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STRUCTURAL FEATURES AND PROPERTIES OF NÄGELI AMYLODEXTRIN FROM WAXY-MAIZE, SWEET-POTATO, AND POTATO STARCHES*

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ABSTRACT

Nägeli amylodextrins were prepared from various types of starch (waxy maize, sweet potato, and potato) by prolonged treatment of the granules with 15% sulfuric acid. Each product was separated, by gel filtration on a column of Bio-Gel P-6, into two fractions, which were purified by repeated gel-filtration on the same column. The fraction having the lower molecular weight (d.p. 15–18, Fraction III) was completely hydrolyzed by beta amylase, regardless of the origin of the starch, suggesting that this product has a linear structure. The λ_{max} value of the iodine complex of Fraction III from waxy-maize amylodextrin was, however, smaller than those of the counterparts from other origins. This result suggests that the chain length of Fraction III from the waxy-maize amylodextrin is slightly shorter than those of the others. The elution profiles of the amylodextrins, as well as those of their beta-amylolysis products, were different from each other. These results, and the different iodine-staining activities of the amylodextrins or their fractions of higher molecular weight (d.p. 30–33, Fraction II), suggest that the structural features of the fractions differ from each other.

INTRODUCTION

Watanabe and French[1] examined the structure of waxy-maize and potato amylodextrin by fractionation on Sephadex G-50 and by analysis of the fractions by using porcine-pancreatic alpha amylase, sweet-potato beta amylase, and pullulanase, and the results indicated that there are three classes of polymer in amylodextrin. Fraction I, of high molecular weight, is multiply branched; Fraction II, d.p. ~25, is singly branched; and Fraction III, d.p. ~12, is linear.

Kikumoto *et al.*[2] prepared amylodextrin from waxy-maize starch by treating it in 16% sulfuric acid for 50 days at 37°, and succeeded in separating Fraction II

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.

from Fraction III by precipitation of the amylodextrin with pyridine. Umeki and Kainuma[3] also prepared Nägeli amylodextrin from the same starch by treating it in 16% sulfuric acid for 13 days at 38°; they fractionated the amylodextrin by multiple, descending paper-chromatography, and analyzed the fine structure of each fraction by enzymic degradation. They found that there were two main groups of polysaccharide in the amylodextrin, one (d.p. 12–16) was linear, or was branched near the reducing end with D-glucosyl or maltosyl stubs, and the other (d.p. 28–30) consisted of two unit chains, of d.p. 14–16, linked together by an α-D-(1→6) bond near the reducing end of one.

In this study, we have prepared Nägeli amylodextrin by a modification of the method of Kikumoto *et al.*[2], and examined the structural features and properties of the Nägeli amylodextrins from various types of starch (waxy maize, sweet potato, and potato) by gel filtration of the original dextrins on Bio-Gel P-6, gel filtration of their beta-limit dextrins on Bio-Gel P-2, analysis of the beta-amylolysis limit, and determination of the value of λ_{max} of the iodine complex of each amylodextrin, as well as of its Fractions II and III. X-Ray diffraction patterns have been obtained, and determination of the d.p. has been made, of Fraction II and Fraction III.

EXPERIMENTAL

Native, starch granules. — Waxy-maize starch was kindly provided by Dr. T. Miwa, Nihon Shokuhin Kako Co., Ltd., Fuji. Sweet-potato starch was a generous gift from Mr. K. Honbo, Nihon Denpun Kogyo Co., Ltd., Kagoshima. Potato starch was obtained from a commercial source. Each starch (100 g) was defatted by extraction, five times, with 85% methanol (300 mL) for several hours, according to the procedure of Schoch[4].

