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Accumulation of Proteins and the Polypeptide Composition of the Glutelin in Ancient Cultivars of Rice Seed

Junko UDAKA, Kenji TAKUMI, Hideaki TSUJI* and Tadashi OGAWA*

Faculty of Education, Ehime University, Matsuyama 790, Japan
** School of Medicine, Tokushima University, Tokushima 770, Japan*

The accumulation of storage proteins in ancient rice cultivars during seed development and the polypeptide composition of the glutelins were studied by SDS-PAGE and two-dimensional electrophoresis for comparison with modern rice cultivars. With the general pattern of protein accumulation, a massive deposition of two glutelins and one prolamin polypeptide with respective MW's of 21, 32 and 15 kDa occurred about 7 to 10 days after flowering (DAF) and steadily increased until reaching at a plateau at about 15 to 25 DAF. In an ancient cultivar, however, the polypeptide deposition was different, a sharp increment in the proteins beginning at 7 DAF and reaching a maximum at 20 DAF, before rapidly decreasing. The maximum amount of deposited proteins at 20 DAF was nearly twice that for the general accumulation pattern with modern and other ancient rice cultivars. The glutelin protein was found to be selectively extractable with SDS solution more than with other conventional solvents including acidic, basic and urea solutions. Some ancient black rice glutelins shared a polypeptide with MW of 19 kDa which was absent or handly detectable in the other glutelins, suggesting the 19 kDa polypeptide to be an inherent protein species of these ancient black cultivars. Two-dimensional electrophoresis of the ancient glutelins revealed two rice glutelin subunits (α and β), but the 19 kDa polypeptide remained unidentified.

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Keywords: rice, ancient cultivar, modern cultivar, seed protein, glutelin, polypeptide composition.

INTRODUCTION

Ancient rice cultivars (*Oryza sativa* L.) are very important in terms of gene preservation, because they have not been subjected to any genetic improvement or reform. Nevertheless, they have received less attention for studies on the physicochemical and biochemical properties of the seed proteins than many other cereals and modern rice cultivars.

Unlike the case of many cereal grains, the major storage proteins of rice seeds are glutelins, which account for about 80% of the total seed proteins.[1,2] It has been established that rice glutelins begin to accumulate in the starchy endosperm at 5-7 days[3,4] after flowering during seed development, before exclusive deposition in the protein body.[5] Li *et al.*[6] have recently demonstrated that rice storage proteins comprising prolamin and glutelin were first detected in 10-day-old seeds and that their amounts steadily increased through seed development with variation in the relative proportions of glutelin and prolamin.

On the other hand, despite their potentially important role in genetics, the biophysical aspects for ancient rice cultivars of storage protein accumulation during seed development have remained uncertain.

In this study, we describe some differences and similarities in the protein accumulation profiles between ancient and modern rice cultivars, together with the glutelin polypeptide composition, which were determined by SDS-PAGE and 2-dimensional electrophoresis. The extraction efficiency for rice glutelin of conventional solvents is also discussed.

MATERIALS AND METHODS

Plant materials

All the rice cultivars used in this study were taken from the collection at Uwa Rice Museum (Uwa-cho, Ehime 797, Japan) and are summarized in Table 1. Each cultivar was grown during the summer (from the middle of June to the end of October in 1994) in pots in a garden at Laboratory of Food Science in Faculty of Education at Ehime University (Matsuyama, Ehime 790, Japan). The individual panicles

Table 1. Rice cultivars

Classification	Cultivar
Ancient non-glutinous Japonica	*Tsushimazairai*
	Takaramitsu
	Benimiyako
	Kunitsukasa-chuukann
	Kunitsukasa-shiro
Ancient glutinous Japonica	*Kuro-mochi*
	Akita-kuro
	Murasaki-kuro
	Kuro-1
	Kuro-2
	Kuro-62
	Kuro-73
Other ancient Japonica	*Midorimai*
	Kaori-eigou
	Kaori-mochi
Modern non-glutinous Japanica	*Koshihikari*
Modern glutinous Japonica	*Takasago-mochi*
Modern long-grain white rice	*Bluebonnet*
	Mitsuyou

All cultivars were supplied by Uwa Rice Museum (Uwa-cho, Ehime 797, Japan).

were tagged at mid-panicle when anthesis occurred. Maturation in the tasted cultivars occurred about 45 days after flowering (DAF). After flowering, the developing panicles were harvested at appropriate intervals during the period of 3 to 45 DAF (Fig. 1). The harvested panicles were immediately frozen at −20℃ until needed.

Assay for protein accumulation

Rice seeds from each sampling date were hulled by hand and ground with a hand grinder. Each resulting flour sample was suspended in an Eppendorf tube at a concentration of 62 mg/700 μl with mixed solution composed of equal volumes of 2% SDS in 5 mM Tris/HCl buffer (pH 8.3) and the sample buffer (×2) of Laemmli.[7] The protein sample in the tube was dissolved by boiling for 15 min and then centrifuged. The resulting clear supernatant was analyzed by SDS-PAGE as described later. The relative amounts of polypeptides in the sample protein were evaluated by the method of Yamagata *et al.*,[3] in which the peak heights by densitometric scanning of the electrophoregram were evaluated by a dual-wavelength flying-spot scanner (model CS-9000, Shimadzu Corp., Tokyo, Japan).

