

Note

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

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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.^{1,2} 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.³ IgE antibodies reactive to a number of wheat-flour components have been detected in sera from allergic bakers.^{4–7} 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.⁸ Maruyama *et al.* also showed that the motif reacted with patients' sera.⁹ 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.^{10,11} 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.¹² Among those allergens, Tri a Bd 17K has been identified as α -amylase inhibitor CM16, with an Asn-linked sugar chain.¹² Weiss *et al.* reported that reactivity was strongest with the fraction containing albumin and globulins⁶ 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% $(\text{NH}_4)_2\text{SO}_4$ from 200 mg of albumin was collected by centrifugation at $10,000 \times g$ 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 50 mM, 0.1 M, 0.2 M, and 1 M NaCl. $(\text{NH}_4)_2\text{SO}_4$ was added to the active fraction to a final concentration of 1 M, and the mixture was put on a Phenyl-Sepharose column (4 ml) equilibrated with 10 mM sodium phosphate buffer (pH 7.4) (buffer B) containing 1 M $(\text{NH}_4)_2\text{SO}_4$. After being washed with the same buffer, the proteins on the column were eluted successively with buffer B containing 0.9, 0.8, 0.7, 0.5, 0.3 M $(\text{NH}_4)_2\text{SO}_4$, or without $(\text{NH}_4)_2\text{SO}_4$. 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, AEPPVAPGLSFDFYR.¹³ We then assayed the purified allergen by the method of Putter,¹⁴ and found it to have peroxidase activity (0.21 unit/mg protein). Sanchez-Monge *et al.*¹⁵ reported this allergen previously, but it has not been characterized in detail. Many allergens from major

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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

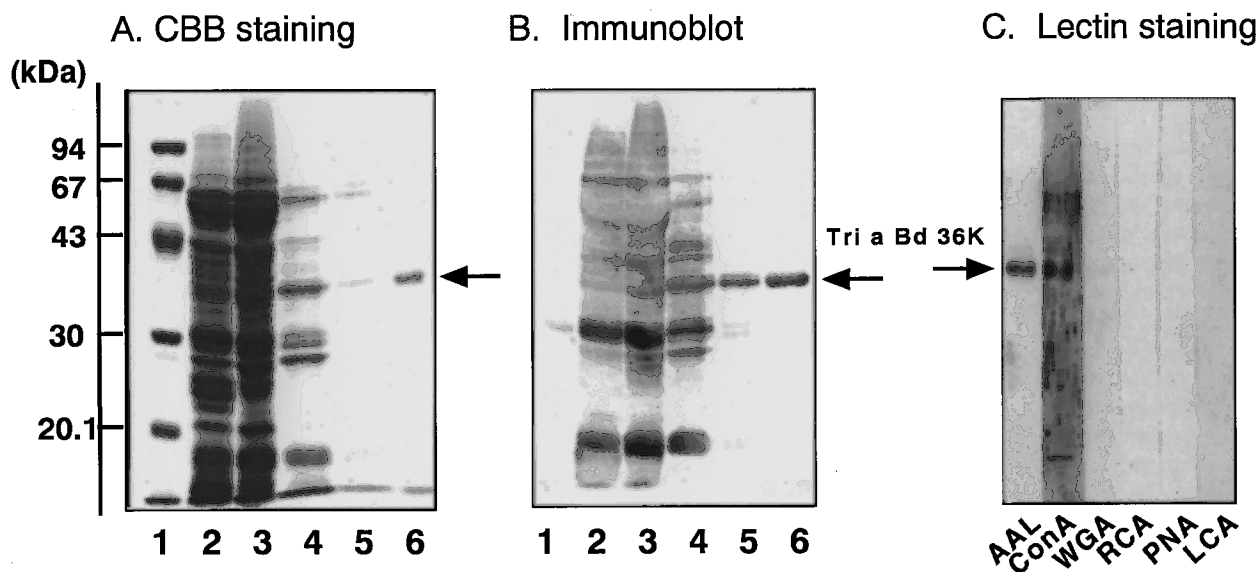


Fig. 1. SDS-PAGE (A), Immunoblotting of the Purified Allergen with Serum of a Wheat-sensitive Patient (B), and the Reactivity with Various Lectins (C).

