

Short communication

Mitigation of the action of wheat allergen by acidic oxidative potential water

Background: Acidic oxidative potential water (AOPW) is strongly acidic, and contains active oxygen species and thus exhibits an oxidative potential to cause biochemical alterations of wheat proteins.

Methods: A commercial wheat allergen extract was treated with AOPW, and then analysed by means of SDS-PAGE and the skin prick test in IgE-mediated wheat allergic patients.

Results: The wheat extract comprised 70 kDa, 32 kDa and 30 kDa fractions, and the protein in the 70 kDa fraction was broken down into less than 14 kDa fractions by AOPW. AOPW mitigated 39% of the wheal reaction to the wheat extract in the skin prick test.

Conclusion: AOPW may be useful for enhancing the digestibility and lowering the allergenicity of wheat proteins.

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Cereal grains constitute the staple food for most of the world's population, providing approximately half of the world's supply of human dietary protein. For many years cereal grains have been recognized as the cause of adverse reactions in some human beings. Several primary disease processes have been recognized to be associated with wheat exposure: gluten-sensitive enteropathy (coeliac disease), baker's allergic asthma, and food hypersensitivity. Coeliac disease is a non-IgE-mediated enteropathy caused by the gliadin fraction of wheat. Baker's allergic asthma has been well studied and demonstrated to be an IgE-mediated allergic reaction to inhaled flour primarily seen in patients with occupational exposure. The third hypersensitivity category is related to the ingestion of cereal grains, that is, IgE-mediated food allergy (1). Since allergic reactions to foods, consisting of cutaneous, respiratory and gastrointestinal symptoms, have long been an important public health problem, some attempts have been made to change the allergenic properties of wheat proteins by altering their biochemical properties (2–4).

Wheat flour consists of very complicated allergens of different molecular weights ranging from 16 to 47 kDa (5). Wheat proteins are classified into two fractions according to their solubility: the salt-soluble (albumin and globulin) and salt-insoluble (gluten: glutenin and gliadin) fractions (6). Although the allergen when wheat causes symptoms after ingestion has been shown to be the salt-insoluble gluten fraction (7–9), immunoblotting studies have shown that IgE antibodies from patients with

baker's allergic asthma (10, 11) and patients experiencing hypersensitivity reactions immediately after the ingestion of wheat (12) bind to a number of salt-soluble fraction components including the α -amylase/trypsin inhibitor family, belonging to the defence-related system in plants (9–12). Furthermore, Tanabe et al. (13) have reported that non-proteinaceous, polysaccharide substances in wheat flour would be more stable in the body, possibly remaining as allergens that cause a long-lasting allergic reaction. Therefore, a method for producing hypoallergenic wheat proteins should not be limited to only certain components.

Many cereal proteins, such as wheat, rice and maize proteins, contain high levels of amide-containing amino acids, i.e., glutamine and asparagine. These side chains are susceptible to hydrolysis of their amide bonds, which leads to the release of ammonia and the transformation to acidic groups. In addition, wheat proteins contain intramolecular disulphide bonds (14). Some attempts have been made to change the allergenic properties of wheat proteins by altering these biochemical properties. Maruyama et al. (2) reported that the reactivity of IgE antibody to wheat proteins dramatically decreased with the degree of deamination of gluten by hydrochloric acid. Thioredoxin, a redox-regulatory disulphide protein, has been reported to reduce intramolecular disulphide bonds, and Buchanan et al. (1) indicated that thioredoxin, in a canine model system, significantly decreased the allergenicity of wheat proteins.

Watanabe et al. (4) reported that enzymatic processing of gluten with actinase, bromelain, cellulase, collagenase or transglutaminase effectively decreased the reactivity of IgE antibody to wheat proteins. On the enzymatic processing of wheat proteins, however, the original dough-forming properties were lost (4), and these enzymes themselves may cross-react with human tissues. Bromelain and transglutaminase left in the wheat proteins have been confirmed to cross-react with human serum (4).