Preparation of the alcohol-insoluble protein residue

Rice seeds of *Akitakomachi* c.v. were hulled and ground in a grinder to pass through a 60-mesh sieve. The flour samples were defatted by stirring with two volumes of acetone for 30 min at room temperature 3 times and then dried in air, before being stored at 4℃. Each defatted flour sample was sequentially extracted twice with 0.5 M NaCl in 10 mM Tris/HCl buffer (pH 7.5) and then with 50% propanol in the same Tris buffer by stirring for 2 h at room temperature as described previously.[3] The resulting alcohol-insoluble residue was washed twice with cold acetone and finally dried in air.

Assay for the extraction efficiency of various solvents for glutelin

In order to look for a more suitable solvent than the conventional ones for rice glutelin extraction, the alcohol-insoluble residue just obtained was separately extracted with various solvents comprising of 1–5 M urea, 0.5–2% SDS and mixture of 4 M urea and 2% SDS in 10 mM Tris/HCl buffer (pH 8.5), mixture of 4 M urea and 2% SDS in 62.5 mM Tris/HCl buffer (pH 6.8), 0.05 M acetic acid, 0.2–0.4% NaOH, and 0.1% KOH. The alcohol-insoluble residue was suspended in each solvent (1 g/20 ml) and stirred for 2 h at 30℃. The reaction mixture was with drawn and centrifuged to gain both the supernatant and residue, which were subjected to an assay of protein content (Fig. 2) and polypeptide composition (Fig. 3A–C), respectively. Protein content was calculated from the volume of the glutelin fraction, and protein concentration by the Lowry method.[4]

Preparation of glutelin

Glutelin from each rice cultivar was extracted with 2% SDS in 10 mM Tris/HCl buffer (pH 8.5) (1 g/10 ml) from the individual alcohol-iusoluble residue by stirring for 2 h at room temperature. After centrifuging at 21,000×*g* for 20 min, the supernatant was filtered through a Millipore membrane (Nihon Millipore Kogyo, Tokyo, Japan) with a pore size of 1.2 μm. To the filtrate was added 2 volumes of cold acetone and the solution kept overnight at 4℃. The precipitated glutelin was centrifuged and dried in air.

SDS-PAGE

SDS polyacrylamide gel electrophoresis (PAGE) was done by the method of Laemmli,[7] using 15% acrylamide gel, with slight modifications. Aliquots (5 mg) of each glutelin sample were dissolved in 200 μl of the sample buffer (20% glycerol, 10% 2-mercaptoethanol, 2% SDS, and 0.002% BPB in 0.125 M

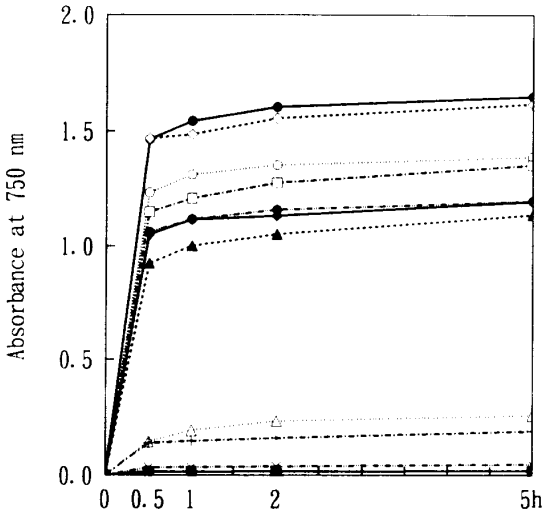

Fig. 2. Solubility of the rice glutelin fractions as a function of stirring time at 30℃ in various solvents and solvent systems

One gram of the 50 % propanol-insoluble residue from rice flour (*O. sativa* L. c.v. *Koshihikari*) was separately suspended in 20 ml of each solvent or solvent system and stirred by a magnetic stirrer at 30 ℃. At appropriate intervals, about 1 ml of the suspension was collected, and a part of the supernatant was assayed for protein content by the Lowry method.[9] Solvents: 0.05 M acetic acid (■); 0.1% KOH (△); 0.2% NaOH (□); 0.4% NaOH (◇); 1 M urea (×); 5 M urea (+); 0.5% SDS (▲); 1% SDS (◆); 2% SDS (●). Urea or SDS solvents were prepared in 10 mM Tris/HCl buffer (pH 8.5). Solvent system 1 (●): 2% SDS plus 4 M urea in 10 mM Tris/HCl buffer (pH 8.5). Solvent system 2 (○): 2% SDS plus 4 M urea in 62.5 mM Tris/HCl buffer (pH 6.8).[3]

Tris/HCl buffer (pH 6.8)) by heating for 5 min in boiling water in an Eppendorf tube. The tube was centrifuged to remove a clear supernatant, a portion of which (2-10 μl) was applied to the gel. Electrophoresis was conducted for about 1.5 h at 30 mA per slab (5.5×8.5×0.075 cm), the gel being stained with 0.05 % Coomassie Brilliant Blue R-250 in 10% acetic acid and 25% isopropanol, and destained with 10 % acetic acid.[8] The molecular weight of each protein was determined by reference to the migration distance of the marker protein in a low-molecular-weight protein kit (Pharmacia LKB Biotechnology, Uppsala, Sweden): myoglobin, 94,000; IBSA, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean inhibitor, 20,100; α-lactoalbumin, 14,400.

