The storage proteins of rice seed are accumulated in two types of particles called PBs. The type I PB (PB-I) stores indigestible protein prolamin, whereas the type II PB (PB-II) mainly contains digestible protein glutelin (Bechthel and Juliano 1980; Tanaka et al. 1980). There are morphological differences between PB-I and PB-II. PB-I is a small spherical particle with a lamellar structure of 1–2 μm in diameter, and PB-II is an amorphous and highly electron-dense structure of 2–3 μm in diameter (Tanaka et al. 1980; Yamagata and Tanaka 1986).

In the low-glutelin rice LGC1, the content of prolamin increased, while the content of glutelin decreased (Iida et al. 1993, 1998). In sake brewing, proteins in rice seed are an important factor imparting the sake flavor, but too much protein produces an undesirable flavor. Furthermore, a morphological characteristic of brewer’s rice is its white core structure, referred to as ‘shinpaku’ in Japanese. Previously, the hybrid rice Tashu-kei 1001, which has a low content of digestible protein glutelin and a white core structure, was crossed between the low-glutelin rice LGC1 and brewer’s rice by WeNARC, and its use as a raw material for sake brewing was investigated (Mizuma et al. 2002).

Furthermore, using immunofluorescent-labeling with anti-glutelin and anti-prolamin antibodies with a confocal laser scanning microscope, we previously visualized the distribution of rice storage proteins and showed that low-glutelin rice differs from general cultivar rice not only in the composition of major storage proteins but also in the transgranular distribution of storage proteins (Furukawa et al. 2003). However, there have been no reports related to the differences in the morphology and/or distribution of storage proteins (protein bodies) between the endosperm cells of low-glutelin rice and general rice cultivars. The objective of this study was to obtain further knowledge about low-glutelin rice with the purpose of increasing its use in sake brewing. Therefore, we observed the morphological characteristics of the storage proteins in endosperm cells of polished rice (degree of milling, 70%; used as raw material for sake brewing) of two low-glutelin rice varieties, LGC1 and Tashu-kei 1001, and two general rice cultivars (brewer’s rice), Hyogokitanishiki and Yamadanishiki, using a transmission electron microscope.

The four cultivars of *Oryza sativa* L. used in this study were harvested in 2000. Brown rice was polished to 70% of its original weight using a milling machine (THE GRAIN-TESTING MILL; Satake, Higashhihiroshima). The content of crude protein in brown rice and polished rice (degree of milling, 70%) was analyzed using a near-
infrared reflectance analyzer (InfraAlyzer 500; Bran+Lubbe, Tokyo). As shown in Table 1, the content of crude protein in the brown rice of the low-glutelin rice LGC1 was as low as that of the brewer’s rice cv. Yamadanishiki. The content of crude protein in the brown rice of Tashu-kei 1001 (a hybrid between LGC1 and Hyogokitanishiki) was close to the median value of LGC1 and Hyogokitanishiki. The content of crude protein in polished rice was the lowest in Yamadanishiki and about 5% in the other cultivars.

Polished rice grains were crushed with a roller mill (Retsch; Nihonseiki Kaisha Ltd., Tokyo), and rice storage proteins were extracted with 500 μl of an SDS-urea solution (8 M urea, 4% SDS, 5% 2-mercaptoethanol, 20% glycerol, and a 50 mM Tris-HCl buffer (pH 6.8)) at 60°C for 2 h. SDS-PAGE was carried out according to the Laemmli method (Laemmli 1970). The gels were stained with Coomassie Brilliant Blue R-250. The content of PBs was determined using a densitometer (Densitograph AE-6920M; ATTO, Tokyo). The content of PB-II (glutelin and globulin) in two varieties of low-glutelin rice, Tashu-kei 1001 and LGC1, was approximately reduced to two-thirds that of Hyogokitanishiki and Yamadanishiki. The content of PB-I (prolamin) was approximately twice that of Hyogokitanishiki and Yamadanishiki, in accordance with a previous report (Iida et al. 1993).

For TEM, the outer layer of polished rice grains (degree of milling, 70%) was sliced with a razor blade and fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) at room temperature for 3 h. The fixed samples were rinsed several times with a 0.1 M phosphate buffer (pH 7.4) and dehydrated in a graded series of ethanol concentrations. The samples were then embedded in acrylic resin (LR white Resin; Okenshoji Co., Ltd., Tokyo). Thin sections were prepared with an ultramicrotome (ULTRA CUT; LEICA, Wetzlar, DE.) equipped with a glass knife and stained with 2% uranyl acetate and lead citrate. The stained sections corresponding to the surface layers of the grains were examined using a TEM (JEM-1220; JEOL, Tokyo).

Figure 2 shows photographs of the TEM observations and a schematic of the observation area. In the outer layer of polished rice of Hyogokitanishiki (Figure 2C) and Yamadanishiki (Figure 2D), PB-IIs could be mainly observed in endosperm cells. In contrast, there were few PB-IIs in the low-glutelin rice LGC1, whereas there were many PB-Is (Figure 2B), in this observation. PB-I in LGC1, seen as small spherical particles of less than 2 μm in diameter, was slightly smaller than those in other cultivars. The ultrastructure of Tashu-kei 1001 of low-glutelin rice for sake brewing (Figure 2A) differed from that of Hyogokitanishiki as a paternal line. In Tashu-kei 1001, PB-IIs were barely evident; however, PB-Is were quite evident as small spherical particles, as in the case of the LGC1 maternal line, in this observation. PBs in LGC1 and Tashu-kei 1001 had different electron density, however we had no known its cause. Further studies are needed to clarify it.

This study demonstrates that low-glutelin rice has a different composition of major storage proteins and a different transgranular distribution of storage proteins, as well as a different distribution of PBs in the endosperm cells from those of general cultivars. The most remarkable difference is that low-glutelin rice has a

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Locality</th>
<th>Class</th>
<th>Content of crude protein (g/100 g dry seed)</th>
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<td></td>
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<td>Polished rice</td>
</tr>
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<td>Low-glutelin rice for sake brewing</td>
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</tr>
<tr>
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<td>WeNARC</td>
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<tr>
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<td>Yamadanishiki</td>
<td>Hyogo</td>
<td>Brewer’s rice</td>
<td>7.12</td>
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</table>

Figure 1. Content of protein bodies in polished rice (degree of milling, 70%). White bars, content of PB-I (summation of 10kDa, 13kDa, 16kDa prolamin polypeptides); black bars, content of PB-II (summation of 22–23 kDa, 37–39 kDa glutelin polypeptides and 26 kDa globulin polypeptide). Error bars indicate SD with five times measurements.
specific endosperm cell structure containing many PB-Is even in the outer layer of polished rice grains (degree of milling, 70%). Tashu-kei 1001 of low-glutelin rice for sake brewing inherits morphological traits from a paternal line, cv. Hyogokitanishiki, but it inherits the trait of the composition of major storage proteins (Mizuma et al. 2002) and the transgranular distribution of storage proteins from LGC1. From this study, it was confirmed that Tashu-kei 1001 also inherits the trait of distribution of PBs in endosperm cells from LGC1.

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References


Figure 2. TEM observation of PBs of rice endosperm cells. (A) Tashukei-1001; (B) LGC1; (C) Hyogokitanishiki; (D) Yamadanishiki. The schematic indicates the observation area.