“Acidic oxidative potential water (AOPW)” (otherwise known as electrolyzed strong acid solution or function water) is a strong acid formed at the anode on the electrolysis of water containing a small amount of salt. AOPW has recently been used extensively in Japan for agricultural and medical disinfection purposes due to its safety and bactericidal effectiveness (15). AOPW has the following physical properties: pH, 2.3–2.7; oxidative-reduction potential, 1050–1150 mV; concentration of dissolved chlorine, 30–50 p.p.m.; and concentration of dissolved oxygen, 10–30 p.p.m. Because of these properties, AOPW may cause biochemical alterations of cereal proteins. The high level of hydrogen ions in AOPW may induce hydrolysis of the amide bonds in wheat proteins, and the oxidative potential of AOPW may lead to the formation of sulphenic acid at cysteine residues, which would reduce disulphide bonds in wheat proteins.

AOPW is a strong acid, however, it is different from hydrochloric acid or sulphuric acid. Both of the last two acids exhibit a strong degree of ionization. When oxidation occurs, hydrogen ions are consumed and new hydrogen ions are generated. In the case of AOPW, hydrogen ions are not generated because they are only produced through electrolysis of saline. Thus, full-strength AOPW is not corrosive to proteins. Arai (16) recently reported a new attempt to bake wheat bread with AOPW, and confirmed that the dough-forming properties, flavour and odour of wheat bread made with AOPW were as good as those of wheat bread made with city water. We now report that AOPW reduces the allergenicity of wheat proteins, thereby opening the door to further testing of AOPW for the improvement of cereal products.

Material and Methods

Cereal materials

The commercial allergen preparation solutions were purchased from Torii Pharmaceutical Co., Tokyo. In brief, wheat grain mix, rice grain mix, maize grain mix and buckwheat grain mix were defatted with ether and then extracted with a 10-times volume of 5% saline containing 50% glycerin.

AOPW treatment

The AOPW was generated with a model NIX-60 KH AOPW generator (Nihon Akua Co., Ltd, Osaka, Japan) supplied with a 5%

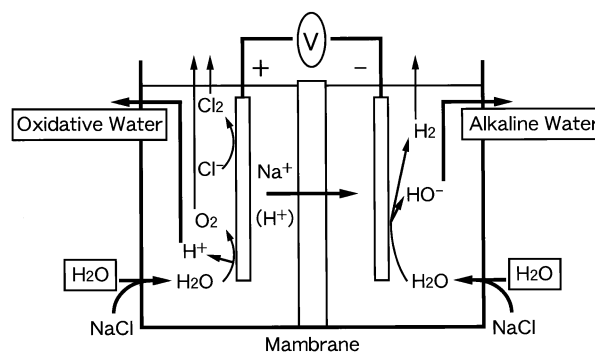


Figure 1. Production of acidic oxidative potential water. Strongly acidic water is generated by electrolysis of a 5% saline solution. Cited from (15). Copyright 1999, with permission from Cognizant Comm Corp.

saline solution. A schematic diagram of the generator is presented in Fig. 1 (14), and the physical properties of the AOPW were described in the introduction. Equal volumes of AOPW (pH, 2.6; oxidative-reduction potential, 1040 mV; chloride concentration, 38 p.p.m) and the allergen extract solutions were mixed and then incubated for 60 min at room temperature. Equal volumes of the alkaline water obtained at the cathode (pH, 11.8; oxidative-reduction potential, -870 mV) and a 5% saline solution were mixed, respectively, with the allergen extract solutions, and then used as controls.

Sds-page

Ten-microlitre aliquots of the samples were loaded on a slab gel (12.5% acrylamide), and then a constant current of 20 mA was applied. The migration buffer comprised 25 mM Tris-HCl, 192 mM glycine, 15% methanol and 0.1% SDS. The separated proteins were fixed by incubation of the gel in 12% trichloroacetic acid for 90 min and visualized with rapid Coomassie stain. Protein markers (Pharmacia Biotech, Tokyo) were used for estimating the molecular weights of the electrophoresed proteins. The proteins were analysed by gel scanning densitometry (Pharmacia Biotech, Tokyo). The distance travelled by each protein was compared with those by protein markers to determine the approximate molecular weight of the protein.