Preparation of amylodextrin. — Each starch type (waxy maize, sweet potato, and potato starch) was treated by suspending the starch (45 g, dry basis) in 15% sulfuric acid (1.8 L). The mixtures were kept at 40°, and resuspended daily. Every 10 days, a portion (50 mL) of the reaction mixture was removed, the insoluble residue was separated from it by centrifugation, and the precipitate was washed with de-ionized water until free of acid. The used sulfuric acid was siphoned off every 10 days, and replaced by fresh, 15% sulfuric acid. After 40 days, the acid-resistant residue (amylodextrin) was collected by filtration, and washed with de-ionized water until the pH of the washings was neutral; yield: waxy maize 8.5 g, sweet potato 10.3 g, and potato 11.4 g.

Enzymes. — Purified, sweet-potato beta-amylase (Sigma No. A 7005) was obtained from a commercial source. Pullulanase was a generous gift from Dr. K. Sugimoto, Hayashibara Biochemical Laboratories, Inc., Okayama.

General methods. — The total carbohydrates and the reducing sugars were determined by the phenol–sulfuric acid method[5] and the Somogyi–Nelson method[6], respectively. The degree of polymerization (d.p.) was determined by gel filtration on Toyopearl HW-40 F, and by the ratio of the total carbohydrate to the reducing sugar.

Glucose was used as the standard for both methods. The amylose content of the original starch was determined by the method of McCready and Hassid[7]. X-Ray diffraction was recorded with a Rigaku Denki Geiger-Flex diffractometer. The packed discs were mounted on a diffractometer, exposed to Ni-filtered CuKα radiation, and operated at 45 kV, 30 mA. The wavelength of the maximum absorbance (λ_{max}) of the iodine complex of the original amylodextrin, as well as those of Fractions II and III, was determined by a modification of the method of Bailey and Whelan[8].

Beta-amylolysis limit. — To a solution (80 μL) of amylodextrin (containing 80 μg of amylodextrin) were added 40 μL of 0.02M acetate buffer, pH 4.8 (containing 0.4 unit of beta amylase) and 80 μL of 0.02M acetate buffer, pH 4.8. After incubation for 24 h at 38°, the enzyme reaction was stopped by heating in a boiling-water bath, and the maltose produced was determined by the Somogyi–Nelson method[6]. The analysis of the beta-amylolysis limit of Fractions II and III from each amylodextrin was made in exactly the same way.

Elution profile of amylodextrin on Bio-Gel P-6. — Nägeli amylodextrin (5 mg) 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, at 50°) of Bio-Gel P-6. 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[5].

RESULTS

Solubilization of starches during treatment with sulfuric acid. — Each starch type (waxy maize, sweet potato, and potato starch) was treated at 40°, at a concentration of 45 g of starch (dry basis) in 1.8 L of 15% sulfuric acid, and resuspended daily. Every 10 days, a portion of the suspension (50 mL) was removed, and the insoluble residue was precipitated from it by centrifugation (5,000 r.p.m., 20 min), and washed with de-ionized water. The supernatant liquor and washings were combined, made neutral with barium carbonate, and treated with Amberlite IR-120 (H^+) resin. The extent of starch hydrolysis with time was monitored by measuring the total carbohydrate in the supernatant liquor by the phenol–sulfuric acid method; the results are expressed as percent of the initial starch. The degrees of hydrolysis of various types of starch are shown in Fig. 1.

The degrees of hydrolysis of waxy-maize, sweet-potato, and potato starch, after treatment with 15% sulfuric acid for 40 days at 40°, were, respectively, 76.2, 79.1, and 72.8%. After this period, the degree of hydrolysis no longer increased in any of them.

Changes of elution profile of waxy-maize, sweet-potato, and potato starch on Bio-Gel P-6 during treatment with 15% sulfuric acid. — Each starch type (waxy maize, sweet potato, and potato starch) was treated by suspending the starch (60 g) in 15% sulfuric acid (2.4 L). The mixtures were kept at 40°, and resuspended daily.

