



Note

Characterization of New 18-kDa IgE-Binding Proteins in Beer

Miki HIEMORI,^{1,†} Yuka EGUCHI,¹ Masumi KIMOTO,¹ Hiromi YAMASITA,¹
Kyoko TAKAHASHI,² Kiyoshi TAKAHASHI,³ and Hideaki TSUJI¹

¹Department of Nutritional Science, Faculty of Health and Welfare Science,
Okayama Prefectural University, Soja, Okayama 719-1197, Japan

²Department of Food Science and Nutrition, School of Human Environmental Sciences,
Mukogawa Women's University, Nishinomiya, Hyogo 663-8558, Japan

³Department of Allergy, National Hospital Organization Minami-Okayama Medical Center,
Tukubo, Okayama 701-0304, Japan

Received September 12, 2007; Accepted December 12, 2007; Online Publication, April 7, 2008
[doi:10.1271/bbb.70584]

The IgE-binding proteins in beer were examined by immunoblotting analysis with sera of patients sensitive to beer. Several proteins were immunoblotted with the sera, and among these, 18-kDa proteins were identified as new IgE-binding proteins in beer. Perhaps they originated from barley as a raw material.

Key words: new IgE-binding protein; 18-kDa proteins; beer; barley; IgE antibody

In recent years, only a small number of allergic patients have been reported to be sensitive to beer. The proteins responsible for the allergic symptoms induced by beer have been reported to exist in malt, barley, rice, corn, and wheat.¹⁻⁵⁾ 10-kDa, 16-kDa, and 45-kDa proteins from barley were found to be allergens in beer.¹⁻³⁾ Among these, the 10-kDa and 16-kDa allergens have been investigated, and were identified as lipid transfer protein I and α -amylase inhibitor respectively,¹⁻³⁾ but other allergens in beer, including a 45-kDa protein, are not well understood. In the present study, we identified new IgE-binding proteins in beer with sera of patients sensitive to beer.

Beer samples (500 ml), purchased from four representative brewer companies in Japan, were degassed, and solid ammonium sulfate was added to the beer samples to 100% saturation in order to concentrate the proteins in the samples. The precipitate formed was collected by centrifugation at $10,000 \times g$ for 30 min. The precipitate was dissolved in 25 ml of 10 mM Tris-HCl buffer, pH 8.6 (buffer A), and then dialyzed against buffer A. For identification of the IgE-binding proteins in the beer, the proteins in the dialysate were concentrated by fractionation with ammonium sulfate and by Q sepharose chromatography. Briefly, solid ammonium sulfate was added to the dialysate to 40% saturation.

The precipitate formed, in which the IgE-binding proteins were confirmed to be localized by immunoblotting with the sera of beer-sensitive patients, as described below. It was collected by centrifugation, dissolved in buffer A, and dialyzed against the same buffer. The dialysate was applied to a Q sepharose column (2.0×50 cm) equilibrated with buffer A. The column was washed with 300 ml of buffer A, and then the proteins adsorbed on the column were eluted with the same buffer, containing 100 mM NaCl. The eluate containing the IgE-binding proteins was pooled and concentrated by ultrafiltration with a PM-10 membrane.

The proteins obtained from the four beer samples were separated by SDS-PAGE by the method of Laemmli and transferred onto nitrocellulose membranes.^{6,7)} Subsequently, the IgE-binding proteins on the membrane were detected by immunostaining in the manner described in a previous paper.⁷⁾

The sera of three patients sensitive to beer and/or wheat, with RAST values of 1-4, which were used in this work, were obtained from National Hospital Organization at the Minami-Okayama Medical Center (Okayama, Japan) and from the University of Tokushima Faculty of Medicine (Tokushima, Japan). These patients suffered from urticaria. The above sera were diluted to between 1/5 and 1/20 and used in the subsequent experiments. Myeloma IgE antibody was utilized as a negative control. Informed consent was obtained from the patients. The experiments were done in accordance with ethical standards as formulated in the Helsinki Declaration.

