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MOLECULAR STRUCTURES OF RICE STARCH

SUSUMU HIZUKURI, YASUHITO TAKEDA, NOBUHISA MARUTA,
Department of Agricultural Chemistry, Faculty of Agriculture, Kagoshima University, Korimoto-1, Kagoshima 890 (Japan)

AND BIENVENIDO O. JULIANO
Cereal Chemistry Department, International Rice Research Institute, Los Baños, Laguna (Philippines)

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ABSTRACT

New procedures for the structural analysis of starches are described. The beta-amylolysis limit of the branched component of seven rice amyloses was estimated to be 39% from the relationship between the beta-amylolysis limit and the number of branched molecules. The $\overline{\text{d.p.}}_n$ and the proportions of the linear and branched molecules were estimated on the basis of the structures of the amyloses and their beta-limit dextrins. The limiting viscosity numbers $[\eta]$ showed good correlation with the proportions of the linear and branched molecules, but not with their molecular sizes. The iodine affinity and the $[\eta]$ of eight rice amylopectins were dependent on the longest chain component, which is lacking in waxy amylopectin but abundant in some indica varieties. The $[\eta]$ of rice amylopectin showed a good correlation with the proportions of the components with the longest and shortest chains, but not with the molecular size.

INTRODUCTION

Rice (*Oryzae sativa* L.) is classified into two sub-species, indica and japonica, and there are many cultivars in each sub-species, which show distinct cooking and processing characteristics. Typical indica rices give dry and hard cooked grains which tend to remain separate and to retrograde rapidly. On the other hand, typical japonica rices give soft and moist cooked grains which tend to stick together and to retrograde slowly. These characteristics are considered to be mainly due to structural differences in the starch, because ~90% of milled rice is starch.

Starch is usually considered to be a mixture of amylose [(1→4)-α-D-glucan] and amylopectin [(1→4)-α-D-glucan with ~5% of α-(1→6)-linked branches]. These molecules are usually easily distinguished since only amylose gives a blue inclusion complex with iodine. Theoretically, amylose is hydrolyzed completely into maltose by beta-amylase, whereas amylopectin is hydrolyzed only partially. Recently, however, it has become clear that amylose isolated and purified by conventional methods contains linear and slightly branched molecules[1-7], because they are

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hydrolyzed partially with beta-amylase and completely with beta-amylase and debranching enzymes[1–10]. The quantitative separation of the linear and branched molecules of amylose has not been accomplished, but the molar fractions have been measured through the determination of branch linkages of amyloses and their beta-limit dextrins[2–7].

We have investigated[7,10–13] the structures of rice starches having iodine affinities (g/100 g) ranging from 3.69 (Koshihikari) to 5.27 (IR42). Although the iodine affinity of starch is thought to be a measure of the amylose content, these rice starches contained similar proportions of amylose and amylopectin[11,12]. The iodine affinities of the latter varied in the range of 0.39 (Koshihikari)–2.57 (IR42), because of the variation in chain lengths[11,12]. The amyloses of these starches were similar in molecular size, but contained various proportions of linear and branched molecules[2,7,12].

We now describe methods for estimating the beta-amylolysis limits of branched amyloses, and the $\overline{\text{d.p.}}_n$ and the proportions of the linear and branched molecules based on data for rice starches[7,11,12], and also discuss some properties of starch molecules.

DISCUSSION

Beta-amylolysis limit ($\beta_{a.l.}$) *of branched amylose.* — The average numbers of chains per molecule ($\overline{\text{n.c.}}$), determined by assaying the reducing and non-reducing terminal residues, of seven amyloses were in the range 2.5–4.3, and the values for their beta-limit dextrins (β-LD) were in the range 5.6–9.0, which is ~2-fold that of the parent amyloses[7,11,12]. These data suggest that the amyloses comprise a mixture of linear and branched molecules, the number proportions [%, N_l and N_b, respectively] of which are calculated with the following equations[14].