Two-dimensional electrophoresis (2-DE)

2-DE was carried out by the method of O'Farrell.[10] The first dimension was conducted by non-equilibrium pH gradient electrophoresis (NEPHGE) in vertical tube gel (10×0.2 cm), using ampholine of pH 3.5 to 10 (Pharmacia LKB Biotechnology). The gels were adapted to vertical electrophoresis apparatus, and NEPHGE was run at 400 V for 2.5 h (1,000 V-hours). The second dimension was conducted by SDS-PAGE with 15 % polyacrylamide gel and run at a constant current of 30 mA for 1.5 h. Staining and destaining of the gel were done as already described.

Chemical analysis

The protein content of each sample solution was assayed by the Lowry method.[9] Amino acid composition was determined by a Hitachi 835 amino acid analyzer after each sample had been hydrolyzed with 6 N HCl at 105℃ for 24 h in a tube sealed under vacuum.[8]

RESULTS AND DISCUSSION

Protein accumulation patterns

For a comparison of the accumulation profiles of rice protein during seed development, panicles were harvested at appropriate intervals (2-10 days) and the isolated proteins were analyzed by SDS-PAGE (Fig. 1). The rice cultivars used in this experiment were modern rice c.v., *Akitakomachi* (panels A and a) and *Takasago-mochi* (panels B and b), and ancient c.v., *Tsushimazairai* (panels C and c) and *Kuro-mochi* (panels D and d). The relative amounts of polypeptides from the panicles were obtained as peak heights evaluated by densitomatric scanning of the electrophoregram, in which the peak height of the polypeptide with a molecular weight (MW) of 87 kDa was designated as 100%.

During the grain-filling period (about 3-45 DAF), the accumulation patterns for the seed protein polypeptides of all the cultivars tested were roughly similar to one another, except for *Tsushimazairai* c.v. At 3-5 DAF, 2 polypeptide subunits with rather high molecular weights (MW's) of 87 and 75 kDa first appeared. Following at 7 DAF, 4 distinct polypeptides with MW's of 52, 32, 21 and 15 kDa, which are major polypeptides of rice glutelin, began to be visible. The amount of accumulated protein increased gradually during the 10-20 DAF period in the three cultivars (panels a, b and d), and after 15-20 DAF, little change in the deposition patterns was seen. These findings agree well with those reported in the literature.[2,4] In the case of ancient rice *Tsushimazairai* c.v. (panel c), however, the protein accumulation profile was significantly different from those of the other three cultivars already cited. Although massive protein accumulation in this cultivar began at 7-10 DAF, the

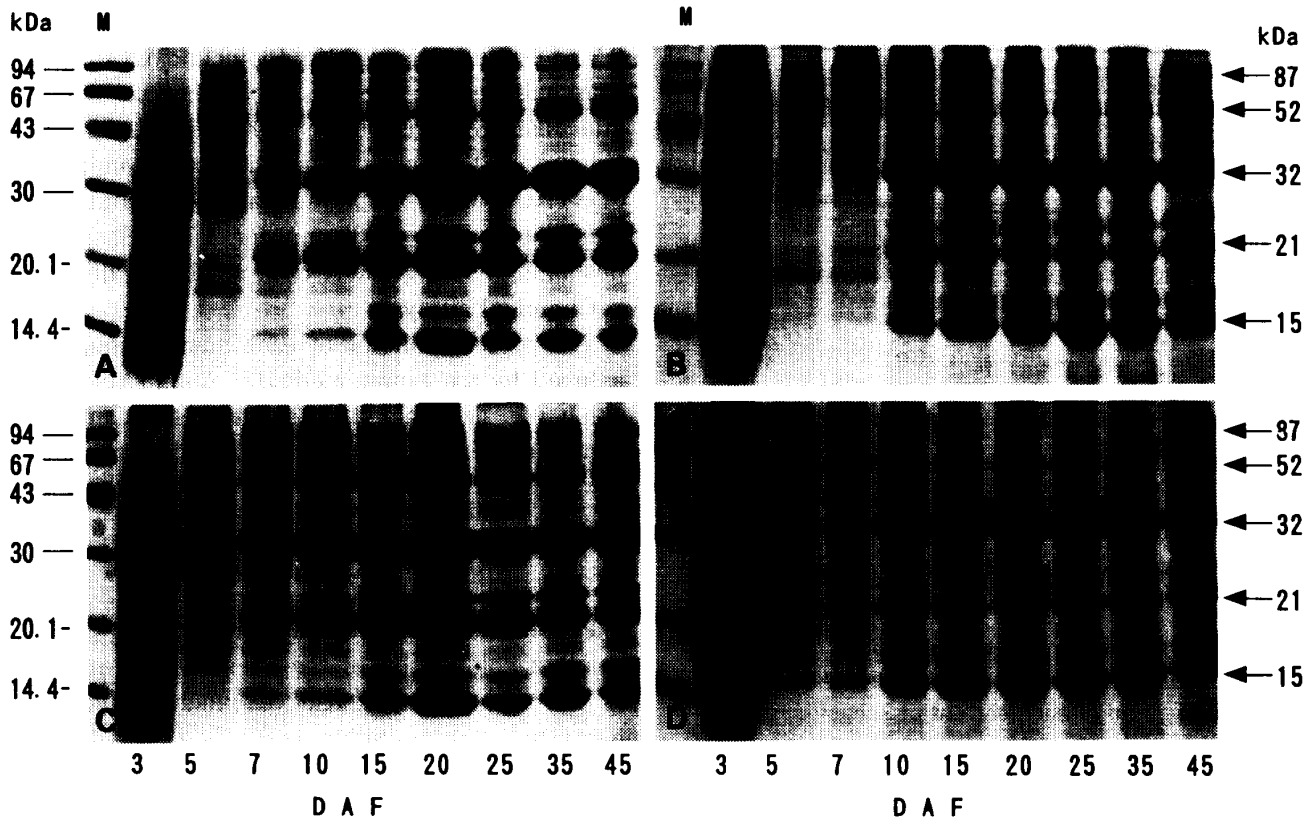

Fig. 1. SDS-PAGE patterns of proteins extracted from developing rice seeds harvested at appropriate days after flowering (DAF)