The IgE antibodies in the serum of a representative beer-sensitive patient gave an intensive band corresponding to a protein with a molecular mass of 18 kDa, and a faint band corresponding to a protein with a molecular mass of 10 kDa (Fig. 1). These beer samples

[†] To whom correspondence should be addressed. Tel/Fax: +81-86-694-2143; E-mail: hiemori@fhw.oka-pu.ac.jp

Abbreviations: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride

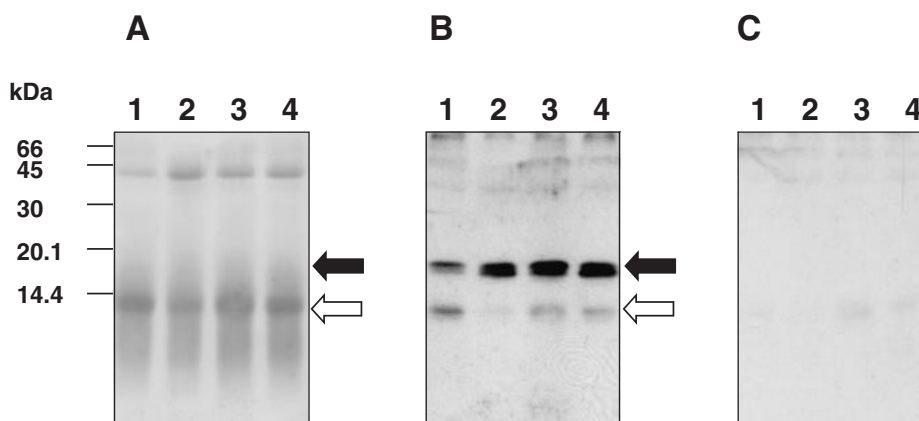


Fig. 1. Immunoblot of Proteins in Commercial Beers with Serum of a Patient Sensitive to Beer.

The proteins obtained from beers were electrophoresed on 17.5% polyacrylamide gels and electroblotted to two nitrocellulose membranes. Then the membranes were stained with Ponceau S (A), and immunoblotted with serum of a patient sensitive to beer (B) and myeloma human IgE antibodies (C), as described in the text. Lanes 1–4 correspond to the proteins in each beer from four domestic brewing companies. The closed arrows represent bands corresponding to the 18-kDa protein recognized by the IgE antibodies in the serum. The open arrows represent bands corresponding to the 10-kDa protein detected by the IgE antibodies in the serum. Standard protein markers are shown on the left side of panel A.

also gave similar patterns on immunoblot with the sera of the other two beer-sensitive patients. These two proteins were found to be recognized by both the sera of the three beer-sensitive patients and those of cereals-sensitive patients (data not shown). Among the beer samples, sample 1 was the beer consumed in the largest quantity in Japan recently. Hence sample 1 was used as the representative sample in the subsequent experiments. The 18-kDa protein moved above the position corresponding to the α -amylase inhibitor, with a molecular mass of about 16 kDa on SDS-PAGE, suggesting that it might have been different from the inhibitor.

In order to elucidate further the properties of these proteins, the IgE-binding proteins were subjected to two-dimensional PAGE (2D-PAGE). This was performed according to the manual of Multiphor II 2-D of Pharmacia (Uppsala, Sweden). Briefly, proteins were separated on short instruction immobiline dry strips, pH 3–10, by isoelectrophoresis focusing with the Multiphor II Electrophoresis Unit, after which the strips were subjected to SDS-PAGE, as described above.

