



Fast ω -gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis

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KEYWORDS

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Summary Background: Wheat-dependent exercise-induced anaphylaxis is an anaphylaxis induced by physical exercise after ingestion of wheat. An immediate-type hypersensitivity to water/salt-insoluble fraction of wheat proteins (gluten) has been considered to underlie in this disease. **Objective:** The aim of the study is to determine the major allergen in Japanese patients with wheat-dependent exercise-induced anaphylaxis by using a panel of purified wheat gliadins and glutenins. **Methods:** Water/salt-insoluble wheat proteins, α -gliadin, β -gliadin, γ -gliadin, fast ω -gliadin, slow ω -gliadin, high molecular weight glutenin and low molecular weight glutenin, were purified, and five patients with wheat-dependent exercise-induced anaphylaxis, whose diagnose had been determined by positive-challenge test, were evaluated for skin prick test, dot-blotting test and CAP–RAST inhibition test by using these purified wheat proteins. **Results:** The fast ω -gliadin was the most potent allergen among these water/salt-insoluble proteins when evaluated by skin prick test and dot-blotting test. Fast and slow ω -gliadin, and γ -gliadin caused dose-dependent inhibition of the serum IgE-binding to solid-phase gluten in the patients. The incubation with fast ω -gliadin of the patient's serum caused dose-dependent inhibition in the IgE-binding to γ -gliadin as well as slow ω -gliadin, indicating a cross-reactivity of these proteins in IgE-binding. **Conclusion:** We concluded that fast ω -gliadin is a major allergen among these water/salt-insoluble proteins for wheat-dependent exercise-induced anaphylaxis in Japanese patients, and IgE against fast ω -gliadin cross-reacts to γ -gliadin and slow ω -gliadin.

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1. Introduction

Wheat-dependent exercise-induced anaphylaxis is a distinct form of wheat-allergy, which is induced by physical exercise after ingestion of wheat. The

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typical symptom is generalized urticaria associated with shock or hypotension. Although the mechanism of physical exercise-induction in the anaphylaxis is unclear, an immediate-type hypersensitivity to water/salt-insoluble fraction of wheat proteins (gluten), has been considered to underlie in this disease. Among the wheat water/salt-insoluble proteins, in our previous study we identified wheat γ -gliadin as a presumptive major allergen by immunoblotting as well as RAST inhibition test [1,2]. Recently Palosuo et al. have identified ω -5 gliadin, which had been reported γ -gliadin-like protein in their previous report [3], as a major allergen in children with wheat-dependent exercise-induced anaphylaxis [4]. In the present study we tested a panel of purified gliadins and glutenins for allergens in the patients with wheat-dependent exercise-induced anaphylaxis, and determined that fast ω -gliadin (contains ω -5 gliadin) is a major allergen in wheat-dependent exercise-induced anaphylaxis, and IgE against fast ω -gliadin cross-reacts to other gliadins.

2. Patients and methods

2.1. Patients

Five patients with wheat-dependent exercise-induced anaphylaxis, whose diagnose had been determined by positive-challenge test, were enrolled in this study (challenge test data shown in Table 1). The challenge test was carried out as described previously [1]. Initially 6-h fasting 100 or 150 g of wheat flour was ingested. Exercise challenge was undertaken with the standard Bruce protocol. In some cases 500 mg of aspirin was ingested 30 min before wheat-ingestion. One healthy control was enrolled in the study.

2.2. Preparation of gliadins and glutenins

Wheat gluten, α -gliadin, β -gliadin, γ -gliadin, fast ω -gliadin, slow ω -gliadin, high molecular weight glutenin (HMW) and low molecular weight glutenin (LMW), were prepared as previously described [1,5,6]. Briefly, wheat flour was defatted and water-soluble proteins were extracted in 0.5 M NaCl. After centrifugation, the pellet was stirred with 70% (v/v) aqueous ethanol. The supernatant was freeze-dried and dissolved in 6M urea. α -Gliadin, β -gliadin, γ -gliadin, fast ω -gliadin, slow ω -gliadin and LMW were purified on size exclusion chromatography followed by ion-exchange chromatography. The pellet of 70% ethanol preparation was extracted in 1% acetic acid for HMW preparation. The purity of these fractions was determined by SDS-PAGE.

2.3. Skin prick testing

For skin prick-test, each protein was dissolved in 50% glycerol at a concentration of 100 μ g/ml. After 15 min of the test, reaction was evaluated for wheal and erythema diameter.

