

IgE-binding Abilities of Pentapeptides, QQPF and PQPF, in Wheat Gliadin

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Summary Wheat gliadin is known mainly as the causative substance for enteropathy accompanied by diarrhea (celiac disease) and food-dependent exercise-induced anaphylaxis. However, little is known in regards to the allergenicity of gliadin in atopic dermatitis. In this study, the allergenicity of gliadin was demonstrated using sera of wheat-allergic patients with atopic dermatitis. Since there are many repeated sequences in gliadin, peptides containing each motif were synthesized to evaluate their IgE-binding abilities. As a result, QQPF and PQPF were found to be epitopes (minimum structures for the IgE-binding). Gliadin might crosslink patient's IgE-antibodies via these epitopes and trigger subsequent allergic reactions.

Key Words allergen, epitope, wheat gliadin, repeated-sequence

Hypersensitive responses to wheat flour have long been an important public health problem. Wheat seeds are composed of four protein classes, such as water-soluble "albumin", salt-soluble "globulin", aqueous ethanol-soluble "gliadin", and urea-, detergent-, or alkali-soluble "glutenin". Wheat gluten is a complex of gliadin and glutenin. Gluten is the elastic rubbery protein that becomes dough in breads and other bakery products.

The adverse reactions to wheat flour develop at least four different phenomena, enteropathy (diarrhea), asthma, food-dependent exercise-induced anaphylaxis (FDEIA) and atopic dermatitis. Gluten-sensitive enteropathy is called "celiac disease", and is caused by ingestion of gliadin fraction (1). The reported prevalence of this disease is 1 : 300 to 1 : 1000 in European countries (2). The amino acid sequences responsible for the disease have been characterized, and the minimum epitope structures were found to be Pro-Ser-Gln-Gln (PSQQ) and Gln-Gln-Gln-Pro (QQQP) in gliadin (1).

The inhalation of wheat flour also often causes baker's asthma (3), a typical occupational allergic disease that has been known since ancient Roman times. Extensive studies identified some proteins as allergens associated with asthma. Among them, alpha-amylase inhibitors (AI) from globulin fraction were identified as major allergens (3–6). Acyl-CoA oxidase (7, 8), peroxidase (9), and fructose-bisphosphate aldolase (8) were identified as other allergens.

FDEIA is a severe (life-threatening) form of allergy in which the ingestion of a specific food before physical

exercise induces symptoms of anaphylaxis. Although various foods (including shellfish, celery, hazelnut, peanut, soy, pea, and banana) have been associated with FDEIA, the most frequently reported cause of these reactions seems to be wheat (10). The fraction responsible for FDEIA is thought to be gliadin (10).

The other wheat-associated phenomenon is skin inflammation, atopic dermatitis, which develops shortly after cereal-based products are ingested, usually resulting in eruption and itching. Wheat allergens associated with atopic dermatitis are so heterogeneous that many attempts had been made internationally to identify them (11–15). We have reported that a major epitope structure of gluten responsible for atopic dermatitis is Gln-Gln-Gln-Pro-Pro (QQQPP) motif in glutenin fraction (13, 14). However, the allergenicity of gliadin fraction and its epitope(s) is not clarified.

We report here the allergenicity of gliadin and IgE-binding abilities of pentapeptides, QQPF and PQPF, in gliadin sequence.

MATERIALS AND METHODS

Reagents

All reagents used in this study were of high grade.

Patients

Peripheral blood samples were obtained from eleven patients with atopic dermatitis and wheat allergy. Wheat allergy was proven by clinical symptoms and a positive radioallergosorbent test (CAP-RAST score: 5–6 for wheat). Informed consent was obtained from all donors. Serum from a non-allergic adult was also used as a negative control. In Figs. 1 and 2, pooled sera were used for enzyme-linked immunosorbent assay (ELISA) after appropriate dilution with pH 7.4 phosphate-buffered saline (PBS) containing 0.05% Tween 80.

Extraction of wheat allergens from wheat flour

Gluten was prepared from soft flour by usual method.

