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Carbohydrate Polymers 38 (1999) 337–347

Carbohydrate Polymers

Structure of rice starch and its relation to cooked-rice texture ☆

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Received 25 February 1998; received in revised form 1 June 1998; accepted 14 June 1998

Abstract

Starch from seven varieties of rice, known to cook from very soft to very hard texture, was fractionated by gel-permeation chromatography on Sepharose CL-2B column. The high-molecular-weight fraction (Sepharose FRI) and the low-molecular-weight fraction (Sepharose FRII, further sub-divided into FRIIa, IIb and IIc) were debranched using isoamylase and fractionated on Biogel P-10. All the four fractions in all the different varieties of rice gave a similar trimodal chain profile, indicating the presence of branched molecules in all of them. Clearly, the branched component of starch ('amylopectin') is not necessarily big in size, but includes very small to very big molecules. The presence or absence of the largely linear, and relatively small molecule, 'amylose', could not be settled either way with the technique employed. However, based on certain assumptions, amylose content was calculated to be in the range of 7%–11% in the samples, much less than generally thought. The content of long-B chains of the branched molecule in the four Sepharose fractions individually and in aggregate, as well as the calculated amylose content, correlated well with the sensory tenderness of cooked rice. It was observed that the content of *all* long linear chains, including amylose if any, govern the texture of cooked rice.

1. Introduction

For close to three and a half decades, up to the mid-eighties, the texture of cooked rice was ascribed (Juliano, 1985) primarily to the content of amylose in starch (now better called amylose-equivalent (AE) in the light of recent findings that long chains of amylopectin react equally well with iodine and thus contribute to the estimated amylose value (see Radhika Reddy et al., 1993). This experiment showed, on the basis of repeated observation that the hot-water-insoluble part of AE (insoluble AE) was the primary determinant of texture soluble AE, being largely unrelated to texture (Bhattacharya and Sowbhagya, 1972, 1978, 1982; Bhattacharya and Sowbhagya, 1979, 1980 Sowbhagya et al., 1987).

Further, a dramatic shift of focus came after the mid-eighties. Chinnaswamy and Bhattacharya (1986) separated rice starch by gel-permeation chromatography (GPC) over Sepharose 2B and noted that the high-molecular-weight branched fraction (FRI) of starch, in terms of its iodine reaction, correlated well with the insoluble AE of rice. The low-molecular-weight fraction (FRII) seemed to correlate with the apparently texturally unimportant soluble AE. These findings were later confirmed by Radhika Reddy et al. (1993). Meanwhile Takeda et al., 1987, 1989) and Hizukuri et al. (1989) observed that chemically isolated rice amylopectin, upon debranching, yielded three groups of anhydroglucose chain populations (long-B, intermediate-B and A plus short-B). High-AE rices had more long-B chains than low-AE rices. Radhika Reddy et al. (1993) took the GPC-separated FRI ('amylopectin') of several rice varieties of graded AE contents and studied their chain profile. They found that the proportion of long-B chains, as well as their external location, in the molecule were strongly positively correlated with the reputed insoluble AE contents of the parent rice varieties. On this basis, the authors proposed that the content and disposition of the long-B chains of amylopectin was the key determinant of rice texture. The FRII was assumed by the authors to be primarily a reflection of the soluble AE or the true amylose content of the starch, and hence not involved in rice quality. On the basis of their own (Radhika Reddy et al., 1994) and other parallel rheological and microscopical studies (Sandhya Rani and Bhattacharya, 1995a, 1995b), they concluded that the long-B chains, by virtue of intermolecular interaction, rendered the starch granule strong and resilient, thus leading to the firm texture of the cooked rice. Shortage of such chains led to weak starch granules and hence, to soft cooked rice (Radhika Reddy et al., 1994). Recently Ong and Blanshard (1995) have confirmed that rice varieties with more long chains in the amylopectin gave hard-cooking parboiled rice, and vice-versa.

☆ Preliminary results of this study were presented in the IX-World Congress of Food Science and Technology, Budapest, Hungary, July 30–August 7, 1995.

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0144-8617/99/$ - see front matter
PII: S0144-8617(98)00125-8

Table 1
Characteristics of experimental rice varieties

Rice quality type[a]	Variety	Amylose equivalent (% dry basis)		Tenderness score
		Total	Hot-water insoluble	
I	Taichung (Native) 1	28.2	17.0	2.1
II	Co 32	26.8	13.1	2.8
III	Jhona 20	29.9	12.6	3.2
IV	Basmati 370	25.4	11.0	4.3
VI	Sukanandi	24.3	7.9	4.8
VII	Taichung 65	20.3	6.9	5.8
VIII	Asm 44	4.8	3.5	8.3
r[b]		937**	964***	

[a] As per Bhattacharya and Sowbhagya, 1982
[b] Coefficient of correlation (× 1000) with tenderness score of the rices.
(*, **, *** = significant at 5%, 1% and 0.1% levels, respectively.)

The one inconsistency in the previous argument was that the broad GPC FRII fraction showed a relatively low λ_{max} (< 630 nm) of the polysaccharide–iodine complex, except for the peak tubes (Chinnaswamy and Bhattacharya, 1986; Radhika Reddy et al., 1993). The question that then arose was, if this fraction were to be true amylose, why was its λ_{max} so low? There seemed to be more to the low-molecular-weight GPC starch fraction than observed earlier.

Here we report the results of studies that answer the previous question and elucidate related issues.

2. Materials and methods

2.1. Rice

Rice belonging to seven quality types (Table 1), classified on the basis of their total and insoluble AE contents (Bhattacharya and Sowbhagya, 1982), were raised at the University of Agricultural Sciences Experimental Station at Nagenahalli (Karnataka, India) from laboratory stock seeds. Paddy was collected shortly after harvest, dried, cleaned, fumigated, aged at room temperature (25 ± 3°C) in closed container for about three months and transferred to low temperature (4°C–6°C), and stored further until the time of experiment.

