

Effects of addition of salt to bread on IgE antibody responses

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Abstract

The effect of the addition of NaCl to bread on antigen-antibody reaction involving IgE was examined. We compared the antigen-antibody reaction involving IgE between dough including NaCl and that not including NaCl. Crude proteins extracted from the dough including NaCl showed weaker antigen-antibody reactions than proteins from the dough no added NaCl on allergic tests such as precipitin ring test with human-specific IgE, and the IgE binding activity on ELISA. The crude proteins extracted from the dough with NaCl or without NaCl were applied to an affinity chromatography column of immobilized-trypsin chitin. Then proteins having affinity were recovered. The recovered proteins were separated by SDS-PAGE. Each protein was examined for the IgE binding activity on ELISA. When the amount of protein in dough including NaCl was compared with that in dough without NaCl, a significant difference in the amount of ovomucoid between the two samples were confirmed. The IgE binding activity of baked bread was studied as well. Crude proteins of baked bread made from dough including NaCl showed weaker IgE binding activity on ELISA than proteins of baked bread made from dough without NaCl. The importance of NaCl as an ingredient of baked bread was confirmed.

Keywords: *NaCl, allergy, dough, baked bread, ovomucoid.*

Introduction

Over the last several years, numerous researchers have studied food allergies from a variety of angles and clarified various facts (Sampson & McCaskoll 1985; Buck & Hefle 1995; Crespo et al. 1995; Fuchs & Astwood 1996; Sampson 1999). Various foods show food allergies, and it is not easy to find safe foods for allergy sufferers. In addition, most of the allergens in food allergies are proteins, and various proteins are known as allergens, but detection of specific allergens in foods is difficult.

Proteins in normal bread also cause allergies for allergy sufferers. Allergens are primarily thought to be proteins and gluten of origin from wheat flour or additional ingredients such as hen eggs in the bread-making process. Most allergy sufferers require commercially prepared allergen-free bread because of the allergy risk. To overcome this issue, development of allergy-free bread has been studied from various

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viewpoints: the interaction between the allergens in hen eggs (mainly ovomucoid) and gluten in the bread effects on allergy. Herein, the details are described.

Materials and methods

Materials

The ovomucoid (Type III), ovomacroglobulin (Type II), ovomucin (Type I), ovalbumin (Type VII), ovotransferrin (Type I), and lysozyme were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Gluten and other reagents were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Formula for dough

Conditions were as follows. Fresh yeast (2 g), gluten (200 g), ovomucoid (1 g), ovomucin (1 g), ovalbumin (1 g), ovotransferrin (1 g), ovomacroglobulin (1 g), and lysozyme (1 g) were added to water (100 ml), the materials were mixed using a mixer. Then, NaCl [0 g (0 mM), 0.24 g (20 mM), 0.48 g (40 mM), 0.72 g (60 mM), 0.96 g (80 mM) or 1.20 g (100 mM)] was added into the mixture and the mixture was kneaded for 20 min using the mixer. Butter (60 g) and shortening (60 g) were added in the mixture, and the mixture was kneaded for 10 min to make dough. The dough was used for each experiment.

Separation of human serum fraction

Human blood was collected from ten females with their permission. The human serum was separated in accordance with method of Mirella et al. (1995). Human serum containing K-EDTA (1.6 mg/ml) was separated with a centrifugal separator (2000 g, 8 min) and objective serum fractions were obtained.

Extract of crude protein from dough

Phosphate buffer (0.1 M) (pH 6.5, 30 ml) was added into dough (50 g) and crude proteins in it were extracted. The mixture was filtered. The filtrate, crude proteins, was used for each experiment.

Measurement of antigen-antibody reaction by single-radial immunodiffusion

Single-radial immunodiffusion was performed for measurement of antigen-antibody reaction of crude proteins obtained from the dough including 100 mM NaCl and the dough without added NaCl against human-specific IgE. Human serum was added into 1% agar solution, the agar solution was coated to a thickness of 4 mm on a slide glass surface. Holes (2.5 mm diameter) were formed in the agar. The proteins obtained from the dough were added into the hole. After the glass was maintained overnight at 37°C, the diameter of the precipitin ring obtained was measured.

Measurement by enzyme-linked immunosorbent assay (ELISA)

For measurement of IgE binding activity of protein, ELISA was conducted according to a method of Engvall and Perlmann (1972).

