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Structure and Properties of Endosperm Starches from Cultivated Rice of Asia and Other Countries*

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Abstract: Starch granules were prepared from mature grains of 75 cultivars (23 *indica*, 27 *Chinese indica*, 6 *japonica* and 19 *javanica*) of rice originating in Asia and the other countries, including Brazil (4), China (25), India (10), Indonesia (3), Japan (8), Korea (2), Laos (3), Myanmar (3), Nepal (4), Pakistan (1), the Philippines (1), Russia (1), Taiwan (3), Thailand (1) and the USA (6). They were cultivated and harvested in the paddy field of Prefectural University of Hiroshima in 2001. We showed that starches of non-waxy cultivars of the *indica* and *Chinese indica*, in general, had higher contents of the apparent amylose (AAM) and super-long chains (SLC) of amylopectin by GPC of *Pseudomonas* isoamylase-debranched starches and amylopectins through Toyopearl columns. High performance anion exchange chromatography with a pulsed amperometric detection (HPAEC-PAD) of isoamylase-debranched starches showed that the starches of non-waxy cultivars of the *indica* and *Chinese indica*, in general, had decreased amounts of branch chains with DP 6–12 (Fr. A). The Fr. A contents correlated positively with the alkali spreading score (ASS) of rice grains and negatively with the peak temperature (T_p) of gelatinization of the rice starches. Among the pasting characteristics of the starches measured using a Rapid Visco Analyser (RVA), setback (*SB*) and breakdown (*BD*) showed high positive and negative correlations with SLC contents, respectively, and both peak top viscosity (*PV*) and *BD* negatively correlated to AAM contents. There was a high positive relationship between amounts of Waxy (*Wx*) protein and SLC contents in starch. This appears to show that *Wx* protein is concerned with synthesis of SLC. SLC contents in starches of rice originating in Asia and the other countries were evenly observed in the range of 0.0–13.4%.

Key words: rice starch, *indica*, *japonica*, *javanica*, super-long chain.

Rice is one of the most important cereals in the world, especially in Asia. There are two cultivated species of rice, *Oryza sativa* L. and *O. glaberrima* Steud, and *Oryza sativa* is distributed throughout many countries, while *O. glaberrima* is endemic to West Africa. Oka[1)] reported that *indica* and *japonica* types differ in several characteristics and genes and that *javanica* may be regarded as a tropical subgroup of the *japonica* type. Nakagahra[2)] proposed four varietal groups: *indica*, *sinica* (*Chinese indica*), *japonica* and *javanica* based on different esterase isozymes of rice types of *Oryza sativa.*

Starch is the main component of rice, and is composed of linear amylose and branched amylopectin. Amylose content is usually higher in endosperm starch of *indica* rice than that of *japonica* rice. This has been explained by the presence of two types of *waxy* alleles, Wx^a and Wx^b. Nakamura *et al.*[3)] have classified the starches of 129 rice varieties cultivated in Asia into two types, *L* and *S*, based on the differences in the chain length of the amylopectin cluster. The *L*-type rice mainly belonged to *indica*, and the *S*-type rice to *japonica.* They determined the relationship between the amylopectin fine structure (characterization using the ratio of the short chains of DP≦10 to the short and intermediate chains of DP≦24) and thermal properties measured by DSC of endosperm starches from Asian cultivated rice. Umemoto *et al.*[4)] showed that the fine structure of amylopectin of the *japonica* rice variety, Kinmaze, differs distinctly from that of the *indica* rice variety, IR36, in that the former is enriched in short chains of DP≦10. We reported the structural and physicochemical characteristics of endosperm starches of rice cultivars recently bred in the agricultural experiment station of the Ministry of Agriculture, Forestry, and Fisheries of Japan, and proposed a scheme for the relationship between the super-long chains (SLC) and Fr. A (DP 6–12) contents of amylopectin as a classification of endosperm starches of rice cultivars.[5)] There were no rice starch samples in either range of 3–4% or 8–12% for SLC contents in starches among the investigated 59 rice cultivars in Japan.

The numbers of rice cultivars grown in Japan are decreasing year after year. Monoculture of Koshihikari is not preferable as it was to lead a poor crop under unnatural weather conditions, and it prevents the introduction of good characteristics of many foreign rice cultivars. Therefore it appeared to be important for rice breeding using foreign rice cultivars to investigate various properties for

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[*] This paper is dedicated to our mentors, the late Professors Dr. Michinori Nakamura, Dr. Susumu Hizukuri and Dr. Toshiaki Komaki, in memory of their numerous pioneering works and leadership in the field of starch and its related science.

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many rice cultivars in the world.

The objective of this study was to investigate the structural and physicochemical characteristics of endosperm starches of rice originating in Asia and other countries, and to propose a scheme for the relationship between the SLC and Fr. A (DP 6–12) contents of amylopectin as a classification of endosperm starches of their rice cultivars. In this paper, rice cultivars are classified into four varietal groups based on the different esterase isozymes of rice types proposed by Nakagahra.

MATERIALS AND METHODS

Sample seeds. Mature grains of 75 cultivars (23 *indica*, 27 *Chinese indica*, 6 *japonica* and 19 *javanica*) of rice originating in Asian and other countries, including Brazil (4), China (25), India (10), Indonesia (3), Japan (8), Korea (2), Laos (3), Myanmar (3), Nepal (4), Pakistan (1), the Philippines (1), Russia (1), Taiwan (3), Thailand (1) and the USA (6) were cultivated and harvested in the paddy field of Prefectural University of Hiroshima in 2001. They were dried and stored at 5°C until used. Table 1 shows the sample name, place of origin and some characteristics of brown rice grains and rice starches of four subspecies (*indica*, *Chinese indica*, *japonica* and *javanica*).