Shelling and milling of the paddy was carried out under standard conditions using Laboratory McGill equipment. Milled rice was ground in a Buhler laboratory disc grinder (type MLI-204) to 30-mesh flour and further in a Fritsch Pulverisette-14 pin mill to pass through a 100-mesh screen. The flour was defatted by refluxing with 85% methanol in a Soxhlet apparatus for 18–20 h, equilibrated for moisture to around 12% (wet basis) and stored at 4°C–6°C.

2.2. Fractionation of starch

Preparation of sample and fractionation were as described by Chinnaswamy and Bhattacharya (1986) and Radhika

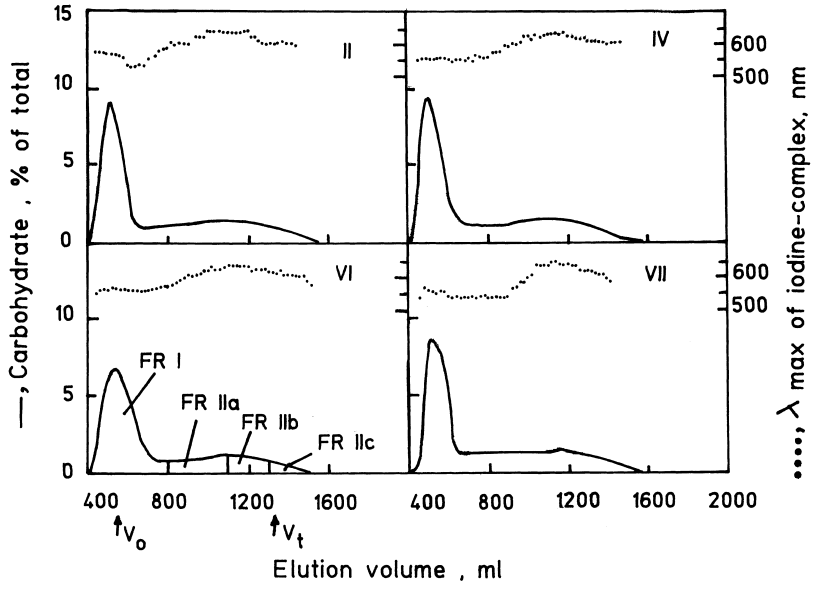

Fig. 1. Representative chromatograms of rice starch on Sepharose CL-2B (preparatory column). The rice quality type (II, IV, VI and VII) and division of different fractions (FRI, IIa, IIb and IIC) are shown.

Table 2
Gel-permeation chromatographic profile of carbohydrate (CHO) and AE of rice starch on Sepharose CL-2B column[a]

Rice Type	CHO (% of total[b]) in FR					AE[c] in FR				
	I	IIa	IIb	IIc	Total	I	IIa	IIb	IIc	Total
I	63.7	14.6	18.3	3.4	100	19.1	6.8	10.2	1.4	37.7
II	64.0	14.8	17.3	3.9	100	14.0	8.2	10.4	1.9	34.6
III	58.7	16.2	18.3	6.8	100	14.3	6.4	3.0	3.7	37.4
IV	63.1	17.5	15.3	4.1	100	8.1	3.9	7.5	0.8	20.4
VI	67.4	15.1	13.4	4.1	100	8.5	3.9	4.7	1.2	18.4
VII	64.7	16.4	15.0	3.9	100	6.4	2.6	5.3	0.7	15.1
VIII	70.6	15.8	10.8	2.8	100	3.5	0.6	0.4	0.1	4.6
r[d]						− 928**	− 945**	− 815*	− 655	− 958***

[a] Preparatory column.
[b] Total of the four fractions.
[c] Expressed in mg per 100 mg carbohydrate injected. Amounts of AE in all tubes so obtained were added together to get the total value, which was then divided into four fractions by their % proportion in each fraction.
[d] Correlation coefficient: see footnote b of Table 1.

Reddy et al. (1993) with minor changes. Rice flour (400 mg) was dispersed in 30 ml 1 N NaOH. A portion of the dispersion, containing about 200 mg (dry basis) of carbohydrate, was fractionated by ascending GPC on a Sepharose CL-2B (Pharmacia Fine Chemicals, Sweden) preparatory column (5 × 72 cm), using azide water (0.02% sodium azide) as an eluent at a flow rate of 1.25 ml min^{-1}. Sub-fractions of 20 ml were collected. Carbohydrate recovery varied between 90% and 108%.

The sub-fractions were pooled into FRl (No. 23–41), FRlla (42–55), FRllb (56–67), FRllc (68–78) (Fig. 1). The fractions were concentrated (to about 1 mg solids/ml) in vacuum at 40°C (Rotavapor, Buchi RE III, Switzerland).

2.3. Debranching with isoamylase

An aliquot of 6 ml of each fraction (Frl, Frlla, Frllb, and Frllc) was debranched with 25 u isoamylase from *Pseudomonas amylodermosa* (Sigma Chemical Co., USA) dispersed in 1 ml of acetate buffer (30 mM, pH 3.5), by incubating at 40°C for 24 h (Ikawa et al., 1981). The enzyme action was arrested by boiling for 5 min. After cooling, the debranched material was made alkaline (pH 8.0) using 1 N NaOH and stored at room temperature (25 ± 3°C) until fractionation.

2.4. Fractionation of debranched starch components on Biogel P-10

Debranched starch components were filtered through G-3 sintered glass filter and an aliquot containing about 5 mg carbohydrate was chromatographed on a Biogel P-10 (Bio-Rad Laboratories, USA) column (1.6 × 60 cm) with double-distilled water containing 0.02% sodium azide as eluent. Three millilitres of sub-fractions were collected. Carbohydrate recoveries were 93(±6)%.