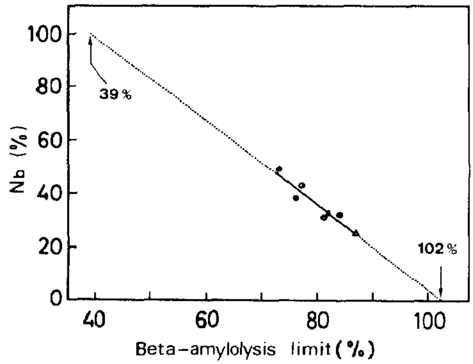

Fig. 1. Relationship between the amount of branched molecules, by number (N_b), and the beta-amylolysis limit of seven rice amyloses: ○, japonica varieties; △, indica varieties (IR48 and IR64) with intermediate i.a.; ●, indica varieties (IR32, IR36, and IR42) with high i.a.

$$\mathrm{N_b}\,(\%) = 100\cdot[(\overline{\mathrm{n.c.}}_{\mathrm{amylose}} - 1)/(\overline{\mathrm{n.c.}}_{\beta\text{-LD}} - 1)] \qquad (1)$$

$$\mathrm{N_l}\,(\%) = 100 - \mathrm{N_b} \qquad (2)$$

The $\beta_{\mathrm{a.l.}}$ of amylose showed good correlation with $\mathrm{N_b}$, as shown in Fig. 1 and equation *3* where γ is a correlation coefficient.

$$\mathrm{N_b}\,(\%) = -1.57\cdot\beta_{\mathrm{a.l.}} + 161\ (\gamma = -0.94 \text{ for seven rice amyloses}) \qquad (3)$$

From equation *3*, the $\beta_{\mathrm{a.l.}}$ of the branched molecules of rice amylose is suggested to be 39%, which is considerably below that (55–60%) of amylopectin. The value (102%) for the linear molecule agrees with the theoretical value within experimental error, suggesting that the relationship is reasonable.

A similar equation (*4*) was obtained for amyloses from corn[3],

$$\mathrm{N_b}\,(\%) = -1.86\cdot\beta_{\mathrm{a.l.}} + 194\ (\gamma = 0.85,\ n = 13) \qquad (4)$$

amylomaize (laboratory-made[6]), wheat[2], water chestnut[4], chestnut[2], two kinds of sago[5], kuzu[2], two kinds of yam (nagaimo[2] and jinenjo[15]), tapioca[2], lily[2], and sweet potato[2]. The average $\beta_{\mathrm{a.l.}}$ of these branched amyloses was suggested to be 50.5%, which is considerably higher than that (39%) of branched amylose from rice, but still lower than those (55–60%) of amylopectin. The $\beta_{\mathrm{a.l.}}$ of a branched amylose having a small number of branches is lower than that of highly branched amylopectin, suggesting different modes of branching. In addition, the $\overline{\mathrm{n.c.}}$ of branched amyloses (β-LDs in Table I) differs with their origin, so that the structure is characteristic of each variety.

TABLE I

MOLECULAR PROPERTIES OF RICE AMYLOSES[a]

Variety	*Amylose*							*Beta-limit dextrin*		*Linear molecule*	*Branched molecule*
	$[\eta]$ *(mL/g)*	$\beta_{a.l.}$ *(%)*	$\overline{D.p.}_n$	$\overline{D.p.}_w$	$\overline{N.c.}$	N_b *(%)*	W_b *(%)*	$\overline{D.p.}_n$	$\overline{N.c.}$	$\overline{D.p.}_n$	$\overline{D.p.}_n$
Japonica											
Sasanishiki	216	81	1110	3090	3.5	31	46	1030	9.0	860	1660
Hokkaido[b]	208	77	1100	3230	4.2	43	56	890	8.5	840	1440
Indica											
IR48	243	82	930	3420	2.5	32	39	700	5.7	830	1130
IR64	249	87	1020	3300	2.3	25	33	840	6.1	910	1350
IR32	180	73	1040	2750	4.2	49	60	790	7.5	820	1270
IR36	208	84	920	2810	2.5	32	50	890	5.7	680	1440
IR42	192	76	980	3320	4.3	38	52	840	9.7	750	1350
Average	214	80	1010	3130	3.4	36	48	850	7.5	810	1380

[a] The $\overline{\mathrm{d.p.}}_{\mathrm{n}}$ of linear and branched molecules and $\mathrm{W_b}$ were newly obtained, and the other values are cited from refs. 7, 10, and 12. Abbreviations are given in the text. [b] Unknown variety, produced in Hokkaido.

