

《Review》

Rice Allergenic Proteins

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Food allergy is an adverse reaction to food defined as the reaction in which an immune response can be demonstrated,¹⁾ and allergens can be defined as substances that provoke the allergic reactions. In IgE-mediated food allergy, the allergens are proteins found in the food. The allergenic foods tend to be commonly consumed with comparatively high protein contents, especially food of animal or marine origin. However, not all proteins contained in food can play a part as allergens. The major factors allotting the allergic sensitization to a particular food protein are the characteristics of the protein itself.

Rice is a grain produced and consumed in large quantities around the world, especially in Asia, and is staple food for Japanese. But there have been only a few reports on rice allergy. Some atopic patients show positive RAST values for rice grain proteins.²⁾ Several clinical studies have suggested that rice grains were responsible for severe atopic dermatitis in some patients.³⁻⁵⁾

Rice grain contains proteins accounting for 8% of the dried endosperm, most of which are storage proteins accumulated in protein bodies. Most plant storage proteins in seed tissues are used as nitrogen, sulfur and carbon sources during the germination of development.

These proteins can be classified into four groups based on their solubility. Depending on the analytical method, genotype and environmental condition, rice seeds contain 4-10% salt-soluble proteins (globulin and albumin), 5-10% alcohol soluble proteins (prolamin) and 80-90% alkali soluble proteins (glutelin).⁶⁾ Fig.1 shows a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of fractionated rice seed proteins. Comparison of the total proteins with the proteins in each fraction demonstrates that glutelin is the major constituent of rice-endosperm proteins. The electrophoretogram also shows that the soluble proteins in the albumin and globulin fractions contain more diverse protein components than the insoluble proteins in prolamin and glutelin fractions. Shibasaki *et al.*⁷⁾ first showed that a high degree of allergenicity was found in a globulin fraction of rice seed

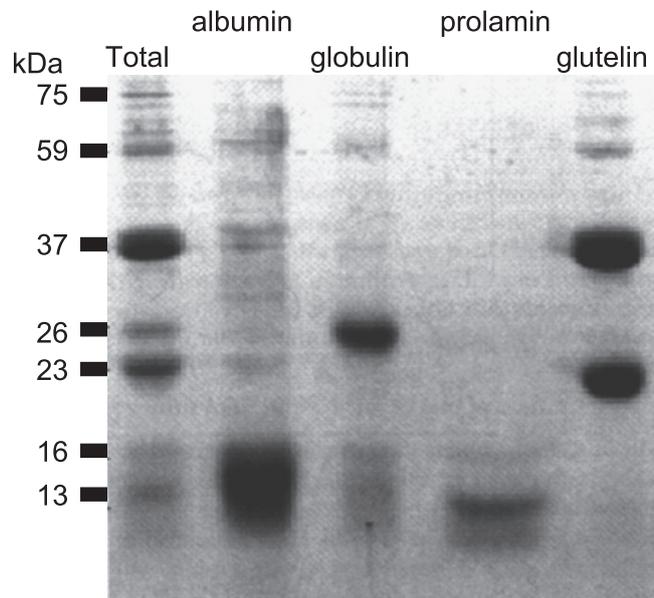


Fig. 1. Solubility-dependent fractionation of rice-seed proteins. Rice-endosperm proteins were fractionation into water-soluble (albumin), salt-soluble (globulin), alcohol-soluble (prolamin), and alkali-soluble (glutelin) fractions. The component proteins in each fraction, as well as seed total proteins, were analyzed by SDS-PAGE (CBB-staining).

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proteins, but the exact properties of rice allergenic proteins had not been clarified.

1. Separation of the Rice Allergenic Protein

To isolate the allergenic protein in rice grain, polished rice grain was extracted with 1M NaCl solution. The rice allergenic proteins were isolated from the extract by ion-exchange chromatography on DEAE cellulose column and gel filtration chromatography on Sephadex G-100, based on the reactivity with specific IgE from patients allergic to rice. The molecular weight of the purified allergenic protein was estimated to be about 16,000, named 16-kDa allergen.⁸⁾

The reactivity of the 16-kDa allergen was tested about 31 sera with positive RAST values for rice grain extract.⁹⁾ The sera examined all gave positive RAST values for the purified this 16-kDa allergen. Furthermore, there was a close correlation between RAST values for rice grain extract and those for purified 16-kDa allergen. These indicated that the 16-kDa allergen is the major allergenic protein in rice grain with response to IgE binding activity. The same result was indicated by the close correlation in the histamine-release activities between the 16-kDa allergen and rice grain extract.