Measurements of characteristic values of brown rice grains. Weight of 1000 grains of brown rice was measured. Ratio of length to width of brown rice grains was measured using a set of dial calipers (Kori Seiki Co.). Five grains of brown rice halved vertically by a razor were soaked in 10 mL of 1.7% (w/v) KOH solution in a plastic cup for 24 h at room temperature. Degree of alkali disintegration for each grain was classified into five kinds of scores (alkali spreading score: ASS; 1: no decay, 2: slight dilation, 3: dilation, 4: slight decay and 5: decay) according to the method described by Little *et al.*[6] with a minor modification, and expressed as an average.

Preparation of starch granules and amylopectins. Starch granules were prepared from polished rice by the cold alkali method.[7,8] Rice amylopectins were isolated and purified from the rice starch by the modification[9] of Schoch's method.[10]

Measurement of iodine absorption spectra. The iodine absorption spectra of the rice starches or amylopectins were measured using a Hitachi U-3210 spectrophotometer as reported previously.[11]

Debranching of starch and amylopectin with isoamylase and fractionation of debranched materials by gel permeation chromatography (GPC). Starches and amylopectins were debranched with crystalline *Pseudomonas* isoamylase by the method of Ikawa *et al.*[12] Debranched materials were fractionated by gel filtration on a column (300×20 mm) of Toyopearl HW55S connected in series to three columns (300×20 mm) of Toyopearl HW50S. Each fraction (Fr.) (Fr. 1, 2 and 3) was divided at the bottom between two vertexes of each elution curve of isoamylase-debranched starches and amylopectins.[5]

Contents of apparent amylose (AAM), true amylose (TAM) and SLC in starches were calculated from the contents of Fr. 1, Fr. 2 and Fr. 3 obtained from the GPC experiments of debranched materials of starches and amylopectins purified from starches using the method of Horibata *et al.*[5]

HPAEC-PAD of isoamylase-debranched materials of starch. Gelatinized starch (5 mg) in 4.96 mL of Milli Q water at 100°C for 10 min was added to 100 mL of 1 M acetate buffer (pH 3.5) and 10 μL of *Pseudomonas* isoamylase (10 μg protein/10 μL, 590 U mg^{-1} protein), and incubated at 45°C for 2.5 h. The reaction mixture was added to 200 μL of 1 N sodium hydroxide solution and supplemented with 5 mL of water, and then filtered through a 0.20 μm filter (Millipore). HPAEC-PAD was performed using a Dionex model DX-300 system (Dionex Corp., Sunnyvale, CA, USA) and a Model SC-PAD II pulsed amperometric detector (PAD-SC cell) consisting of an amperometric flow-through cell with a gold working electrode, a silver-silver reference electrode, and potentiostat according to the method described by Koizumi *et al.*[13] with a minor modification.[11]

Rapid Visco Analyser (RVA) viscograms. The viscosity of a 10% aqueous suspension of starch was measured using a RVA (Model RVA-3D, Newport Scientific, Co., Ltd., Tokyo, Japan). The power demanded of the motor to spin (160 rpm) and the paddle in the slurry was monitored as the slurry was heated and cooled. The temperature conditions during the measurement were as follows: retention at 30°C for 1 min, heating from 30 to 95°C at 5°C/min, retention at 95°C for 6 min, cooling from 95 to 50°C at 5°C/min, and retention 50°C for 10 min. The temperatures at initial viscosity rising (T_v), peak top viscosity (PV), breakdown (BD) and setback (SB) of starch samples were obtained from the viscograms.[14,15] The T_v was measured as the temperature when viscosity exceeded a calculated viscosity [base viscosity+(1/20) (peak top viscosity−base viscosity)], the peak viscosity was the maximum value during heating time from 30 to 95°C, the breakdown was measured as the viscosity of [PV−the minimum viscosity (MV) (the minimum value during holding time at 95°C or cooling time from 95 to 50°C)], the setback was measured as the viscosity of [the final peak viscosity (FV) (the maximum viscosity during cooling time from 95 to 50°C or holding time at 50°C)−MV].

Thermal analysis of starch. The thermograms of starch granules were recorded on a differential scanning calorimeter (DSC) (Rigaku 8240D) by modification of the method of Inouchi *et al.*[16]

Identification and quantification of Waxy (Wx) protein by SDS-PAGE. The *Wx* protein compositions of rice starch granules were analyzed on SDS-polyacrylamide gels containing 10% (w/v) acrylamide.[17,18] Samples were prepared as follows: starch granules (usually 20 mg by dry weight) were suspended in 670 μL of Milli Q water, 100 μL of 10% SDS, 10 μL of 2-mercaptoethanol, 20 μL of 0.5 M Tris-HCl buffer (pH 6.8), 200 μL of glycerol, and bromophenol blue and extracted by boiling for 5 min. Samples were centrifuged (15,000× g, 3 min). The supernatant was subjected to SDS-gel electrophoresis. Proteins were stained with Coomassie Brilliant Blue. The stained protein bands on the dried gel were quantified by a scanner. The area of each band was digitized using a

NIH image program, and then converted into values relative to the band of commercial normal maize starch that was electrophoresed on the same gel as a standard.

RESULTS

Some characteristics of brown rice grains.

Some characteristic values of brown rice grains are summarized in Table 1. Values of average ± standard deviation of weight of 1000 grains of *indica*, *Chinese indica*, *japonica* and *javanica* were (19.4 ± 3.4) g, (20.0 ± 2.6) g, (21.7 ± 2.4) g and (22.7 ± 3.8) g, respectively. Those of the ratio of length to width of brown rice grains were 2.3 ± 0.5, 2.1 ± 0.3, 1.9 ± 0.3 and 2.1 ± 0.3, respectively. Those of ASS of brown rice grains were 2.1 ± 1.3, 2.3 ± 1.2, 2.5 ± 2.0 and 2.8 ± 1.5, respectively. These results appear to show some general characteristics of rice seeds in four subspecies, namely, *javanica* rice grains are heavy, *indica* and *Chinese indica* rice grains are light, *indica* rice grains are thin, and *japonica* rice grains are thick. Each brown rice of four subspecies showed various values of ASS with the range of 1–5.