2.4. Dot-blotting

Serum samples were obtained from the patients and the healthy subject, and stored at -70°C until use. Gluten, α -gliadin, β -gliadin, γ -gliadin, ω -gliadins, HMW and LMW were dissolved in buffer (50 mM sodium acetate, 25 mM phosphate buffer) at a concentration of 1 mg/ml. Each protein (10 μ g/spot) was blotted on nitrocellulose sheets (Schleicher & Schnell, 0.2 μ m, Kassel, Germany), and incubated with serum (final concentration 10%) for 30 min. The IgE antibodies reacting the water/salt-insoluble wheat proteins were visualized by using ECLTM Western Blotting Analysis System (Amersham Pharmacia Biotech, UK) after incubating with peroxidase-labelled anti-human IgE antibodies

Table 1 Challenge test in the patients with wheat-dependent exercise-induced anaphylaxis

Patient no.	Age (years)/sex	Challenge test				
		Wheat alone	Exercise alone	Wheat + exercise	Aspirin + wheat	Aspirin + wheat + exercise
1 ^a	44/M	–	–	Urticaria	Not done	Not done
2 ^a	43/M	–	–	Urticaria	Not done	Not done
3	54/M	–	–	Urticaria	Not done	Not done
4	17/F	–	–	Erythema	Urticaria	Not done
5	54/M	–	–	–	–	Urticaria, shock

^a Patient 1 and 2 were reported previously [1].

(Biosource, CA). The intensity of the chemiluminescence on the dots was calculated as integrated optical density, which was subtracted by the value against BSA in each patient using an image-analyzing soft (Gel-Pro analyzer, Media Cybernetics, Inc., MD). Cross-reactivity of IgE against gliadins was determined as inhibition for dot-blotting. The patients' sera were mixed with a various amount of purified fast ω -gliadin, incubated at 37 °C for 30 min, and reacted with ω -gliadins and γ -gliadin blotted on the nitrocellulose sheets. The inhibition was expressed as percentage of values obtained from buffer-treated patients' sera.

2.5. CAP-RAST inhibition

Specific IgE antibodies for gluten in serum were determined by a fluoroenzyme immunoassay (CAP system, Pharmacia Upjohn, Uppsala, Sweden) [7]. In inhibition test for gluten specific IgE-titer by γ -gliadin or ω -gliadins, the patients' sera were mixed with a various amount of purified γ -gliadin or ω -gliadins, and incubated at 37 °C for 30 min before determination of specific IgE to gluten. The inhibition was expressed as percentage of values obtained from buffer-treated patients' sera as previously described [2].

3. Results

The data of skin prick test and dot-blotting were summarized in Tables 2 and 3. In skin prick test all patients reacted with gluten and at least two types of gliadins or glutenins. Four of five patients reacted with fast ω -gliadin, three reacted with γ -gliadin or slow ω -gliadin, however, α -gliadin and β -gliadin were found to be less allergenic. Specific IgE-reacting with these water/salt-insoluble wheat proteins in the patients' sera was determined by dot-blotting (the reaction data in patient 3 was representatively shown in Fig. 1). The patients' sera reacted with these proteins almost parallel to the skin-reaction with a few exceptions. In this dot-blotting test high titer of IgE reacting to gluten was detected in all patients' sera, and fast ω -gliadin was the most potent allergen among these water/salt-insoluble proteins.

Then we examined whether the IgE reacting to gluten is inhibited by preincubation with ω -gliadins and γ -gliadin by using CAP system as previously described [2]. The titer of IgE reacting to gluten enough to inhibition-test was obtained in three patients (patient 3, 4 and 5), and the results were shown in Fig. 2. The ω -gliadins and γ -gliadin caused dose-dependent inhibition of the patients' serum

Table 2 Skin prick test in the patients with wheat-dependent exercise-induced anaphylaxis^a