A, B: Lane 1, molecular weight markers; lane 2, wheat albumin fraction; lane 3, 30–70% ammonium sulfate precipitate; lane 4, SP-Sepharose column eluate; lane 5, Phenyl-Sepharose column eluate; lane 6, P-30 gel filtration fraction. (A) Proteins in the samples were separated on a 12% polyacrylamide gel and stained with Coomassie brilliant blue (CBB). (B) After electrophoresis, the proteins on the gel were electroblotted onto nitrocellulose membranes. After blocking of the membranes with 1% bovine serum albumin (BSA) in 20 mM Tris-HCl buffer (pH 7.3) containing 0.9% NaCl and 0.05% Tween 20 (TBS-T) at room temperature for 1 h, the membranes were incubated with a patient's serum diluted 1:4 with TBS-T containing 1% BSA for 16 h at 4°C. The membranes were next treated with biotinylated anti-human IgE for 1 h at room temperature, and then treated with peroxidase-conjugated avidin at room temperature for 1 h. The immunocomplexes on the membranes were detected by treatment with 4-chloro-1-naphthol and H₂O₂ as the substrates. (C) After SDS-PAGE, a nitrocellulose membrane onto which Tri a Bd 36K was electroblotted was cut into several strips, and the allergen on each strip was reacted with one of the biotinylated lectins (2 µg/ml) indicated in the figure (AAL, *Aleuria aurantia* lectin; ConA, concanavalin A; WGA, *Triticum vulgaris* lectin; RCA, *Ricinus communis* lectin; PNA, *Arachis hypogaea* lectin; LCA, *Lens culinaris* lectin). The membranes were treated with peroxidase-conjugated avidin at room temperature for 1 h, and then the immunocomplexes on the membranes were detected by treatment with 4-chloro-1-naphthol and H₂O₂.

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 α 1,3-fucose branch, to Tri a Bd 36K was also inhibited by preincubation with the patient's serum^{21–23} (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 (Fc ϵ RI) 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.

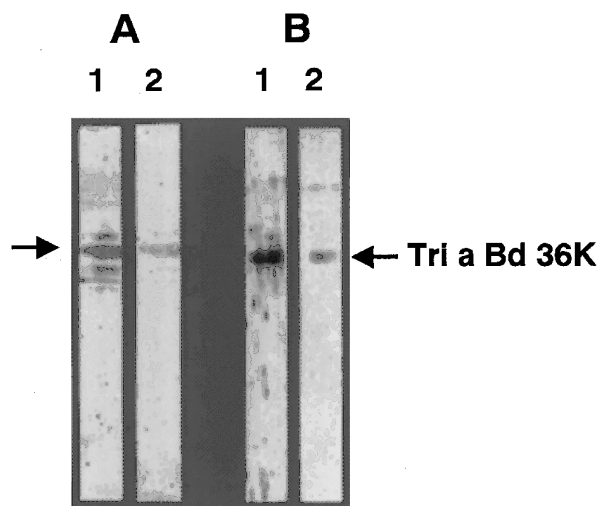


Fig. 2. Inhibition by Patient's Serum of Reactivity of Tri a Bd 36K with AAL and Anti-Horseradish Peroxidase.

Tri a Bd 36K was electroblotted onto a nitrocellulose membrane and the membrane was cut into four strips. Two of the strips were treated with AAL (A1) or anti-horseradish peroxidase (HRP) (B1), and the other two were incubated with patient's serum and then treated with AAL (A2) or anti-HRP (B2). The reaction with AAL was as in the legend of Fig. 1-C. For the reaction with anti-HRP, the membranes were incubated with anti-HRP (20 μ g/ml) at room temperature for 1 h, and then with peroxidase-conjugated anti-rabbit IgG at room temperature for 1 h. The immunocomplexes on the membranes were detected by treatment with 4-chloro-1-naphthol and H_2O_2 .

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