

Note

Effects of Heat and High-Pressure Treatments on Antigenicity of Beef Extract

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The sera of bovine gamma globulin (BGG) positive beef allergic patients were used in this study in order to investigate changes in IgE-specific binding activity with regard to beef extract altered by heat or high-pressure treatment. In inhibition-ELISA, the sample treated at 60°C did not show any significant changes in the antigenicity of BGG, but the sample treated at 100°C showed a decrease of the antigenicity. In the case of the treatment with heating at 100°C, heat-coagulation occurred in the beef extract. The resulting supernatant and precipitate of the sample by centrifugation were analyzed by immunoblotting. Only the fraction of precipitate showed a specific binding activity with the sera. Based on this result, it was speculated that the persistent antigenicity found even after the treatment at 100°C in inhibition-ELISA remained principally in the heat-coagulated fraction, which indicated the importance of the method of handling the heat-coagulation in heat treatment. High-pressure treatments (200 MPa–600 MPa) of beef extract did not show any significant changes in the binding with the sera.

Key words: antigenicity/allergenicity; heat treatment; high-pressure treatment; bovine gamma globulin; beef extract

Various food-processing techniques have been applied to foods in order to eliminate their allergenic proteins or to reduce their levels. The effects of heat treatment on food allergenic proteins have been widely studied by many research groups. Heat treatment reduced the sensitization of beef, even if the treatment was less effective on pure BSA under domestic heating conditions.^{1,2)} On the contrary, in some cases, heat treatment showed negative results. For example, the allergenicity of cow's milk³⁾ and shrimp allergens⁴⁾ increased as a result of heat treat-

ment. Heat treatment of milk in the presence of lactose is known as the browning reaction.⁵⁾ It is speculated that this reaction can increase the allergenicity of beta-lactoglobulin.³⁾ Restani *et al.*⁶⁾ have reported that heat treatment was not able to decrease the BSA capability to bind to IgE. On the other hand, Urisu *et al.*⁷⁾ have suggested the combination use of heat treatment with other procedures, which was useful in eliminating allergenic proteins in egg white. The allergenicity of the egg white was not eliminated completely by heat treatment alone, but the allergenic protein (ovomucoid) was completely eliminated by rinsing the egg white in a saline solution and distilled water after heat treatment.

High-pressure treatment has recently been considered a useful food processing technique and the efficiency of this treatment on meat has been reported by several research groups including that of Suzuki.⁸⁾ However, to date studies on the effects of the high-pressure treatment on food allergenicity have not been done.

In our previous study, we indicated bovine gamma globulin (BGG) played an important role in the allergenicity of beef.⁹⁾ Therefore investigation of the characteristics of BGG and further elimination techniques for its allergenicity are needed. In this study, the effects of heat and high-pressure treatment on antigenicity of BGG in beef extract were investigated with regard to the BGG-positive beef allergic patient. The allergenicity of the sample proteins could be indirectly evaluated from the investigation of their antigenicity (IgE-specific binding activity) to the sera of allergic patients.

Sera of BGG-positive beef allergic patients were obtained from Yoshida Hospital (Niigata) for this study. Beef extract was prepared with 20 mM sodium phosphate buffer (pH 7.4) according to the general

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procedures described previously.⁹⁾ The protein concentration of the extract was measured by the biuret method with BSA as the standard¹⁰⁾ and was adjusted to 5 mg/ml.

Heat treatment of beef extract was done in a captube at 60°C or 100°C for a specified time. High-pressure treatment of beef extract was done by the procedure of Homma *et al.*¹¹⁾ The sample sealed in a polyethylene bag was pressurized under the pressures at 200, 400, and 600 MPa at 5–7°C for 5 min using NBIP (Nikkiso Isostatic Processor). Inhibition-ELISA was done with untreated and heat- or high-pressure-treated beef extract as an inhibiting product. The serum (BGG-positive) was diluted (1:5 vol/vol) with various concentrations of the inhibiting product (0–500 µg/ml) and incubated for 2 h at 30°C. The inhibited serum (50 µl) was then used as the antibody for ELISA, which was done according to the method of Engval & Perlmann¹²⁾ with some slight modifications. The percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{OD}_{405\text{nm}} \text{ without inhibiting product} - \text{OD}_{405\text{nm}} \text{ with inhibiting product}}{\text{OD}_{405\text{nm}} \text{ without inhibiting product}} \times 100$$

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli¹³⁾ with some slight modification, using 4% stacking gel and 11% separating gel. The gel was stained and destained by the general procedures previously described.⁹⁾ Immunoblot analyses were done by the method of Towbin¹⁴⁾ with slight modifications. Alkaline phosphatase-conjugated goat *anti*-human IgE (Tagoimmunologicals Co. Ltd., USA) was used as the 2nd-antibody.

