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Note

Measurement of *Gly m* Bd 30K, a Major Soybean Allergen, in Soybean Products by a Sandwich Enzyme-linked Immunosorbent Assay

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By a sandwich enzyme-linked immunosorbent assay, a soybean major allergen, *Gly m* Bd 30K, in soybean products was measured. The allergen occurred at high concentrations in soy milk, *tofu*, *kori-dofu*, and *yuba*, but its content in *kinako* was small. No allergen was found in fermented foods such as *miso*, *shoyu*, and *natto*. The allergen was clearly shown to occur in meat balls, beef croquettes, and fried chicken that contained soybean protein isolate.

Food allergy has become a critical social problem. The allergens in egg and milk have been discovered, but those in soybean have been little studied. In the course of the investigations on soybean allergens with the sera of soybean-sensitive patients with atopic dermatitis, we found 16 allergenic proteins in the soybean.[1] About two-thirds of soybean-sensitive patients examined was shown to have IgE antibodies binding to the 30-kDa protein named *Gly m* Bd 30K. This finding suggests that *Gly m* Bd 30K is a major allergen in the soybean and that it may be a target allergen for hypoallergenization of soybean products. *Gly m* Bd 30K has been purified to homogeneity and shown to be identical with the 34-kDa oil body-associated protein.[2-5] For the development of hypoallergenic soybean products, it is essential to establish a method for the detection and measurement of the allergen in the products. We prepared two monoclonal antibodies (mAbs, F5 and H6) against the allergen and used them in a sandwich enzyme-linked immunosorbent assay (ELISA) system of the allergen.[6] In this paper, we describe the measurement of a major soybean allergen, *Gly m* Bd 30K in various soybean products using the sandwich ELISA.

Soybean and its products used in this work were soybean seed (*nagayukiha*), soy milk, *tofu* (*kinugoshi*), *tofu* (*momen*), *kori-dofu*, *kinako*, *abura-age*, *yuba*, *miso*, *natto*, and *shoyu*. Processed foods containing soygean protein isolate (SPI) such as meat balls, beef croquettes, fried chicken, fish sausage, and hamburger were obtained from commercial sources. The preparation of the two mAbs (F5 and H6) was described in our previous paper.[6] Peroxidase-conjugated F5 was prepared by the method of Nakane and Kawaoi.[7] *Gly m* Bd 30K was prepared from soybean seeds as described previously.[2] Soybean products (5 g) were homogenized in 50 ml of 50 mM sodium phosphate buffer (pH 8.0) containing 1% sodium dodecyl sulfate (SDS) and 20 mM mercaptoethanol (buffer A) with a Polytron homogenizer and centrifuged at $10,000 \times g$ for 30 min. The supernatants were used as the extracts of the soybean products. SDS in the extracts was shown to inhibit the antibody-antigen reaction in the ELISA system. Therefore, the extracts were further incubated with 1 M KCl and then cooled in an ice bath. After removal of crystalline potassium dodecyl sulfate by centrifugation at $10,000 \times g$ for 10 min, the supernatants were used for the sandwich ELISA. The sandwich ELISA was done in the same manner as described earlier.[6] The extracts obtained from *yuba*, meat balls, and beef croquettes has some interference of the assay of their *Gly m* Bd 30 K. When these extracts were filtered on a Sephacryl S-200 gel column (0.7×75 cm), most of the interferring materials were eluted more slowly than *Gly m* Bd 30 K and the remainder was eluted faster than the allergen. Therefore, the extracts were put on the Sephacryl S-200 column, the fractions including the allergen were pooled, and the allergen in the fractions was assayed by the sandwich ELISA. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was done by the method of Laemmli.[8] Immunoblotting was done in the same manner as described in our previous paper.[9]

The amounts of the allergen in the soybean products were measured as described above and are summarized in the Table. Of the products tested, soybean seed, soy milk, *tofu* (*kinugoshi*), *tofu* (*momen*), and *kori-dofu* were shown to contain high concentrations of the allergen. The fact that the allergen occurs at high concentrations in the products suggests that most of the allergen from soybean remains unchanged during the processing. The native allergen was shown to be insensitive to trypsin and chymotrypsin and heat treatment did not reduce its allergenicity (data not shown). This is compatible with the observation that the IgE-binding ability of the allergen is very stable during the

