

Note

Primary Structure of an Allergenic Peptide Occurring in the Chymotryptic Hydrolysate of Gluten

Michiko WATANABE, Soichi TANABE, Takashi SUZUKI, Zenro IKEZAWA,* and Soichi ARAI**

Food Science Laboratory, Faculty of Education, Tokyo Gakugei University, Koganei-shi, Tokyo 184, Japan

*Department of Dermatology, Yokohama City University School of Medicine, Minami-ku, Yokohama 232, Japan

**Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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An allergenic peptide was isolated from the chymotryptic hydrolysate of gluten by gel filtration and HPLC. The primary structure of the peptide was determined as Ser-Gln-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Pro-Pro-Phe. The amino acid sequence similarity shows that the peptide originated from a low-molecular-weight glutenin chain.

The number of patients suffering from food allergy is increasing throughout the world.¹⁾ Cereal-associated allergy is particularly considered to be a serious problem, because cereals are consumed as the staple food in most countries. To better understand this problem, we first developed a process for producing a hypoallergenic rice preparation.^{2–4)} This products has been approved as a physiologically functional food for specified health use by the Japanese Ministry of Health and Welfare, and is being commercially produced for patents with rice-associated allergy. Subsequently, we proposed a procedure for producing hypoallergenic wheat flour by the enzymatic decomposition of gluten, to which many patents with wheat-associated allergy are sensitive.⁵⁾ The hypoallergenic flour produced by the enzymatic process, however, had a poor food functionality and needed to be processed into an easy-to-eat food. We then developed methods to prepare noodles and bread from the hypoallergenic flour.⁶⁾ In that study, baker's yeast could not be used, because it seems that many patients with wheat-associated allergy are also sensitive to yeast.

The objectives of this present work are to clarify the primary structure of at least one of the allergens and to show a structural homology between the allergen and a protein from baker's yeast.

Gluten from soft flour (Showa Sangyo Co.; commercial name: Cleopatra) was freeze-dried and then pulverized. The gluten powder was autoclaved before use. As reported in our previous paper,⁵⁾ a chymotryptic hydrolysate of gluten showed allergenicity, so we used an α -chymotrypsin preparation (0.5 g, Sigma Chemical Co., type II, 40–60 units/mg of protein) to obtain peptide fragments with allergenicity. The enzyme preparation was dissolved in water (10 ml) and then filtered through a 0.45 μ m pore-sized filter to obtain a cell-free filtrate. The filtrate was mixed with autoclaved water (2.5 liters) and the gluten powder (50 g). A hydrolytic reaction of the mixture was carried out at 37°C for 18 h by adjusting to pH 7 with 0.1 N NaOH. The resulting reaction product was heated at 60°C for 5 min to inactivate the enzyme and then centrifuged at 1000 $\times g$ for 20 min to obtain a supernatant. This supernatant, after vacuum concentration, was submitted to gel filtration.

A Sephadex G-50 column (4.9 $\times$ 45 cm, V_0 = 255 ml) was used for the gel filtration, the chymotryptic hydrolysate (3 g on a dry matter basis) being loaded for each run and eluted with 10% ethanol. Figure 1 shows a representative gel filtration pattern of

the chymotryptic hydrolysate of gluten. The eluate in 50-ml portions was collected as one fraction. Each fraction was freeze-dried, and the dried powder (50 mg) was dissolved in 0.1 M Tris-HCl (pH 8.6) containing 4 M urea (1 ml). The solution was then submitted to allergenicity measurement by ELISA, using the serum of a patient with wheat-associated allergy at Yokohama City University Hospital as mentioned in the previous paper.⁵⁾ The fraction indicated with an asterisk (Fig. 1) was found to be allergenic.

This allergenic fraction was chromatographed by the first HPLC run in an ODS column (Japan Spectroscopic Co.) at 22°C. A linear gradient of 0.1% trifluoroacetic acid-methanol (0 to 75% in methanol concentration) was used as the eluting solvent, and UV at 210 nm was used for detection. The ELISA data from the peaks in Fig. 2A indicated that the numbered peaks were allergenic. Peak 2 (with higher allergenicity) in Fig. 2A was further fractionated by the second HPLC run by modifying the solvent system in a gradient manner (45 to 60% with respect to methanol concentration). The asterisked peak (Fig. 2B) obtained by the second HPLC run was judged to be allergenic. This peak was chromatographed three times under the same conditions as those under which the second HPLC run was carried out.