Patients	Control	α	β	γ	Fast ω	Slow ω	HMW	LMW	Gluten
1	0 × 0/0 × 0	3 × 3/3 × 3	4 × 4/4 × 4	3 × 3/4 × 4	6 × 6/25 × 20	5 × 5/15 × 15	5 × 5/5 × 5	6 × 3/15 × 10	7 × 5/20 × 15
2	0 × 0/0 × 0	3 × 3/10 × 10	3 × 3/7 × 7	5 × 5/30 × 20	3 × 3/25 × 20	8 × 6/30 × 25	5 × 5/15 × 10	5 × 3/8 × 5	7 × 5/35 × 28
3	0 × 0/1 × 1	3 × 3/10 × 10	4 × 4/10 × 10	6 × 6/25 × 20	5 × 5/20 × 20	3 × 3/15 × 10	2 × 2/5 × 5	2 × 2/3 × 3	4 × 4/20 × 20
4	0 × 0/0 × 0	2 × 2/6 × 5	0 × 0/0 × 0	0 × 0/0 × 0	2 × 2/13 × 11	2 × 2/5 × 5	5 × 5/38 × 25	4 × 3/50 × 24	5 × 4/45 × 30
5	0 × 0/0 × 0	4 × 3/12 × 12	5 × 3/35 × 30	4 × 4/20 × 19	5 × 4/32 × 25	5 × 5/30 × 25	4 × 4/4 × 4	5 × 4/15 × 9	7 × 4/40 × 35
Control	0 × 0/0 × 0	0 × 0/0 × 0	0 × 0/0 × 0	0 × 0/0 × 0	0 × 0/0 × 0	0 × 0/0 × 0	0 × 0/0 × 0	0 × 0/0 × 0	0 × 0/0 × 0

^a Wheal/erythema diameter (mm).

Table 3 Dot-blot with sera of the patients with wheat-dependent exercise-induced anaphylaxis

Patients	α	β	γ	Fast ω	Slow ω	HMW	LMW	Gluten
1	42	26	96	845	223	590	504	1982
2	395	119	52	2867	606	2162	795	3304
3	27	3	4	2205	232	181	43	8228
4	83	0	18	333	0	1218	159	1084
5	409	24	0	628	77	362	256	1249
Control	34	6	35	36	202	38	34	79

Data was expressed as integrated optical density.

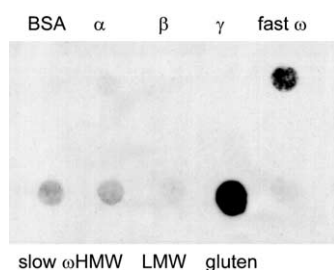


Fig. 1 Dot-blotting with wheat gliadins and glutenins. Gluten, purified α -gliadin (α), β -gliadin (β), γ -gliadin (γ), fast ω -gliadin (fast ω), slow ω -gliadin (slow ω), HMW glutenin and LMW glutenin were prepared as previously described [2,5,6]. The result of patient 3 was shown.

IgE-binding to solid-phase gluten proteins in all three patients. Almost 100% inhibition was obtained at 100 μ g/ml of fast ω -gliadin (final concentration), however, more of 60% inhibition and somewhat less inhibition were seen with slow ω -gliadin as well as γ -gliadin. This result indicates that fast ω -gliadin has the highest affinity to the patients' sera. To determine the cross-reactivity of IgE against these gliadins, the patient's serum (patient 3) were preincubated with fast ω -gliadin at a various concentration for 30 min and then determined the reactivity to ω -gliadins and γ -gliadin by dot-blotting. The incubation with fast ω -gliadin of the patient's serum caused dose-