The 16-kDa allergen was measured by using monoclonal antibody against that about various rice strains mainly provided by IRRI (International Rice Research Institute in Philippine).¹⁰⁾ All of the screened Japanese cultivars contain nearly the same amount of this protein.

2. Property of Rice Allergenic Protein

Since allergenic proteins obtained earlier often have high heat stability, experiments have been done about the effects of heating against the antigenicity of the 16-kDa allergen.⁸⁾ Antigenicity was measured using both human sera of rice allergenic patients and rabbit antisera against the 16-kDa allergen. Almost the same results were obtained from both experiments. No less than 60% of the allergenicity was still remained even after heating at 100°C for 60min. This high resistance for heat treatment may allow this protein to be a food allergen.

3. Primary Structure of Rice Allergenic Protein

A cDNA clone encoding the 16-kDa allergen was first isolated from cDNA libraries of maturing rice seeds by screening with a ³²P-labeled synthetic oligonucleotide mixture corresponding to N-terminal amino acid of the 16-kDa allergen and its nucleotide sequence was analyzed.¹¹⁾ The deduced amino acid sequence of 16-kDa allergen showed a similarity to the members of cereal α -amylase/trypsin inhibitor family. In Fig.2, the deduced amino acid sequence of 16-kDa allergen is compared with two members of family. The most prominent feature of the amino acid sequence of this family is the ten conserved cysteine residues that are all considered to be present as cystine in 16-kDa allergen. Among them, both wheat and barely α -amylase/trypsin inhibitors were recently identified major allergens associated with baker's asthma.^{12,13)} This seems to show that the proteins belonging to α -amylase/trypsin inhibitor family may be potential allergens.

4. Structural Characterization of Rice Allergenic Protein

A 16-kDa allergen belonging to the α -amylase/trypsin inhibitor family was isolated from rice seed and structurally characterized by identifying cystine-containing peptides and predicting secondary structure and

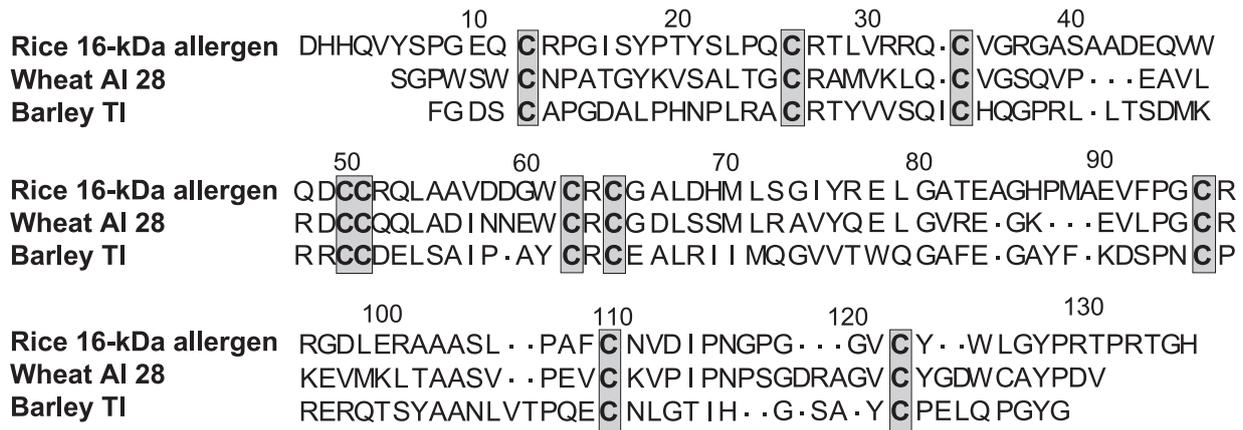


Fig. 2. Alignment of the deduced amino acid sequence of Rice 16-kDa allergen with sequences of α -amylase/trypsin inhibitor family proteins, namely, wheat α -amylase inhibitor (AI 28), barley trypsin inhibitor (TI). The sequences aligned for maximum homology, resulting in several gaps (shown as a dot) which may represent insertions/deletions. Conserved residues are shown in shaded region.

hydrophobic regions.¹⁴⁾ Eight peptides, which constitute three sets of cystine-containing peptide, were purified by HPLC from thermolytic digest of 16-kDa allergen and identified by amino acid-sequence and -composition analyses, indicating five intramolecular disulfide bridges; Cys³⁴-Cys⁹⁴, Cys²⁶-(Cys⁵⁰ or Cys⁵¹)-Cys¹¹⁰ and Cys¹²-(Cys⁶² or Cys⁶⁴)-Cys¹²².