Modern non-glutinous c.v.: *Akitakomachi* (panels A and a); modern glutinous c.v.: *Takasago-mochi* (panels B and b); ancient non-glutinous c.v.: *Tsushimazairai* (panels C and c); and ancient glutinous c.v.: *Kuro-mochi* (panels D and d). Sample proteins from panicles were dissolved in 2% SDS-5 mM Tris/HCl buffer (pH 8.3) and in sample buffer (×2 concentration, 1:1 v/v, 62 mg/700 μl) in an Eppendorf tube as described in the MATERIALS AND METHODS section. After centrifuging the tube, each clear supernatant (10 μl) was loaded on the lane. Numbers in the left margins refer to the apparent molecular weights of the standard marker proteins. Numbers in the right margins refer to the apparent molecular weights of the major polypeptides. Panels a-d show changes in the relative amounts of polypeptides deposited in endosperm during seed development, values being calculated as peak heights by densitometric scanning of the electrophoregram. The peak height of the 87 kDa polypeptide is designated 100%. Polypeptides: ●, 87 kDa; ○, 52 kDa; □, 32 kDa; △, 21 kDa; ■, 19 kDa; ▲, 15 kDa.

highest value for accumulated protein content was gained at 20 DAF, the relative peak height of the proteins at that time being found to be nearly twice that of the other cultivars. Moreover, this maximum protein value was followed by a rapid reduction to a generally low level. Although there is limited data at present, this accumulation pattern for rice protein in the endosperm seems likely to be peculiar to some ancient rice cultivars. We are thus going to attempt to clone the 19 kDa subunit to study the molecular characteristics of the genetic structure of ancient rice.

Glutelin extraction

To examine the extractability of rice glutelin by different solvents or solvent systems, the relative absorbance values at 750 nm for the supernatants of the individual solvents or solvent systems were first compared with each other, after being extracted from the 50% propanol-insoluble residue by stirring for 2 h (Fig. 2). Next, both the supernatants and insoluble residues were analyzed by SDS-PAGE (Fig. 3). As shown in Fig. 2, of all the solvents used, 0.4% NaOH (◇) or solvent system 1 (●, a mixture of 2% SDS and 4 M urea) revealed the highest extraction efficiency in terms of the absorbance at 750 nm (1.2-1.5) in the supernatant. SDS-PAGE of the 0.4% NaOH fraction (panel A ; lanes 3, 3′), however, shows that both the supernatant and residue contained only faint bands,