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Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; FDEIA, food-dependent exercise-induced anaphylaxis; PBS, phosphate-buffered saline (pH 7.4); UTH, 0.1 M Tris-HCl buffer containing 4 M urea (pH 8.6)

Table 1. List of gliadin peptides used in this study.

Sequence synthesized	Frequency (pentapeptide)*
PQQPF	14
PQQPQ	11
QPPQP	21
PQQP	—
QQFPF	17
QPF PQ	15
QQPF	—

As candidate epitopic peptides of gliadin, 7 peptides were synthesized essentially according to the solid phase method using a peptide synthesizer. Synthesized peptides were dissolved in DMSO at a concentration of 5 mg/mL for storage.

*The numbers indicate how many times the pentapeptides occur in gliadin sequence.

Subsequently, three volumes of 50% ethanol was added to the gluten sample and shaken for 1 h, followed by centrifugation at $1,000\times g$ for 20 min. After the supernatant was dialyzed against distilled water, the resulting product was freeze-dried and used as gliadin fraction. Glutenin was extracted from the pellet by use of 0.1 M, pH 8.6, Tris-HCl buffer containing 4 M urea (UTH buffer) in the same manner as done for gliadin preparation. *Synthesis of gliadin-derived peptides* (Table 1)

As candidates for epitopic peptides of gliadin, 7 peptides were synthesized essentially according to the solid phase method using a peptide synthesizer (PSSM-8, Shimadzu, Japan). QQQPP in glutenin and LILNR in bovine serum albumin (BSA) were also synthesized as positive and negative controls, respectively (14, 16). The synthesized peptides were applied to reversed-phase HPLC, and the purified peptides were lyophilized, dissolved in dimethylsulfoxide (DMSO) at a concentration of 5 mg/mL, and stored at -80°C until use.

ELISA for specific IgE antibodies

Gluten, gliadin and glutenin (0.1 mg each) were dissolved in 1.0 mL of UTH buffer, and their allergenicity was tested by ELISA (14, 17). Briefly, polystyrene micro-titer plates (96 wells, Nunc, Denmark) were coated with 100 μL of the above-mentioned solutions overnight at 4°C . Blocking was done with 200 μL of 2% BSA at 37°C for 1 h. A ten-fold diluted solution of the pooled sera was applied for assay, and IgE antibodies to peptides were detected using biotinylated anti-human IgE (Kirkegaard & Perry Lab. Inc., USA), streptavidin-peroxidase conjugate (Boehringer Mannheim, Germany), and *o*-phenylenediamine (Wako Pure Chemical Industries, Japan). Data in Figs. 1 and 2 were expressed in terms of absorbance at 492 nm.

For the evaluation of the synthetic peptides, their peptide solutions were diluted five-fold with distilled water, and then diluted ten-fold with 0.1 M carbonate buffer (pH 9). The resulting peptide solutions (100 μL) were covalently immobilized in the wells of plates (AquaBind, M&E, Denmark) as in our previous paper (16). After blocking with 200 μL of 2% BSA, ten-fold



Fig. 1. The allergenicity of gluten, gliadin and glutenin IgE-ELISA was performed using pooled serum of wheat-allergic patients. After the coloring reaction using *o*-phenylenediamine as a substrate, the OD value was read by an ELISA plate reader. Data were expressed in terms of absorbance at 492 nm. The allergenicity of gliadin was evaluated to be essentially same as that of glutenin.

diluted pooled serum (Fig. 2) or the individual serum (Table 2) was applied for assay in the same way as described above.

Inhibition ELISA

Inhibition ELISA was performed by the usual method (14). Briefly, peptides (40 $\mu\text{g}/\text{mL}$ at a final concentration) were added to the individual serum samples (Patient Nos. 3, 10, and 11) diluted five-fold in PBS containing 0.05% Tween 80. After 1 h preincubation at ambient temperature, the peptide-serum mixture was applied to polystyrene micro-titer plates (Nunc, Denmark) coated with gliadin (0.25 mg/well) as an antigen and blocked with 2% BSA in PBS. Serum untreated with peptides was used as a control. The binding of IgE-antibody to gliadin was determined by sequential incubations with biotinylated anti-human IgE, streptavidin-peroxidase conjugate, and *o*-phenylenediamine, as described above. Then, data were represented in terms of relative ELISA value when the value for control sample was taken as 100.