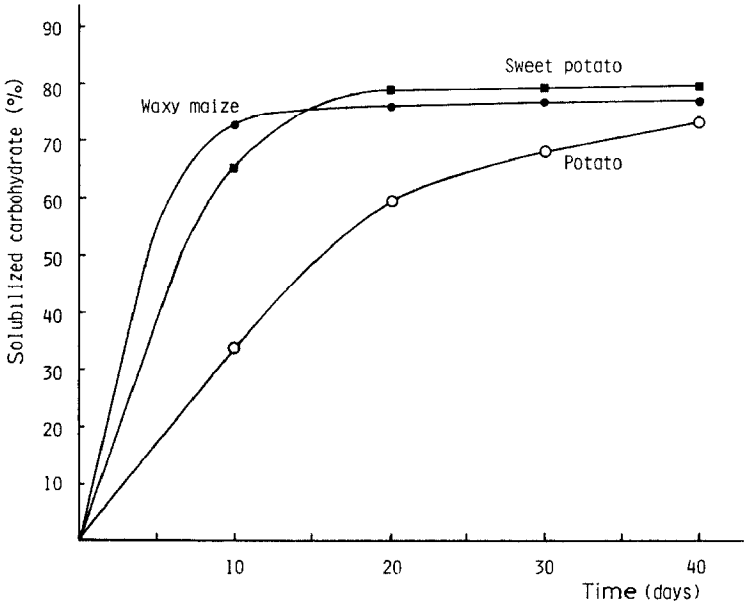


Fig. 1. Solubilization of starches during treatment with sulfuric acid.

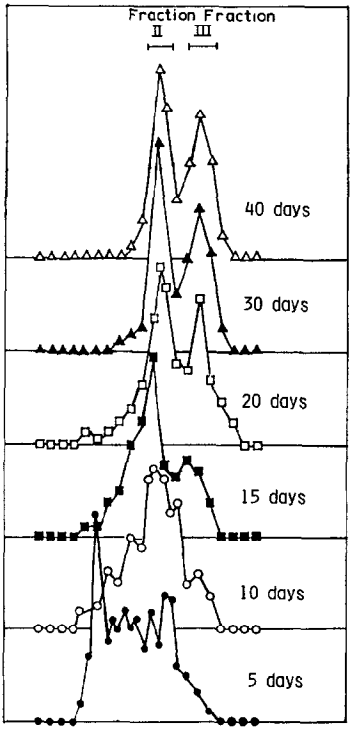
Every 5 days, a portion (10 mL) of the suspension was withdrawn, the supernatant liquor was removed by centrifugation, and the precipitate was washed with deionized water until free of acid. The precipitate (5 mg) was chromatographed on a column of Bio-Gel P-6, as already described. The changes in the elution profiles of each starch type during acid treatment are shown in Figs. 2–4.

After treatment with 15% sulfuric acid for 40 days at 40°, the ratios of Fraction II and Fraction III from waxy-maize, sweet-potato, and potato amylodextrin were 1.1 : 1; 1 : 1, and 1 : 4, respectively.

TABLE I

PROPERTIES OF NATIVE STARCHES AND NÄGELI AMYLODEXTRINS FROM VARIOUS TYPES OF STARCH

Carbohydrate	*Amylose (%)*		*Beta-amylolysis limit (%)*		
Starch from					
Waxy maize	0		56.1		
Sweet potato	22		63.8		
Potato	19		62.5		
Amylodextrin from	*% of Original starch*	*d.p.*		λ_{max}	*X-Ray diffractogram*
Waxy maize	23.8	20	86.8	420	A
Sweet potato	20.9	18	81.0	490	C
Potato	27.2	20	80.6	500	B