Molecular sizes of linear and branched molecules of amylose. — From the $\beta_{\text{a.l.}}$ values of the branched molecules and the $\overline{\text{d.p.}}_n$ of the β-LDs, the $\overline{\text{d.p.}}_n$ of the branched and linear molecules can be calculated with equations *5* and *6*, respectively.

$$\overline{\text{D.p.}}_{n\ \text{branched}} = \overline{\text{d.p.}}_{n\ \beta\text{-LD}}/(1 - \beta_{\text{a.l.}}/100) \qquad (5)$$

$$\overline{\text{D.p.}}_{n\ \text{linear}} = (100 \cdot \overline{\text{d.p.}}_{n\ \text{whole}} - N_b \cdot \overline{\text{d.p.}}_{n\ \text{branched}})/N_l \qquad (6)$$

Thus, the calculated molecular sizes of the branched molecules were 1.4–2.2-fold those of the linear molecules (1.8-fold on average, Table I).

Proportions of linear and branched molecules of amylose. — The proportions of the branched (W_b) and linear molecules (W_l) were calculated from the $\overline{\text{d.p.}}_n$ and N_b of each molecule with equations *7* and *8*, and are listed in Table I.

$$W_b\ (\%) = \overline{\text{d.p.}}_{n\ \text{branched}} \cdot N_b/\overline{\text{d.p.}}_{n\ \text{whole}} \qquad (7)$$

$$W_l\ (\%) = 100 - W_b \qquad (8)$$

The branched molecule was found to be minor in number but major in proportion in some specimens, comprising ~50% on average.

Implication of the limiting viscosity number of amylose. — The $\overline{\text{d.p.}}_n$ and $\overline{\text{d.p.}}_w$ of all amylose specimens were similar, being ~1000 and ~3100, respectively, but their limiting viscosity numbers $[\eta]$ (M KOH, 22.5°) varied in the range 180–249 (mL/g)[11,12]. The following Mark–Houwink–Sakurada equation *9* was obtained from these data but the correlation was poor, because the amyloses were mixtures of linear and

$$[\eta] = 0.22 \cdot (\overline{\text{d.p.}}_w)^{0.85}\ (\gamma = 0.62 \text{ for seven rice amyloses}) \qquad (9)$$

branched molecules with different proportions in each specimen. Good correlations between $[\eta]$ and the proportions of branched molecules, by number (N) and by weight (W), especially the latter, were found, as shown below:

$$[\eta] = -0.25 \cdot N_b + 90\ (\gamma = 0.81, n = 7) \qquad (10)$$

$$[\eta] = -0.35 \cdot W_b + 123\ (\gamma = 0.94, n = 7) \qquad (11)$$

Thus, $[\eta]$ appears to be a useful index of the proportions of linear and branched molecules for rice amyloses, because their molecular sizes are similar.

In general, however, a good correlationship between $[\eta]$ and $\overline{\text{d.p.}}_w$ or $\overline{\text{d.p.}}_n$ (equations *12* and *13*) was found for 19 kinds of amyloses, namely, corn (alkali-steeping[3]), three kinds of amylomaize[6], chestnut[16], yam (jinenjo[15]), two kinds of sago[5], kuzu[17], sweet potato (Koganesengan[18,19]), tapioca[17], potato[17], and seven rice varieties. Therefore, the $\overline{\text{d.p.}}_w$ and $\overline{\text{d.p.}}_n$ of amyloses may be estimated from $[\eta]$ with equations *12* and *13*. This means that $[\eta]$ is dependent on the molecular size rather than the proportions of the linear and branched molecules.

$$[\eta] = 0.41 \cdot (\overline{\text{d.p.}}_w)^{0.78} \; (\gamma = 0.96, n = 19) \qquad (12)$$

$$[\eta] = 0.51 \cdot (\overline{\text{d.p.}}_n)^{0.53} \; (\gamma = 0.88, n = 19) \qquad (13)$$

The exponent value of equation *12*, which is almost identical to the value (0.77) reported by Banks and Greenwood[20] and smaller than that (0.89) reported by Cowie[21], implies that the amylose in alkaline solution is a random coil.

