, the resistant portion was not cleaved by further treatment with isoamylase (see Figs. 10 and 11). These results indicate that

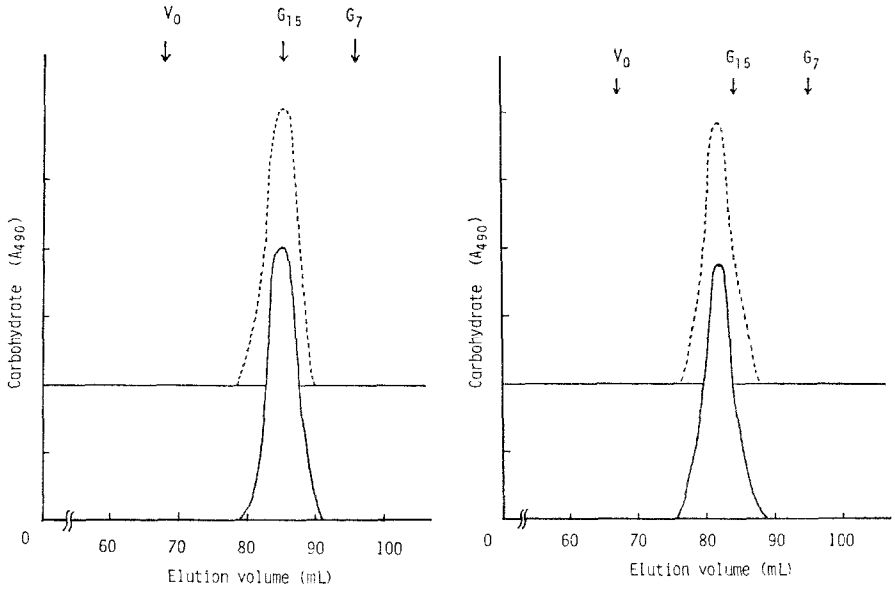

Fig. 7. Elution profile of Fraction III from waxy-maize amylodextrin, before and after simultaneous treatment with pullulanase and isoamylase on Toyopearl HW-40 F. (——, Before treatment; – – – –, after treatment.)

Fig. 8. Elution profile of Fraction III from sweet-potato amylodextrin, before and after simultaneous treatment with pullulanase and isoamylase on Toyopearl HW-40 F. (——, Before treatment; – – – –, after treatment.)

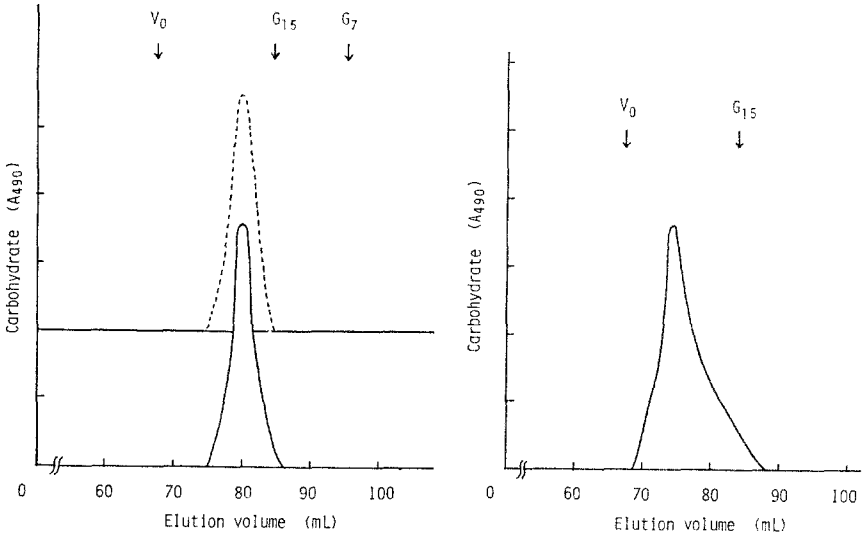

Fig. 9. Elution profile of Fraction III from potato amylodextrin, before and after simultaneous treatment with pullulanase and isoamylase on Toyopearl HW-40 F. (——, Before treatment; – – – –, after treatment.)

Fig. 10. Elution profile of the portion resistant to debranching enzymes of Fraction II from sweet-potato amylodextrin after further treatment with isoamylase on Toyopearl HW-40 F.