RESULTS

We have previously succeeded in isolation of Ser-Gln-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Pro-Pro-Phe-Ser-Gln-Gln-Gln-Gln-Pro-Pro-Phe as a 30-mer IgE-binding peptide from glutenin (13). In addition, we identified Gln-Gln-Gln-Pro-Pro (QQQPP) as an epitope structure (minimum sequence for the IgE-binding) (14). At first in this study, we tried to compare the allergenicity of gliadin with that of glutenin. Consequently, the allergenicity was evaluated to be essentially in common with gliadin and glutenin by ELISA using pooled sera of wheat-allergic patients (Fig. 1).

In gliadin sequence, there are many kinds of repeated motifs. As for amino acid sequence of ω -gliadin, there appear QPPQP and QQFPF sequences 14 times, PQQPF sequence 11 times, QPF PQ sequence 10 times, and PQQPQ and QPPQP sequences 7 times. We next syn-

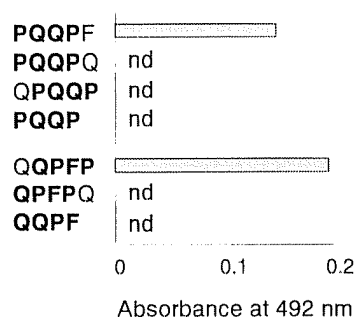


Fig. 2. IgE-binding abilities of the synthesized peptides. As candidate epitopic peptides of gliadin, 7 peptides were synthesized (Table 1), coated on a solid support (AquaBind), and applied to IgE-ELISA. QQPFP and PQQPF were found to be epitopes.

Table 2. IgE-binding abilities of QQPFP and PQQPF.

Patient No.	QQPFP	PQQPF
1	0.340	0.150
2	0.640	0.420
3	1.105	0.235
4	0.495	0.280
5	0.745	0.415
6	0.400	0.050
7	0.270	0.140
8	0.295	nd*
9	0.215	0.135
10	1.725	1.715
11	1.430	1.470

QQPFP and PQQPF were coated on AquaBind and applied to IgE-ELISA using individual sera of 11 patients. The values were expressed as relative values based on absorbance at 490 nm. *, not detected (<0.026).

thesized these sorts of peptides as candidates for gliadin epitope(s) (Table 1) and evaluated their IgE-binding abilities using the pooled sera (Fig. 2).

For this purpose, we adopted the solid phase method using AquaBind. In this method, the synthesized peptides were coated on a solid support, and an aliquot of the pooled sera was tested for IgE binding to the peptide fragments. As expected, at least two antibody binding regions such as QQPFP and PQQPF were found out among the synthesized peptides (Fig. 2). Among them, QQPFP was the highest in ELISA value. Since other QQPFP-containing peptides such as QPFPQ and QPFP could not bind to the antibody, N-terminal Gln residue in QQPFP might be essential for IgE-binding. It is thus concluded that QQPFP is one of the epitope (minimum IgE-binding) structure of gliadin. The ELISA value for PQQPF was the second highest among the peptides tested. Since other PQQP-containing peptides such as PQQPQ, QPQQP, and PQQP could not bind to the antibody, it is concluded that C-terminal Phe residue might be necessary for IgE-binding. In addition, QQPF which lacks N terminal of PQQPF was far lower in ELISA value than PQQPF. Therefore, we judged that PQQPF is one of