Patients

Ten patients with IgE-mediated wheat allergy were enrolled in this study (Table 1). Nine of them had experienced systemic urticaria, angioedema or respiratory distress immediately after the ingestion of wheat. Three of them had collapsed, however, two of these had no symptoms if ingestion was not followed by exercise. The foods causing severe reactions included noodles, wheat bread and cookies. One patient was a baker with generalized chronic dermatitis, which improved to a great extent within 2 weeks after suspension of work, followed by aggravation again after resumption of work. Although all patients had eaten rice, seven (cases 1–6, 10) had eaten maize-containing foods and four (cases 2, 4–6) had eaten buckwheat, nobody had suffered from any allergic disorders after eating these cereal products. The study was approved by the Ethics Committee of the Kumamoto Society for Paediatric Allergy. Informed consent for the investigation was obtained from all patients, the parents' consent being obtained for children.

Table 1. Clinical features, IgE antibodies in serum and skin prick test results in patients with IgE-mediated wheat allergy

Case	Age (years)	Sex	Clinical features†	Specific IgE (kU/l)				Skin prick test to wheat (mm)		Inhibition of wheal (%)‡
				Wheat	Rice	Maize	Buckwheat	Saline	AOPW	
1	1	F	SU/A	1.82	< 0.35	< 0.35	< 0.35	10/30§	1/7	90
2	8	M	A	10.30	0.87	< 0.35	< 0.35	10/34	4/24	60
3	2	M	A	2.17	< 0.35	< 0.35	< 0.35	6/13	5/16	17
4	20	M	WDEIA	1.95	< 0.35	< 0.35	< 0.35	5/9	2/5	60
5	42	F	WDEIA	3.08	< 0.35	< 0.35	< 0.35	7/42	6/24	14
6	55	M	CD	97.40	2.11	3.23	7.56	6/26	6/16	0
7	1	F	SA	17.40	< 0.35	< 0.35	< 0.35	4/18	4/22	0
8	0	F	A	1.54	< 0.35	< 0.35	< 0.35	11/20	4/11	64
9	0	F	SU	0.63	< 0.35	< 0.35	< 0.35	15/24	9/19	40
10	1	M	SU/A	29.00	≤ 0.35	≤ 0.35	≤ 0.435	16/35	9/26	44
								9 ± 4/25 ± 10¶	5 ± 3/17 ± 7	39 ± 29

† SU, systemic urticaria; A, angioedema; WDEIA, wheat-dependent exercise-induced anaphylaxis; CD, chronic dermatitis; SA, systemic anaphylaxis.

‡ $(1 - (\text{wheal with AOPW}/\text{wheal with saline})) \times 100$.

§ Diameter of wheal (mm)/diameter of erythema (mm).

¶ Mean ± 1 standard deviation.

Skin prick test

The skin prick test (SPT) was performed with plastic bifurcate needles (DUOTIP-TEST; Lincoln Diagnostics, IL), and the diameters of the wheals and the erythema were determined after 15 min. Five percent saline with or without 0.1% histamine hydrochloride was applied as a control. The skin reactions to 0.1% histamine hydrochloride dissolved in AOPW and alkaline water were also measured, respectively.

Determination of IgE antibodies

IgE antibody in serum was titrated by means of a fluoroenzyme immunoassay using the Pharmacia CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden). The detection limit of the CAP system is 0.35 kU/l IgE.

Statistical analyses

Group means were compared by means of Student's paired *t*-test. The analyses were performed with the StatView Statistical Package 4.5 (SAS Institute, Cary, NC). Probability (*P*) values of less than 0.05 were considered significant.