Table Contents of *Gly m* Bd 30 K in Soybean Products

Product	*Gly m* Bd 30 K	
	mg/g fresh weight	mg/g nitrogen
Soybean seed	7.29±0.66	126±12
Soy milk	0.67±0.03	108±5
Tofu (*kinugoshi*)	0.94±0.11	89±5
Tofu (*momen*)	0.82±0.06	65±5
Kori-dofu	5.54±0.69	64±8
Kinako	1.82±0.11	29±2
Abura-age	2.53±0.43	59±9
Yuba	4.68±0.54	66±8
Miso	n.d.[a]	n.d.
Shoyu	n.d.	n.d.
Natto	n.d.	n.d.
Meat balls[b]	0.30±0.11	17±6
Beef croquettes[b]	0.20±0.05	21±5
Fried chicken[b]	0.14±0.17	9±11
Fish sausage[b]	n.d.	n.d.
Hamburger[b]	n.d.	n.d.

[a] Not detected.
[b] The manufacturers informed that these products contained soybean material (SPI), except for hamburger.

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SPI, soybean protein isolate.

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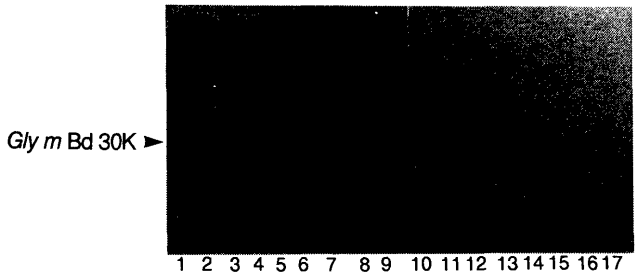

Fig. Immunoblots of *Gly m* Bd 30 K in Soybean Products.

Proteins (3 μg/lane) in the extracts of soybean products and authentic *Gly m* Bd 30 K (0.1 μg/lane) were separated by SDS–PAGE and then the proteins on the gels were electrophoretically transferred on nitrocellulose membranes. The allergen on the membranes was detected with F5 as described in the text. Samples: 1, authentic *Gly m* Bd 30 K; 2, soybean seed; 3, *soy milk*; 4, *tofu* (*kinugoshi*); 5, *tofu* (*momen*); 6, *kori-dofu*; 7, *kinako*; 8, *abura-age*; 9, *yuba*; 10, *miso*; 11, *shoyu*; 12, *natto*; 13, meat ball; 14, beef croquette; 15, fried chicken; 16, fish sausage; 17, hamburger.

processing of the products. *Kinako* contained less allergen than the above-mentioned soybean products, although it is produced by parching soybean seeds. SDS–PAGE of the proteins in *kinako* showed that most of the proteins in the soybean seeds were polymerized by parching, indicating that the epitopes on the allergen against the mAbs might be modified during the polymerization of the proteins in soybean seeds and that the mAbs could not bind to the modified epitopes. Fermented soybean products such as *miso*, *shoyu*, and *natto* had no allergen. These results suggest that the allergen in the products has been rapidly hydrolyzed by the proteinases of microorganisms during the fermentation. On the other hand, interestingly, the allergen could be detected in meat balls, beef croquettes, and fried chicken that contained SPI, but the other SPI-containing processed foods, fish sausage, and hamburger, were shown to contain no allergen. The manufacturers informed us that meat balls, beef croquettes, fried chicken, and fish sausage, but not hamburger were supplemented with soybean materials as an ingredient. The findings that meat ball, beef croquette, and fried chicken contained definite amounts of the allergen is compatiple with the manufactures' information. However, the fact that the allergen could not be detected in the fish sausage suggests that the allergen from soybean may be destroyed during the processing, although it is uncertain whether the soybean materials added to be the product were free from the allergen.

We further examined the allergen in the soybean products by an immunoblotting technique with one of our mAbs, F5. As shown in the Fig. soybean seed, *tofus*, *kori-dofu*, *kinako*, and *yuba* gave intensive bands corresponding to the allergen, but the allergen could not be detected in *miso*, *shoyu*, *natto*, hamburger, and fish sausage. Definite bands of the allergen were clearly observed in the immunoblots of meat balls, beef croquettes, and fried chicken. These results confirm the results obtained with the sandwich ELISA. This study provides useful information not only to soybean-sensitive patients with regard to the estimation of the allergenicity of soybean products but also in the development of hypoallergenic soybean products.

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