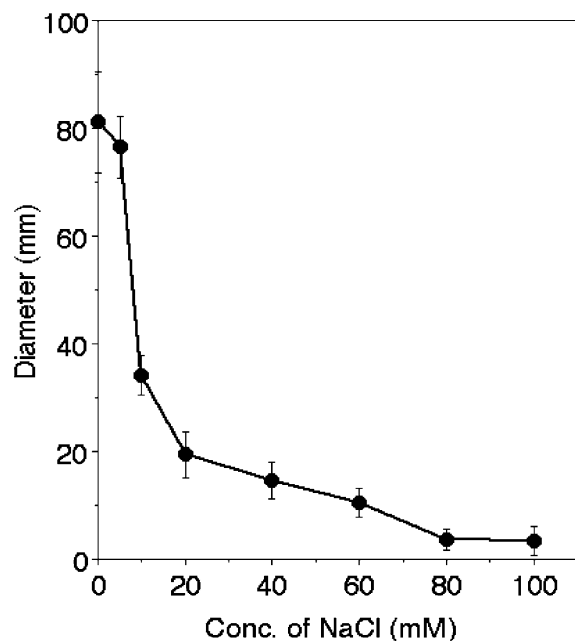


Figure 1. Evaluation of binding activity of human-specific IgE with dough added various concentrations of NaCl by single-radial immunodiffusion. Each value represents the mean $\pm$ standard error in four trials.

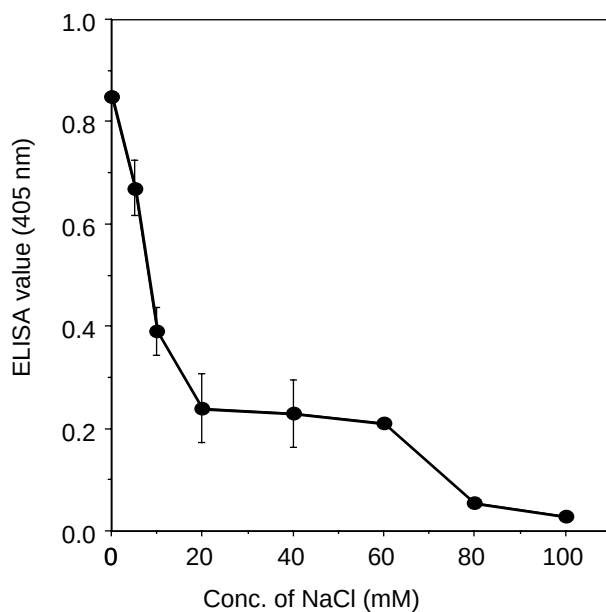


Figure 2. Evaluation by ELISA of human-specific IgE binding to dough with various concentrations of NaCl. Each value represents the mean $\pm$ standard error in four trials.

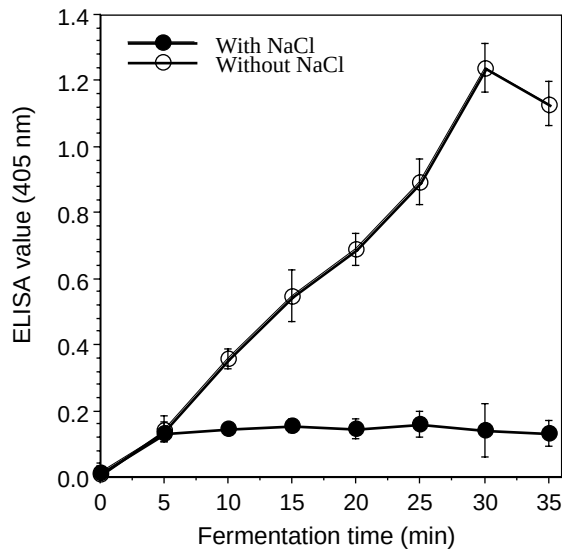


Figure 3. Time-course of the binding activity of human-specific IgE with dough during fermentation process. —○—: dough including 100 mM NaCl, —●—: dough and no added NaCl. Each value represents the mean $\pm$ standard error in four trials.

Separation of proteins by affinity chromatography

Protein fractions extracted from the dough including 100 mM NaCl and no added NaCl were separated by immobilized-trypsin affinity column chromatography (Mine & Zhang 2001). The adsorbed substances were eluted by the linear gradient of pH 6.5–7.5 in 1.0 M phosphate buffer. The non-affinity fraction eluted in the flow-through void volume solution, whilst the bound fractions were eluted from the column by the buffer linear gradient elution. Latter fraction was further purified by SDS-polyacrylamide gel electrophoresis (PAGE). Amounts of protein in each fraction obtained by affinity column chromatography were measured by the Lowry et al. (1951) method.

SDS-polyacrylamide gel electrophoresis (PAGE)

Electrophoresis was performed in accordance with a method of Laemmli (1979) using Ready Gel J 18mA/gel (gradient gel) using a Mini-Protean II Electrophoresis Cell (Bio-rad Laboratories, Inc, Tokyo, Japan). After electrophoresis, the gel was stained using Coomassie brilliant blue R250. The HAST System automated electrophoresis device Cell was used as well.