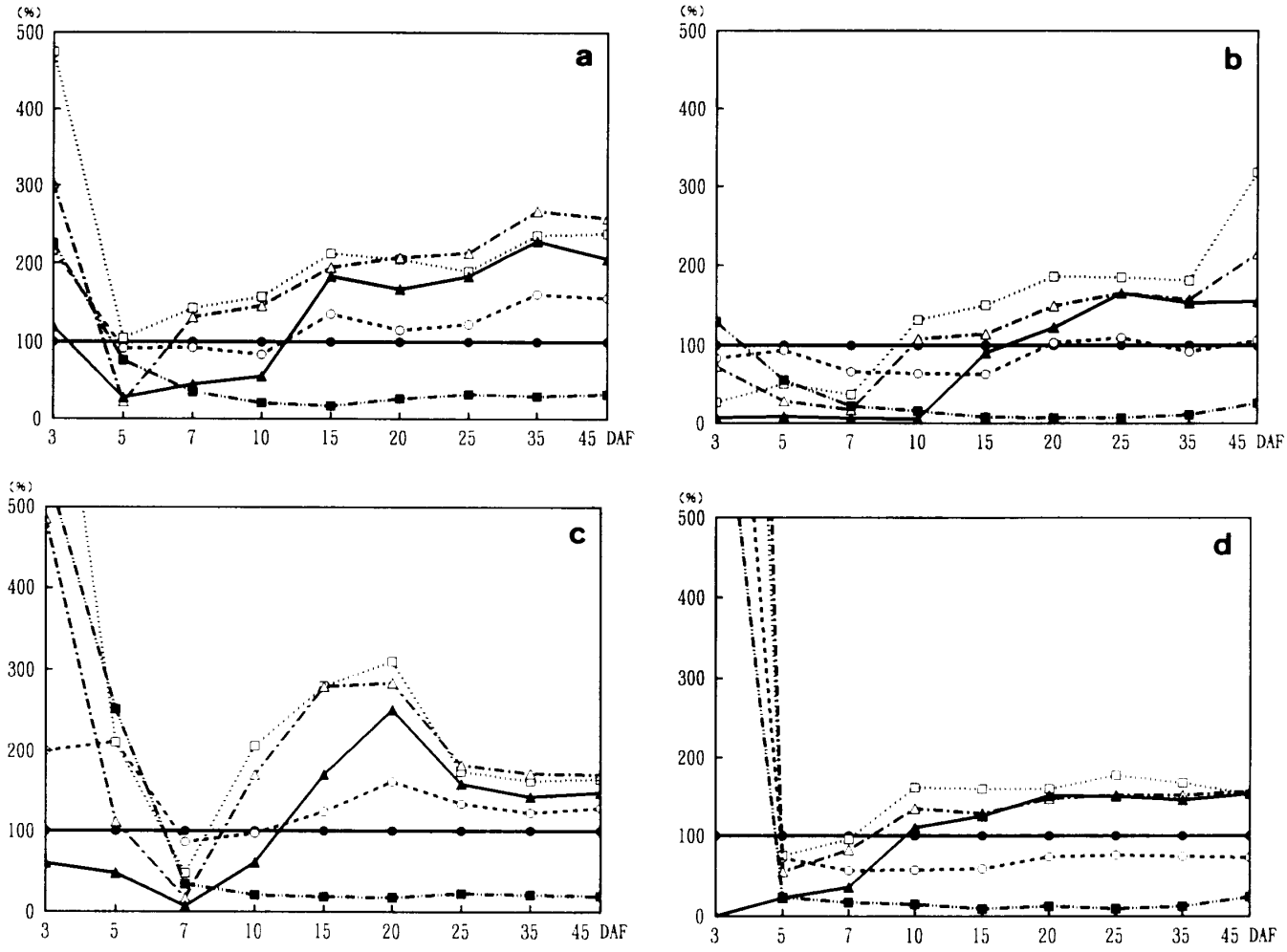

Fig. 1. Continued

suggesting alkaline degradation of the protein. The acidic solvent (panel A; lanes 1, 1′) was likely to be ineffective. With solvent system 1 (panel C; lanes 3, 3′), two major glutelin subunits with MW's of 32 and 21 kDa were found in the supernatant, while significant amounts of the bands corresponding to both subunits were left in the residue, indicating insufficient extraction of the protein. The SDS solvents, especially its 1 or 2 % solvents, however, extracted most efficiently the two major glutelin subunits, little evidence of protein bands being left in the residues (panel B; lanes 4, 4′ or 5, 5′), demonstrating that the SDS solvents had prominent selectivity for rice glutelin together with high extraction efficiency. On the other hand, urea solvents (panel B; lanes 1, 1′ and 2, 2′), which have generally been used in preceding papers,[3,4] were found to be less useful. Although several available solvent systems for rice glutelin extraction have been developed, *i.e.*, 0.5% SDS-0.6% mercaptoethanol,[11] 6 M urea in phosphate buffer (pH 6.5),[4] and 6 or 8 M urea in Tris/HCl buffer (pH 8.0),[3] their extraction efficiency for rice glutelin has varied according to the individual reports. It hitherto indicates a lack of standardization for rice glutelin extraction. The findings obtained here can be applied as a principle for rice glutelin extraction.

Polypeptide composition of the ancient rice glutelins

Glutelin fractions from 4 modern rice cultivars and from 11 ancient cultivars (4 red rice and 7 black rice), together with 3 specific ancient rice cultivars, were analyzed either by SDS-PAGE (Fig. 4) or by 2-DE (Fig. 5; A–D).

There was no insignificant difference in polypeptide patterns between the ancient and modern rice cultivars tested: 4 major subunits of polypeptides with MW's of 52, 32, 21 and 15 kDa were common to all the glutelin fractions. This polypeptide pattern has already been established by a number of preceding