Wavelength at maximum absorption (λ_{max}) and blue value of the iodine complexes of rice starches and amylopectins.

λ_{max} and blue value (absorption at 680 nm) of the iodine complexes of rice starches are shown in Table 1. Seven rice starches [*indica* (1), *Chinese indica* (2) and *javanica* (4)] with λ_{max} in the range of 520–531 nm and blue value in the range of 0.06–0.10 appeared to be waxy rice starch judging from their values. The λ_{max} and blue value for non-waxy rice starches are in the following ranges; 569–600 nm and 0.24–0.40 for *indica* rice, 572–599 nm and 0.23–0.40 for *Chinese indica* rice, 556–581 nm and 0.19–0.28 for *japonica* rice, and 570–593 nm and 0.23–0.38 for *javanica* rice, respectively. The fact that amylose content is usually higher in endosperm starch of *indica* rice than that of *japonica* rice has been explained by the presence of two types of waxy alleles, Wx^a and Wx^b.[18,19] Our results corresponded to these reports.

There was a high relationship (correlation coefficient; $r = 0.98$, $n = 75$) between λ_{max} and blue value of iodine absorption spectra of starches. The λ_{max} and blue value of iodine absorption spectra of amylopectins isolated and purified from rice starches were in the range of 526–591 nm and 0.08–0.33, respectively (Table 2). A high correlation ($r = 0.98$, $n = 23$) between λ_{max} and blue value of amylopectins was also observed.

Contents of AAM, TAM and SLC in rice starches.

Table 2 shows contents of AAM, TAM and SLC in rice starches. Starches of two waxy cultivars, Hongxienuo and Jaguary, comprised 100% amylopectin and neither TAM nor SLC appeared in the amylopectin, since there was no Fr. 1 composed of AAM, namely, total of TAM and debranched SLC in the elution patterns. Contents of AAM, TAM and SLC were in the following ranges for 25 non-waxy starches; 18.1–34.3, 17.7–28.2 and 0.4–13.4%, respectively.

Table 1. Sample name and place of origin of rice seeds, weight of 1000 grains, ratio of length to width, degree of alkali disintegration of brown rice, λ_{max} and blue value of iodine absorption spectra, Fr. A contents and group of amylopectin fine structures, pasting and thermal properties of endosperm starches of rice cultivars in Asia and other countries, cultivated and harvested in Hiroshima, Japan.

Ⅰ. *Indica* rice

Sample name	Place of origin	Brown rice			Starch									
		Weight of 1000 grains (g)	Length / Width	ASS*[1]	Iodine absorption spectra*[2]		HPAEC-PAD*[3]		RVA*[4]				DSC*[5]	
					λ_{max} (nm)	Blue value	Fr. A (%)	Group	T_v (℃)	*PV* (RVU)	*BD* (RVU)	*SB* (RVU)	T_p (℃)	ΔH (J/g)
Basmati 370	Pakistan	14.1	3.7	4	588	0.33	26.6	(c)	74.4	235	123	122	67.7	10.5
Chinsurah Boro 2	India	19.1	2.2	1	600	0.39	26.0	(c)	75.0	216	116	186	72.6	13.2
Co 13	India	20.2	2.0	2	595	0.40	27.2	(c)	73.7	237	137	203	72.2	12.6
Dakanalo	India	23.3	2.0	2	599	0.38	26.2	(c)	73.8	210	125	155	73.1	13.1
Della	USA	18.0	3.2	1	582	0.32	24.8	(b)	77.9	250	164	98	75.5	12.4
Dianyu 1	China	18.9	1.6	3	578	0.29	32.7	(f)	69.5	244	163	112	64.2	9.2
Dular	India	19.1	2.6	1	594	0.37	25.6	(b)	75.2	220	135	141	74.1	12.6
IR 28	Philippines	18.8	2.9	4	594	0.37	29.0	(d)	75.4	277	124	310	65.4	9.8
Jaguary	Brazil	28.6	2.4	4	522	0.06	32.3	(f)	62.3	185	106	40	66.4	13.7
Jhona 2	India	22.2	2.7	1	594	0.40	26.6	(c)	74.3	232	130	181	72.2	13.1
Kasalath	India	15.3	2.3	1	599	0.37	26.3	(c)	74.3	217	121	164	71.9	13.5
Ma Sho	Myanmar	15.3	2.0	3	591	0.36	27.4	(c)	73.3	228	141	134	70.7	12.4
Muha	India	22.2	2.5	2	596	0.38	26.8	(c)	73.3	189	124	112	73.0	12.4
Nepal 1	Nepal	21.4	2.1	1	594	0.37	26.4	(c)	73.2	305	134	204	72.2	12.7
Nepal 18	Nepal	18.4	2.1	3	595	0.37	27.0	(c)	73.6	289	139	224	72.2	12.6
Nepal 555	Nepal	17.1	2.1	1	595	0.37	25.9	(b)	75.7	214	130	148	74.5	12.9
Nepal 8	Nepal	17.1	2.0	1	594	0.38	25.7	(b)	75.3	209	136	150	74.0	13.2
Pusur	India	21.5	2.4	1	598	0.39	25.3	(b)	74.9	230	149	138	73.5	13.4
Sari Queen	Japan	15.7	2.6	2	569	0.24	29.3	(d)	69.4	307	218	85	67.3	10.1
Surjamkhi	India	14.7	2.4	1	595	0.40	24.3	(b)	76.1	238	166	133	75.8	11.9
Suweon 258	Korea	20.8	2.0	4	576	0.24	32.4	(f)	74.0	290	175	83	64.3	9.8
T 246	India	21.3	2.1	1	599	0.40	25.7	(b)	74.1	224	104	138	73.2	13.6
Tongil	Korea	22.6	2.1	5	583	0.32	31.8	(e)	70.2	259	162	118	65.1	10.0

Table 1. Continued.

