

Cereal allergens: rice-seed allergens with structural similarity to wheat and barley allergens

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Cereals are food or food materials produced and consumed in large quantities in the world. Cereal allergy is a serious problem in that cereals are a staple food and a major nutrient source of not only carbohydrate but also protein, especially in Asian countries such as Japan. Many structural data including cDNA sequences on several cereal allergens have recently accumulated. We principally describe here the structural and immunologic properties of major rice allergens, and discuss the structural similarity of the rice allergens to wheat and barley allergens in the α -amylase inhibitor family.

Material and methods

For the isolation of rice allergens, commercially available dehulled grains of rice (*Oryza sativa* L. Japonica var. Nipponbare) were used. Rice proteins were extracted with 1 M NaCl solution, and allergenic proteins were isolated by ion-exchange chromatography, as described previously (1). The sera showing positive RAST values for rice, which were higher than the mean plus two standard deviations (SD) of those of control sera from healthy volunteers, were obtained from patients

asthma (2). A λ gt11 cDNA library was constructed with RNA extracted from maturing rice seeds harvested at 20 days after flowering (3).

Results and Discussion

Structure and IgE reactivity of rice 14–16-kDa allergens

Using an oligonucleotide probe to the N-terminal amino-acid sequence of the isolated allergen, cDNA, RA17, encoding the 16-kDa protein, was obtained from the cDNA library of maturing rice seeds (3). Furthermore, by persistent screening of rice seed cDNA libraries and using RA17 as a probe, we have isolated so far more than 10 independent positive cDNA clones, and have shown the sequences of these clones to be similar, encoding proteins consisting of 150–170 amino-acid residues. These results indicate that the 14–16-kDa proteins in the rice salt-soluble fraction are the products of a multigene family (4, 5). The percentage of identities among these sequences was 70–95% at the amino-acid level, and changes in amino-acid residues, including deletions and insertions, occurred more frequently in the upper

rice RA17	DHSG...VYS	PSEUGP...S	YVYSLPQCR	L...VPRQGV	RGASAGED	AVDGGWRCG	ALDHMLSGY	76
rice RA14	DHSGGVVYS	LSGRCQPGG	YVYSLPQCR	L...VPRQGV	TRSPGAVED	AVDGGWRCG	ALDHMLSGY	79
barley BDAI-1	SG	PNMWCAPG	HKVSLPQCR	L...VPRQGV	NRPG...ED	VLRDQDQEA	VYSNWDGCS	87
wheat WMAI-1	SG	PNMWCAPG	HKVSLPQCR	L...VPRQGV	SGVP...SEA	VLRDQDQEA	VYSNWDGCS	87
barley TI	SF	SDS-CAPGA	LPMPLPQCR	YVYSLPQCR	HQGRLLTSD	VRRDQDQEA	ALP-AVQDCE	88
maize TI	SA	GTS-CYDPA	LPMPLPQCR	YVYSLPQCR	GVRPLATSE	VRRDQDQEA	DIP-AVQDCE	88
Indian millet A/TI	SV	GTS-CYDPA	LPMPLPQCR	YVYSLPQCR	GVRPLATSE	VRRDQDQEA	DIP-AVQDCE	88

Fig. 1. Alignment of amino-acid sequences of seven cereal proteins in α -amylase/trypsin inhibitor family. AI, TI, and A/TI represent α -amylase inhibitor, trypsin inhibitor, and inhibitor of both enzymes. Amino-acid residues conserved among seven sequences are shaded.

rice 14–16-kDa potent allergens showed limited similarities to wheat and barley allergens belonging to a family of α -amylase inhibitors (Fig. 2), but their 10-cysteine residues were well conserved (Fig. 1).

The 14–16-kDa proteins were fractionated by ion-exchange chromatography, of which five proteins were further purified by reverse-phase HPLC (1). The determined N-terminal amino-acid sequences of these proteins were similar to each other, indicating that these 14–16-kDa proteins have structural similarity with microheterogeneity. Four of the five N-terminal sequences were identical to the deduced amino-acid sequences for the cDNA clones, while no cDNA clone has yet been obtained for the most basic component of about 14 kDa.

Three monoclonal antibodies, 25B9, 4D9, and 5D3, were obtained by immunizing mice with the rice 14–16-kDa proteins. The antibody 25B9 reacts to all of the five proteins isolated, while the other two antibodies do so with four of the five proteins. The reactivity to three independent monoclonal antibodies clearly indicates that these proteins are immunologically cross-reactive.