Antigen-antibody reactions of baked bread

The dough including 100 mM NaCl and no added NaCl were baked for 30 min at 180°C. 0.1 M Phosphate buffer (pH 6.5, 30 ml) was added into baked bread (50 g) and crude proteins in it were extracted. The mixture was filtered. The filtrate including crude proteins was used for the experiment of IgE binding activity on ELISA.

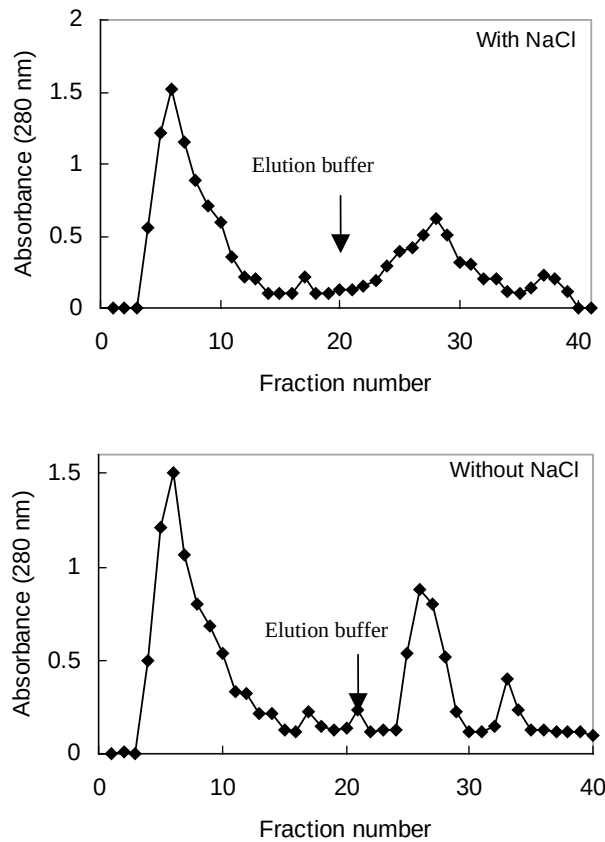


Figure 4. Elution profiles of proteins extracted from dough by affinity chromatography using immobilized-trypsin chitin. (Upper: Crude proteins from dough with 100 mM NaCl, lower: crude proteins from dough without NaCl).

Results and discussion

Measurement of antigen-antibody reaction of crude proteins extracted from dough of different NaCl concentration using single-radial immunodiffusion and ELISA

Crude proteins in dough were extracted using phosphate buffer. Crude protein solution extracted from dough without NaCl showed a concentration dependent antigen-antibody reaction (diameter of precipitin ring: 81 mm) against IgE in the single-radial immunodiffusion method. In contrast, crude protein solution extracted from dough added NaCl decreased precipitin ring. The diameter of the precipitin ring of crude protein extracted from dough having 20 mM NaCl was 19 mm. The diameter of the precipitin ring of dough having a higher concentration of NaCl decreased drastically (Figure 1).

The IgE binding activity of crude proteins using ELISA was confirmed. The reaction of dough extracts of a higher concentration of NaCl against IgE got weaker. Crude protein extracted from dough having more than 20 mM NaCl decreased antigen-antibody reaction against IgE drastically. It was concentration-dependent (Figure 2) and these results consisted with the results in Figure 1. These facts strongly

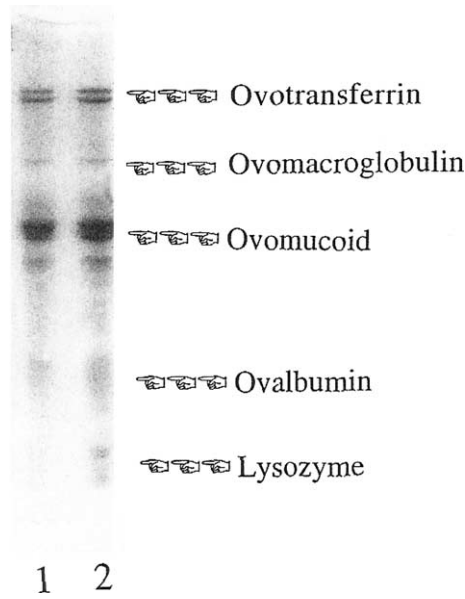


Figure 5. SDS-PAGE of 20–40 fractions numbers from the affinity chromatography studies. Lane 1, With 100 mM NaCl; L 2, without NaCl.

suggested that the addition of NaCl affected antigen-antibody reaction of dough crude protein.