Figure 1 shows inhibition-ELISA results of the heat-treated beef extracts. The untreated sample (control) strongly inhibited the IgE-specific binding activity of the sera of BGG-positive beef allergic patients to beef extract. The inhibition rate gradually rose depending on the protein concentration of the sample to ~87% at 500 µg/ml. The sample treated at 60°C showed similar patterns. On the contrary, when beef extract was treated at 100°C, the inhibition rate decreased. The inhibition rate was ~35% after 10 min and ~32% after 30 min of heating at 500 µg/ml. Figure 2(A) shows the SDS-PAGE patterns of the control and heat-treated samples. As shown in Fig. 2(A), the SDS-PAGE patterns of the control and the samples treated at 60°C were generally similar except for their BGG band. The BGG band of the samples of 60°C was not clearly visible in comparison to the control. When the samples were heated at 100°C, the patterns were very different from both the control and sample of 60°C. The BGG band of the sample started to become faint and some unclear bands appeared at the start lines of both the stacking and separating gel (Fig. 2(A)-100°C). As the heating

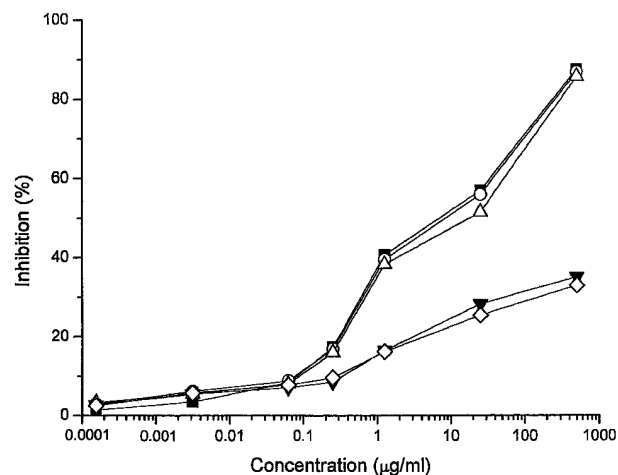


Fig. 1. IgE-specific Inhibition-ELISA with Beef Extracts Treated by Heat.

■ control; ○ 60°C for 10 min; △ 60°C for 30 min; ▼ 100°C for 10 min; ◇ 100°C for 30 min. Graphs plot percent inhibition versus log concentration of the inhibitor.

time became longer, this phenomenon became more evident. This could be a result of heat-coagulation in the samples. Both samples were immunoblotted to investigate their antigenicity. From Fig. 2(B), we can observe that all samples showed specific binding for BGG band. The binding activity of the sample treated at 100°C seemed weak in comparison to the control and the sample treated at 60°C. The sample of 100°C showed additional bindings to tailing bands on both stacking and separating gels. It was suspected from this result that the antigenicity of BGG remains in the aggregated fractions. At this point, another immunoblotting for both a resulting supernatant and precipitate by centrifugation of the sample was done. As shown in Fig. 3, only the precipitate showed specific binding at all treating times. This suggested that persistent antigenicity found even after treatment at 100°C in inhibition-ELISA remained principally in the heat-coagulated fraction, indicating the importance of the method of handling the heat-coagulation.

Figure 4 shows the inhibition-ELISA results of the beef extracts treated with high-pressure. There were few differences in inhibition rate between the untreated and the treated samples. However, the sample treated at 600 MPa, showed a slight decrease in its binding to the sera of beef allergic patients. This result might be due mainly to the decrease in protein solubility. No differences in SDS-PAGE patterns and immunoblotting were observed between the control and samples treated at pressures from 200 MPa to 600 MPa (data not shown). Together, these results showed that high-pressure treatments did not affect the antigenicity of BGG. Further study concerning the effects of high-pressure treatment and its combination with other food-processing techniques is