Ⅱ. Chinese *indica* rice

Sample name	Place of origin	Brown rice			Starch									
		Weight of 1000 grains (g)	Length/Width	ASS^{*1}	Iodine absorption spectra^{*2}		HPAEC-PAD^{*3}		RVA^{*4}				DSC^{*5}	
					$\lambda_{\max}$ (nm)	Blue value	Fr. A (%)	Group	T_v (°C)	PV (RVU)	BD (RVU)	SB (RVU)	T_p (°C)	ΔH (J/g)
Aijiaonante	China	16.1	2.0	2	593	0.38	25.6	(b)	75.1	302	144	236	72.6	11.8
Bayuenuo	China	27.1	2.1	4	523	0.06	32.9	(f)	60.8	219	149	42	62.9	12.4
China 830	China	25.4	1.7	2	598	0.39	nd	nd	74.8	202	108	180	73.0	11.9
Chixandao	China	22.0	2.1	2	592	0.38	26.6	(c)	75.3	299	153	192	72.6	12.4
Daorenqiao	China	19.5	1.8	1	593	0.37	26.2	(c)	74.4	312	155	178	73.9	12.2
Deegeowoogen	China	18.8	2.0	2	593	0.35	nd	nd	74.4	302	154	198	72.6	10.8
Deejiaohualuo	China	20.0	1.9	2	590	0.37	27.5	(c)	74.3	292	134	205	71.9	12.1
Deng Pao Zhai	China	18.3	2.0	2	590	0.37	26.1	(c)	74.4	318	153	208	72.9	12.1
Duanguanhualuo	China	19.0	1.7	4	593	0.36	25.8	(b)	74.3	294	126	233	73.7	12.2
Guangluai 4	China	19.5	1.8	1	589	0.35	26.3	(c)	76.9	308	168	191	74.0	12.3
Hong Cheuh Zhai	China	17.5	2.4	1	591	0.38	27.1	(c)	74.8	246	140	202	73.1	11.9
Hongmi	China	20.6	1.8	1	593	0.37	27.1	(c)	74.1	301	141	213	72.0	11.3
Hongxienuo	China	18.1	2.4	2	531	0.10	33.0	(f)	63.2	179	102	47	66.5	10.7
Hunanxian	China	18.3	2.0	2	591	0.35	26.0	(c)	75.7	303	144	203	73.0	12.0
Liuzhoubaoyazao	China	21.1	2.1	1	592	0.37	27.4	(c)	73.9	279	124	194	71.4	12.3
Milyang 23	China	20.5	2.2	3	575	0.27	29.7	(d)	67.6	317	225	91	66.2	10.9
Nanjing 11	China	21.7	2.0	1	599	0.38	25.8	(b)	74.9	291	120	238	72.5	12.4
Qingyu	China	18.4	1.9	2	591	0.38	25.5	(b)	75.3	301	141	191	73.1	12.7
Taichungxian 3	Taiwan	22.7	2.9	5	581	0.27	32.0	(f)	73.9	269	185	105	64.6	10.3
Taichungyu 204	Taiwan	23.5	2.9	4	572	0.23	33.1	(f)	68.3	348	269	74	67.1	10.4
Taichungzailai 1	Taiwan	18.1	2.0	5	592	0.35	31.9	(e)	72.8	271	100	300	63.4	8.7
Toboshi	Japan	17.4	2.3	2	596	0.39	28.2	(c)	74.4	192	114	160	71.9	11.0
Wuguhualuo	China	19.1	1.8	3	590	0.38	26.6	(c)	75.7	302	146	204	72.8	12.4
Xiligu	China	22.0	2.2	1	593	0.38	25.3	(b)	73.4	292	133	213	72.1	11.9
Xuanchangmi	China	16.8	2.2	2	591	0.37	25.5	(b)	75.4	275	114	231	72.6	12.3
Zhaiyeqing 8	China	19.9	2.2	2	590	0.38	25.5	(b)	75.5	321	160	208	74.2	12.3
Zhamianni	China	18.5	1.6	3	592	0.40	27.6	(c)	74.8	306	128	223	71.4	12.1

Ⅲ. *Japonica* rice

Sample name	Place of origin	Brown rice			Starch									
		Weight of 1000 grains (g)	Length/Width	ASS^{*1}	Iodine absorption spectra^{*2}		HPAEC-PAD^{*3}		RVA^{*4}				DSC^{*5}	
					$\lambda_{\max}$ (nm)	Blue value	Fr. A (%)	Group	T_v (°C)	PV (RVU)	BD (RVU)	SB (RVU)	T_p (°C)	ΔH (J/g)
Hieri	Japan	25.4	1.5	1	572	0.26	30.4	(d)	72.2	291	176	97	67.6	11.7
Houmanjinja	Japan	19.6	1.8	5	581	0.28	33.3	(f)	67.3	236	116	98	nd	nd
Koshihikari	Japan	20.7	1.7	2	564	0.22	30.3	(d)	70.2	314	238	64	69.1	11.6
Ou 368	Japan	23.6	1.8	1	579	0.25	30.3	(d)	70.2	285	201	89	67.6	11.7
Souzyajinja	Japan	21.4	1.9	5	570	0.26	nd	nd	68.6	278	176	108	nd	nd
Yakan	Japan	19.2	2.4	1	556	0.19	24.2	(b)	76.5	353	274	55	78.0	13.6

Unit-chain length distributions of debranched materials of starches measured by HPAEC-PAD.