2.5. Analytical methods

Carbohydrate (glucose × 0.9) in each sub-fraction was measured by phenol sulphuric acid method (Dubois et al., 1956). Number average degree of polymerisation $\overline{dpn}$ was of derived from reducing-end value, as determined by the modified Park Jhonson's method described by Hizukuri et al. (1981). AE and the absorption maxima of the iodine–polysaccharide complex were measured as reported earlier (Radhika Reddy et al., 1993).

2.6. Measurement of cooked-rice texture

Rice (approximately two grains thick layer), with 2.5 times its weight of water, was cooked in flat shallow stainless steel dishes by open steaming in a loose autoclave for 45 min (Deshpande and Bhattacharya, 1982). The cooked rice was placed in a Petri dish, stirred gently to prevent mat formation and to break any lump, and covered with a lid containing a filter paper. The samples were cooled for 15 min in an air-conditioned room (25°C). Sensory tenderness was tested (Sowbhagya et al., 1987) by a panel of 10 judges and the mean of all the scores for a sample was calculated.

GPC runs were usually not replicated, except when in doubt, but all analysis were done at least in duplicate and the mean values used.

3. Results

3.1. Composition of GPC-separated fractions

Upon GPC on Sepharose CL-2B, the starch separated into the usual two fractions (Fig. 1): fraction 1 (Sepharose FRI) eluted at the void volume, showing that it constituted of very high-molecular-weight components ($>20 \times 10^6$ Da); the other was a broad fraction (Sepharose FRII) which entered the gel and was eluted over the range between void and total

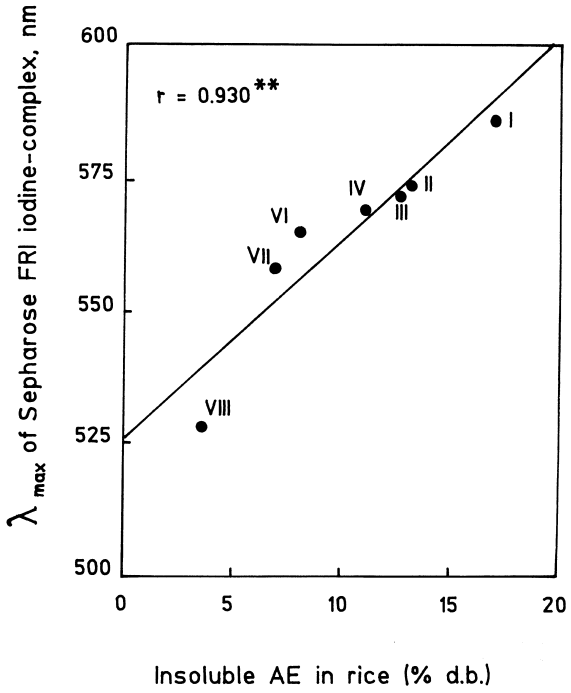

Fig. 2. Relationship between insoluble AE in rice flour with the λ_{max} of the iodine complex of Sepharose FRI of rice starch. The rice quality types have been identified alongside the points.

volumes. This pattern is in conformity with earlier work (Chinnaswamy and Bhattacharya, 1986; Radhika Reddy et al., 1993). The Sepharose FRII was further sub-divided arbitrarily into three parts: Sepharose FRIIa, IIb and IIc, as shown. The distribution of carbohydrate and AE in the four fractions (Sepharose FRI, IIa, IIb and IIc) are given in Table 2.

The absorption maxima (λ_{max})of the polysaccharide–iodine complex of the starch components in the individual tubes are recorded in Fig. 1. The λ_{max} of Sepharose FRI, presumably amylopectin, decreased from type I to type VIII rice (Fig. 2). Further, the values correlated well with the insoluble (AE) contents of the rice samples shown in Table 1 (Fig. 2), confirming the earlier results from this laboratory (Chinnaswamy and Bhattacharya, 1986, Radhika Reddy et al., 1993).

3.2. Fine structure of separated fractions

The four Sepharose fractions were treated with isoamylase. The debranched mixtures on fractionation over Biogel P-10 was divided into the usual three fractions: Biogel fr1, fr2 and fr3 (Fig. 3). The Biogel fr1 eluted at the void volume, showing that it was relatively high in molecular weight. The other two fractions were obviously smaller and entered the gel. The polysaccharide–iodine complex λ_{max} was high (>600 nm) at the beginning and then declined steadily as the elution volume increased. The last few elution tubes had no measurable blue colour. This pattern of chain distribution in debranched rice starch is in conformity with those obtained earlier in our laboratory (Radhika Reddy et al., 1993), as well as other laboratories (Takeda et al., 1987, 1989; Hizukuri et al., 1989; Ong and Blanshard, 1995).

What is noteworthy is that the previous profile was true not only of Sepharose FRI, but also of FRIIa, IIb, IIc. Clearly, branched starch molecules were present not only in Sepharose FRI, as expected, but also in Sepharose FRIIa, IIb and IIc.

The average molecular weights of the linear chains in each elution tubes were estimated by reducing-end value analysis (Fig. 4). As expected, the chain length or $\overline{dpn}$ decreased steadily from the first to the last tube and the corresponding iodine-complex λ_{max} values followed the expected trend (Fig. 4). The relationship between the $\overline{dpn}$ and λ_{max} determined earlier, as shown in Fig. 5, is similar to that shown by Banks et al. (1971) in their study of the iodine complex λ_{max} of synthetically prepared linear anhydroglucose chains. The anhydroglucose chains in the three Biogel fractions had $\overline{dpn}$ values as shown in Table 3. As suggested by Hizukuri (1986) and Manners (1989), the relatively long chains in Biogel fr1 can be identified as long-B chains of amylopectin, but it would also include any amylose in the parent starch in the present work as the four Sepharose fractions were debranched without prior separation of amylose, if any. The two remaining fractions, Biogel fr2 and fr3, can be identified as intermediate-B chains and A plus short-B chains of branched molecules, respectively (Table 3). The percentage distribution of linear branches and the proportion of AE in three Biogel fractions are shown in Tables 4 and 5 respectively.