The 18-kDa IgE-binding protein was found to give six spots, corresponding to proteins (proteins 1–6) with different isoelectric points (pIs) and different molecular masses, and the 10-kDa protein was also found to separate into two components (proteins 7 and 8), with pI values of 8.3 and 7.3 (Fig. 2). Proteins 1–3 and 4–6 appeared to belong to two groups with the pI values of 7.3 and 8.3 respectively. Of the six proteins, proteins 1, 2, 4, 5 were found shown to have sugar moieties by glycan analysis using G. P. Sensore (Honen, Tokyo), a kit for the detection of the sugar moiety of glycoprotein (data not shown). The above-mentioned heterogeneity of the 18-kDa IgE-binding proteins can be explained by the occurrence of the glycan moieties, but the heterogeneity of the IgE-binding proteins should be further elucidated.

In order to determine the N-terminal amino acid

sequences of proteins 1–8, they were separated into clear, distinct spots by 2D-PAGE with larger slab gels, as described in Fig. 2, after which the proteins separated were electrophoretically transferred onto PVDF membrane in the manner described above. The spots corresponding to the proteins positive to the sera of allergic patients were cut off, and the N-terminal amino acid sequences of the proteins on the spots were analyzed with an Applied Biosystems, 473 protein sequencer (Applied Biosystems, Foster City, CA). The N-terminal amino acid sequences of proteins 1–8 were as follows: Protein 1, LDAAGHQLMR; 2, LDAAHGQLYR; 3, LDAAHGQLYR; 4, LQAAGHQLMR; 5, LQAAGHQLMR; 6, LQAAGHQLMR; 7, ADGHQLMRQQ; and 8, LNHGQVDSKMKP-QLTYVQGG. The results indicate that all of the 18-kDa proteins (proteins 1–6) and the 10-kDa protein with a pI value of 8.3 (protein 7) were highly homologous with one another, but homology analysis with FAST software indicated that these seven proteins did not exhibit significant homology with any known proteins. These findings indicate that these components are unknown IgE-binding proteins and that they belong to the same protein family. On the other hand, protein 8, the 10-kDa protein with a pI value of 7.3, was highly homologous (90%) with that of the lipid transfer protein 1 (LTP1: accession no. X60292) from barley, suggesting that protein 8 is likely to be LTP 1.

It is very important to determine from which raw material, barley or yeast, the 18-kDa IgE-binding proteins originate. In the present study, the proteins in wheat were also examined with regard to their allergenicity using the sera of beer-sensitive patients, because such sera have been reported to react with the proteins in wheat,⁴⁾ and because barley grains been found to contain allergens similar to those in wheat, such as α -amylase inhibitor nakan⁸⁾ and peroxidase.⁹⁾ As shown in Fig. 3,

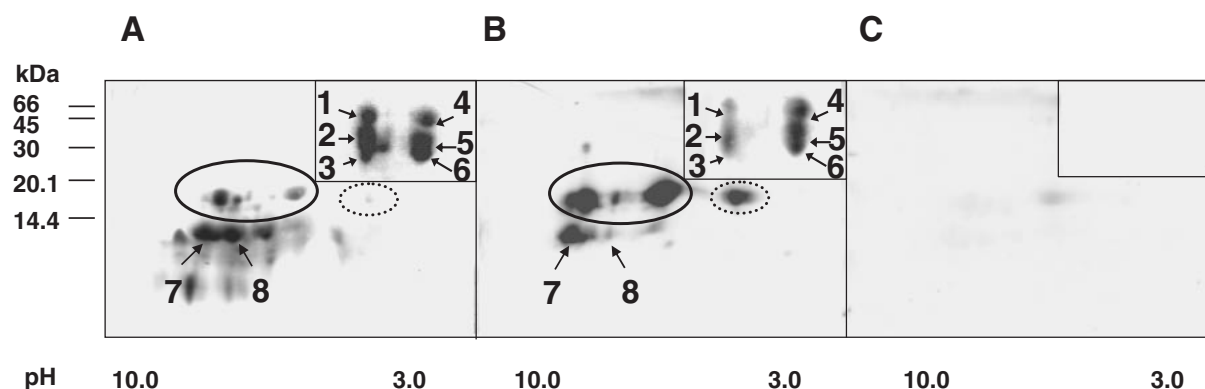


Fig. 2. 2D-PAGE and Immunoblot of the Proteins in Beer.

