Abbreviations: AAL, *Aleuria aurantia* lectin; HRP, horseradish peroxidase; CBB, Coomassie brilliant blue; BSA, bovine serum albumin; TBS-T, 20 mM Tris-HCl buffer (pH 7.3) containing 0.9% NaCl and 0.05% Tween 20

**Note**

Identification of a Wheat Allergen, Tri a Bd 36K, as a Peroxidase

Hiromi YAMASHITA,† Yoko NANBA, Miki ONISHI, Masumi KIMOTO, Miki HIEMORI, and Hideaki TSUJI

Department of Nutritional Science, Faculty of Health and Welfare Science, Okayama Prefectural University, 111 Kuboki, Soja-shi, Okayama 719-1197, Japan

Received May 9, 2002; Accepted July 5, 2002

A 36-kDa allergen, Tri a Bd 36K, was purified from wheat albumin and characterized. The protein was similar to barley peroxidase BP-1 both in its amino acid sequence and peroxidase activity. The enzyme seemed to contain L-fucose and D-mannose and the glycan moiety reacted with IgE antibodies in a patient’s serum.

Key words: wheat allergen; Tri a Bd 36K; lectin; peroxidase

Food allergy is a critical social problem. Egg, milk, wheat, buckwheat, and shrimp have been reported to be representative allergy-inducing foods in Japan. The allergens in eggs and milk have been characterized. Inhalation of wheat flour can cause baker's asthma, an occupational allergic disease. Baker's asthma is mainly a type I allergy in which IgE antibodies specific to the allergen appear. James et al. has found that a 15-kDa protein responsible for allergic reaction after ingestion of wheat is an α-amylase inhibitor; it is a sensitizing allergen whether it is ingested or inhaled. IgE antibodies reactive to a number of wheat-flour components have been detected in sera from allergic bakers. Tanabe et al. has shown that the allergens are in the gluten fraction of wheat and that an epitope of the allergens contains a QQQPP motif. Maruyama et al. also showed that the motif reacted with patients’ sera. Salcedo and colleagues have reported that a strong major allergen that elicits anaphylactic symptoms found in some bakers belongs to the family of α-amylase inhibitors and that a glycosylated form of some members may be more potent than the nonglycosylated form. Earlier, we examined wheat proteins responsible of wheat allergy in 65 wheat-sensitive patients and found 15 antigens recognized by IgE antibodies in the patients’ sera. Among those allergens, Tri a Bd 17K has been identified as α-amylase inhibitor CM16, with an Asn-linked sugar chain. Weiss et al. reported that reactivity was strongest with the fraction containing albumin and globulins in which the α-amylase inhibitor family is included; however, characterization of the albumin and globulins has not been reported. More information about these allergens is of importance, not only for the development of better diagnostic methods, but also for the prevention of this disease by the use of less allergenic flour.

In this study, we focused on wheat albumin, purifying a 36-kDa allergen from this fraction and characterizing it. The precipitate that formed with 30–70% (NH₄)₂SO₄ from 200 mg of albumin was collected by centrifugation at 10,000 × g for 30 min, dissolved in 20 mM Tris-HCl buffer (pH 8.0; buffer A), dialyzed against buffer A, and put on an SP-Sepharose column (5 ml) equilibrated with the same buffer. After being washed with buffer A, the proteins on the column were eluted successively with buffer A containing 0.9, 0.8, 0.7, 0.5, 0.3 M (NH₄)₂SO₄, or without (NH₄)₂SO₄. The fractions containing the allergen were pooled, dialyzed against 50 mM sodium phosphate buffer (pH 7.4) containing 0.15 M NaCl, concentrated with an Amicon filter, and put on a gel-filtration column (P30) equilibrated with the same buffer. After gel filtration, the 36-kDa protein was purified to near homogeneity (Fig. 1-A, B). The N-terminal amino acid residues of the purified allergen obtained were found to be AEPPVARGLXFDFYR, which was similar to the N-terminal amino acid sequence of barley peroxidase BP-1, AEPPVARGLXFDFYR. We then assayed the purified allergen by the method of Putter, and found it to have peroxidase activity (0.21 unit/mg protein). Sanchez-Monge et al. reported this allergen previously, but it has not been characterized in detail. Many allergens from major
Crops are defence-related proteins such as the members of the α-amylase/trypsin inhibitor family. The wheat peroxidase might function in defence-related system as well.

Garcia-Casado et al.16) showed that the glycan moieties of α-amylase inhibitors are involved in their binding to IgE antibodies in the sera of patients. Some pollens have antigenicity or allergenicity because of the glycan moieties of their glycoproteins.17–20) The glycan moieties of Tri a Bd 36K was examined with several lectins that recognize specific glycan moieties, and this allergen reacted with Aleuria aurantia lectin (AAL), specific for α-L-fucose, and concanavalin A (ConA), specific for α-D-mannose (Fig. 1-C), suggesting that this 36-kDa protein is a glycoprotein and may contain L-fucose and D-mannose. To find if the glycan moiety of Tri a Bd 36K was involved in its binding activity to IgE antibodies, we investigated the effects of a patient's serum on the binding of AAL with Tri a Bd 36K. AAL recognized the allergen (Fig. 2-A1), but the binding of AAL to Tri a Bd 36K was reduced when the membrane was preincubated with the patient's serum (Fig. 2-A2). The binding of rabbit polyclonal anti-horseradish peroxidase (HRP) antibodies, which recognize N-linked glycan moieties with an α1,3-fucose branch, to Tri a Bd 36K was also inhibited by preincubation with the patient's serum21–23) (Fig. 2-B). These findings indicate that the binding of IgE antibodies in patient's serum might be closely related to a glycan moiety containing L-fucosyl residue in the Asn-linked sugar chain of the protein as was seen for α-amylase inhibitor CM16.

Food-specific IgE antibodies bind to high-affinity IgE receptors (FcεRI) on mast cells or basophils. When food allergens bind to IgE antibodies on these cells, mediators like histamine that elicit symptoms of immediate hypersensitivity are released in a type I IgE-mediated reaction. For IgE-mediated reactions by a type I allergy, allergens that bind with IgE antibodies on the cells must contain at least two epitopes.24) Whether one or all of these epitopes are related to the glycan moiety is not known. Elucidation of the chemical structure of these epitopes in major crops would help to achieve the hypoallergenization of these crops for susceptible subjects.
References


23) Wilson, I. B., Harthill, J. E., Mullin, N. P., Ashford, D. A., and Altmann, F., Core α1,3-fucose is a key part of the epitope recognized by antibodies reacting against plant N-linked oligosaccharides and is present in a wide variety of plant extracts. *Glycobiology*, 8, 651–661 (1998).