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EFFECT OF HIGH HYDROSTATIC PRESSURE ON STRUCTURE AND ALLERGENICITY OF BOVINE GAMMA GLOBULIN

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Introduction

Meat is an important food because of its high nutritional value and functionality. Although meat allergy is considered a rare condition, recent reports have demonstrated that it is a serious problem, particularly in children, which can strike unexpectedly. In our previous study, we identified beef allergens as bovine serum albumin (BSA) and bovine gamma globulin (BGG) (Han *et al*., 2000).

High pressure treatment is considered a useful food-processing technique, and the efficacy of this treatment for meat has been also reported by several research groups. However, to date there is a paucity of data on the effects of high pressure treatment on food allergenicity. We have demonstrated that high pressure could reduce the allergenicity of BSA evaluated by histamine release assay using sera from allergic patients and human basophilic KU812F cells (Suzuki *et al*., 2004). The objective of the present study was to investigate the effect of high pressure on the structure and allergenicity of BGG.

Materials and Methods

High pressure treatment. BGG solutions sealed in polyethylene bags were pressurized to 100, 200, 300, 400, 500 and 600 MPa at 5-7ºC for 5 min using a Nikkiso Isostatic Processor.

Inhibition ELISA. To evaluate the IgE binding activity of sera from patients with food allergies, ELISA was performed in accordance with the method of Han *et al.* (2000) with slight modifications. Fifty microliters of BGG solution in 50 mM sodium carbonate buffer (pH 9.6) was added to a 96-well ELISA plate. The plate was incubated at 30ºC for 2 hr, washed with PBS (pH 7.4), blocked at 30ºC for 1 hr with a 2% gelatin-PBS, and then washed with PBS-T (pH 7.4). Fifty microliters of serum (diluted 20-fold in PBS-T) was added to the plate, which was incubated at 30ºC for 1 hr. The plate was washed with PBS-T and then the second antibody (alkaline phosphatase-conjugated anti-human IgE) was added. The plate was washed with PBS-T. One hundred microliters of p-nitrophenyl phosphate in 10% diethanolamine buffer (pH 9.8) was added to the plate. After the plate was incubated at 30ºC for 60 min, absorbance was measured using a microplate reader with a 405 nm filter. To serum (diluted 10-fold in PBS-T) from a patient with an allergy to BGG was added an equal amount of pressurized BGG solution (0.2 µg/ml in PBS-T), and the mixture was incubated at 30ºC for 2 hr. The inhibited serum was then used as the primary antibody for ELISA. The percentage inhibition was calculated.

Measurement of β-hexosaminidase release activity. Human basophilic KU812F cells, obtained from the Cell Resource Center for Biomedical Research, Tohoku University (Japan), were washed in Tyrode buffer (pH 7.2) and resuspended in RPMI-1640 medium (1.0×10^6 cells/100 µl). In order to permit IgE to bind to the high-affinity IgE receptor FcεRI on the surface of KU812F cells, the cells were incubated with serum (diluted 1:100) exhibiting IgE binding activity to BGG at 37ºC for 2 hr. Then, the cells were washed and resuspended in Tyrode buffer including Ca^{2+} and Mg^{2+}. β-Hexosaminidase release was measured after stimulation of serum-sensitized cells with pressurized BGG at 37ºC for 40 min. Total β-hexosaminidase release was determined using cells treated with 0.1% Triton X-100. Stimulation of the cells was stopped by placing the cells on ice. After centrifugation, the amount of β-hexosaminidase activity released from the cells in the supernatant was measured in accordance with the method of Shibata and Yagi (1996) and Matsuda *et al*. (2002).

Determination of the secondary structure. Circular dichroism (CD) spectra for a pressurized BGG solution (0.2 mg/ml in 20 mM Tris-HCl, pH 7.4) were recorded by using a Jasco J-725 spectropolarimeter. The mean residual ellipticity is expressed in degrees·cm^2·$dmol^{-1}$, and was calculated using a molecular weight of 160,000 and 918 residues. The secondary structure of BGG was analyzed using the program developed by Yang *et al*. (1986).

Measurements of fluorescence spectra and center of spectral mass. Fluorescence spectra were obtained using a Hitachi F-2500 spectrofluorometer fitted with a high-pressure vessel and pump. A BGG solution (0.2 mg/ml in 20 mM Tris-HCl) was subjected to pressures of 0.1-400 MPa, and fluorescence spectra were measured after maintaining

each pressure for 5 min. After a pressure of 400 MPa was reached, the BGG sample was decompressed in stages. Fluorescence spectra between 300 and 450 nm were recorded for BGG during the pressurization and depressurization, with excitation at 280 nm. Changes in the center of spectral mass were calculated in accordance with the method of Ruan *et al*. (1998).

Measurement of the surface hydrophobicity. The surface hydrophobicity of pressurized BGG was measured in accordance with the method of Boyer *et al*. (1996). Either 10 µl of 5 mM 8-anilino-1-naphthalene-sulphonic acid (ANS) or 15 µl of 1 mM cis-parinaric acid (cPA) was added to 3 ml of BGG solution (0.05 mg/ml in 20 mM Tris-HCl). The fluorescence intensity of the ANS-protein conjugates was measured at 475 nm, with excitation at 380 nm. The excitation and emission wavelengths were 325 nm and 410 nm, respectively, for the cPA-protein conjugates.

Results & Discussion

IgE binding activity to BGG of sera from patients with food allergies was evaluated using ELISA. Sera from patients 5 and 64, which had high IgE binding activity, were used in this study. The IgE-specific binding activity of BGG to sera from patients allergic to BGG gradually decreased with increases in pressure.

BGG-induced b-hexosaminidase release from KU812F human basophilic cells sensitized with sera from allergic patients was inhibited by high pressure treatment of BGG to above 300 MPa for patient 5 or 100 MPa for patient 64, and was profoundly inhibited by high pressure treatment of BGG to 400 MPa for both patients. These results suggest that the allergenicity of BGG decreases after high pressure treatment.

Changes in the secondary structure of BGG after pressurization were investigated. Except for the slight increase in the b-structure after treatment at 600 MPa, almost no significant changes in the secondary structure were observed after high pressure treatment.

In the measurement of the fluorescence spectra of BGG after pressurization, the red shift and decrease in fluorescence gradually progressed with increases in the pressure applied. These observations indicate that the environment polarity of the tryptophan and tyrosine residues became stronger due to a pressure-induced change in the tertiary structure of the BGG molecule. Values of the center of spectral mass decreased gradually with increasing pressure. Furthermore, the values during decompression were lower than those at the same pressures during compression, and the value after release of pressure returned to the initial value. The values therefore suggest that the high pressure-induced structural changes in BGG are irreversible.

The aromatic and aliphatic surface hydrophobicity of BGG as measured using ANS and cPA increased slightly at pressures up to 500 MPa and then increased markedly when pressurized to 600 MPa.

Conclusions

From the results of this study, it is clear that high hydrostatic pressure affects the structure of BGG, especially the tertiary structure. Therefore, decreases in the IgE-specific binding activity and allergenicity of BGG after pressurization are probably due to the change in the tertiary structure that pressurization causes.

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