The reactivity of these five proteins to serum IgE antibodies was analyzed for patients with a high RAST score to rice (13). The sera from four patients contained IgE antibodies reacting to all of the five proteins, suggesting that these patients had been sensitized to each of the five proteins or had IgE antibodies specific for epitopes common to these allergens. On the other hand, the serum IgE

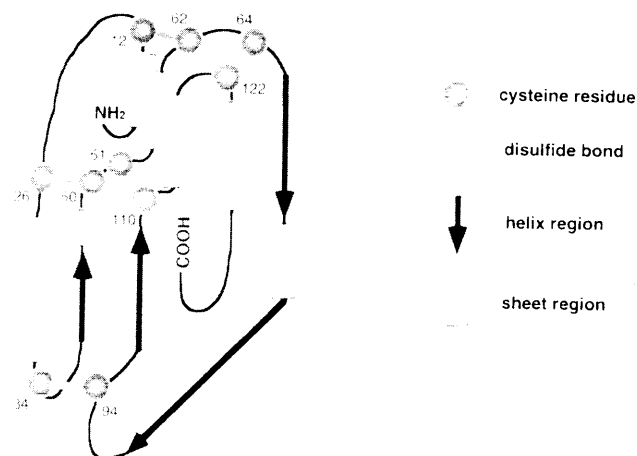
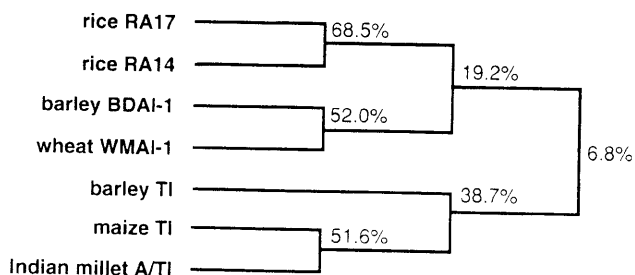


Fig. 3. Schematic view of rice 16-kDa allergen.

antibodies from seven patients reacted to the most basic protein but not to the other proteins, indicating that these patients' IgE antibodies distinguished minor structural differences between the most basic protein and the other ones. This result is in contrast to the fact that these 14–16-kDa proteins, including the most basic one, reacted to three monoclonal antibodies. The human polyclonal IgE antibodies in some patients might react to epitopes different from those recognized by these mouse monoclonal antibodies, or might recognize delicate distinctions in epitope structures between these 14–16-kDa proteins.

Structural similarity between rice and wheat allergens

Rice grains are the source of food allergens in atopic dermatitis, whereas wheat and barley flours are the source of inhalant allergens in asthma. Nevertheless, several proteins with a sequence similarity in a single protein family were identified as major allergens in rice, wheat, and barley. These proteins all contain 10 well-conserved cysteine residues, which form five intramolecular disulfide bonds. The polypeptide folding pattern, including disulfide bridges, predicted for the rice RA17 protein suggested that this allergen is a compactly folded protein with a small hydrophobic core, which is rich in sheet and helix structures (Fig. 3). The five disulfide cross-linkages would limit a degree of conformational freedom for the major part of the polypeptide backbone, and at the same time stabilize the allergen molecule against heat- and acid-induced denaturation.

to isolate other allergens in the salt-soluble fraction of rice proteins. A 33-kDa protein was isolated by a combination of chromatographic separations, and reactivity of the isolated protein to IgE antibodies was analyzed for 34 rice-sensitive patients. Most of the patients showed positive reaction to the 33-kDa protein, and a high correlation in the IgE reactivity was obtained between the 33-kDa protein and total salt-soluble proteins of rice, suggesting that the 33-kDa protein is one of the major allergens in rice grains.

Some partial amino-acid sequences were determined for proteolytic fragments of the isolated protein. By screening a maturing rice seed cDNA library with degenerated oligonucleotides as probes, we isolated a cDNA clone encoding the 33-kDa allergen (unpublished data). The amino-acid sequence deduced from the cDNA sequence showed a unique structure consisting of two tandemly repeated homologous domains with some well-conserved sequences. Several sequences for plant glyoxalase I were retrieved by a search of sequence databases, as these exhibit sequence similarity to the 33-kDa allergen.

Acknowledgments

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