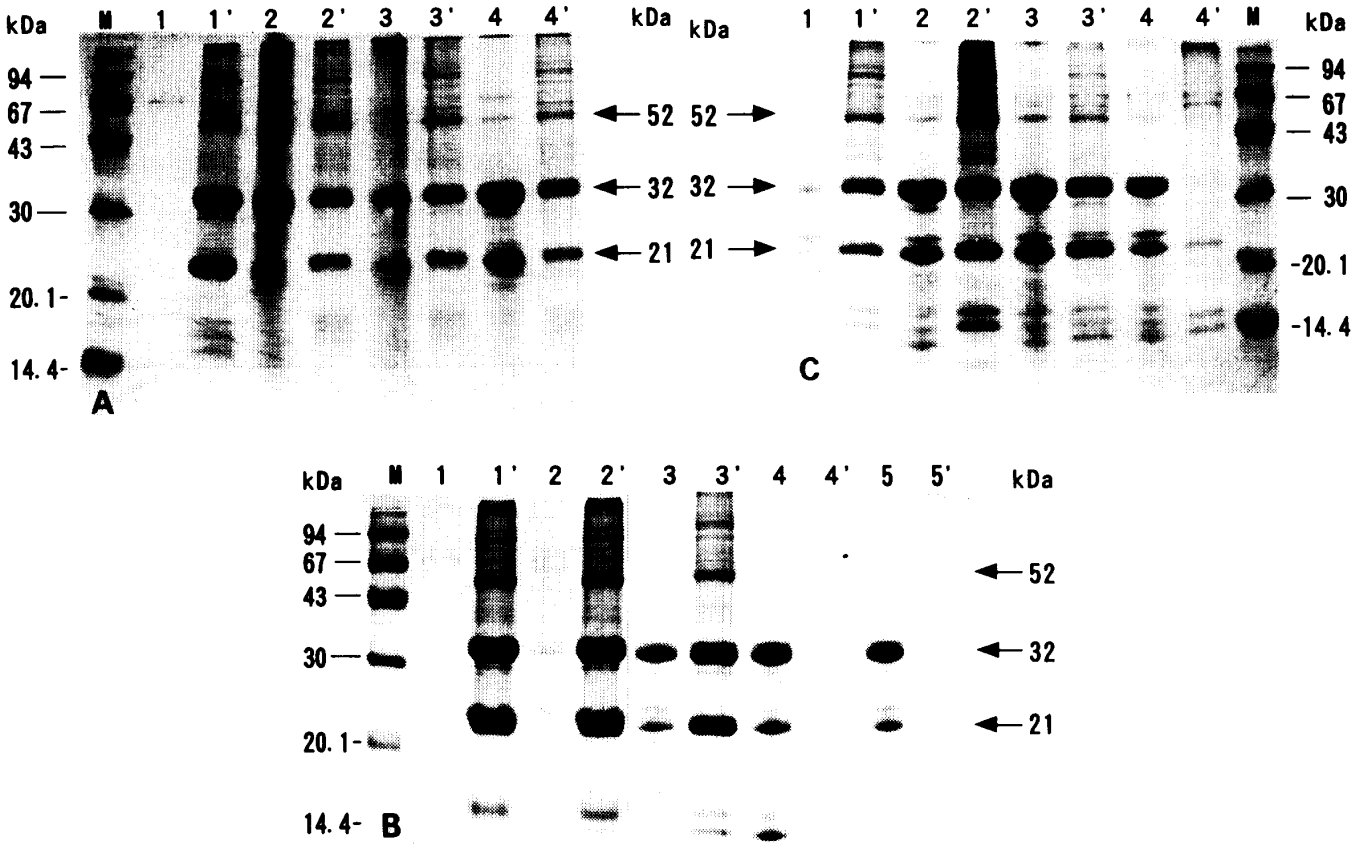

Fig. 3. SDS-PAGE patterns of the supernatants and insoluble residues described in Fig. 2

Samples from the supernatants (lanes 1-4 or 5) and insoluble residues (lanes 1'-4' or 5') were adjusted to give the best separation of each fraction and loaded on to the gels. Panel A: lanes 1 and 1', 0.05 M acetic acid; lanes 2 and 2', 0.2% NaOH; lanes 3 and 3', 0.4% NaOH; lanes 4 and 4', 0.1% KOH. Panel B: The solvents were prepared in 10 mM Tris/HCl buffer (pH 8.5). Lanes 1 and 1', 1 M urea; lanes 2 and 2', 5 M urea; lanes 3 and 3', 0.5% SDS; lanes 4 and 4', 1% SDS; lanes 5 and 5', 2% SDS. Panel C: lanes 1 and 1', 5 M urea in 10 mM Tris/HCl (pH 8.5); lanes 2 and 2', 4 M urea plus 2% SDS in 62.5 mM Tris/HCl (pH 6.8); lanes 3 and 3', 4 M urea plus 2% SDS in 10 mM Tris/HCl (pH 8.5); lanes 4 and 4', 2% SDS in 10 mM Tris/HCl (pH 8.5). Lanes M are the same protein markers as those described in Fig. 1. Numbers in the left and right margins refer to MW's of the marker proteins and of the major polypeptides of the rice glutelin, respectively.

works.[2-4] However, ancient black glutinous rice cultivars *Kuro*-2, *Kuro-mochi* and *Murasaki-kuro* (Fig. 4; lanes 8, 11 and 13) displayed one unique subunit (head arrows) with MW of 19 kDa, which also emerged faintly in some ancient red rice cultivars (lanes 2, 3, 4 and 5). On the other hand, few subunits corresponding to 19 kDa were detected in the modern rice cultivars (lanes 1, 6, 15 and 16) or in the other ancient cultivars (lanes 14, 17 and 18). These results suggest that the 19-kDa subunit may be an inherent protein species of the ancient black rice cultivars.

We therefore attempted to detect the 19 kDa subunit by 2-DE with NGPHGE/SDS-PAGE (Fig. 5; A-D), because the polypeptide subunits of rice glutelin have been characterized on the basis of their mobility in a 2-DE system, by which the glutelin is separable into acidic (α) and basic (β) subunits.[14,15] Both the subunit groups (α and β) could be separated in this experiment, but identification of the 19 kDa subunit was unsuccessful. The glutelin specimens tested here did not respond to alkylation with 4-vinylpyridine,[12] while the NGPHGE system used was composed of only one ampholine (pH 3.5-10), rather than the multi-system used by Luthe.[16] It will be necessary to re-examine by the 2-ME multisystem, using alkylated glutelin samples.