Next, the generation of crude proteins showing antigen-antibody reaction during the fermentation process of dough with 100 mM NaCl and without NaCl was studied. The dough was fermented for 35 min in a drier (humidity 90%, 30°C). Proteins were extracted from the dough every 5 min during the fermentation process. The dough without NaCl tended to increase drastically the reaction against IgE during the

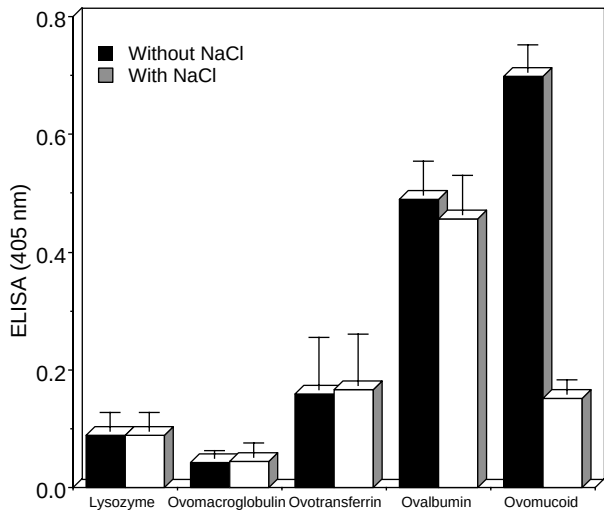


Figure 6. Comparison of the binding activity of human-specific IgE to proteins from dough with 100 mM NaCl and without NaCl. Each value represents the mean $\pm$ standard error in four trials.

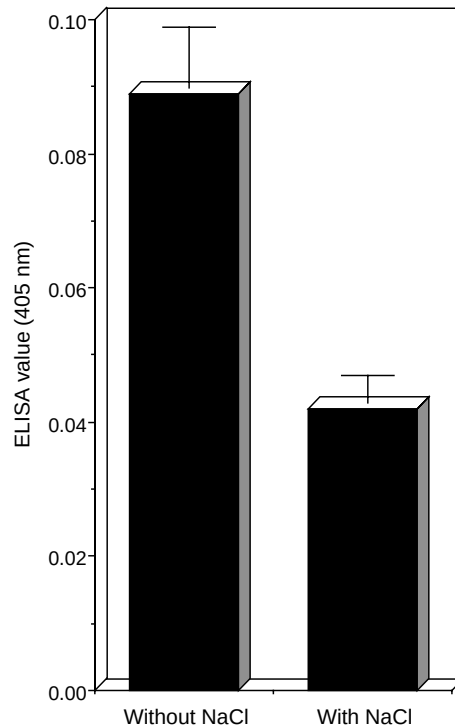


Figure 7. Comparison of the binding activity of human-specific IgE to crude proteins from baked bread made from dough with 100 mM NaCl and baked bread made from dough without NaCl. Each value represents the mean $\pm$ standard error in four trials.

fermentation process (Figure 3). The addition of 100 mM NaCl inhibited the production of proteins having a reaction with IgE in dough during the fermentation process.

Collection and identification of proteins by affinity chromatography

The crude proteins extracted from dough with 100 mM NaCl or without NaCl were separated using affinity chromatography of an immobilized-trypsin chitin. The crude proteins were applied on the affinity chromatography. The non-affinity fraction came into the flow-through solution, while affinity fractions of proteins are eluted from the column by the buffer linear gradient elution and the fraction numbers 20–40 were recovered (Figure 4). Proteins of both affinity fractions were identified by SDS-PAGE. Ovotransferrin, ovomacroglobulin, ovomucoid and ovalbumin were present in both affinity fractions of the dough with 100 mM NaCl and without NaCl (Figure 5).

Antigen-antibody reaction of proteins isolated by SDS-PAGE

Antigen-antibody reaction of each isolated protein against IgE was measured using ELISA (Figure 6). Ovalbumin isolated from both dough with 100 mM NaCl or without NaCl and ovomucoid isolated from dough without NaCl showed strong antigen-antibody reaction against IgE. In contrast, the antigen-antibody reaction of

ovomucoid isolated from the dough including NaCl was markedly weak as compared with dough without NaCl.

Antigen-antibody reactions of baked bread

Bread using the dough with 100 mM NaCl or without NaCl was actually made. Antigen-antibody reactions of crude proteins extracted from both bread samples were studied. Crude proteins extracted from bread using dough with 100 mM NaCl showed weaker antigen-antibody reactions with IgE than crude proteins extracted from dough without NaCl (Figure 7).

Thus, the addition of NaCl into dough weakens the antigen-antibody reaction of dough itself and the baked bread as compared with NaCl-free dough. We could confirm here the importance of NaCl as an ingredient of baked bread.

Acknowledgements

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