The purified compound was submitted to a primary structural determination, using an amino acid sequencer (Applied Biosystems 477 A), the determined primary structure being Ser-Gln-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Pro-Pro-Phe. The amino acid sequence similarity between the allergenic peptide and other naturally occurring proteins was checked by using a search program (Fast Homology Search CDS, GenBank). Similarities of 70–90% were obtained between the sequence of the allergenic peptide and that of the low-molecular-weight

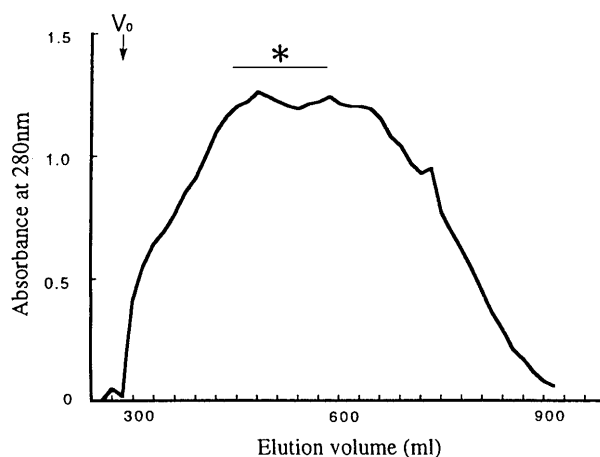


Fig. 1. Gel Filtration Pattern for the Chymotryptic Hydrolysate of Gluten.

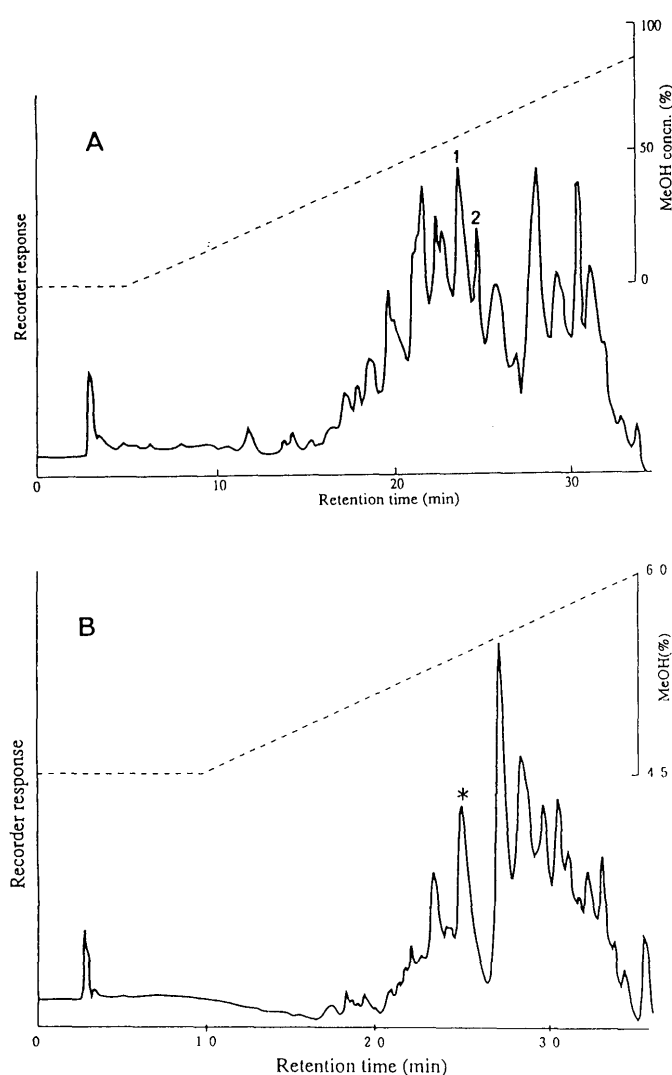


Fig. 2. HPLC of the Gel Filtrate (A) and of the First HPLC Eluate (B). ELISA values for peaks 1 and 2 in A were 0.08 and 0.16, respectively, and those of other peaks were less than 0.03. Only asterisk peak in B showed allergenicity.

glutenin precursor.⁷⁻¹⁰⁾ A high degree of similarity (53.6%) was also found between the sequence of the allergenic peptide and that of a *Saccharomyces cerevisiae* protein.¹¹⁾ This result suggests that many patients with wheat-associated allergy are sensitive to the baker's yeast.⁶⁾

We have previously reported that collagenase and transglutaminase were effective enzymes to produce a hypoallergenic wheat flour.⁵⁾ During the incubation of wheat flour in the presence of one of the enzymes, the peptide bond(s) involving Pro-Pro and/or Pro-Phe and the amide of the Gln residue(s) would be respectively hydrolyzed by collagenase and transglutaminase. Identification of the amino acid residues necessary as the epitope awaits further investigation.

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