Amino acid composition of the ancient rice glutelins

The amino acid composition of the glutelins from 3 black glutinous and 3 red non-glutinous ancient rice cultivars are summarized in Table 2. There was little difference in the amino acid profiles among the

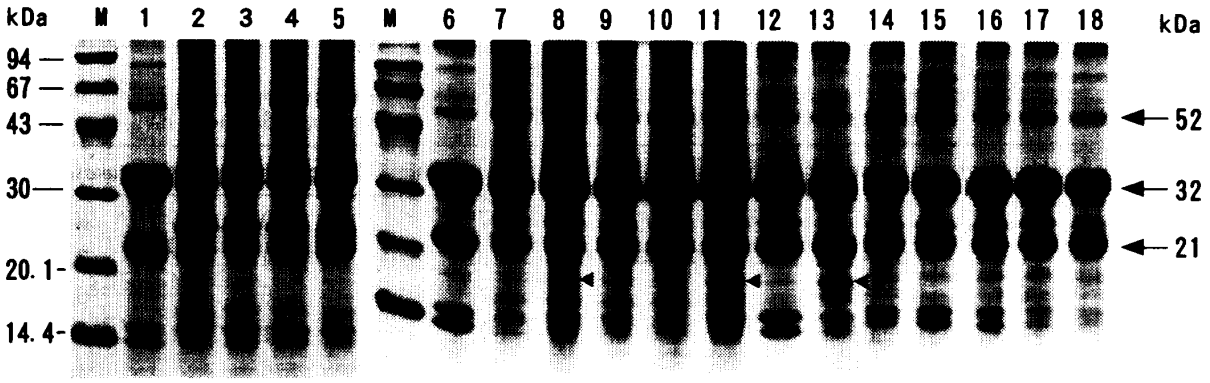

Fig. 4. SDS-PAGE patterns of the glutelin fractions extracted with 2% SDS in Tris buffer from various rice cultivars

An aliquot (1 g) of the 50% propanol-insoluble residue from each rice cultivar flour was suspended (100 mg/ml) by 2% SDS in 10 mM Tris/HCl buffer (pH 8.5), stirred for 2 h and then centrifuged at 21,000×*g* for 20 min. To the supernatant was added 2 volumes of cold acetone, and the precipitated glutelin was recovered by centrifugation. The glutelin was then washed twice with acetone and dried in air. Each dried glutelin was dissolved in the SDS sample buffer (25 μg/μl) and heated at 100℃ for 5 min, before 2 μl of the heated sample was loaded on to the gel. Numbers of the left and right margins are the same as those in Fig. 3. Lane 1, *Koshihikari* c.v.; lane 2, *Takaramitsu* c.v.; lane 3, *Benimiyako* c.v.; lane 4, *Kunitsukasa-chuukann* c.v.; lane 5, *Kunitsukasa-shiro* c.v.; lane 6, *Takasago-mochi* c.v.; lane 7, *Kuro*-1 c.v.; lane 8, *Kuro*-2 c.v.; lane 9, *Kuro*-62 c.v.; lane 10, *Kuro*-73 c.v.; lane 11, *Kuro-mochi* c.v.; lane 12, *Akita-kuro* c.v.; lane 13, *Murasaki-kuro* c.v.; lane 14, *Midorimai* c.v.; lane 15, *Bluebonnet* c.v.; lane 16, *Mitsuyou* c.v.; lane 17, *Kaori-eigou* c.v.; lane 18, *Kaori-mochi* c.v.; lane M, protein markers. Numbers in both margins are the same as those shown in Fig. 3.