Multiplying the proportions of carbohydrate and AE in the three Biogel fractions (Tables 4 and 5) by the respective weight of the components in the corresponding parent Sepharose fraction (Table 2), the actual amounts of the previous chain fractions (Biogel fr1, fr2, fr3) in the respective Sepharose fraction were calculated. Then by adding up the respective Biogel fractions in the four Sepharose fractions, the aggregate amounts of the three chain fractions in the total starch were calculated. These values are shown in Table 6.

To confirm these results further, flour from three representative varieties of rice (types I, III and VII) was directly debranched with isoamylase. The hydrolysed mixture was fractionated on Biogel P-10, yielding the usual three fractions (not shown). The amount of carbohydrate and AE in the three fractions so determined are shown in Table 6, in parentheses. The sum of the proportions of Biogel P-10 fractions found in the separated starch components and the respective fractions of directly debranched components matched almost exactly, showing the validity of both the results.

3.3. Content of true 'amylose'

As mentioned earlier, under the experimental procedure

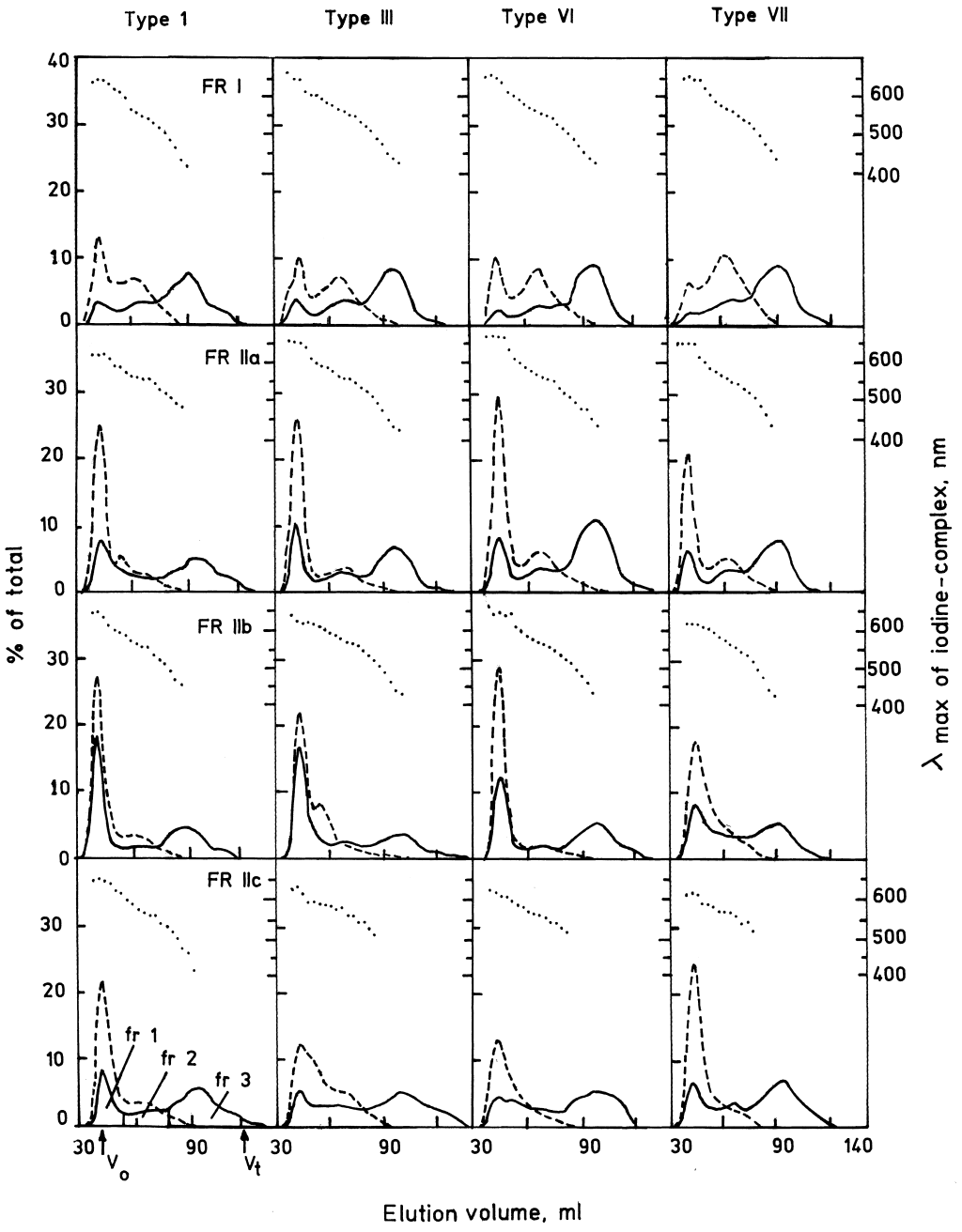

Fig. 3. Representative Biogel P-10 chromatograms of the debranched separated components of rice starch. (—) Carbohydrate; (–-) AE; (…) λ_{max} of iodine complex. The rice quality type, Sepharose fractions (FRI, FRIIa, FRIIb and FRIIc) and division of different Biogel fractions (fr1, fr2 and fr3) are shown.

adopted, the Biogel fr1 would contain not only long-B chains of branched starch but also very long linear chains, namely amylose, if any, in the parent starch. Therefore, an indirect approach was adopted to calculate the approximate amount of amylose in Biogel fr1. This approach was based on the following facts and assumptions.

First the facts. Rice flour from a high-AE rice (type I) was treated with isoamylase and the resultant debranched mixture was chromatographed over Sepharose CL-2B. The elution patterns before and after debranching (Fig. 6) showed that no carbohydrate material was left in the void volume after debranching, the entire material having entered the gel. Chinnaswamy (1985), too had observed in two rice samples that when Sepharose FRI was debranched and rechromatographed over Sepharose 2B, the entire amount entered the gel and eluted towards the end. What these mean is that the entire Sepharose FRI fraction consisted of branched molecules. It could not contain very long linear chains, viz, amylose, for in that case those chains would have been retained at the void volume after debranching. In other words, amylose if any in starch was present only in Sepharose FRIIa, IIb and possibly IIc fractions.