According to the results obtained in this study and to the previously reported disulfide cross-linking of wheat α -amylase inhibitor,¹⁵⁾ the polypeptide folding of the 16-kDa allergen was predicted and schematically drawn in Fig.3. The 16-kDa allergen appeared to be a globular and compactly folded molecule with five intramolecular disulfide bridges. The N- and C-terminal regions might cover the hydrophobic core region, and contribute to antigenic and/or allergenic epitopes of the 16-kDa allergen. Such a prediction would agree with the result that rabbit and mouse antibodies against the 16-kDa allergen recognized the N-terminal region of recombinant the 16-kDa allergen molecules.¹⁶⁾

The 16-kDa allergen is the member of a plant α -amylase/trypsin inhibitor family. Ten cysteine residues are conserved well within this family.¹¹⁾ The similarity of intramolecular disulfide bridges in addition to the sequence homology suggests that the proteins in this inhibitor family have a common folding profile, and conformational and physio-chemical properties. The 16-kDa allergen has been identified as an allergen in severe atopic dermatitis caused by rice ingestion,^{3,7)} and α -amylase inhibitors of wheat and barley seeds have also been identified as major allergens associated with baker's asthma caused by the inhalation of cereal flour.^{12,13)} Allergenic proteins in food have been suggested to be stable against heat and proteases.⁸⁾ The five intramolecular disulfide bridges of the 16-kDa allergen would stabilize the folding of the ordered structures, resulting in heat

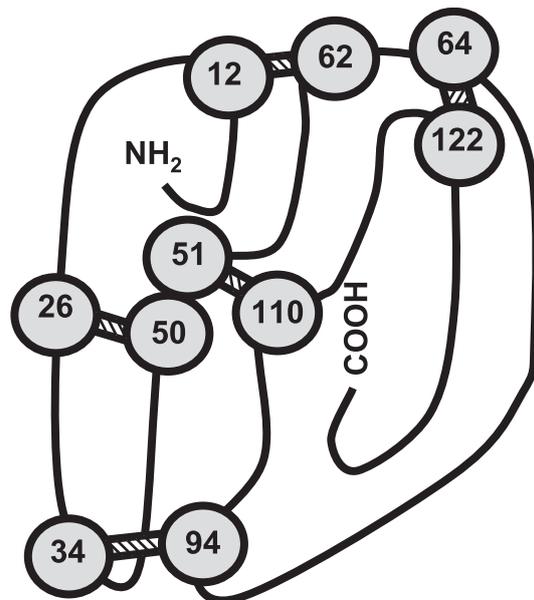


Fig. 3. Speculated folding profile of rice 16 kDa allergen. Cysteine residues are shown as open circles with residue numbers, and disulfide bridges are represented by striped bar between two circles.

stability and reversibility in heat-induced denaturation. The compactly folded structure of the 16-kDa allergen might also contribute to resistance to proteolytic degradation in alimentary tract. This stability against heat denaturation and proteolytic degradation would increase the allergenic potential of the 16-kDa allergen.

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和文抄録

米アレルゲンタンパク質

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近年、アレルギー患者が増加しており食物アレルギーも例外ではない。古くからよく知られる卵、牛乳に加えて最近では、植物性食品である大豆、米、小麦などに対するアレルギー症が増加している。

米アレルゲンについては、米タンパク質と米アレルギー患者血清中に存在する特異 IgE 抗体との反応性が調べられた。その結果、可溶性画分中の16-kDa のタンパク質と強く反応することが見出され、これを米主要アレルゲンとした。これまでに、この16-kDa アレルゲンについて構造解析を行った結果、16-kDa アレルゲンは、穀類や豆類の α -アミラーゼ/トリプシンインヒビターファミリーに属し、分子内に存在する10残基のシステインはすべて S-S 結合を形成し、小さく折りたたまれた構造をとっていることが明らかとなった。その構造安定性が、アレルゲン特有の性質であるプロテアーゼ耐性や熱安定性に寄与していることが示唆された。

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