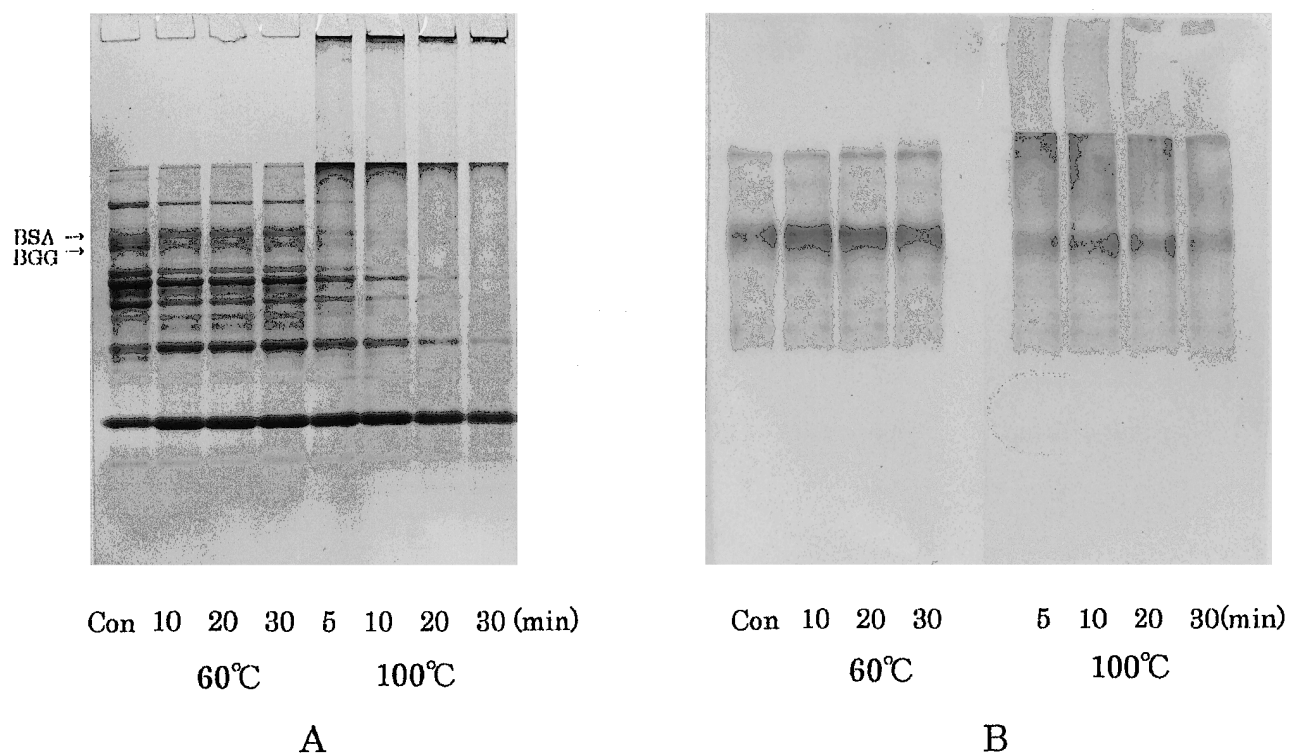


Fig. 2. SDS-PAGE and Immunoblot Analyses of the Beef Extracts Treated by Heat.
(A) SDS-PAGE; (B) immunoblotting to (A) with serum of BGG-positive beef allergic patient. Lane Con shows the control sample.

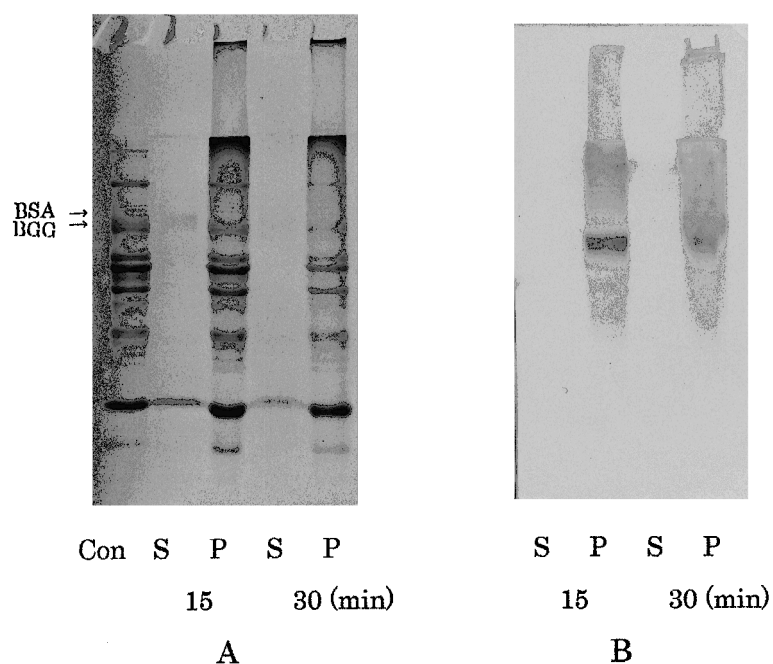


Fig. 3. SDS-PAGE and Immunoblot analyses of Resulting Supernatants and Precipitations of the Beef Extracts treated at 100°C.
(A) SDS-PAGE; (B) immunoblotting to (A) with serum of BGG-positive beef allergic patient. Lane Con shows the control sample. Lane S and P show supernatant and precipitation of the beef extracts treated at 100°C respectively.

necessary even if the treatment showed no positive result concerning the elimination of antigenicity of BGG in this study.

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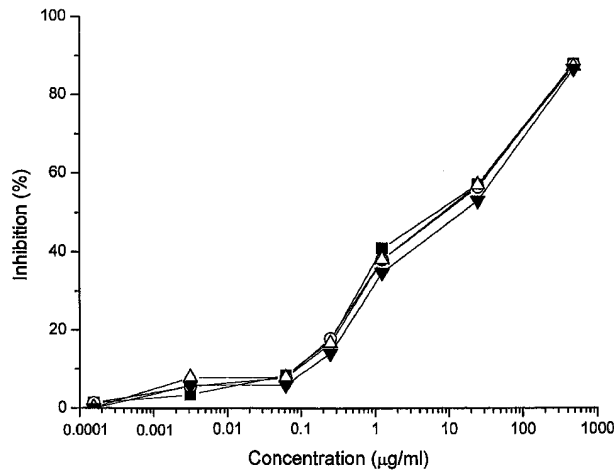


Fig. 4. IgE-specific Inhibition- ELISA with Beef Extracts Treated with High-Pressure.

■ control; ○ 200 MPa; △ 400 MPa; ▼ 600 MPa.

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