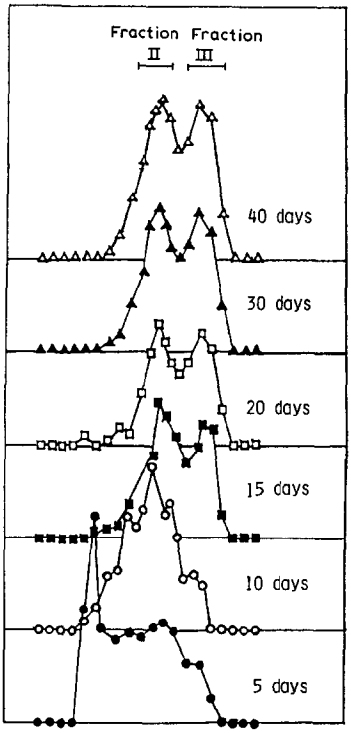


Fig. 2. Changes of elution profile of waxy-maize starch on Bio-Gel P-6 during treatment with 15% sulfuric acid.

Fig. 3. Changes of elution profile of sweet-potato starch on Bio-Gel P-6 during treatment with 15% sulfuric acid.

Properties of native starches and Nägeli amylodextrins from various starch types. — The Nägeli amylodextrin from each starch type (waxy maize, sweet potato, and potato starch) was prepared as already described. The properties of the native starches and Nägeli amylodextrins from various starch types are shown in Table I.

The yields of amylodextrins from the various types of starch differed. The degrees of polymerization of waxy-maize, sweet-potato, and potato amylodextrins are 20, 18, and 20, respectively. On treatment with beta amylase, waxy-maize, sweet-potato, and potato amylodextrins were ~80% hydrolyzed to maltose. The maximum absorbance (λ_{max}) of waxy-maize amylodextrin–iodine complex was observed at 420 nm, whereas that of sweet-potato and potato amylodextrin–iodine complex respectively lay at 490 nm and 500 nm.

The waxy-maize, sweet-potato, and potato amylodextrins respectively gave X-ray diffraction patterns of the A, C, and B type (see Figs. 5–7). In that of waxy-maize amylodextrin, a peak at 4.9 Å (4b) was observed, but there was no peak at 15.8 (1), 6.3 (3a), or 4.4 Å (5b); in that of potato amylodextrin, there was no peak at

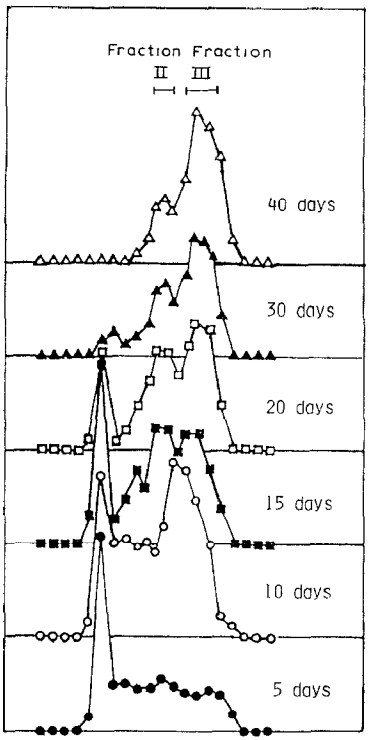


Fig. 4. Changes of elution profile of potato starch on Bio-Gel P-6 during treatment with 15% sulfuric acid.

