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Note

Decreased IgE-binding with Wheat Gluten by Deamidation

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The major wheat allergens contain a number of glutamine residues, suggesting that deamidation would be a promising method to produce hypoallergenic wheat proteins. Gluten was deamidated by acid under various conditions. The 30%-, 50%- and 90%-deamidated glutes were reacted with sera of patients allergic to wheat proteins. The results indicate that the reactivity was dramatically decreased according to the degree of deamidation.

Key words: deamidation; gluten; hypoallergenic wheat proteins

Wheat is one of the major allergenic foods. Identification of the proteins responsible for the allergenicity of wheat and determination of their epitopes are important steps to develop hypoallergenic wheat products. 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.¹⁾ Ikezawa *et al.*²⁾ have reported that a major wheat allergen existed in a salt-soluble fraction. On the contrary, Watanabe *et al.*³⁾ have observed that most patients with wheat allergy were sensitive to gluten, especially to an LMW-glutenin subunit containing a number of the pentapeptide repeat motif, Gln-Gln-Gln-Pro-Pro,⁴⁾ which has been identified as an IgE-binding epitope.⁵⁾ Moreover, we have confirmed that the LMW-glutenin subunit was the major allergen and also observed that α - and γ -gliadins were the secondary allergens.⁶⁾ Therefore, a method to produce hypoallergenic wheat proteins should not be limited to only a certain component. In this respect, the method based on proteases developed by Watanabe and co-workers^{3,7,8)} is very desirable. An alternative method that would be effective for any allergen may be deamidation, since the epitope of the major allergen, LMW-glutenin, contains three glutamine residues, and the secondary allergens (α - and γ -gliadins) also contain a number of glutamine residues.¹⁾ Since gluten is produced by washing wheat flour with a salt solution, it contains only a small amount of salt-soluble proteins. Therefore, deamidation of gluten would probably result in hypoallergenic wheat proteins that could be eaten by any patients allergic to wheat. In this paper, we assess the reactivity of wheat glutes having different degrees of deamidation by testing on the sera of patients with

wheat-associated allergies.

Raw gluten (Glico Food, 200 g) was mixed little by little with 400 ml of 25 mM lactic acid containing 0.002% of sodium hydrogen sulfite and then completely homogenized. Twenty five gram of HCl was added to the homogenate while stirring. After adding HCl, samples were kept for one to four hours at 60°C or 80°C. After cooling, each solution was adjusted to pH 4 and then centrifuged to separate the precipitate and the supernatant. The precipitate was dispersed in three times its volume of distilled water and dissolved by adjusting to pH 6.5. The resultant solution was spray-dried. The degree of deamidation was determined by comparing the amount of ammonia released from the sample with that by excessive hydrolysis (100°C, 6 h) of the original sample. The amount of ammonia released from each sample was determined by the microdiffusion method of Conway and O'Malley.⁹⁾ Hydrolysis at 60°C for 3 h, 80°C for 1 h and 80°C for 3 h gave 30%-, 50%- and 90%-deamidation, respectively.

Ten serum samples with significant RAST scores against wheat proteins were selected from the sera of patients with a known clinical history of allergies. Informed consent was obtained from all donors or their parents. Control sera were obtained from a healthy adult volunteer who had no clinical history of allergic diseases and from an adult volunteer who is allergic to house dust. The RAST scores against wheat proteins and RIST scores of ten serum samples and two serum controls are listed in the Table.

The native gluten and the acid-treated gluten samples with 30%-, 50%- and 90%-deamidation were dissolved in 2% SDS containing 1% 2-mercaptoethanol and a 0.1 M Tris-HCl buffer (pH 8.0) containing 8 M urea and 1% 2-mercaptoethanol. After adjusting the protein content to 4 mg/ml, 1.25 μ l each of a sample was spotted on to a nitrocellulose membrane. The reactivity of the protein sample with the patients' sera was measured by using a goat anti-human IgE-alkaline phosphatase conjugate (Sigma) as described previously.⁶⁾ However, we employed a dilution ratio (1:10) for the sera instead of 1:30 to detect reactivity with high sensitivity. The intensity of the colour of the spot was evaluated by densitometry, using transmitted light and an NIH image program. The intensity derived from non-specific binding of the human

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Abbreviations: RAST, radioallergosorbent test; RIST, radioimmunosorbent test; LMW, low molecular weight; SDS, sodium dodecyl sulfate

Table 1. RAST and RIST Values for the Serum Samples

Patient	Age	RAST (UA/ml)	RIST (IU/ml)
A	19	2.37	N.D. ^a
B	27	0.44	239
C	33	0.38	411
D	29	1.15	1,052
E	28	4.84	972
F	3	5.56	N.D.
G	4	29.5	4,400
H	1	19.6	N.D.
I	17	6.69	25,528
J	3	4.11	7,980
Control 1	45	<0.34	19
Control 2	23	<0.34	890