Proteins obtained from beer sample 1 were separated by two-dimensional electrophoresis (pH 3.0–10.0), as described in the text. The proteins on these slab gels were electroblotted to nitrocellulose membranes, and the membranes were stained with Ponceau S (A) and then immunostained with IgE antibodies in the serum of the beer-sensitive patient (B), or with myeloma IgE antibody, used as a control (C). The spots corresponding to the 18-kDa proteins are enclosed by the solid circle. The spots corresponding to the 10-kDa proteins are indicated by arrows. In order to separate more clearly the protein spots corresponding to the 18-kDa protein, SDS-PAGE of the proteins in sample 1 was done with larger slab gels (160 × 160 mm) than the ones (106 × 100 mm) used in SDS-PAGE. The patterns obtained are shown in the inside panels in panels A, B, and C. Spots 1–8 in panel A, corresponding to spots 1–8 in panel B respectively, were detected by the serum of the beer-sensitive patient. Spots 7 and 8 in panel A correspond to the respective 10-kDa proteins positive to the serum, which are shown in panel B. The proteins corresponding to spots 1–8 were electroblotted onto a PVDF membrane, cut out, and subjected to N-terminal amino acid sequencing. Standard protein markers are shown on the left side of panel A.

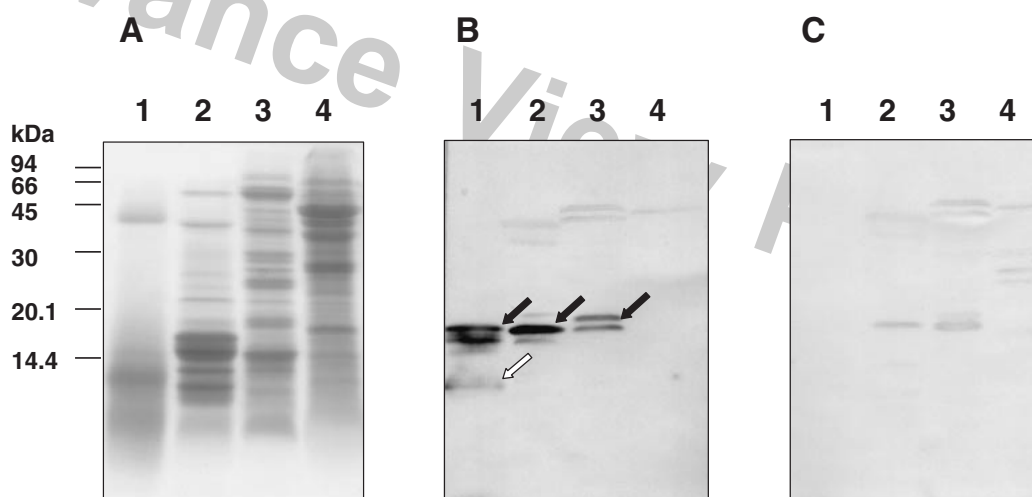


Fig. 3. Immunoblot of Proteins in the Raw Materials of Beer with the Serum of the Beer-Sensitive Patient.

Proteins extracted from the raw materials of the beer were electrophoresed on 17.5% polyacrylamide gels and electroblotted onto nitrocellulose membranes. Then the membranes were stained with Ponceau S (A), and immunostained with the serum of the beer-sensitive patient (B) or myeloma human IgE antibodies (C). Lane 1, beer; lane 2, barley; lane 3, wheat; lane 4, beer yeast. The closed arrows represent the 18-kDa proteins. The open arrow represents the 10-kDa protein. Standard protein markers are shown on the left side of panel A.

the occurrence of a protein with a molecular mass of 18 kDa was observed in immunoblots obtained with both barley proteins and wheat proteins. When the barley proteins were applied to the Q sepharose column, the 18-kDa protein was adsorbed on the column like that in beer, whereas that in wheat flew through without adsorption on the column. These observations suggest that the 18-kDa IgE-binding proteins in beer might originate in barley.