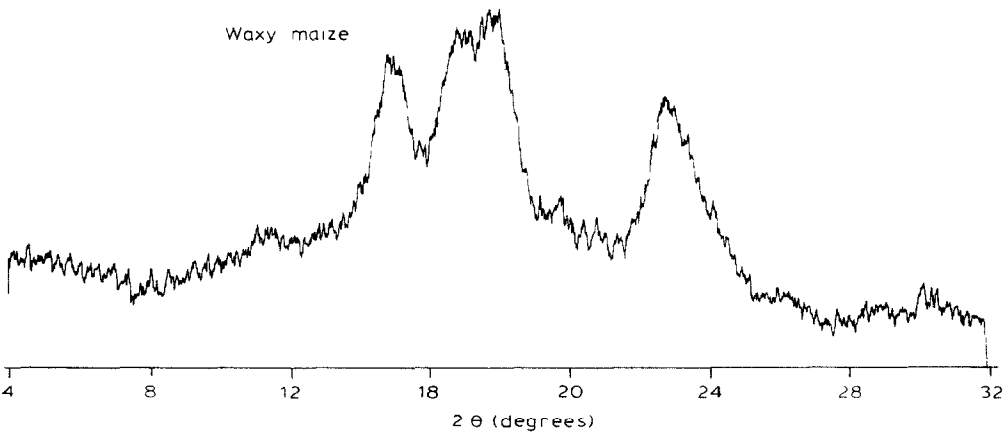


Fig. 5. X-Ray diffraction pattern of waxy-maize amylodextrin.

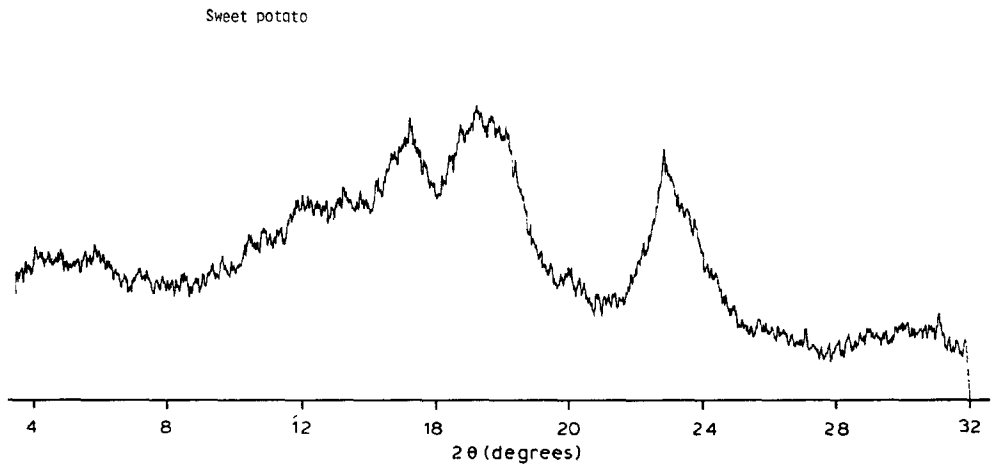


Fig. 6. X-Ray diffraction pattern of sweet-potato amylodextrin.

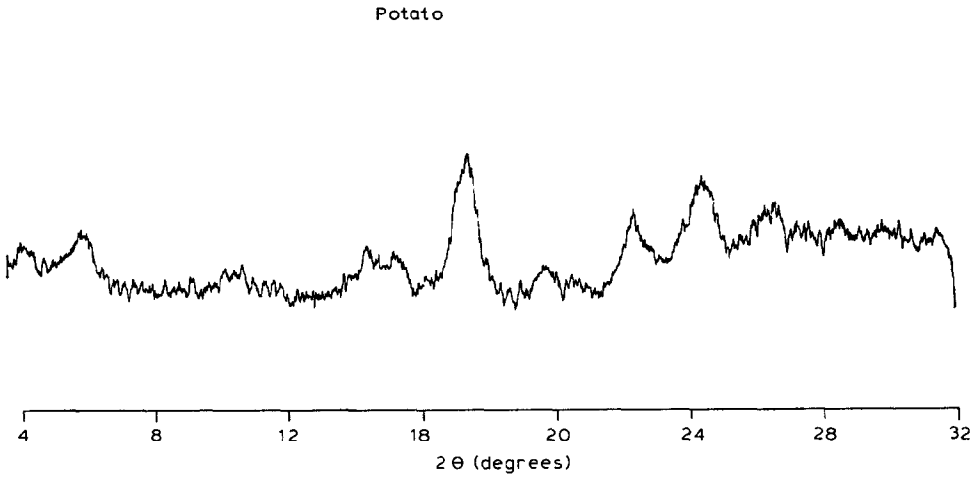


Fig. 7. X-Ray diffraction pattern of potato amylodextrin.