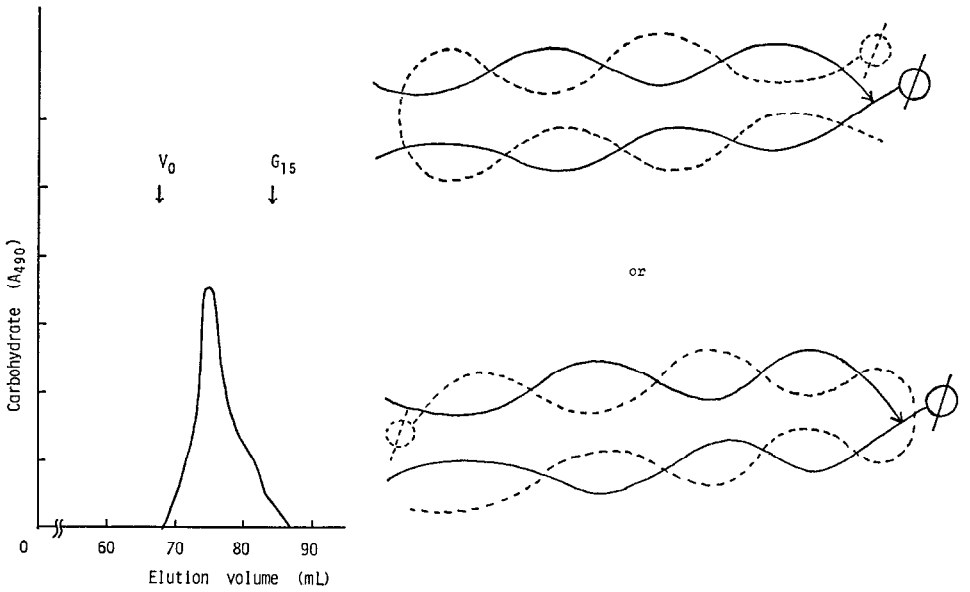

Fig. 11. Elution profile of the portion resistant to debranching enzymes of Fraction II from potato amylodextrin after further treatment with isoamylase on Toyopearl HW-40 F.

Fig. 12. Tentative structure of Fraction II of sweet-potato and potato amylodextrin. (——, Singly branched polysaccharide; ----, linear polysaccharide; Ø reducing end.)

Fraction II from sweet-potato or potato amylodextrin contains a linear portion resistant to pullulanase and isoamylase.

The structure of Fraction II has been considered to be that of a singly branched polysaccharide[2]. The foregoing data, however, indicate that the purified Fraction II from sweet-potato or potato amylodextrin is a mixture of a singly branched polysaccharide having d.p. 31–32 (sweet potato) or d.p. 33 (potato) with a linear polysaccharide. The proportion of the part resistant to debranching enzymes in Fraction II was different, as between sweet-potato and potato amylodextrin.

Although the fine structure of Fraction II is as yet unknown, purified Fraction II consists of two polysaccharides, as already mentioned, and the linear polysaccharide may possibly be twisted onto the singly branched polysaccharide (see Fig. 12).

As already reported[1], the maximum absorbance (λ_{max}) of the iodine-stained Fraction II from waxy-maize amylodextrin was observed at 420 nm, whereas the values for the corresponding fractions from sweet-potato and potato amylodextrins were 515 and 520 nm, respectively. Bailey and Whelan[3] reported that a linear relationship was observed between iodine staining and chain length of a synthetic amylose. According to their results, λ_{max} increases linearly with chain length between d.p. 12 and 50. The λ_{max} values of the Fraction II from sweet-potato and potato amylodextrins, higher than that of the counterpart from waxy-maize amylodextrin, also support the presence of a linear polysaccharide in the former two products.

EXPERIMENTAL

Native-starch granules, and amylodextrins. — Native-starch granules (waxy-maize, sweet-potato, and potato starches) were the same as reported previously[1]. The amylodextrins from these three starch types were prepared as reported previously[1].

Enzymes. — Purified, sweet-potato beta-amylase (Sigma No. A 7005) and pullulanase (crystalline, Nakarai Chemicals, Ltd.) were obtained from commercial sources. Isoamylase was a generous gift from Dr. K. Sugimoto, Hayashibara Biochemical Laboratories, Inc., Okayama. To remove insoluble materials, the isoamylase preparation (100 mg; 140 × 10^4 U) was mixed with 0.3M acetate buffer, pH 5.5 (5 mL), and centrifuged before use; the supernatant liquor was used as the enzyme solution.