To investigate the detailed chain-length distributions in the short region of amylopectin unit-chains, the debranched materials of rice amylopectin were detected in the range of DP 6–48 by HPAED-PAD. The fraction of peak areas of unit-chains of DP 6–12 detected by PAD was named "Fr. A", the ratio of Fr. A to the peak areas of unit-chains of DP 6–48 was calculated as Fr. A content.

Table 1 shows the Fr. A contents and the groups of rice samples classified into six groups (a)–(f) based on the same conditions as in our previous study.[5)] We have reported that Koshihikari starch belonged to group (d), Kenkei 2064 (*ae* mutant rice) starch with a high gelatinization temperature belonged to group (a) while the other starches with a high gelatinization temperature belonged to group (b), and the many other starches with a low or medium gelatinization temperature belonged to groups (d)–(f) among starches of rice cultivars recently bred in Japan. There were many starches belonging to groups (b) and (c) among the rice starches of *indica* and *Chinese indica.* This result means that unit-chain lengths of amylopectin of *indica* and *Chinese indica* rice generally longer than that of *japonica* rice, and is consistent with the results reported by Nakamura *et al.*[3)]

Amylopectins of the *indica* cultivars except for Dianyu 1, Jaguary, Suweon 258 and Tongil had lower Fr. A contents compared with that of Koshihikari (the most popular variety in Japan). Amylopectins of the *Chinese indica* cultivars except for Bayuenuo, Hongxienuo, Taichungxian 3, Taichungyu 204 and Taichungzailai 1, had lower Fr. A

Table 1. Continued.

IV. *Javanica* rice

Sample name	Place of origin	Brown rice			Starch									
					Iodine absorption spectra[*2]		HPAEC-PAD[*3]		RVA[*4]				DSC[*5]	
		Weight of 1000 grains (g)	Length/Width	ASS[*1]	λ_{max} (nm)	Blue value	Fr. A (%)	Group	T_v (°C)	*PV* (RVU)	*BD* (RVU)	*SB* (RVU)	T_p (°C)	ΔH (J/g)
Afgha WYR-5088	Russia	23.5	1.8	5	588	0.38	32.5	(f)	74.2	242	85	181	59.0	9.2
Basilanon	Brazil	16.0	2.5	1	590	0.37	nd	nd	73.4	283	178	134	nd	nd
Bodat Mayang	Indonesia	24.7	2.2	1	587	0.36	nd	nd	74.9	238	115	133	nd	nd
CG-S4	USA	24.0	1.7	3	579	0.26	31.1	(e)	nd	268	174	110	65.8	11.1
Dam Ngo	Laos	26.4	2.0	5	522	0.07	31.4	(e)	60.0	209	142	46	nd	nd
Deng Mack Tek	Laos	26.0	1.7	5	520	0.07	32.3	(f)	60.6	224	157	42	nd	nd
Dourado Precoce	Brazil	26.6	2.0	1	593	0.38	nd	nd	74.7	237	114	130	nd	nd
In Sitt	Myanmar	20.3	2.3	3	589	0.34	nd	nd	73.6	248	152	145	69.7	11.0
Khauk Yoe	Myanmar	18.3	1.9	3	585	0.35	nd	nd	76.7	239	149	134	72.0	12.0
Ku 70-1	Thailand	25.5	2.1	4	520	0.06	33.4	(f)	59.0	220	151	39	nd	nd
Labell	USA	16.6	2.9	1	586	0.34	26.0	(c)	75.0	282	204	103	75.7	11.5
Mack Kheua	Laos	26.1	1.9	5	521	0.07	33.6	(c)	58.0	215	148	42	61.5	11.1
Naxi	China	21.8	1.9	3	591	0.34	28.1	(c)	72.9	258	163	137	71.8	11.4
North Rose	USA	19.1	2.2	2	570	0.23	28.7	(c)	nd	302	160	72	67.6	10.3
Padi Cici	Indonesia	26.5	1.9	4	589	0.34	nd	nd	72.2	253	148	110	nd	nd
Simedel	Indonesia	26.8	1.8	2	588	0.33	nd	nd	73.5	300	202	129	72.6	12.4
Tambo	Brazil	25.1	2.4	1	589	0.34	nd	nd	77.1	258	129	141	nd	nd
Texas Fortuna	USA	21.8	2.1	1	585	0.31	27.7	(c)	nd	303	180	148	71.8	12.8
Vista	USA	16.9	2.2	3	570	0.23	29.0	(d)	nd	346	190	96	66.6	12.9

[*1] Alkali spreading score of rice grains, 1: no decay, 2: slight dilation, 3: dilation, 4: slight decay, 5: decay. [*2] λ_{max}, Maximum absorption wavelength of spectra of iodine-starch complexes; Blue value, Absorbance at 680 nm of spectra of iodine-starch complexes. [*3] Chain-lengths of debranched rice amylopectin were classified into six groups based on Fr. A content. Group (a), Fr. A$<$20.0%; Group (b), 20.0$\leqq$Fr. A$<$26.0%; Group (c), 26.0%$\leqq$Fr. A$<$29.0%; Group (d), 29.0%$\leqq$Fr. A$<$30.5%; Group (e), 30.5%$\leqq$Fr. A$<$32.0%; Group (f), 32.0%$\leqq$Fr. A. [*4] Rapid Visco Analyser: T_v, Temperature at initial viscosity rising; *PV*, Peak top viscosity; *BD*, Breakdown; *SB*, Setback. [*5] Differential scanning calorimeter: T_p, peak temperature; ΔH, enthalpy change of gelatinization.

Table 2. Contents of apparent and true amylose and super-long chain and λ_{max} and blue value of amylopectin.