4.9 Å (4b), but there were peaks at 15.8 (1), 6.3 (3a), and 4.4 Å (5b); and that of sweet-potato amylodextrin appeared to be that of a mixture of types A and B.

Elution profiles of waxy-maize, sweet-potato, and potato amylodextrins on Bio-Gel P-6. — Waxy-maize, sweet-potato, and potato amylodextrins (5 mg each) were chromatographed on a column of Bio-Gel P-6. Fractions II and III of waxy-maize amylodextrin were separated by fractionation on Bio-Gel P-6, and purified by repeated gel-filtration on the same column. Fractions II and III from sweet-potato and from potato amylodextrin were similarly separated. The elution profiles of the amylodextrins were different from each other, as shown in Figs. 2–4.

Determination of the wavelength of the maximum absorbance (λ_{max}) of the iodine complex of original amylodextrin, Fraction II, and Fraction III from waxy-maize,

TABLE II

DEGREE OF POLYMERIZATION AND BETA-AMYLOLYSIS LIMIT (% OF TOTAL MALTOSE) OF FRACTION II AND FRACTION III

Amylodextrin from	*Fraction*	*d.p.*	*Beta-amylolysis limit (%)*
Waxy maize	II	30	71
	III	15	100
Sweet potato	II	32	76
	III	17	102
Potato	II	33	61
	III	18	98

sweet-potato, and potato starch amylodextrin. — Amylodextrin, Fraction II, or Fraction III (2 mg) was dissolved in de-ionized water (0.2 mL) by boiling, the solution cooled, 0.004% I_2–0.04% KI solution (10 mL) was added, and after 15 min, the absorbance at 400–650 nm was measured with a Beckman ACTA CIII spectrophotometer. 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. The λ_{max} value of the iodine-stained Fraction III from waxy-maize amylodextrin was 405 nm, whereas those of the corresponding fractions from sweet-potato and potato amylodextrins were 415 and 430 nm, respectively.

Molecular size of Fraction II and Fraction III from waxy-maize, sweet-potato, and potato amylodextrins. — The d.p. of Fraction II and Fraction III of the three starch types were determined by gel filtration, and from the ratio of the total carbohydrate and the reducing sugar. The d.p. of Fraction II and Fraction III of waxy-maize, sweet-potato, and potato starch amylodextrin were respectively estimated to be ~30, 32, and 33; and 15, 17, and 18.

Beta-amylolysis limit of Fraction II and Fraction III from waxy-maize, sweet-potato, and potato amylodextrins. — The beta-amylolysis limits of Fraction II and Fraction III from waxy-maize, sweet-potato, and potato amylodextrins were determined as already described. The results are shown in Table II. The degrees of beta amylolysis (%) of Fraction III from waxy-maize, sweet-potato, and potato amylodextrins are 100, 102 and 98, respectively. These results suggest that the structure of Fraction III is linear in each case. The degree of beta amylolysis of Fraction II from potato amylodextrin is 61, which is lower than those of waxy-maize and sweet-potato amylodextrin.

Elution profiles of beta-limit dextrin of amylodextrin from waxy-maize, sweet-potato, and potato starch on Bio-Gel P-2. — A mixture of amylodextrin solution (45 mL, containing 200 mg of carbohydrate) from waxy-maize starch and 5 mL of 0.2M acetate buffer, pH 4.8, containing beta amylase, was incubated for 24 h at 38°. Enzyme action was stopped when the reducing power became constant (24 h).