Carbohydrate analysis. — Total carbohydrates and reducing sugars were determined by the phenol–sulfuric acid method[4] and the Nelson–Somogyi method[5], respectively. The degree of polymerization (d.p.) was determined by the ratio of the total carbohydrate to the reducing sugar. D-Glucose was used as the standard for both methods.

Paper chromatography. — Paper chromatography was performed on Toyo No. 50 filter paper by the multiple-ascending method, with the following solvent systems: (*A*) 6:4:3 (v/v) 1-butanol–pyridine–water, and (*B*) 7:3 1-propanol–water. Sugar spots were detected by the silver nitrate-dip method[6], after treatment with glucoamylase.

Elution profiles of waxy-maize, sweet-potato, and potato amylodextrins on Bio-Gel P-6. — The Nägeli amylodextrin (5 mg each) was dissolved in 0.5M sodium hydroxide (0.3 mL), and the base was neutralized with acetic acid. The solution was diluted with de-ionized water (0.2 mL), and applied to a column (1.5 × 140 cm) of Bio-Gel P-6 at 50°. The column was eluted with de-ionized water, and the eluate was separated by a fraction collector into tubes containing 2 mL each. The carbohydrate content of each tube was determined by the phenol–sulfuric acid method.

Purification of Fraction II and Fraction III of waxy-maize, sweet-potato, and potato amylodextrins. — Fraction II and Fraction III of waxy-maize, sweet-potato, and potato amylodextrin were purified by repeated gel-filtration on a column of Bio-Gel P-6.

Action of pullulanase and isoamylase (simultaneous treatment) on Fraction II and Fraction III of waxy-maize, sweet-potato, and potato amylodextrin. — Fraction II (3 mg) and Fraction III (3 mg) from each of the three starch types were each dissolved in distilled water (1.8 mL). To the solution of Fraction II, and of Fraction III, was added 0.2 mL of 0.3M acetate buffer, pH 5.5, containing pullulanase (8 U) and isoamylase (14 U). After incubation for 8–12 h at 40°, the enzyme reaction was stopped by heating in a boiling-water bath for 5 min, and each digest was evaporated to dryness. Each dry digestion-product (0.8 mg) was dissolved in distilled water (0.3 mL), and the solution was applied to a column (1.75 × 80 cm) of Toyopearl HW-40 F. The column was eluted with distilled water, and the carbohydrate content of each

tube (1 mL) was determined by the phenol–sulfuric acid method. The degree of polymerization of Fraction II and Fraction III was determined by gel filtration on a column of Toyopearl HW-40 F calibrated with the standard oligosaccharides (G_2, G_4, G_6, G_7, and G_{15}).

Action of beta amylase on Fraction III from waxy-maize, sweet-potato, and potato amylodextrin. — Fraction III (1 mg each) from waxy-maize, sweet-potato, or potato amylodextrin was dissolved in distilled water (0.1 mL), and to the solution was added 100 μL of 0.3M acetate buffer, pH 4.8, containing beta amylase (12 U). After incubation for 8 h at 40°, the enzyme reaction was stopped by heating in a boiling-water bath for 5 min, and each digest was examined by paper chromatography.

Beta-amylolysis of the portion of Fraction II from sweet-potato or potato amylodextrin resistant to debranching enzymes. — Fraction II (10 mg) of sweet-potato and potato amylodextrin was treated with pullulanase and isoamylase as already described. The portion resistant to debranching enzymes was collected, evaporated to dryness, and the residue dissolved in distilled water (1 mL). To 30 μL of this solution (containing 96 μg of carbohydrate) were added 30 μL of distilled water and 40 μL of 0.3M acetate buffer, pH 4.8, containing beta-amylase (0.4 U). After incubation for 12 h at 40°, distilled water (0.9 mL) and alkaline copper reagent (1 mL) were added to the enzyme digest, and the reducing sugar produced was determined by the Nelson–Somogyi method.

Further treatment with isoamylase of the portion resistant to debranching enzymes. — The portion of Fraction II (300 μL, containing 960 μg of carbohydrate) resistant to debranching enzymes was evaporated to dryness, and the residue was dissolved in distilled water (100 μL) by boiling. After cooling, 100 μL of 0.01M acetate buffer, pH 6.2, containing purified isoamylase (0.09 U) and 100 μL of 0.1M acetate buffer, pH 3.5, was added. Following incubation for 16 h at 40°, the enzyme reaction was stopped by heating in a boiling-water bath for 5 min, and the solution was applied to a column of Toyopearl HW-40 F.

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