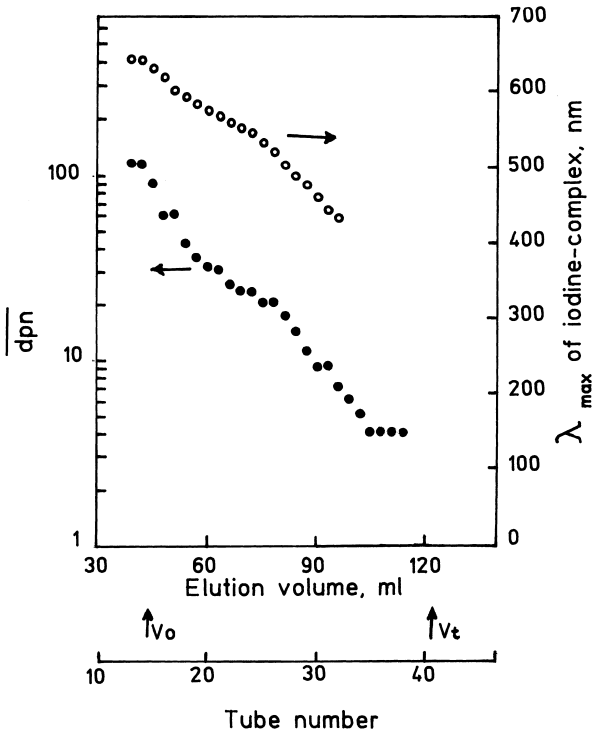

Fig. 4. The average iodine-complex λ_{max} and average $\overline{dpn}$ of released chains in individual tubes of Biogel chromatogram. (○) λ_{max}. Values are averages of all the seven varieties of rice used. Each value represents an average of 28 tubes. (●)$\overline{dpn}$. Values are averages of rice type I, III and VII. Each value represents an average of 12 tubes.

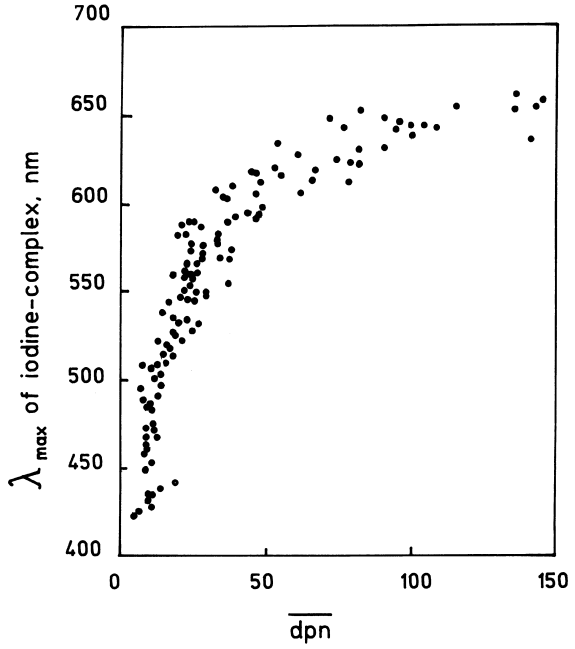

Fig. 5. Relationship between the absorption maxima (λ_{max}) of the polysaccharide–iodine complex and number average degree of polymerisation ($\overline{dpn}$) of debranched chains of rice starch. Values are for type I, III and VII rices. $\overline{dpn}$ was determined by reducing-end group analysis.

Table 3
Length of different chains released by debranching of Sepharose fractions and their identification

Biogel fraction	$\overline{dpn}$[a]	Chain identification[b]
fr1	42– > 110	Long-B ± Amylose
fr2	23–35	Intermediate-B
fr3	4–20	A and short-B

[a] Mean number-average degree of polymerisation determined by reducing-end estimation.
[b] Identification of branched-molecule chains are as per Hizukuri et al. (1989).

Secondly, amylose, if any, could be present only in Biogel fr1 and not in fr2 and fr3, for the chain length of the latter two fractions (Table 3) was obviously too small.

Now the assumption. If one could estimate the amount of long-B chains in Biogel fr1, the amount of amylose could be calculated by the difference. But an assumption had to be made for the former purpose. One had to assume that the pattern of branching in the branched molecules of Sepharose FRIIa and IIb and IIc, was essentially similar to that in Sepharose FRI. In other words, one had to assume that the following ratio in the case of Sepharose FRI, viz.

$$\frac{\text{Long-B chains}}{\text{Shorter chains}} = \frac{\text{Biogel fr1}}{\text{Biogel fr2} + \text{fr3}}$$

applied to the branched molecules in Sepharose FRIIa, IIb and IIc as well. One should note that this assumption, regardless of its validity, would not introduce a large degree of error in any calculation. This was because, as shown in Table 2, the material in Sepharose FRI contained roughly two-thirds of the total carbohydrate, and surely a still greater proportion of the total branched molecules if indeed there was any amylose in the parent starch.

The amount of long-B chains in the Sepharose FRI, IIa, IIb and IIc fractions were thus calculated by applying the earlier ratio. Subtracting these values from their total Biogel fr1 values gave their approximate amylose contents. The resulting calculated values of long-B and amylose chains are shown in Table 7.

3.4. Relation with rice texture

The tenderness scores of the seven cooked rice samples are shown in Table 1. The correlation coefficient of these values with various parameters are shown in the respective Tables. The tenderness score was well correlated, inversely, with the total as well as with the insoluble AE contents of the rice samples (Table 1). This relationship is nothing new, having been repeatedly shown from this laboratory (Bhattacharya et al., 1972, 1978, 1982; Bhattacharya and Sowbhagya, 1979, 1980; Sowbhagya et al., 1987).