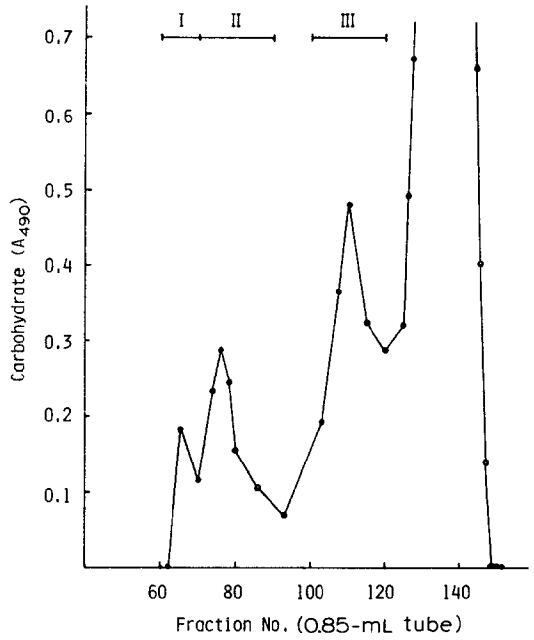


Fig. 8. Elution profile of beta-amylolyzate of waxy-maize amylodextrin on Bio-Gel P-2 (1.5 × 90 cm) at 50°.

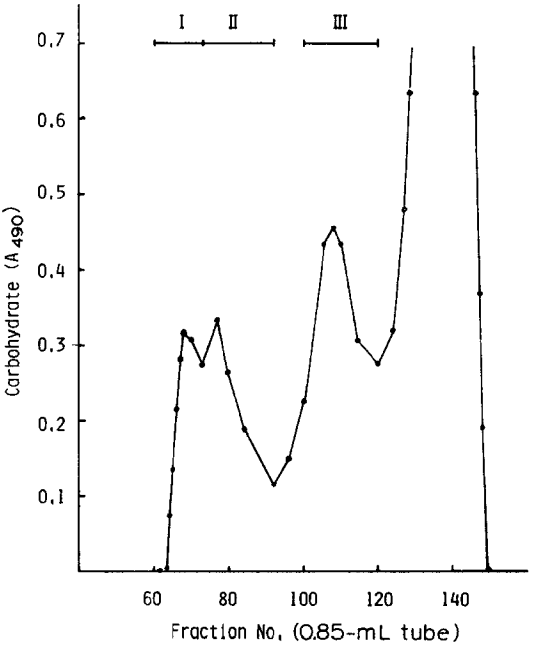


Fig. 9. Elution profile of beta-amylolyzate of sweet-potato amylodextrin on Bio-Gel P-2 (1.5 × 90 cm) at 50°.

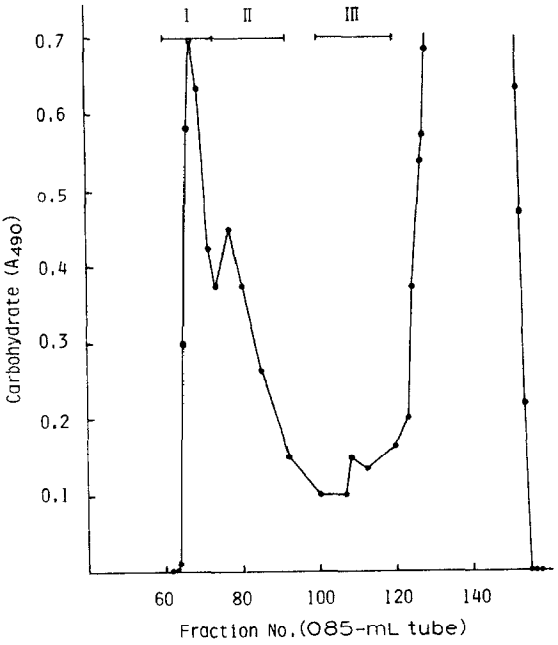


Fig. 10. Elution profile of beta-amylolyzate of potato amylodextrin on Bio-Gel P-2 (1.5 × 90 cm) at 50°.