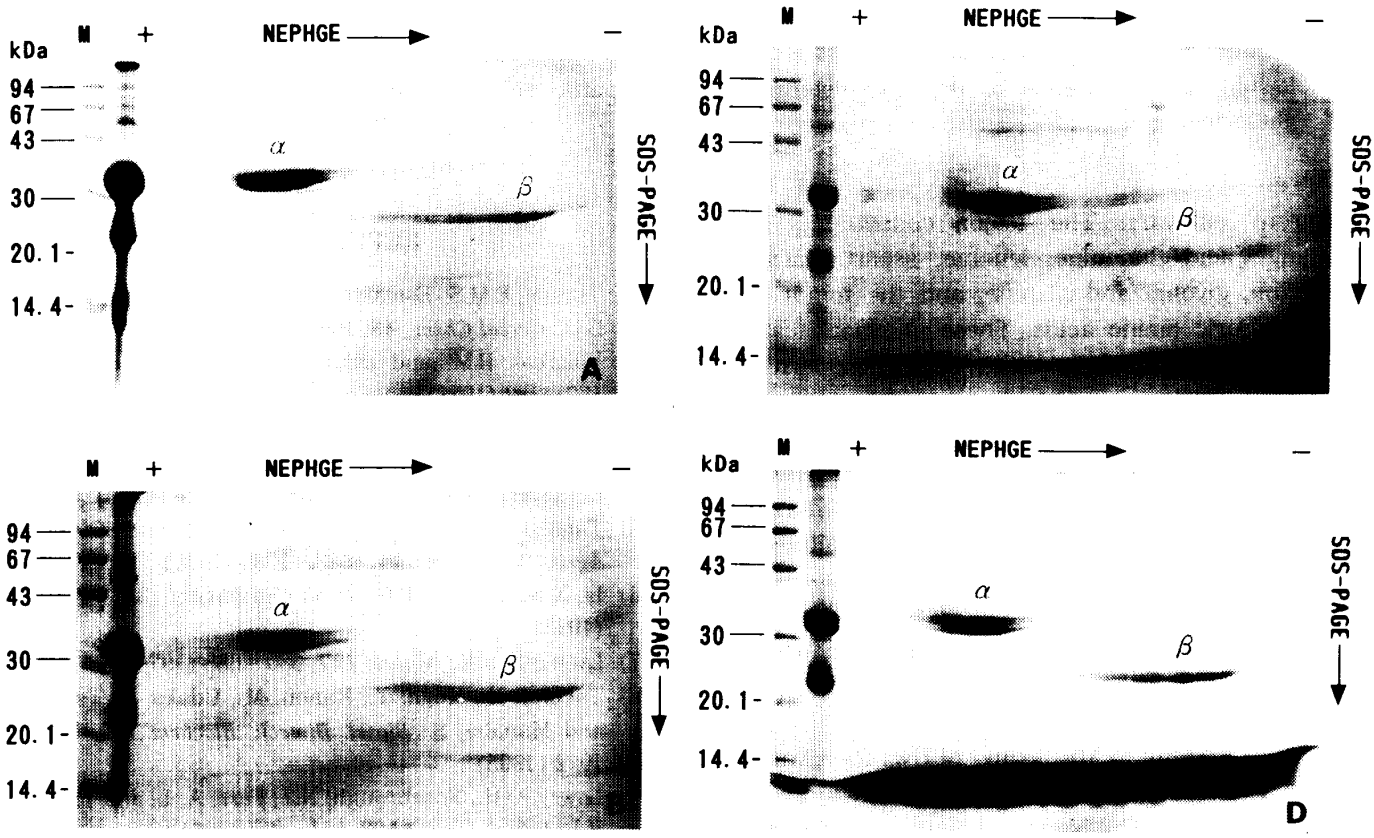

Fig. 5. Two-dimensional electrophoresis of rice glutelins extracted with 2% SDS from the 50% propanol-insoluble residue of various cultivars

Each glutelin was dissolved in the lysis buffer (5 mg/300 μl), and centrifuged, the resulting supernatant (20 μl) then being applied to a vertical gel. NEPHGE was conducted in the first-dimension, and second-dimension electrophoresis was carried out by SDS-PAGE. Panel A: *Koshihikari* c.v.; panel B: *Benimiyako* c.v.; panel C: *Murasaki-kuro* c.v.; panel D: Akita-kuro c.v.

Table 2. Amino acid compositions of the glutelins from defatted ancient rice flour

Amino acid	mol%					
	Black glutinous c.v.			Red non-glutinous c.v.		
	Akita-kuro	*Murasaki-kuro*	*Kuro*-K	*Takaramichi*	*Benimiyako*	*Kunitsukasa-chuukann*
Asp/Asn*	8.2	8.7	8.4	7.7	8.0	8.0
Thr	4.0	4.5	5.8	3.8	4.4	3.9
Ser	6.4	6.2	6.5	7.1	6.6	6.8
Glu/Gln**	17.9	17.7	17.3	17.4	17.1	18.7
Pro	5.8	4.3	7.0	6.2	5.6	4.6
Gly	8.3	8.8	8.0	7.9	9.0	7.8
Ala	8.4	7.7	7.2	7.9	8.2	8.5
Val	7.2	7.2	7.0	7.0	6.8	7.1
Cys	0.3	0.2	0.2	0.2	0.2	0.1
Met	0.2	0.2	0.3	0.3	0.2	0.1
Ile	4.9	5.3	4.7	5.0	4.8	5.1
Leu	8.7	8.4	7.8	9.1	8.1	9.4
Tyr	2.6	2.6	2.3	3.4	2.7	2.6
Phe	5.1	5.4	5.0	5.2	4.8	5.2
Lys	2.8	3.4	3.5	2.6	3.3	2.5
His	1.8	1.9	2.0	2.1	2.2	2.0
Arg	7.5	7.6	7.1	7.2	8.1	7.5

Results are the means of duplicate determinations. *Aspartic acid plus arginine. **Glutamic acid plus glutamine.

different cultivars. The major constituents were glutamic acid/glutamine, leucine, aspartic acid/asparagine, glycine and alanine, and the minor ones were sulfuric amino acids. These chemical features are almost identical to those of the modern rice glutelins.[1,2,15]

The present work has identified a variable pattern in the rate of accumulation of storage proteins during seed development between ancient and modern rice cultivars, with a few ancient cultivars containing one apparently unique polypeptide subunit. It was also found that rice glutelins were more selectively soluble in SDS solvents than in urea or in acidic or basic agents.

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古代米種子蛋白質の蓄積とグルテリンのポリペプチド組成

宇高順子，宅見賢二，辻　英明*，小川　正*
（愛媛大学教育学部，*徳島大学医学部）

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種子生育期間における古代米貯蔵蛋白質の蓄積とグルテリンのポリペプチド組成について，SDS-PAGE と2次元電気泳動により現代米との比較研究を行った．種子胚乳への蛋白質の一般的な蓄積パターンを，87 kDa ペプチドに対する量比でみた場合，分子量 21，32 kDa の二つのグルテリンと 15 kDa プロラミンが開花後7～10日で現れた後，徐々に増加し開花後 15～25 日で平衡に達した．しかし，古代米の1品種では異なるパターンを示し，開花後7日でそれらの蛋白質が急速に増加しはじめ，20日で最高に達しその後急速に減少した．その際，開花後 20 日目における蓄積蛋白質の最高値は他の米の蓄積パターンのそれの約2倍であった．米グルテリン画分の可溶化では，酸，塩基，尿素溶液よりも SDS 溶液でより選択的に溶出された．いくつかの古代黒米グルテリンには他品種では欠損または僅少なポリペプチド（19 kDa）がみられ，古代黒米に固有の蛋白質種であることが示唆された．古代米グルテリンの2次元電気泳動を行った結果，古代米，現代米共に α（酸性）および β（塩基性）の二つのサブユニットを確認した．19 kDa は検出できなかった．

キーワード：米，古代米，現代米，種子蛋白質，グルテリン，ポリペプチド組成．