The cooked-rice texture was strongly correlated with the long chains (Biogel fr1) of the Sepharose void volume FRI (Table 4). The same correlation remained when the AE

Table 4
Proportion of different anhydroglucose chains in different fractions of rice starch[a]

Sepharose CL-2B fraction	Biogel P-10 fraction	Carbohydrate, % of total, in rice of type							r^b
		I	II	III	IV	VI	VII	VIII	
FRI	fr1	16.5	15.7	14.7	10.6	9.8	8.9	3.5	−987***
	fr2	26.9	21.6	24.3	19.8	20.8	28.0	24.0	+045
	fr3	56.6	62.7	61.0	69.6	69.4	63.1	72.5	+779**
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
FRIIa	fr1	32.1	28.9	28.7	29.4	20.6	22.2	2.9	−939**
	fr2	18.6	17.9	20.0	16.0	17.2	26.8	21.9	+498
	fr3	49.3	53.2	51.3	54.6	62.2	51.0	75.2	+825*
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
FRIIb	fr1	50.8	47.3	49.2	44.0	40.4	34.3	5.0	−954***
	fr2	14.3	13.2	17.4	12.8	13.4	29.4	21.0	+568
	fr3	34.9	39.5	33.4	43.2	46.2	36.3	74.0	+836*
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
FRIIc	fr1	28.2	22.9	21.3	18.9	18.9	25.2	24.3	−055
	fr2	19.2	22.0	23.0	20.8	22.8	25.5	21.7	+377
	fr3	52.6	55.1	55.7	60.3	58.3	49.3	54.0	−144
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	

[a] Rice starch was first fractionated over Sepharose into four fractions (FRI, IIa, IIb, IIc). Each fraction was debranched and the released chains were fractionated over Biogel P-10 into three fractions (fr1, fr2, fr3).
[b] Correlation coefficient: see footnote b of Table 1.

distribution and not the carbohydrate content, was considered (Table 5). Radhika Reddy et al. (1993) reported similar correlation of long chains of Sepharose FRI with insoluble AE of rice, and hence by implication with rice texture. Interestingly, long chains (Biogel fr1) of Sepharose FRIIa and IIb fractions also showed good correlation with cooked-rice texture (Table 4 and 5). In fact when all long chains were aggregated, as shown in Table 6, the strong correlation remained valid. Further, rice texture strongly correlated with the calculated long-B chain content, both in the individual and aggregated Sepharose fractions (Table 7). Surprisingly, even the calculated amylose contents in Sepharose FRIIa, and especially IIb, as well as in the aggregated fraction showed reasonable correlation with rice texture (Table 7).

We can thus say that rice texture is related not only to the long-B chains of the high-molecular-weight part of the amylopectin, but also to all the long chains in the entire starch, perhaps including the amylose if any.

Values in Tables 4, 5, and 6 suggest that the short chains (Biogel fr3) also had a weak positive correlation with rice texture. But that could be an artefact. For one thing, Biogel

Table 5
Proportion of AE in different chain fractions of rice starch[a]

Sepharose CL-2B fraction	Biogel fraction	AE, % of total, in rice of type							r^b
		I	II	III	IV	VI	VII	VIII	
FRI	fr1	53.2	45.6	47.9	45.7	38.4	33.0	21.4	−977***
	fr2	42.1	50.9	44.6	46.3	50.4	60.8	64.2	+894**
	fr3	4.7	3.5	7.5	8.0	11.2	6.2	14.4	+820*
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
FRIIa	fr1	77.0	73.3	73.7	72.0	66.2	66.6	12.9	−886**
	fr2	21.3	21.4	21.7	24.0	27.6	30.3	72.3	+894**
	fr3	1.7	5.3	4.6	4.0	6.2	3.1	14.8	+802*
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
FRIIb	fr1	77.6	85.9	72.7	85.7	82.0	72.5	16.0	−805*
	fr2	20.8	11.8	23.3	11.1	14.5	26.7	68.2	+800*
	fr3	1.6	2.3	4.0	3.2	3.5	0.8	15.8	+764*
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
FRIIc	fr1	71.1	70.4	57.4	66.5	49.2	81.8	62.9	−038
	fr2	24.9	26.7	38.2	30.8	22.8	18.2	37.1	+173
	fr3	4.0	2.9	4.4	2.7	0.0	0.0	0.0	−834*
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	

[a] See footnote a in Table 4 for identification of the various fractions.
[b] Correlation coefficient: see footnote b of Table 1.

Table 6
Distribution of different anhydroglucose chains and AE in whole rice starch

Rice Type	Biogel P-10 fraction							
	fr1	fr2	fr3	Total	fr1	fr2	fr3	Total
	Carbohydrate% of total starch [a,b]				AE[b,c] mg/100 mg carbohydrate injected			
I	25.5	23.0	51.5	100	24.3	11.9	1.3	37.5
	(25.0)	(23.5)	(51.5)	(100)	(24.4)	(11.8)	(1.3)	(37.5)
II	23.3	19.6	56.8	100	22.6	10.6	1.2	34.4
III	23.6	22.3	54.0	100	23.0	12.3	2.0	37.3
	(23.2)	(23.7)	(53.1)	(100)	(22.2)	(12.3)	(2.3)	(36.8)
IV	19.2	18.1	62.5	100	13.4	5.8	1.1	20.3
VI	15.8	19.3	64.6	100	10.6	6.4	1.3	18.3
VII	15.4	27.9	56.5	100	8.2	6.2	0.6	15.1
	(15.9)	(28.8)	(55.3)	(100)	(8.6)	(5.5)	(0.9)	(15.0)
VIII	4.0	23.2	72.8	100	1.1	2.8	0.7	4.6
r[d]								
($n = 7$)	− 987***	+ 323	+ 849*		− 970***	− 910**	− 672	− 957***
($n = 6$)	− 970**	+ 314	+ 597		− 969**	− 869*	− 583	− 946**