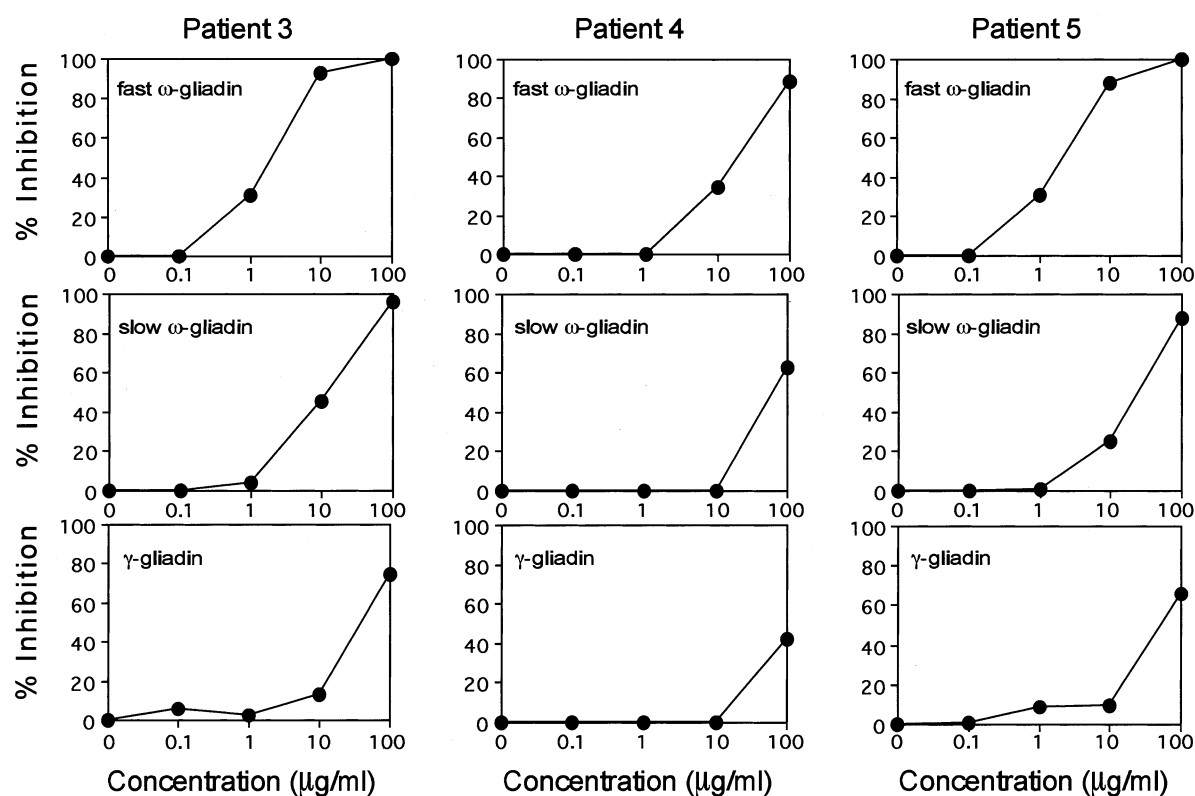


Fig. 2 Inhibition of IgE binding to solid-phase wheat gluten following preincubation of serum with purified fast ω -gliadin, slow ω -gliadin and γ -gliadin in three patients.

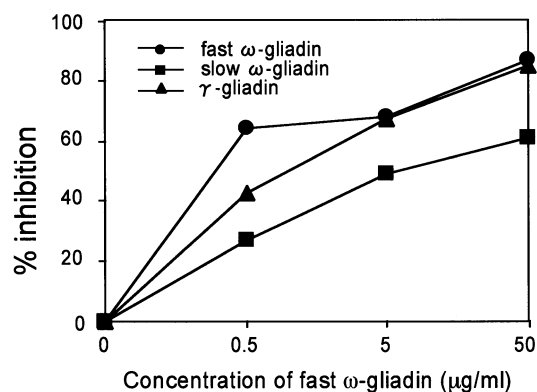


Fig. 3 Inhibition of IgE binding to ω -gliadins and γ -gliadin dot-blotted on nitrocellulose sheets following preincubation of serum with a various concentration of fast ω -gliadin. The result of patient 3 was shown.

dependent inhibition in the IgE-binding to γ -gliadin and slow ω -gliadin as shown in Fig. 3.

4. Discussion

Various foods have been reported to be associated with exercise-induced anaphylaxis as allergens. These include wheat, shellfish, vegetables, nuts, and fish [8,9]. Among them wheat is the most frequent causative food in Japan [9]. The water/salt-insoluble wheat proteins, termed as gluten, have been considered to be causative allergens for the wheat-dependent exercise-induced anaphylaxis. The wheat gluten is categorized as gliadins and glutenins. The former consists of α -gliadin, β -gliadin, γ -gliadin and ω -gliadins, and the latter divided into HMW and LMW. In our previous studies we identified γ -gliadin as a presumptive allergen in wheat-dependent exercise-induced anaphylaxis in Western blotting followed by amino acid sequence of the IgE-binding wheat gluten protein [1,2]. On the other hand, Palosuo et al. [3,4] determined α -gliadin and γ -gliadin-like protein (later this was found to be identical to ω -5 gliadin) as major allergen for the anaphylaxis. These studies suggest a diversity of causative allergen in the wheat-dependent exercise-induced anaphylaxis.