The 18-kDa protein in barley was further elucidated by 2D-PAGE and immunoblotting (data not shown). It

was found to give six spots, which corresponded to proteins 1–6 in beer, and were immunostained with the sera of the patients sensitive to beer. The proteins corresponding to the six spots were named proteins 1'–6'. The N-Terminal amino acid sequences of the protein components 1'–6' in barley were as follows: Protein 1', LDDTHHQLYR; 2', LDDTHHQLYR; 3', LDDTHHQLYR; 4', LQAAGHQLMR; 5', LQAAGHQLMR; and 6', LQAAGHQLMR. These IgE-binding proteins in barley showed high homology (70–100%) with proteins 1–6 in beer. The origin of the barleys utilized in the

production of the beer samples remains unknown a manufacturing secret. The difference between the amino acid sequences of the IgE-binding proteins in the beer sample and barley grain would be explained if the barley used in the production of the beer sample was be different from that used in the present study. Although the amino acid sequencing of the proteins in barley and those in beer was done repeatedly, the same results were obtained. These findings suggest, again, that these IgE-binding proteins in beer may originate from barley.

In conclusion, the present study indicates that the 18-kDa proteins are major IgE-binding proteins in beer and that the proteins originate from barley. The 10-kDa protein with a pI value of 7.3 as another major IgE-binding protein was found to belong to the LTP family.

Acknowledgment

We are grateful to the Asahi Brewer Company for financial support of the present study.

References

- 1) Figueredo, E., Quirce, S., del Amo, A., Cuesta, J., Arrieta, I., Lahoz, C., and Sastre, J., Beer-induced anaphylaxis: identification of allergens. *Allergy*, **54**, 630–634 (1999).
- 2) Curioni, A., Santucci, B., Cristaudo, A., Canistraci, C., Pietravallo, M., Simonato, B., and Giannattasio, M., Urticaria from beer: an immediate hypersensitivity reaction due to a 10-kDa protein derived from barley. *Clin. Exp. Allergy*, **29**, 407–413 (1999).
- 3) Garcia-Casado, G., Crespo, J. F., Rodriguez, J., and Salcedo, G., Isolation and characterization of barley lipid transfer protein and protein Z as beer allergens. *J. Allergy Clin. Immunol.*, **108**, 647–649 (2001).
- 4) Herzinger, T., Kick, G., Ludolph-Hauser, D., and Przybilla, B., Anaphylaxis to wheat beer. *Ann. Allergy Asthma Immunol.*, **92**, 673–675 (2004).
- 5) Vally, H., and Thompson, P. J., Allergic and asthmatic reactions to alcoholic drinks. *Addict. Biol.*, **8**, 3–11 (2003).
- 6) Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).
- 7) Hiemori, M., Ito, H., Kimoto, M., Yamashita, H., Nishizawa, K., Maruyama, N., Utsumi, S., and Tsuji, H., Identification of the 23-kDa peptide derived from the precursor of Gly m Bd 28 K, a major soybean allergen, as a new allergen. *Biochim. Biophys. Acta*, **1675**, 174–183 (2004).
- 8) Armetia, A., Sanchez-Monge, R., Gomez, L., Barber, D., and Salcedo, G., *In vivo* allergenic activities of eleven purified members of a major allergen family from wheat and barley flour. *Clin. Exp. Allergy*, **23**, 410–415 (1993).
- 9) Yamashita, H., Nanba, Y., Onishi, M., Kimoto, M., Hiemori, M., and Tsuji, H., Identification of a wheat allergen, Tri a Bd 36 K, as a peroxidase. *Biosci. Biotechnol. Biochem.*, **66**, 2487–2490 (2002).