Sample name	Content in starch (%)			Amylopectin	
	AAM[*1]	TAM[*2]	SLC[*3]	λ_{max} (nm)	blue value
Afgha WYR-5088	29.3	24.5	4.8	nd	nd
Aijiaonante	30.7	20.6	10.1	584	0.23
Basmati 370	28.4	23.3	5.1	558	0.15
Chinsurah Boro 2	34.3	26.6	7.7	571	0.20
Co 13	31.8	22.1	9.7	581	0.24
Dakanalo	32.5	27.1	5.4	564	0.18
Daorenqiao	30.9	20.2	10.7	572	0.22
Della	28.5	26.0	2.5	538	0.11
Dular	32.8	26.7	6.1	564	0.19
Hongxienuo	0.0	0.0	0.0	nd	nd
IR 28	32.6	19.2	13.4	591	0.33
Jaguary	0.0	0.0	0.0	nd	nd
Kasalath	33.3	24.4	8.9	571	0.21
Koshihikari	18.1	17.7	0.4	527	0.09
Labell	27.5	24.3	3.2	541	0.13
Milyang 23	23.9	22.8	1.1	527	0.08
Muha	32.0	26.6	5.4	562	0.18
Nepal 1	31.7	23.0	8.7	583	0.25
North Rose	21.9	20.2	1.7	527	0.09
Pusur	33.5	28.2	5.3	565	0.19
Sari Queen	18.8	18.0	0.8	526	0.08
Surjamkhi	32.4	26.2	6.2	561	0.20
Suweon 258	24.6	23.3	1.3	nd	nd
T 246	32.5	21.7	10.8	581	0.24
Taichungyu 204	20.8	20.4	0.4	526	0.08
Taichungzailai 1	32.7	20.0	12.7	589	0.27
Vista	21.3	20.8	0.5	526	0.09

[*1] Apparent amylose, [*2] true amylose, [*3] super-long chain.

contents compared with that of Koshihikari. Among *japonica* cultivars, Yakan had lower Fr. A content compared with that of Koshihikari. Amylopectins of Labell, Naxi, North Rose, Texas Fortuna and Vista among the *javanica* cultivars had lower Fr. A values compared with that of Koshihikari; however, the other *javanica* cultivars had higher Fr. A values.

Characteristics of RVA viscograms of rice starches.

Characteristics of pasting for the rice starches measured by RVA are shown in Table 1. Values for T_v, *PV*, *BD* and *SB* of all rice starches were in the ranges of 58.0–77.9°C, 179–353 RVU, 85–274 RVU and 39–310 RVU, respectively.

Gelatinization temperature and enthalpy change of gelatinization measured by DSC.

The peak temperature (T_p) and the enthalpy change (ΔH) of the gelatinization of rice starches measured by DSC are shown in Table 1. The T_p of *Indica*, *Chinese indica*, *japonica* and *javanica* rice starches were in the range of 64.2–75.8°C, 62.9–74.2°C, 67.6–78.0°C and 59.0–75.7°C, respectively. The ΔH values of rice starches of the four subspecies were in a range of 8.5–13.5 J/g.

Correlations of the relative amounts of Wx protein with contents of AAM, TAM and SLC.

Figure 1 shows the correlations of relative amounts of *Wx* protein with contents of AAM, TAM and SLC in rice starches of four subspecies. This correlative curve of AAM contents in rice starches against the relative amount

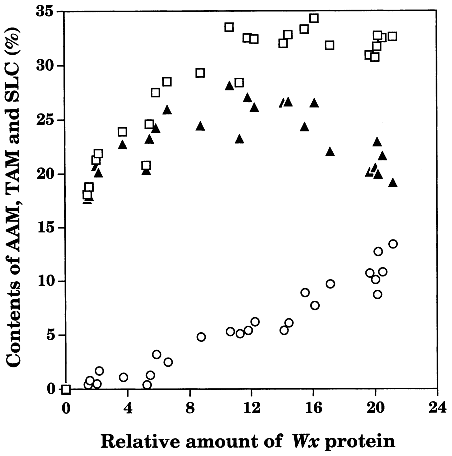

Fig. 1. Correlations of relative amount of *Wx* protein with content of AAM, TAM and SLC in rice starches. open square, AAM content; closed triangle, TAM content; open circle, SLC content.

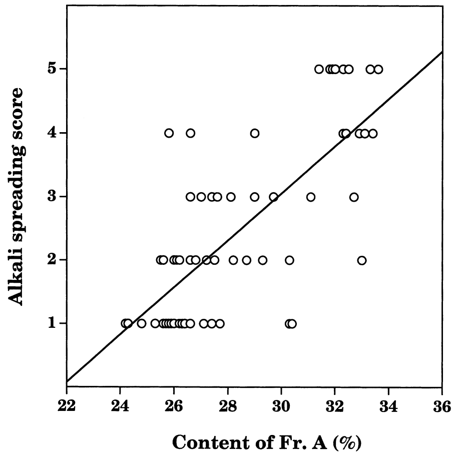

Fig. 2. Correlation of Fr. A (DP 6–12) content in rice starches with alkali spreading score (ASS).

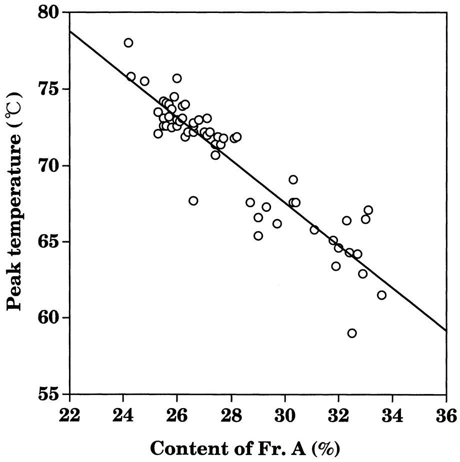

Fig. 3. Correlation of Fr. A (DP 6–12) content with peak temperature of rice starches.

of *Wx* protein were similar to the figure shown by Sano.[18] It was observed that there was a highly significant relationship between relative amounts of *Wx* protein and SLC contents in starches. The TAM contents of rice starches with higher relative amounts of *Wx* protein (more than 15, there is no *Japonica* rice) were low.