^a N.D., not determined.

antibodies of each control serum was subtracted from those of the patients' sera, the results being shown in the Figure. All patients' sera exhibited high reactivity to the native gluten, except that from patient B. Generally, the reactivity of a patient's serum to the native gluten dissolved in 0.1 M Tris-HCl (pH 8.0) containing 8 M urea and 1% 2-mercaptoethanol was higher than that with it dissolved in 2% SDS containing 1% 2-mercaptoethanol. It is known that urea is a stronger denaturant than SDS (in this study, the gluten dissolved in SDS was not

boiled). Therefore, the more denatured the gluten was, the stronger was the reactivity with a patient's serum. This is consistent with the results obtained previously that the LMW-glutenin subunit containing a number of Gln-Gln-Gln-Pro-Pro motifs dissolved in 0.1 M Tris-HCl (pH 8.0) containing 8 M urea and 0.1 M 2-mercaptoethanol exhibited much higher reactivity than the protein in 10 mM aluminum lactate containing 2 M urea and 0.1 M 2-mercaptoethanol.⁶⁾ On the other hand, each deamidated sample exhibited much lower reactivity with the patients' sera as expected. Although six (C, D, E, F, H and I) of ten patients' sera exhibited reactivity with the 30%-deamidated gluten, which could not be considered negligible, all patients' sera exhibited negligible or no reactivity with the 50%- and 90%-deamidated glutes. Consequently, a deamidation degree of more than 50% markedly decreased IgE-binding with gluten.

Glutamine residues are likely to play an important role in the formation of a gluten network through hydrogen bonds.¹⁾ Therefore, excess deamidation results in loss of the intrinsic functional properties of gluten. In fact, even the 30%-deamidated gluten did not exhibit the same functional properties as the native gluten did. However, Sugiura and Tsuda observed that the deamidated glutes have functional properties suitable as ingredients and/or modifiers for producing processed foods.¹⁰⁾ Consequently, such processed foods can probably be eaten by patients allergic to wheat proteins. However, we have to pay attention to the possibility that deamidated gluten could induce a new allergy since it is a novel protein.

Coeliac disease is characterized by injury to the small-intestinal mucosa, and occurs in genetically predisposed individuals after ingestion of the prolamins of the grain of wheat, barley and rye.¹¹⁾ The sequences -Pro-Ser-Gln-Gln- and -Gln-Gln-Gln-Pro- are common in toxic peptides derived from α -gliadin.¹¹⁾ Most of the *in vivo* and *in vitro* studies of synthetic peptides have confirmed the importance of one or both of these sequences.¹²⁾ It has recently been demonstrated that tissue transglutaminase mediated T cell-stimulatory activity through the ordered and specific deamidation of gliadins.^{13,14)} This suggests that the deamidated glutes reported here would not be suitable for patients with coeliac disease.

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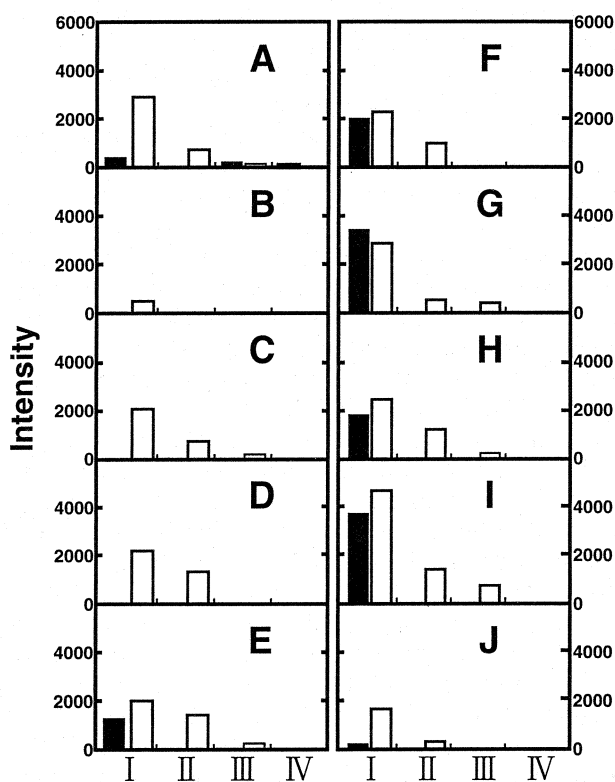


Fig. Reactivity of Native and Deamidated Glutens with Patients' Sera. The reactivity of native (I), and 30%- (II), 50%- (III) and 90%-deamidated (IV) glutes with sera from patients (A-J) was determined by densitometry. Solid bars, dissolved in 2% SDS containing 1% 2-mercaptoethanol; open bars, dissolved in a 0.1 M Tris-HCl buffer (pH 8.0) containing 8 M urea and 1% 2-mercaptoethanol.

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