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Note

Structures of branched molecules of amyloses of various origins, and molar fractions of branched and unbranched molecules

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Pure amyloses obtained from various starches contain limited numbers of branch linkages and are mixtures of branched and unbranched molecules[1–14], although amyloses are defined as unbranched molecules. However, amyloses of various origins have been characterised generally without separation of the branched and unbranched molecules due to the lack of an effective method for their separation. The aqueous leaching of starch granules leads to only partial separation[2–4,15]. In this study, we investigated in detail the structures of beta-limit dextrins of amyloses of various origins in order to elucidate the structures of branched components of the amyloses. In addition, the molar fractions of the branched and unbranched molecules of the amyloses have been determined.

Table I shows the properties of 9 amyloses from cereals, seeds, roots, and tubers. These specimens showed similar values of iodine affinity, blue value, and λ_{max}, and were confirmed to be free from amylopectin by gel chromatography[6,8,10]. The corn, wheat, kuzu, and sweet-potato amyloses were characterised in this work, and the others previously[6,8,10–14]. The molecular sizes of these amyloses ranged from $\overline{d.p.}$ 960 (corn) to 3280 (sweet potato), and the cereal amyloses appear to be smaller molecules than other amyloses. These amyloses were hydrolysed to 75–91% with beta-amylase, and the average chain-lengths ($\overline{c.l.}$) ranged from 270 (wheat) to 525 (nagaimo). Most amyloses comprised 3–5 chains per molecule on average, but the tapioca and sweet-potato amyloses had 7.9 and 9.8 chains, respectively. These chains were joined by α-(1→6) linkages, since the amyloses were completely degraded with beta-amylase and pullulanase. These properties

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TABLE I

PROPERTIES OF AMYLOSES

	Corn	*Rice*[a]	*Wheat*	*Chestnut*[b]	*Kuzu*	*Nagaimo*[c]	*Lily*	*Tapioca*[d]	*Sweet potato*
Iodine affinity (g/100 g)	21.1	20.6	19.9	19.9	20.0	19.9	20.2	20.0	19.7
Blue value	1.39	1.40	1.40	1.41	1.46	1.55	1.49	1.47	1.50
λ_{max} (nm)	644	658	664	655	658	658	648	662	660
$\overline{D.p.}$[e]	960	1110	1290	1690	1460	2000	2300	2660	3280
Chain length	335	320	270	375	310	525	475	340	335
Number of chains per molecule	2.9	3.5	4.8	4.5	4.7	3.8	4.9	7.8	9.8
Beta-amylolysis (%)	82	81	82	91	76	86	89	75	76
Beta-amylolysis with pullulanase (%)	99	103	101	102	100	102	100	99	99

[a-d]Mostly from refs. 11, 14, 13, and 8, respectively. [e]Average degree of polymerisation.

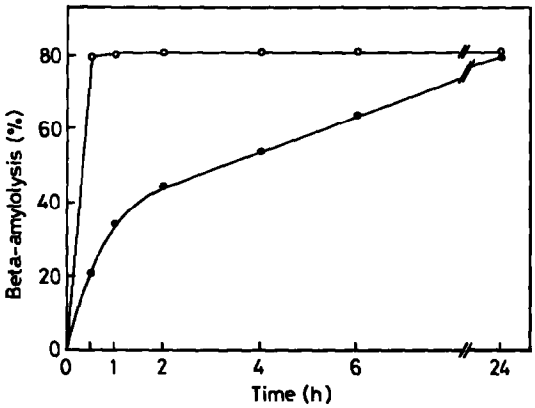

Fig. 1. Degradation of rice amylose with low (1 U/mg of amylose, ●) and high (25 U/mg of amylose, ○) concentrations of beta-amylase.

indicate the average structures of the branched and unbranched molecules of the amyloses, but the branched molecules may be solely characterised by the structures of the beta-limit dextrins. Fig. 1 shows the degradation of rice amylose with low and high concentrations of beta-amylase (1 and 25 U/mg of amylose). With the high concentration, the degradation reached a limit within 1 h; with the low concentration, the hydrolysis proceeded slowly and approached the limit in 24 h. The addition of pullulanase to a beta-amylase hydrolysate (25 U/mg, 3 h), without inactivation of the beta-amylase, resulted in the complete degradation of the amylose. The beta-limit dextrin was isolated from the beta-amylolysate (25 U/mg, 3 h) by chromatography on Bio-Gel P-4.

Table II shows the properties of the beta-limit dextrins. They showed iodine binding and staining properties similar to those of the parent amyloses[4,7,16]. Their $\overline{\text{d.p.}}$ values were in the range 720–1970 and differed with the origin of the amylose. Judging from these values, the wheat, nagaimo, lily, sweet-potato, and tapioca amyloses contain large branched-molecules, and the corn and chestnut amyloses have small branched-molecules.

The average inner chain-lengths of the branched molecules are close to the $\overline{\text{c.l.}}$ values of the beta-limit dextrins, since the inner chain-length is expressed as $\overline{\text{c.l.}} - 3$, in which the 3 D-glucosyl residues comprise a residue with a side chain and the adjoining average 2 residues at the non-reducing side which are not trimmed by beta-amylase[17]. The corn, kuzu, nagaimo, and lily amyloses contain branched molecules with inner chains comprising 147–157 D-glucosyl residues, the chestnut and wheat amyloses have shorter inner-chains of 60–91 D-glucosyl residues, while the remaining amyloses have inner chains of 112–127 D-glucosyl residues. These inner chain-lengths appear to be related to the iodine affinities of the beta-limit dextrins, except for the beta-limit dextrins of nagaimo and lily amyloses, which may have different modes of branching than the others.