Branched amylose as a third component of starch. — From the results of a series of studies[2–7,10,12], it is clear that the branched amylose found in the amylose fraction, which can be isolated by conventional fractionation and has a structure that is intermediate of those of amylose and amylopectin, may be regarded as a third component of starch.

Relationship between the chain-length distribution and iodine affinity (i.a.) of amylopectin. — Amylopectin contains numerous chains of ~20 glucose residues (Table II). Several models have been proposed but, at present, the racemose or cluster models[22–25] are accepted as the most plausible in terms of chain-length distribution[25–28] and other properties[22,23]. Amylopectins comprise 2 main fractions of long and short chains, each of which appears to contain 2 or 3 sub-fractions which are distinguishable by gel-permeation h.p.l.c.[24,25]. Fig. 2 shows the chain-length-distribution chromatograms of three typical rice amylopectins with low, medium, and high affinities for iodine, respectively. The chains are grouped at hollows and a shoulder. Fractions F1a and F1b were combined previously[11,12] as

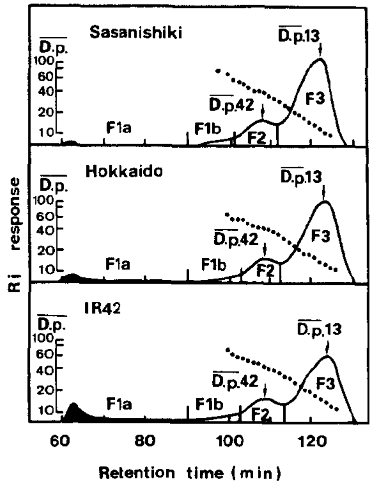

Fig. 2. Chain-length distributions of three rice amylopectins, determined by h.p.l.c., cited from ref. 11 in which F1a (stained black) and F1b were combined as F1.

TABLE II

MOLECULAR PROPERTIES OF RICE AMYLOPECTINS[a]

Variety	*I.a.* *(g/100 g)*	$\beta_{a.l.}$ *(%)*	$[\eta]$ *(mL/g)*	$\overline{D.p.}_n$ *(k)*	$\overline{C.l.}$	$\overline{N.c.}$
Japonica						
Sasanishiki	0.49	58	134	12.8	19	670
Koshihikari	0.39	59	137	8.2	20	410
Hokkaido	0.87	59	137	11.0	19	580
Indica						
IR48	0.86	58	157	9.0	20	450
IR64	0.63	59	152	15.0	20	700
IR32	1.62	56	150	4.7	21	220
IR36	1.62	59	170	5.4	21	260
IR42	2.57	58	165	5.8	22	260

[a] Cited from refs. 11 and 12, except $\overline{\text{n.c.}}$ Abbreviations are given in the text.

F1. Fraction F3 appears to comprise two components in some species[27], but this is not clear in rice. The amounts and $\overline{\text{c.l.}}$ of these fractions are listed in Table III.

The i.a. of rice amylopectin is dependent on the proportion of the longest-chain fraction (F1a) as shown in Fig. 3, and an excellent correlation (equation *14*) was found.

$$\text{F1a}(\%) = 5.4\cdot\text{i.a.} + 0.33 \ (\gamma = 1.0,\ n = 8) \qquad (14)$$

The japonica variety (Koshihikari) with the lowest i.a. contained only 2% of this fraction, and the indica variety (IR42) with the highest i.a. contained as much as 14%. Conversely, the shortest-chain fraction (F3) was found in a larger proportion in an amylopectin having a lower i.a. However, the proportions of the fraction (F2)

TABLE III

CARBOHYDRATE PROPORTIONS AND $\overline{\text{D.P.}}_w$ OF THE FRACTIONS OF ISOAMYLASE-DEBRANCHED RICE AMYLOPECTINS[a]

	Sasanishiki	*Koshihikari*	*Hokkaido*	*IR48*	*IR64*	*IR32*	*IR36*	*IR42*
Carbohydrate (% of by weight)								
F1a[b]	3	2	5	5	4	9	9	14
F1b	4	4	4	5	5	5	6	6
F2	19	19	17	20	21	19	19	19
F3	74	75	74	70	70	67	66	61
$\overline{D.p.}_w$								
F1b	~80	~90	~70	~70	~70	~80	~90	~70
F2	41	44	41	42	40	44	43	42
F3	17	17	16	16	16	17	17	16

[a] Cited from refs. 11 and 12, except F1a and F1b. [b] See Fig. 2.