[a] Respective fr1, fr2 and fr3 amounts of Sepharose FRI, IIa, IIb and IIc added together.
[b] Values shown in parentheses are obtained upon direct debranching of respective rice starch.
[c] Arrived at by dividing the AE values of the four Sepharose fractions in Table 2 into Biogel fr1, fr2 and fr3 as per their % distribution in Table 5; then the respective fr's were added together.
[d] Correlation coefficient: see footnote b of Table 1; $n = 7$ row represents correlation with all rices; $n = 6$ row represents correlation excluding Type VIII (waxy) rice.

fr3 usually increased more or less in the same order as Biogel fr1 decreased among the seven varieties of rice. So the correlation of Biogel fr3 was perhaps nothing more than a mirror image of that of Biogel fr1. Secondly, the amounts of Biogel fr3 increased (or the AE content decreased) sharply in type VIII rice (waxy) compared with the other varieties of rice. This sharp increase (or decrease) at one end tended to create an artificial correlation. This is shown in Table 6, where the waxy rice was excluded from the calculation, when the correlation disappeared. Although the correlation values decreased on exclusion of waxy rice in the case of long chains also, the values still remained highly significant (Tables 6 and 7).

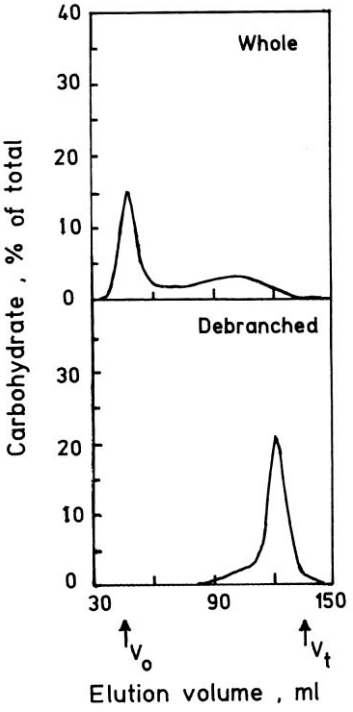

Fig. 6. Elution profile of whole and debranched Jaya (type I) rice starch on Sepharose CL-2B gel column.

4. Discussion

There are two important outcomes of this study. First, it throws some new light on the composition and structure of rice starch, and probably of starches in general. This includes some new hints regarding the presence or absence of amylose as a distinct component of starch. Second, a better insight into the role of starch structure in rice texture has been gained than observed earlier.

4.1. Composition and structure of starch

Theories about starch composition and structure have gone through many twists and turns, but the search is far from concluded. The accepted theory, as it currently exists, is that starch is composed of two somewhat heterogeneous populations, amylopectin and amylose. Amylopectin is thought to be a very large molecule and branched. Amylose, on the other hand is considered to be a much smaller molecule and largely linear; although part of it has been shown to contain branches (Kjolberg and Manners, 1963; Banks and Greenwood, 1966; Manners and Matheson, 1981; Cura et al., 1995; Falk et al., 1996), of which a few are very long branches and perhaps a few are very short branches (Takeda

Table 7
Approximate amount of long-B chains and amylose in rice

Sepharose CL-2B fraction	Chains in Biogel fr1[a]	Approximate amount of LB[b] and AM[b](% of total carbohydrate in rice) Rice type							r[c]	
		I	II	III	IV	VI	VII	VIII	($n = 7$)	($n = 6$)
FRI	LB	10.5	10.0	8.6	6.7	6.6	5.8	2.4		
	Am	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	Total[d]	10.5	10.0	8.6	6.7	6.6	5.8	2.4	−987***	
FRIIa	LB	1.9	1.9	1.9	1.5	1.3	1.3	0.3	−978***	−938**
	Am	2.8	2.4	2.7	3.6	1.8	2.3	0.0	−777*	−264
	Total[d]	4.7	4.3	4.6	5.1	3.1	3.6	0.5	−875**	
FRIIb	LB	1.7	1.6	1.6	1.0	0.8	1.0	0.1	−947**	−891*
	Am	7.6	6.6	7.7	5.7	4.6	4.2	0.2	−969***	−927**
	Total[d]	9.3	8.2	9.0	6.7	5.4	5.2	0.5	−980***	
FRIIc	LB	0.5	0.5	0.8	0.4	0.4	0.3	0.1	−812*	−612
	Am	0.5	0.5	0.6	0.3	0.3	0.7	0.5	+058	−039
	Total[d]	1.0	0.8	1.4	0.7	0.7	1.0	0.6	−507	
Total										
	LB	14.6	14.0	13.1	9.6	9.1	8.4	3.3	−988***	−969**
	Am	10.9	9.3	11.0	9.6	6.7	7.2	0.7	−939**	−827*
LB + Am	(= fr1[e])	25.5	23.3	23.6	19.2	15.8	15.4	4.0		

[a] The LB and Am values are derived by hypothetically dividing the respective Biogel fr1 values into two parts as follows:(1) The Biogel fr1 in Sepharose FRI is considered to be composed of LB only and (2) Carbohydrate in Biogel fr1 in Sepharose FRII a, IIb and IIc was approximately divided into LB and Am chains based on the formulae:

$$[\mathrm{LB}]_{\mathrm{FRIIa,IIb,IIc}} = [\mathrm{fr2} + \mathrm{fr3}]_{\mathrm{FRIIa,IIb,IIc}} \times \frac{[\mathrm{fr1}]_{\mathrm{FRI}}}{[\mathrm{fr2} + \mathrm{fr3}]_{\mathrm{FRI}}}$$

$$[\mathrm{Am}]_{\mathrm{FRIIa,IIb,IIc}} = [\mathrm{fr1}]_{\mathrm{FRIIa,IIb,IIc}} - [\mathrm{LB}]_{\mathrm{FRIIA,IIb,IIc}}$$

[b] LB = Long-B chain of branched starch, Am = Amylose.
[c] Correlation coefficient: see footnote b of Table 1. The ($n = 7$) and ($n = 6$) columns represent correlation with all rices and excluding Type VIII (waxy) rice, respectively.
[d] Actual amount of linear carbohydrate, calculated by multiplying the percentage of carbohydrate in each Sepharose fraction (Table 2) by its percent content of Biogel fr1 (Table 4).
[e] Same as in Table 6.

et al., 1987, 1989, 1990; Hizukuri et al., 1989). Some have even classified the latter materials as an intermediate fraction (Sugimoto et al., 1981; Asaoka et al., 1984, 1986; Inouchi et al., 1987) and Hizukuri et al. (1989) suggested that the branched amylose be considered as a third component of starch.