This beta-amylolysis procedure was repeated three times. After exhaustive treatment with beta amylase, the enzyme reaction was stopped by heating in a boiling-water bath. This digest was concentrated, and the concentrate was centrifuged for 10 min at 3,000 r.p.m. The supernatant liquor (8 mL) was applied to a column (3.5 × 140 cm) of Bio-Gel P-2 at 50°, eluted with de-ionized water, and the eluate separated by a fraction collector into tubes containing 5 mL each. The carbohydrate content of each tube was determined by the phenol–sulfuric acid method. The beta-limit dextrins from sweet-potato and potato starch amylodextrins were chromatographed on Bio-Gel P-2 in the same way. The elution profiles are shown in Figs. 8–10.

TABLE III

RATIO OF BETA-AMYLASE-RESISTANT, OLIGOSACCHARIDE FRACTIONS (I, II, AND III) FROM THE VARIOUS NÄGELI AMYLODEXTRINS

Amylodextrin from	*Fraction*		
	I	*II*	*III*
Waxy maize	1	6.0	10.0
Sweet potato	1	2.5	4.0
Potato	1	1.5	0.75

The beta-limit dextrins from three starch types of amylodextrin gave three peaks, namely, I (V_0), II (d.p. ~10), and III (d.p. ~6), in addition to maltose. The elution profiles of the beta-limit dextrins from various types of amylodextrins were different from one another. The difference in the ratio of beta-amylase-resistant oligosaccharide fractions among the various types of amylodextrins, shown in Table III, suggest different structural features of Fraction II from these amylodextrins.

DISCUSSION

Nägeli amylodextrins from waxy-maize, sweet-potato, and potato starch granules respectively gave X-ray diffraction patterns of the A, C, and B type. In this study, the amylodextrins from three typical starch types (those of waxy maize, sweet potato, and potato) were compared for their structural features.

The X-ray diffraction pattern of waxy-maize amylodextrin prepared by the conventional method of Nägeli[9] was identical with that of waxy-maize amylodextrin prepared by a modification of the method of Kikumoto *et al.*[2]. Therefore, we prepared Nägeli amylodextrin by the latter method. A major part of Fraction I was solubilized under these conditions, and could not be obtained.

The elution profiles of waxy-maize, sweet-potato, and potato amylodextrins on Bio-Gel P-6 were different from one another. Fraction II and Fraction III from the three types of amylodextrin were purified by repeated gel-filtration on the same column.

Fraction III from each amylodextrin was completely hydrolyzed by beta amylase, but was not cleaved by pullulanase (data not shown). These results suggest that the structure of Fraction III is linear, regardless of the origin.

The molecular size of Fraction II and Fraction III from waxy-maize, sweet-potato, and potato amylodextrin was determined by gel-filtration on Toyopearl HW-40 F, and from the ratio of the total carbohydrate to the reducing sugar. The degrees of polymerization of Fraction II and Fraction III of waxy-maize, sweet-potato, and potato starch amylodextrin were estimated to be ~30, 32, and 33; and 15, 17, and 18, respectively.

Bailey and Whelan[8] reported the relationship between iodine staining and chain length of a synthetic amylose prepared by incubation of maltohexaose and α-D-glucosyl phosphate with phosphorylase. According to their results, between d.p. 12 and 50, λ_{max} increases linearly with chain length, but, thereafter, the rate of increase falls and attains a steady value.

The λ_{max} value of the iodine complex of Fraction III from waxy-maize amylodextrin was smaller than those of the counterparts from the other origins. This result suggests that the chain length of Fraction III from waxy-maize amylodextrin is shorter than those of the other counterparts. It appears, therefore, that the chain length of Fraction III differs slightly according to the origin.

The elution profiles of the amylodextrins and their beta-limit dextrins on

Bio-Gel P-6 and P-2, as well as the iodine staining of the amylodextrins and their Fractions II, strongly suggest that the structural features of Fraction II also differ with the origin.

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