TABLE II

PROPERTIES OF BETA-LIMIT DEXTRINS FROM AMYLOSES

	Corn	*Rice*	*Wheat*	*Chestnut*	*Kuzu*	*Nagaimo*	*Lily*	*Tapioca*	*Sweet potato*
Iodine affinity (g/100 g)	19.1	19.5	17.4	15.4	18.9	16.2	16.3	19.1	18.4
Blue value	1.31	1.35	1.37	1.20	1.41	1.40	1.38	1.35	1.39
λ_{max} (nm)	645	659	660	637	660	660	645	653	652
$\overline{D.p.}$	850	1030	1400	720	1170	1690	1670	1970	1770
Chain length	160	115	94	63	150	160	150	115	130
Number of chains per molecule (n.c.)	5.3	9.0	14.9	11.4	7.8	10.6	11.1	17.1	13.6
$\overline{N.c.}/\overline{n.c.}$(amylose)[a]	1.8	2.6	3.1	2.5	1.7	2.8	2.3	2.2	1.4
$\overline{D.p.}/\overline{d.p.}$(amylose)[b]	0.89	0.93	1.08	0.42	0.85	0.85	0.73	0.74	0.54

[a]Number of chains per molecule of amylose. [b]$\overline{D.p.}$ of the parent amylose (see Table I).

TABLE III

MOLAR FRACTIONS OF BRANCHED AND UNBRANCHED MOLECULES IN AMYLOSES

	Corn	*Rice*	*Wheat*	*Chestnut*	*Kuzu*	*Nagaimo*	*Lily*	*Tapioca*	*Sweet potato*
Unbranched molecule	0.56	0.69	0.73	0.66	0.47	0.71	0.61	0.58	0.30
Branched molecule	0.44	0.31	0.27	0.34	0.53	0.29	0.39	0.42	0.70

The corn amylose contains branched molecules with the least ~5 chains, the wheat, tapioca, and sweet-potato amyloses have molecules with 14–17 chains, while the rice, chestnut, kuzu, nagaimo, and lily amyloses have molecules with 8–11 chains. The higher numbers of chains of the beta-limit dextrins (NCb) than those of the parent amyloses (NCa) confirmed that the amyloses are mixtures of branched and unbranched molecules. From these numbers of chains, the molar fraction of branched molecules is given[18] by (NCa − 1)/(NCb − 1). Table III shows that the rice, wheat, chestnut, and nagaimo amyloses contain low molar fractions (less than $^1/_3$) of branched molecules whereas the sweet-potato amylose has a high molar fraction ($^2/_3$), and that the corn, kuzu, lily, and tapioca amyloses are composed of nearly equal numbers of branched and unbranched molecules.

The ratios of $\overline{d.p.}$ values for the beta-limit dextrin and the parent amylose, 0.42–1.08 (Table II), suggest that the branched molecules in most amyloses are larger than the unbranched molecules. The corn, rice, wheat, kuzu, and nagaimo amyloses appear to contain much larger branched molecules, whereas the chestnut and sweet-potato amyloses appear to have slightly larger molecules. Judging from the ratios and molar fractions, the wheat amylose probably contains a small number of very large branched molecules, whereas the sweet-potato amylose has a small number of relatively large unbranched molecules.

These results indicate that the branched molecules of amyloses of various origins have their own characteristic structures in terms of molecular size, inner chain-length, and the number of chains, and that the molar fractions of unbranched and branched molecules vary with the origin of the amylose.

EXPERIMENTAL

Materials. — Corn, wheat[11], kuzu, and sweet-potato[8] amyloses were prepared by the methods described. Rice (Sasanishiki variety)[11], chestnut[14], nagaimo[13], lily, and tapioca amyloses[8] were the same specimens as used previously. All specimens were free from amylopectin, as judged by chromatography[8] on Toyopearl HW-75F (Toyo Soda Manuf. Co. Ltd.) and h.p.l.c.[10].

Beta-amylase was prepared[19] from sweet potatoes and recrystallised from aqueous ammonium sulfate to improve its stability on storage. No maltase activity

was detected in the preparation. Crystalline pullulanase was purchased from Hayashibara Biochemical Lab.

Preparation of beta-limit dextrins from amyloses. — To amylose (500 mg) were added successively ethanol (2 mL), water (5 mL), and 2M NaOH (5 mL). After dissolution of the amylose (0.5–1 h), water (28 mL), M acetate buffer (pH 4.8, 2 mL), and 2M HCl (5 mL) were added successively. The amylose was degraded with 12,500 U (μmol of maltose released per min) of beta-amylase at 37° for 3 h followed by heating in a boiling water bath for 10 min. After centrifugation (10,000*g*, 10 min) in a preheated rotor (50°), the supernatant solution was added immediately to a column (2.6 × 100 cm) of Bio-Gel P-4 (Bio-Rad Laboratories), which was then eluted at 45° with deaerated water at a flow rate of 75 mL/h; fractions of 10 mL were collected. Beta-limit dextrin was separated thoroughly from maltose (Fig. 1). The recoveries from beta-amylolysates were ~90%.

Analytical methods. — Iodine affinities were determined by the amperometric titration procedure of Larson *et al.*[20], and blue values by the method described elsewhere[21]. Carbohydrate was determined by the anthrone–sulfuric acid method[22]. The average degree of polymerisation ($\overline{d.p.}$) and the average chain-length ($\overline{c.l.}$) were determined by the modified Park–Johnson method[5] and the rapid Smith-degradation method[5,8], respectively. Beta-amylolysis and the simultaneous degradation with beta-amylase and pullulanase were performed as described previously[5].

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