Mutants for rice storage proteins

2. Isolation and characterization of protein bodies from rice mutants

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Summary. Rice storage proteins of the endosperm are localized in two types of protein bodies, PB-I and PB-II. Protein bodies were isolated by sucrose density gradient centrifugation from developing endosperm of three rice mutants, CM 21, CM 1675 and CM 1834, and characterized after pepsin-digestion treatment by protein contents determination. Mutant protein bodies (PBs) except for their internal structure, were similar in shape and density to PB-I of the variety Kinmaze. Electrophoretic analysis of PB-I polypeptides revealed that SDS (Sodium dodecysulfate) bands of 13 and 16 kilodaltons consisted, respectively, of four and two individual polypeptides with different pI values, while the 10-kilodalton band behaved as a single polypeptide after isoelectric focussing (IEF) electrophoresis. The differences in the polypeptide composition induced by mutants were due to the decrease and/or increase in the content of specific PB-I polypeptides. Electron microscopic observations revealed that the typical lamellar structure of the PB-I is not visible in CM 1675. On the contrary, the inner portion of PB-I in CM 1834 and CM 21 showed higher electron density than that of the variety Kinmaze. On these two mutants, the content of pepsin-indigestible and -digestible proteins were similar to those of Kinmaze, although the values of the PB-II/PB-I ratio were greater than those for Kinmaze, suggesting that these two mutants are high-glutelin rice mutants.

Key words: Endosperm – Mutant – Oryza sativa L. – Protein body – Storage protein

Introduction

Rice storage proteins in the starchy endosperm are localized in two types of protein bodies called PB-I and PB-II (Tanaka et al. 1980). The major rice storage protein, glutelin, consists of groups of 20- and 40-kilodalton polypeptides and is deposited in PB-II (Tanaka et al. 1980; Zhao et al. 1983; Wen and Luthe 1985; Krishnan and Okita 1986; Krishman et al. 1986; Tanaka and Ogawa 1986). The proteins present in PB-I belong to the 13-kilodalton polypeptide group, and are prolamin in nature (Tanaka et al. 1980; Ogawa et al. 1987). In addition, PB-I contains polypeptides with apparent molecular masses (MMr) of 10 and 16 kilodaltons. Proteins present in PB-I and PB-II account, respectively, for 20% and 60% of the total proteins of rice starchy endosperm (Ogawa et al. 1987).

Because of its high lysine content, the nutritional value of rice protein is superior to other cereal proteins such as those of wheat, barley and maize (Juliano 1985). Rice grains, however, contain indigestible fecal protein particles (FPP) (Tanaka et al. 1975a, b). Recently, Ogawa et al. (1987) found that FPPs are actually PB-I. If the digestibility of PB-I proteins can be improved, this would increase the overall nutritional value of rice protein. Hino et al. (1989) purified the 10-, 13- and 16-kilodalton polypeptides and determined their amino acid compositions. They also found that the 13-kilodalton prolamins consisted of no less than three polypeptides with different pI values, and that one of them contained more lysine than the other two. According to their data, the sulfur-
containing amino acid contents of the 10- and 16-kilodalton polypeptides were higher than that of other rice proteins.

We have screened rice mutants induced by the treatment of fertilized egg cell with N-methyl-N-nitrosourea, focussing on variants affecting protein compositions of PB-I and PB-II. Four mutant lines – CM 21, CM 1675, CM 1834 and CM 1787 – indicated also as 13b-L, 10/13a-L, 10/16-H and 57-H, were isolated (Kumamaru et al. 1988). In the 13b-L mutation, the contents of the 13b polypeptide was relatively low, while 10/13a-L had lower amounts of 10 kilodaltons and 13a polypeptides. In the 10/16-H mutation, the contents of both the 10- and 16-kilodalton polypeptides increased, while 13b decreased. The 57-H mutation showed a higher content of 57-kilodalton polypeptide and lower amounts of glutenin, compared to Kinmaze. The 13b-L and 57-H mutations are controlled by single recessive genes (Kumamaru et al. 1987), while 10/13a-L and 10/16-H mutations show maternal inheritances (Kumamaru et al. 1989). Unlike the case of CM 1787, which affects PB-II, CM 21, CM 1675 and CM 1834 possibly induce modifications of the polypeptides of PB-I.

The present study considered the purification and characterization of PB-I from the mutants CM 21, CM 1675 and CM 1834. The specific proteins in PB-I and PB-II in each mutant were also determined.

Materials and methods

Plant materials

Rice mutants were obtained as described by Satoh and Omura (1979, 1981). Mutants CM 21, CM 1675, and CM 1834 were grown in water culture (Yoshida et al. 1976) and 15 to 20-day-old developing grains were used for the isolation of protein bodies.

PB isolation. PBs were isolated by sucrose density gradient centrifugation and purified by pepsin digestion treatment (Ogawa et al. 1987).

Electrophoretic analysis of proteins in purified PBs. Purified PBs were suspended in IEF solution (O'Farrell 1975), sonicated for several minutes to denature proteins and centrifuged at 15,000 rpm for 15 min. The supernatant was subjected to IEF electrophoresis, and the resultant IEF gel was loaded on to a 14% polyacrylamide gel and electrophoresed, as described elsewhere (Kumamaru et al. 1988).

Electron microscopic observation. Isolated PBs were fixed with 0.2% glutaraldehyde and 4% paraformaldehyde in 20 mM piperazine-N,N-bis (2-ethane sulfonic acid) (PIPES) (pH 7.0) for 2 h on ice, followed by postfixing with 1% osmium tetroxide for 1 h on ice. The fixed samples were rinsed with the same buffer and dehydrated with ethanol solution. These samples were then embedded in Spurr's low viscosity resin after immi-

Protein analysis of PB-I and PB-II by pepsin-digestion treatment.

Results and discussion

The three rice mutants, CM 21, CM 1675 and CM 1834, were known to have different glutenin and prolamin contents when compared to wild types, as well as different polypeptide compositions in a molecular size range of 10, 13 and 16 kilodaltons (Kumamaru et al. 1988). To better clarify these differences, we isolated and purified PBs from each mutant by sucrose density gradient centrifuga-