DISCUSSION

There was a highly significant correlation of AAM contents (Table 2) with $\lambda_{\max}$ and blue value of starches (Table 1) ($r=0.99$ and 0.96, respectively, $n=27$). This result shows that AAM contents (namely, total contents of TAM and SLC) strongly affect $\lambda_{\max}$ and blue value of starches, corresponding to our previous reports.[5,20] There was also a highly significant correlation of SLC contents in starches with $\lambda_{\max}$ and blue value of purified amylopectins (Table 2) ($r=0.97$ and 0.97, respectively, $n=23$). This result means that SLC in purified amylopectin has a great influence on $\lambda_{\max}$ and blue value of the entire purified amylopectin, and that SLC content in starch can be well estimated by the $\lambda_{\max}$ and blue value of purified amylopectin.

Figure 2 shows the relationship between the Fr. A contents in starches and ASS (degree of alkali disintegration) of the brown rice grains. A correlation ($r=0.74$, $n=64$) between them was observed. Furthermore, there was a highly negative relationship ($r=-0.92$, $n=60$) between Fr. A contents and T_p of starches (Fig. 3). These results strongly suggest that shorter branches (Fr. A) of amylopectin decrease the stability of starch granules. Accordingly, the starches with fewer Fr. A contents have higher gelatinization temperatures of starches, and lower ASS of the rice grains.

Little *et al.*[6] and Juliano *et al.*[21] demonstrated that the *japonica* starch granules are more easily disintegrated in alkali solution and exhibit a lower gelatinization temperature than *indica* starch granules. As an interpretation for their demonstration, Umemoto *et al.*[22,23] proposed the difference between the alleles of *starch synthase IIa* (*SSIIa*) in *japonica* and *indica* rice causes the phenotypic difference between the two rice varieties in amylopectin structure. The *SSIIa* gene is responsible for the difference in chain-length distribution of amylopectin between rice varieties, and controlling differences in alkali disintegration of rice grains and chain-length distribution of amylopectin among *japonica* and *indica* cultivars, namely *japonica* amylopectin is enriched in shorter (A and B_1) chains relative to *indica* amylopectin. It is difficult to explain these conceptions from our results (Fig. 3), because the samples of investigated *japonica* rice were few in comparison with the other subspecies in this paper. Umemoto *et al.*[4] also predicted that starch granules containing amylopectin with longer chains were more resistant to gelatinization and were less soluble in alkali solution. Nakamura *et al.*[3] demonstrated a similar relationship between the structure of amylopectin and thermal properties of the endosperm starches from Asian cultivated rice. Our results (Figs. 2 and 3) were also consistent with their reports, and support their conception, namely, the *SSIIa* gene is responsible for the chain length of amylopectin and alkali disintegration of rice grains.

Each correlation between structural parameters (AAM, TAM and SLC contents) and RVA parameters (*PV*, *BD* and *SB*) for non-waxy starches is shown in Fig. 4 (A–I). There were highly negative correlations of *PV* with AAM and TAM contents in non-waxy starches ($r=-0.74$ and -0.73, respectively) (Fig. 4-A, B). This result appears to mean that TAM mainly makes *PV* decrease, because there

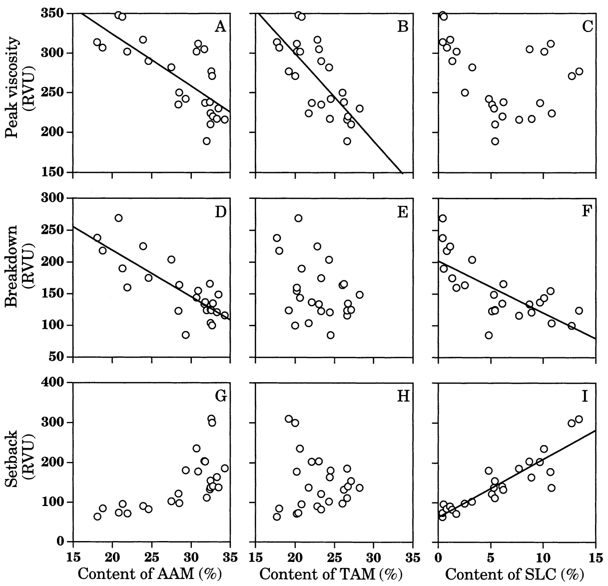

Fig. 4. Correlations of apparent and true amylose, super-long chain content in starches with RVA parameters of starches obtained from rice starches (27 starch samples in Table 2).

RVA parameters are as follows: peak top viscosity, [A: $r=-0.74$], [B: $r=-0.73$], [C: $r=-0.38$]; breakdown, [D: $r=-0.81$], [E: $r=-0.38$], [F: $r=-0.72$]; setback, [G: $r=0.67$], [H: $r=-0.08$], [I: $r=0.90$].