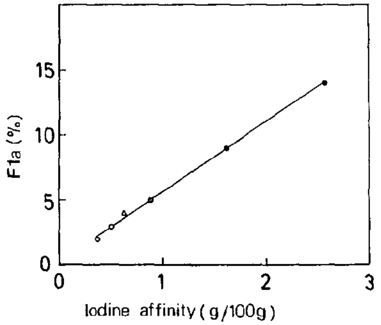

Fig. 3. Relationship between the percentage F1a and the i.a. of rice amylopectin. Symbols as in Fig. 1.

of chains of medium length were similar. Fraction F1a was not found in waxy rice amylopectin. These relationships suggest that there are essential differences in molecular architecture among the amylopectins of these varieties. Fraction F1a could contain the trunk of the amylopectin cluster (probably the C-chain, which has a reducing terminal residue[29]) or a long B-chain[29] and seems to be the basic component of the molecular architecture, although it is present in a minor amount. The amylopectins with higher i.a. contain higher amounts of fraction F1a. Fraction F1a may be long exterior chains or large linear intervals between branches, because the i.a. of some of the amylopectins (IR48, IR64, and IR36) decreased to below those of low i.a. amylopectins (Koshihikari and Sasanishiki) on hydrolysis with beta-amylase, and some (IR32 and IR42) still showed high values (Table IV) after hydrolysis. Therefore, these amylopectins are expected to show functional properties more or less similar to those of amylose. In fact, IR32 amylopectin showed a more marked tendency to rapid retrogradation than Sasanishiki amylopectin[30]. These amylopectins differ in c.l. at most by 2 glucosyl residues but differ significantly in their molecular architecture, especially the waxy variety, which lacks this fraction.

TABLE IV

IODINE AFFINITIES (g/100 g) OF RICE AMYLOPECTINS AND THEIR BETA-LIMIT DEXTRINS[a]

Variety	*Amylopectin (A)*	*Beta-limit dextrin (B)*	*B/A*
Japonica			
Koshihikari	0.39	0.22	0.56
Sasanishiki	0.49	0.33	0.67
Hokkaido	0.87	0.66	0.76
Indica			
IR48	0.86	0.27	0.31
IR64	0.63	0.37	0.59
IR32	1.62	0.86	0.53
IR36	1.62	0.17	0.10
IR42	2.52	0.83	0.33

[a] Cited from refs. 11 and 12.

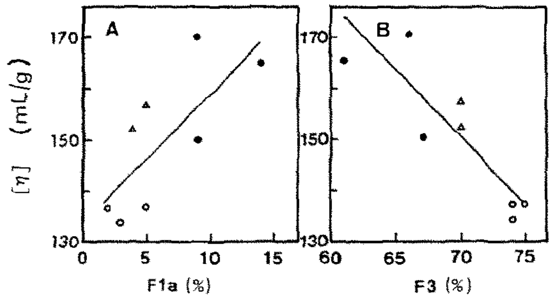

Fig. 4. Relationships between [η] and the percentage F1a (A) and F3 of eight rice amylopectins (B). Symbols as in Fig. 1.

Implication of [η] *of amylopectin.* — The amylopectins (IR32, IR36, and IR42) with high i.a., of which the number-average molecular weights were about half those of other amylopectins[11], showed higher [η] in alkaline solution, so that this parameter was not correlated with molecular size. However, good correlations (equations *15* and *16*) between [η] and the amounts of F1a or F3 were found, as shown in Fig. 4. F1a and F3 increase and decrease [η], respectively.

$$[\eta] = 2.51 \cdot (\mathrm{F1a}, \%) + 134 \; (\gamma = 0.75, \mathrm{n} = 8) \qquad (15)$$

$$[\eta] = -2.44 \cdot (\mathrm{F3}, \%) + 320 \; (\gamma = -0.87, \mathrm{n} = 8) \qquad (16)$$

In the former fraction, the molecules appear to be slender and globular in the latter fraction so that the molecular shape of each amylopectin differs with the specimen, depending upon the amounts of these fractions. Consequently, [η] reflects the profile of the molecular architecture but not the molecular size. It is concluded that, in general, the molecular weight of an amylopectin cannot be estimated from [η].