Results

Figure 2 (A,B) shows the results of SDS-PAGE sequencing of commercially available cereal grain allergen extracts with or without AOPW. The proteins were separated into numerous fractions, ranging from 96 kDa to 10 kDa. The wheat allergen extract gave three bands (70 kDa, 32 kDa and 30 kDa fractions) in the saline lane. The 70 kDa-band was absent in the AOPW lane, and fractions of less than 14 kDa were considerably increased in the AOPW lane, as judged on gel-scanning. Compared with the saline lane, three bands (96 kDa, 60 kDa and 21 kDa fractions) were lost and two bands (24 kDa and 25 kDa fractions) newly appeared in the AOPW lane with

the rice allergen extract. In addition, two bands (96 kDa and 67 kDa fractions) observed in the saline lane with the maize allergen extract were absent in the AOPW lane. The band pattern of the buckwheat allergen extract did not differ between the saline and AOPW lanes. For all the cereal grain allergen extracts, there was no difference between the saline lane and that with alkaline water obtained at the cathode.

In view of the above results, we examined whether or not AOPW treatment caused mitigation of the allergenicity of the wheat extract. The action of AOPW decreased the extent of the allergic skin reaction in eight out of 10 patients with IgE-mediated wheat allergy. As shown in Table 1, AOPW treatment mitigated 39% of the wheal response in the SPT, and the results were statistically significant, the *P*-value being 0.005. In addition, AOPW treatment significantly decreased the erythematous response in the SPT, the *P*-value being 0.013. The wheal and erythematous reactions to 0.1% histamine hydrochloride dissolved in AOPW were not different from those with saline or alkaline water, respectively. All six cases suffering from systemic urticaria and/or angioedema, and both cases with wheat-dependent exercise-induced anaphylaxis showed significant mitigation in the SPT with AOPW treatment, whereas a baker (case 6) with generalized chronic dermatitis and a patient with systemic anaphylaxis immediately after the ingestion of wheat (case 7) did not show any mitigation of the wheal and erythematous reactions in the SPT with AOPW treatment. Finally, the action of AOPW as to wheal and erythematous responses in the SPT was independent of the IgE antibody concentrations as to wheat allergens in serum.

The SPT as to rice grain mix, maize grain mix and buckwheat grain mix was performed in seven patients