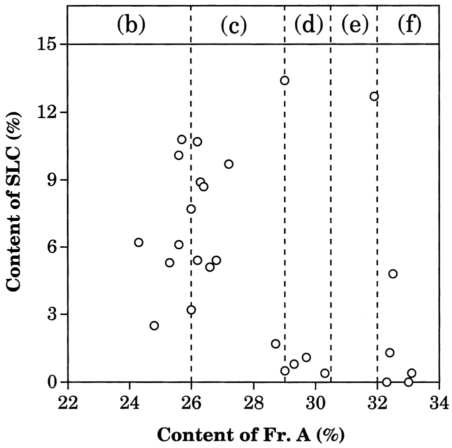

Fig. 5. Scatter plots of Fr. A (DP 6–12) content and super-long chain (SLC) content in the rice starches.

is no relationship between SLC content and *PV* ($r=-0.38$) (Fig. 4-C). There was no significant relationship between TAM content and *BD* ($r=-0.38$) (Fig. 4-E). SLC content also inversely correlated to *BD* ($r=-0.72$) (Fig. 4-F) as reported by Han *et al.*[24] Since the difference between *PV* and *BD* corresponds to *MV*, SLC appears to affect *MV* during the RVA measurement. A highly positive correlation ($r=0.90$) was observed between contents of SLC and *SB* in non-waxy starches (Fig. 4-I). This result means that SLC makes the viscosity of starch paste under the cooling condition (from 95 to 50°C) high. This result corresponded to our reports for starches of rice cultivars recently bred in Japan.[5] Since this result could be observed in many starches of rice cultivars in many countries including Japan, we are confident that the function of the *Wx* gene and the existence of SLC in starch significantly affect the characteristics of gelatinization and retrogradation for rice starches.

It is known that granule-bound starch synthase I (GBSS I) (or Waxy protein) responsible for amylose synthesis in rice endosperm is encoded by the Waxy (*Wx*) gene.[25] There are three major waxy alleles in rice, Wx^{a}, Wx^{b} and *wx*.[17] The Wx^{a} allele is predominant among *indica* subspecies, while Wx^{b} is predominant among *japonica* subspecies. Rice cultivars that possess Wx^{a} produce more GBSS I protein and amylose than those with Wx^{b}, and cultivars with *wx* lack GBSS I protein and amylose in rice grains.[17] Denyer *et al.*[26] demonstrated that GBSSI elongate not only amylose chains but amylopectin chains in starch granules isolated from pea embryos and potato tubers. Since a highly significant relationship between the relative amount of *Wx* protein and SLC content was observed, *Wx* protein appears to be responsible for SLC synthesis in rice endosperm. In contrast to a straight increase of SLC contents with relative amounts of *Wx* protein, the maximum of TAM contents (about 25%) was observed in the middle of the range of observed relative amounts of *Wx* protein (about 10). This seems to indicate that the synthesis of TAM in rice starches is limited to about 30%, even if the amount of *Wx* protein in rice starches increases. Many possibilities such as three-dimensional hindrance of synthesis of glucosidic chains in starch granules and specificity of GBSSI for the substrate can be imagined to explain this result. The exact explanation for this result seems to need further investigation.

Figure 5 shows the scatter plots of Fr. A content and SLC content in the rice starches. Since waxy rice starches did not contain SLC, waxy starch samples were plotted on the *X* axis. The plots in the graph were roughly classified into three groups, namely, group (1) with Fr. A contents in the range of 24–27% and SLC contents in the range of 2–12%, group (2) with Fr. A contents in the range of 28–34% and SLC contents in the range of 0–6% and group (3) with Fr. A contents in the range of 28–34% and SLC contents of the range of 12–15%. The *indica* and Chinese *indica* rice mainly belonged to group (1), *japonica* rice like a Koshihikari variety mainly belonged to group (2), and IR28 (*indica*) and Taichungzailai 1 (*Chinese indica*) belonged to group (3). SLC contents in the range of 0–13.4% were evenly observed in endosperm starches from cultivated rice of Asia and other countries. However we could not observe any starch samples in either ranges of 3–4% or 8–12% for SLC contents in starches among the 59 investigated rice cultivars in Japan.[5] These results show that the SLC contents of starches are even in the range of 0–13% in the cultivated rice of foreign countries. The contents of Fr. A and SLC in rice amylopectin appear to be independently responsible for *SSIIa* and *Wx* genes, respectively. However, the scatter plots of Fr. A and SLC content in the rice starches never evenly distribute in graph of Fig.5. Namely, the cultivated rice with low Fr. A content (<28%) and high SLC content (>12%), and with high Fr. A content (>28%) and middle SLC content (5–12%) were not observed. These results may be basic data for the mutual biosynthesis of A plus B_1 chains and SLC of amylopectin in rice starches in future.

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アジアおよびその他の国の栽培稲の胚乳澱粉の構造と性質

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2001 年に県立広島大学で栽培されたアジアおよびその他の国の栽培米 75 品種（インディカ 23 品種，シニカ（中国産インディカ）27 品種，ジャポニカ 6 品種，ジャバニカ 19 品種）の玄米の性質および胚乳澱粉の構造と物理化学的性質について調べた．測定した項目は，玄米の千粒重，長幅比，アルカリ崩壊性，胚乳澱粉のヨウ素吸収曲線の青価と最大吸収波長，GPC 法による澱粉と精製アミロペクチンの側鎖長分布から求めた真と見かけのアミロース含量およびアミロペクチン中の超長鎖含量，HPAEC-PAD 法によるアミロペクチンの側鎖長分布，DSC による糊化温度と糊化熱量，RVA による粘度特性値，SDS-PAGE による胚乳澱粉中の *Wx* タンパク量である．インディカと中国インディカの胚乳澱粉のアミロペクチン側鎖の重合度 6–12 の割合（Fr. A 含量）は低かった．Fr. A 含量は玄米のアルカリ崩壊度と正の相関，胚乳澱粉の糊化ピーク温度と負の相関関係が観察された．RVA によって測定した澱粉の糊化特性の中で，セットバックとブレークダウンは超長鎖含量とそれぞれ正と負の相関関係，ピーク粘度およびブレークダウンはどちらも見かけのアミロース含量と負の相関関係が観察された．澱粉中の *Wx* タンパク質のタンパク量と超長鎖含量との間には高い正の相関関係が得られた．アジアおよび他の国々の栽培品種の米胚乳澱粉中には，超長鎖含量が 0.0–13.4% の範囲で連続的に分布していた．