Starch gets separated into two broad components by GPC, one a population of very big molecules and another that of small molecules (Ebermann and Schwarz, 1975; Yamada and Taki, 1976; Biliaderis et al., 1979; Boyer et al., 1980; Yeh et al., 1981; Craig and Stark, 1984). Since the first population is branched, it was automatically assumed to be the designated amylopectin of starch. The second, smaller-molecular population was, by inference, thought to be the designated amylose.

The present study shows that the smaller-molecular population of starch is not necessarily amylose, nor that the branched molecule is necessarily big in size. All the subfractions of Sepharose FRII after GPC of rice starch yielded, after treatment with debranching enzyme, short linear branches. Clearly there was 'amylopectin' in all the subfractions of Sepharose FRII. This is quite a surprise, but explains why the Sepharose II fraction showed an iodine complex λ_{max} of well below 630 nm except at its peak (Fig. 1).

We can conclude that the branched molecules of starch are not all big in size but vary from very small to very big molecules.

4.2. The question of amylose

The question that might then arise is whether a long, largely linear, and relatively small molecule, i.e. amylose, really does exist or is an artefact. The present studies could not answer this question, because of limitation of facilities, viz., a suitable chromatographic system for separation of very long from rather long anhydroglucose chains. Recently, Ong and Blanshard (1995) separated a very long linear chain fraction, supposedly amylose, from debranched starch by using a sophisticated size exclusion-HPLC system. In the absence of quantification reported in their article, it is difficult to comment on the result at this time.

The iodine-complex λ_{max} values of some of the fractions no doubt suggested that a small amount of amylose perhaps did exist. Thus the λ_{max} of the peak tubes of Sepharose FRII was 630 nm or more (Fig. 1), even though these

sub-fractions were shown to contain branched molecules also, thus strongly suggesting the partial presence of very long linear chains at least in these peak tubes. Secondly, the λ_{max} of Biogel fr1 and Sepharose FRIIa and FRIIb were usually slightly higher than that in Sepharose FRI (Fig. 3). This suggests that the former contained some very long chains in addition to the usual long-B chains.

In any case certain calculations that were made, based on assumptions that can be considered reasonable although by no means fool-proof, would strongly suggest that amylose, if at all present, was present in much smaller quantities than assumed so far. Takeda et al. (1987), after confirming that the long-B chains of amylopectin contributed some part to the analytically estimated 'amylose' content of starch, as determined by iodine reaction, estimated that the real amylose contents were in the range of 15%–19% and were more or less identical in all non-waxy rice. The values calculated in the present work (7%–11%) are much less than the aforementioned range and also varied somewhat among the different varieties. It is difficult to say at this time which set of values is nearer to truth.

Takeda et al. (1987) calculated the true amylose content from the respective iodine affinities of whole starch, pure amylose and pure amylopectin. These and all similar studies on starch are based on chemically separated 'amylose' and 'amylopectin' from previously isolated starch, in which process both components are separately and rigorously 'purified' by repeated recrystallisation/precipitation with chemicals to free them from presumed contaminants, viz., the other component. One does not know whether the presumed contaminant is really a contaminant or a natural part in the native molecule, which is forcibly detached during repeated 'purification'.

This is not to say that the possibility of artefacts does not exist in the present work. One possible source of artefact lies in the dispersion of starch itself. Although isolation of starch, already difficult especially in the case of rice, was avoided precisely for that reason and rice flour was directly dispersed prior to GPC, one cannot be definite that the process of dispersion involving treatment with hot dilute alkali, for however brief a period, did not affect the native starch in anyway. Nonetheless, it is likely that the chance of creating artefacts would be less in this procedure using GPC fractionation than when starch is first isolated, then separated into two components by using chemicals followed by repeated purification by the same means, and finally dispersed.

All in all, the question of composition of starch and the existence of two distinct populations still remains open.

4.3. Determinants of rice texture

Cooked-rice texture, for long, was thought to be a resultant of its AE content. Based on the work of Chinnaswamy and Bhattacharya (1986), Takeda et al., 1987, 1989), Hizukuri et al. (1989) and their own work, Radhika Reddy et al. (1993, 1994) changed the focus of rice texture from amylose to amylopectin. They concluded that rice texture was determined by the content of externally located long-B chains of the high-molecular-weight branched component, amylopectin, of rice starch. Amylose in this scheme played no role. The work of Ong and Blanshard (1995) more or less confirmed this contention.

There may be more to rice texture than stated previously. It would appear that amylopectin or branched starch exists in very large to very small sizes. Further, that the long-B chains of all the branched starch molecules, big or small, may be involved in rice texture. In fact there was also indirect evidence that even the amylose molecules, if any, were involved in the phenomenon.

It may, therefore, be concluded that starch contains branched molecules or amylopectin of varied sizes, from very big to very small. The content of amylose in starch may be much less than that thought so far. The texture of cooked rice of a variety seems to be mostly determined by the content of all the long linear chains, free (amylose) or bound (to amylopectin), in the starch molecule. May be all these chains participate in intermolecular interaction, thus affecting the rigidity of the starch granule and hence indirectly the texture of the cooked rice.

Acknowledgements

One of the authors (MR) is thankful to the Council of Scientific and Industrial Research (CSIR), India for the award of a Research Fellowship.

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