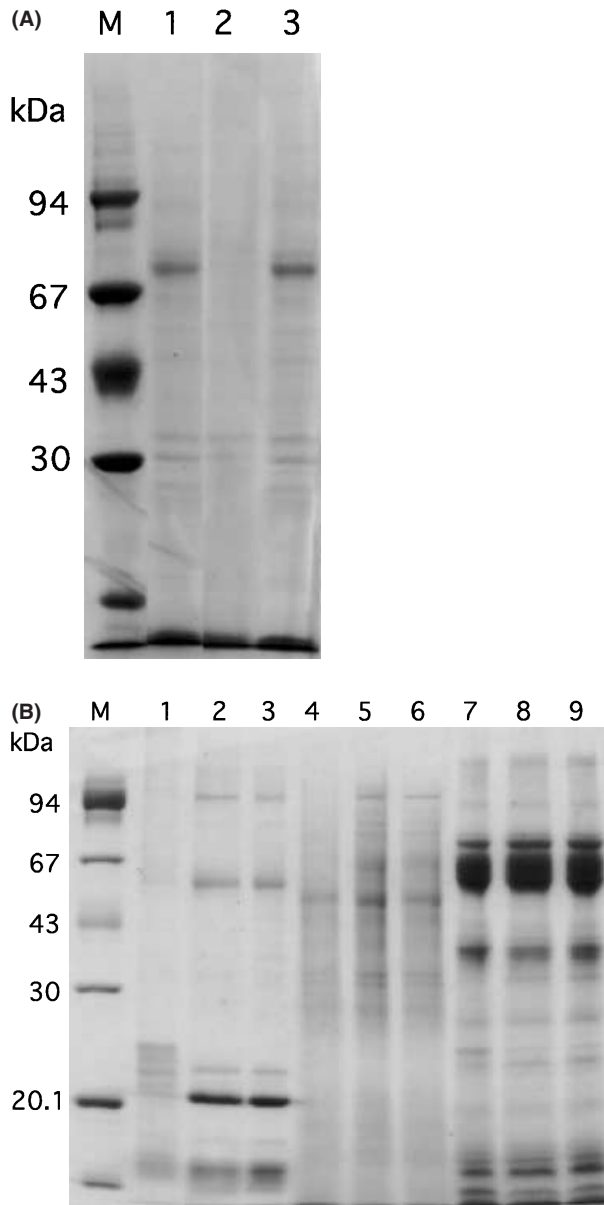


Figure 2. SDS-PAGE fractionation of (A) a commercial wheat allergen extract, and (B) rice, maize and buckwheat allergen extracts. The separated bands were stained with Coomassie blue. Lanes M, protein markers. The wheat extract was treated with 5% saline (A, lane 1), AOPW (A, lane 2), or alkaline water (A, lane 3). The rice allergen extract was treated with AOPW (B, lane 1), saline (B, lane 2), or alkaline water (B, lane 3). The maize extract was treated with AOPW (lane 4), saline (lane 5), or alkaline water (lane 6). The buckwheat extract was treated with AOPW (lane 7), saline (lane 8), or alkaline water (lane 9).

(cases 1–5, 7, 10), and none of them showed any positive reactions in the study. The serum IgE antibody concentrations to rice, maize and buckwheat were measured in all patients. Although the baker (case 6) had considerable amounts of IgE antibodies specific to rice, maize and

buckwheat, and the patient with angioedema after the ingestion of wheat (case 2) had a low level of IgE antibodies to rice, the other eight patients did not have measurable amounts of IgE antibodies to any of these cereals.

Discussion

There was wide variation, from 90% to 0%, in the inhibition of the wheal response to the wheat allergen extract in the SPT with AOPW treatment in patients with IgE-mediated wheat allergy. All six cases suffering from systemic urticaria and/or angioedema, and both cases with wheat-dependent exercise-induced anaphylaxis showed significant mitigation in the SPT with AOPW treatment. However, no mitigation in the SPT was observed in two cases; a patient with generalized chronic dermatitis due to wheat and a patient with a systemic anaphylactic reaction to wheat. Alternatively, the wide deviation of the AOPW action in the SPT may be attributed to the heterogeneity in the clinical features of the patients with IgE-mediated wheat allergy. The close association between baker's allergic asthma and a number of salt-soluble wheat fraction components including α -amylase inhibitor (10) and wheat flour peroxidase (11) has been reported. Wheat-dependent exercise-induced anaphylaxis has been shown to be related to ω -5 gliadin (8), and IgE-mediated wheat-allergic patients with atopic eczema/dermatitis syndrome had IgE antibodies against a low-molecular gliadin fraction of less than 14 kDa (7). Patients with IgE-mediated anaphylaxis after the ingestion of wheat have been associated with α -amylase inhibitor (12) or ω -5 gliadin (9).

Although in the present study we did not examine the exact mechanism of action, we believe that the effect of AOPW in mitigating the allergenicity of wheat protein is due to the combined actions of its high oxidative potential and hydrogen ion concentration. The novelty of AOPW is that the mitigation is achieved through safe and adaptable biochemical alterations consistent with its application to the improvement of foods, in this case wheat. AOPW treatment has advantages over current improvement procedures (2–4) in not involving agents that may lower the nutritional value or alter the taste.

Two patients with IgE-mediated wheat allergy had some IgE antibodies to rice, maize or buckwheat; however, the other eight patients did not show any allergic reactions to these cereals, as judged by the examination of their dietary histories, and from the results of the SPT and measurements of IgE antibody in serum. It seems likely that the allergenic properties of wheat flour do not have cross-reactivities to those from rice, maize or buckwheat. Sequence analysis confirmed that rice and maize proteins are susceptible to AOPW treatment, suggesting that AOPW is also

applicable to the mitigation of the allergenic properties of rice and maize proteins. The present findings open the door to the testing of the AOPW system for the production of hypoallergenic, more digestible cereal foods.

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