In the present study we concluded that fast ω -gliadin is major allergen among these water/salt-insoluble proteins for wheat-dependent exercise-induced anaphylaxis in Japanese patients, and IgE against fast ω -gliadin cross-reacts to other gliadins. In skin prick test three of five patients show positive reaction to γ -gliadin, however, stronger reaction was seen with fast ω -gliadin in these patients. In addition, all patients had IgE antibodies to fast ω -gliadin when tested by dot-blotting

as shown in Table 3. Moreover, in CAP-RAST inhibition studies fast ω -gliadin inhibited over 90% of the binding of IgE antibodies from patients' sera to solid-phase gluten, indicating that the most part of IgE antibodies in the patients' sera reacting to gluten recognized fast ω -gliadin. The titer of IgE reacting to γ -gliadin was very low in patient 2, 3 and 5 in contrast to the reaction in skin prick test. Also the titer of IgE reacting to HMW was high in patient 1, 2 and 5 in contrast to the reaction in skin prick test. The reason for this dissociation is unclear at the moments.

The reactivity of IgE antibodies to solid-phase gluten was also inhibited up to 80–100% with γ -gliadin and slow ω -gliadin, respectively (Fig. 2). These results indicate the cross-reactivity of the IgE antibodies against these gliadins in the patients' sera. The idea was clearly supported by the dose-dependent inhibition of binding to γ -gliadin and slow ω -gliadin of the IgE antibodies of the patient's serum with fast ω -gliadin as shown in Fig. 3. This was also supported by the fact that ω -5 gliadin has high amino acid sequence homology to γ -gliadin [3,4]. In addition, such cross-reactivity was reported between ω -5 gliadin and other cereal proteins, rye secalins and barley hordein [10]. But possible contaminations might still be considered in our gliadin-preparation.

Bread wheat (*Triticum aestivum*) is an allohexaploid with three genomes called A, B and D. Although ω -gliadins are complex polymorphic mixtures of polypeptides, most are encoded by single loci, which are located in similar position on the short arms of the group of chromosome 1 of wheat (1A, 1B and 1D) [11]. The fast ω -gliadin corresponds to the 1B-encoded gliadins and the slow ω -gliadin corresponds to the 1A- and 1D-encoded gliadins. The two groups have similar amino acid compositions, being characterized by high contents of glutamine, proline and phenylalanine, low contents of charged amino acids, and lack of cysteine [11]. The 1A- and 1D-encoded ω -gliadins (slow ω -gliadin) contain about 40 mol.% glutamine and 30 mol.% proline, whereas the 1B-encoded ω -gliadins (fast ω -gliadin) contain higher levels of glutamine (approximately 50 mol.%) and less proline (approximately 20 mol.%). The slow ω -gliadin is homologous to the C hordeins of barley and ω -secalins of rye, and the gene sequences show that amino acid sequences of these proteins are largely repetitive, consisting mainly of octapeptides (consensus PQQPFPQQ) flanked by unique short sequences [11]. This repeat motif is similar to that found in the repetitive domain of γ -gliadins (consensus PQQPFPQ), supporting the immunological cross-reactivity of these proteins. So far there exist no

reported gene sequences for the fast ω -gliadins, the amino acid-compositions would indicate a different repeat motif. DuPont et al. [12] reported repeats of QQXP, QQQXP or QQQQXP for 1B-encoded ω -gliadins (fast ω -gliadin), where X is phenylalanine, isoleucine or leucine in order of predominance, being distinct from the repeat type of the slow ω -gliadin. This might account for the strongest allergenicity of the fast ω -gliadin.

Although the reaction pattern to these purified gliadins/glutenins was varied among the patients with wheat-dependent exercise-induced anaphylaxis, the fast ω -gliadin is commonly allergic, suggesting existence of IgE-binding epitopes in the fast ω -gliadin which are commonly causative for the anaphylaxis. Studies of the IgE-binding epitopes will elucidate the molecular mechanism underlying the wheat-dependent exercise-induced anaphylaxis, and may provide a useful tool for the diagnosis of wheat-allergy. Exercise-challenge test, or alternatively aspirin-challenge test, combined with wheat-ingestion is reliable test for the diagnosis of the wheat-dependent exercise-induced anaphylaxis [13,14], however, such challenge tests sometimes result in so serious reactions that the tests should be limited. In vitro detection of IgE specific for the causative epitopes might replace the challenge tests in diagnosis of the wheat-dependent exercise-induced anaphylaxis.

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