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REFERENCES

1 S. Hizukuri, Y. Takeda, M. Yasuda, and A. Suzuki, *Carbohydr. Res.*, 94 (1981) 205–213.
2 Y. Takeda, S. Hizukuri, C. Takeda, and A. Suzuki, *Carbohydr. Res.*, 165 (1987) 139–145.
3 Y. Takeda, T. Shitaozono, and S. Hizukuri, *Staerke*, 40 (1988) 51–54.
4 S. Hizukuri, Y. Takeda, T. Shitaozono, J. Abe, A. Ohtakara, C. Takeda, and A. Suzuki, *Staerke*, 40 (1988) 165–171.
5 Y. Takeda, C. Takeda, A. Suzuki, and S. Hizukuri, *J. Food Sci.*, in press.

6 C. Takeda, Y. Takeda, and S. Hizukuri, *Cereal Chem.*, in press.
7 Y. Takeda, S. Hizukuri, and B. O. Juliano, *Carbohydr. Res.*, 186 (1989) 163–166.
8 O. Kjølberg and D. J. Manners, *Biochem. J.*, 86 (1963) 258–262.
9 W. Banks and C. T. Greenwood, *Staerke*, 19 (1967) 197–206.
10 Y. Takeda, S. Hizukuri, and B. O. Juliano, *Carbohydr. Res.*, 148 (1986) 299–308.
11 Y. Takeda, S. Hizukuri, and B. O. Juliano, *Carbohydr. Res.*, 168 (1987) 79–88.
12 Y. Takeda, N. Maruta, S. Hizukuri, and B. O. Juliano, *Carbohydr. Res.*, 187 (1989) 287–294.
13 B. O. Juliano, R. M. Villareal, C. M. Perez, C. P. Villareall, Y. Takeda, and S. Hizukuri, *Staerke*, 39 (1987) 390–393.
14 S. Hizukuri, Y. Takeda, and S. Imamura, *Nippon Nôgei Kagaku Kaishi*, 46 (1972) 119–126.
15 S. Hizukuri, unpublished data.
16 C. Takeda, Y. Takeda, and S. Hizukuri, *Denpun Kagaku*, 34 (1987) 31–37.
17 A. Suzuki, Y. Takeda, and S. Hizukuri, *Denpun Kagaku*, 32 (1985) 205–212.
18 S. Hizukuri and T. Takagi, *Carbohydr. Res.*, 134 (1984) 1–10.
19 Y. Takeda, N. Tokunaga, C. Takeda, and S. Hizukuri, *Staerke*, 38 (1986) 345–350.
20 W. Banks and C. T. Greenwood, *Carbohydr. Res.*, 7 (1968) 414–420.
21 J. M. G. Cowie, *Macromol. Chem.*, 42 (1961) 230–247.
22 Z. Nikuni, *Chôri Kagaku*, 2 (1969) 6–14; *Denpun Kagaku*, 22 (1975) 78–92.
23 D. French, *Denpun Kagaku*, 19 (1972) 8–25.
24 D. J. Manners and N. K. Matheson, *Carbohydr. Res.*, 90 (1981) 99–110.
25 J. P. Robin, C. Mercier, R. Charbonnier, and A. Guilbot, *Cereal Chem.*, 51 (1974) 389–406.
26 L. F. Hood and C. Mercier, *Carbohydr. Res.*, 16 (1978) 53–66.
27 S. Hizukuri, *Carbohydr. Res.*, 147 (1986) 342–347.
28 S. Hizukuri, *Carbohydr. Res.*, 141 (1985) 295–306.
29 S. Peat, W. J. Whelan, and G. J. Thomas, *J. Chem. Soc.*, (1952) 4546–4548.
30 A. Suzuki and S. Hizukuri, unpublished data.