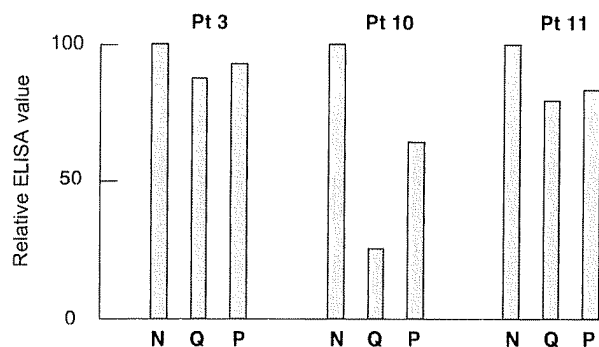


Fig. 3. Inhibition ELISA of QQPFP and PQQPF. IgE-binding abilities of QQPFP and PQQPF were confirmed using 3 individual patients serum: N, none (control); Q, QQPFP (40 µg/mL); P, PQQPF (40 µg/mL).

the epitope structures of gliadin as well.

Incidentally, individual serum samples was checked in order to clarify to what extent the epitopic peptides could be recognized by respective patients. It turned out that QQPFP bound IgE antibodies in all serum samples, and that PQQPF bound IgE antibodies in ten of eleven samples (Table 2). In addition, the ELISA value for QQPFP was generally higher than that for PQQPF. This was comparable to the result of Fig. 2 in which the pooled sera were used.

Inhibition ELISA of these peptides was also performed, since the ELISA values in Fig. 2 and Table 2 might be influenced by the binding affinity of the peptide to the plate in the solid phase method. For this assay, we used the individual serum samples from three patients Nos. 3, 10, and 11 whose IgE titers were higher than others (Table 2). As shown in Fig. 3, QQPFP showed higher inhibition than PQQPF in these three serum samples, which supported the data given in Table 2 and Fig. 2. The ELISA inhibition of these peptides was most pronounced in patient No. 10 among three patients.

DISCUSSION

It is well known that gliadin is causative of allergen for celiac disease (1, 2) and FDEIA (10). Gliadin is also responsible for atopic dermatitis. In this connection, we have already reported that its major epitope structure is attributable to QQQPP motif in glutenin fraction (13, 14). In addition, QQPFP and PQQPF motifs in gliadin sequence, in which they appeared 14–17 times, were revealed to be epitopes in this study. Since the allergic reaction occurs when allergen binds to IgE antibody, it might be easy for gliadin to crosslink IgE antibodies via such epitopes as QQPFP and PQQPF and to trigger the subsequent allergic reaction. These repeated sequences which contain mainly Gln, Phe, and Pro residues might be recognized easily by our IgE-antibodies, since those are unfamiliar in protein sequence in our body. The characteristics of wheat proteins would be probable reasons why wheat allergy is more difficult to outgrow, which is somewhat different from other food-associated

allergies.

Both solid phase ELISA (Fig. 2 and Table 2) and inhibition ELISA (Fig. 3) revealed that the IgE-binding ability of QQPFP was higher than that of PQQPF. In a sense, QQPFP is similar in sequence to QQQPP which is one of the major glutenin epitopes (14). We reported that N-terminal Q and two P residues were essential for IgE-binding (14). It is of much interest whether the QQQPP-sensitive IgE antibodies would also recognize QQPFP or not.

There are two kinds of epitopes; one is the linear (sequential) epitope such as QQPFP and PQQPF in this paper, and the other is conformational epitope. As shown in Fig. 3, the ELISA inhibition by QQPFP or PQQPF was most prominent in patient No. 10 than in other patients. This result might indicate that the linear epitope rather contributes to patient No. 10 more than the conformational epitope does.

How many amino acid residues are minimally required for the recognition by the wheat-specific IgE antibodies? As shown in Fig. 2, both QQPFP and PQQPF were strongly recognized by the IgE-antibodies, but not QPFP- and PQQP-containing pentapeptides. Thus, it is highly probable that at least five residues are required for the IgE-binding. This inference is supported by our previous report (14) in which QQQPP in glutenin was looked upon as the minimum epitope since QQQPG or QQQPP failed to bind IgE-antibodies.

In conclusion, this study has revealed that QQPFP and PQQPF are responsible for the allergenicity of gliadin as epitope structures using sera of wheat-allergic patients with atopic dermatitis.

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