Effects of a High-Pressure Treatment on the Wheat Alpha-Amylase Inhibitor and Its Relationship to Elimination of Allergenicity

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Abstract. In this study, the effects of high-pressure treatment on structure and allergenicity of alpha amylase inhibitor (α-AI) were investigated. The pressure-induced structural changes of α-AI were estimated by fluorescence spectra and by fourth derivative UV-spectroscopy for probed tyrosine residues and by circular dichroism (CD) spectroscopy. The changes in the tertiary structure detected by fluorescence spectra and by fourth derivative UV-spectroscopy under high pressure were indicated at over 300 MPa. Measurements of CD spectroscopy suggested that the effects of a high-pressure treatment on changes in the secondary structure of α-AI were little. From our results, pressure-induced changes of the α-AI structure were not apparent. On the other hands, the IgE-specific binding activities of pressurized α-AI to sera from allergic patients against wheat, which is estimated by observations of dot-blotting, were decreased by high-pressure treatment. It is known that the pressure-induced elimination of allergenicity is related to the tertiary structural changes of allergen molecules. This study are suspected that the epitopes of α-AI do not contain tyrosine residues, and thus the decrease of IgE-specific binding activities is probably caused by the tertiary structural changes of these parts of α-AI.

1. Introduction

Wheat alpha-amylase inhibitor (α-AI) is one of the most frequent elements of immediate food allergic reactions [1]-[2]. Several researchers investigated to development of low allergic wheat foods [3]-[4]. However, elimination of allergenicity is difficult because properties of wheat are modified by low-allergization treatment and wheat α-AI is contained in all protein fractions of wheat.

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High-pressure treatment is considered an useful food-processing technique, and is effective for treated food materials. However, only a few studies on the effects of high-pressure treatment on food allergic reactions have been carried out [5]-[8]. If the biological activities of α-AI can be selectively modified by high-pressure treatment, high-pressure treatment is on useful for the production of low allergenic foods.

In this study, the effects of high-pressure treatment on structure and allergenicity of α-AI were investigated.

2. Materials and methods

2.1 Materials

The method of purification of wheat α-AI was modified by method of Kokiladevi et al. [9]. α-AI (Wako, Tokyo, Japan) solution (20 mM phosphate buffer, pH 7.0) was loaded onto Sephadex G-50 column (30 × 1 cm) equilibrated with the same buffer at flow rate of 0.2 ml min⁻¹. Fractions of α-AI dialyzed against water at 4°C for 48 h. After dialysis, α-AI solution lyophilized and stored at -20°C.

Sera from allergic patients against wheat were provided from Yoshida Hospital, Niigata, Japan. Serum stored at -80°C.

2.2 Methods

2.2.1 Pressurization. Pressurization was performed using the method described by Homma et al. [10]. α-AI solutions were vacuum-sealed in a polyethylene bag, was pressurized at 0.1-600 MPa at about 10°C for 10 min using a Cold Isostatic Press (CIP) apparatus from Nikkiso Co., Ltd., Tokyo, Japan.

2.2.2 Measurements of the fluorescence spectra and center of spectral mass of α-AI under high pressure. The α-AI (0.2 mg/ml) in 20 mM Tris-HCl (pH 7.0) was subjected to a high pressure of 0.1-400 MPa for 10 min. This device consisted of a temperature-controlled high pressure vessel equipped with sapphire windows and a TP-500 high-pressure hydrostatic pump capable of elevating the pressure to 400 MPa (Teramecs Co., Kyoto, Japan). Changes in the fluorescence spectrum of the α-AI were measured with a Hitachi F-2000 spectrofluorometer fitted with a high-pressure vessel. The fluorescence spectra of the proteasome were recorded under pressure between 300-420 nm, with excitation at 280 nm. Changes in the center of spectral mass (<ν>) were calculated according to the method of Ruan et al. [11].

2.2.3 Analysis of fourth derivative UV-spectroscopy under high pressure. The α-AI (1.0 mg/ml) in 20 mM Tris-HCl (pH 7.0) was pressurized by same method as 2.2.2. Changes in the UV-spectrum of the α-AI were measured with a Hitachi U-3350 UV-spectrometer fitted with a high-pressure vessel. The fourth derivative of α-AI was analyzed with the program of Lange et al. [12].

2.2.4 Measurement of the secondary structure of α-AI. The circular dichroism (CD) spectra of pressurized α-AI solutions (0.2 mg/ml) in 20 mM Tris-HCl (pH 7.0) were recorded by a Jasco J-725 spectropolarimeter at 20°C. The secondary structure of α-AI was analyzed with the program of Yang et al. [14].

2.2.5 The IgE-specific binding activities of pressurized α-AI to sera from allergic patients against wheat. The method of dot-blotting was modified by Morita et al. [11]. α-AI was dissolved in phosphate-buffered saline (PBS) at concentration of 1 mg/ml. α-AI (2 µg/spot) was blotted on nitrocellulose sheets (ATTO, Tokyo, Japan), and sheets were blocked for 2 h at 37°C in a buffer containing 1% gelatine and 0.1% Tween in PBS. The blocked sheets were rinsed with Tween/phosphate-buffer (PBST), and were then incubated overnight in PBST containing serum (final concentration of 17 UI/mg for serum IgE from α-AI allergy patients) at 4°C. The sheets were then washed with PBST. Subsequently, the sheets were incubated in PBST containing the second antibody, a 1:2000 dilution of goat anti-human IgE, HRP conjugate (Biosource, California, U.S.A.), for 1 h at 37°C. After being washed with PBST, the proteins on sheets were visualized by chemiluminescence using the ECL Plus kit (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.).
3. Results and Discussion

Changes in the fluorescence spectra are shown in Figure 1. The center of mass ($<\nu>$) calculated from figure 1 is shown in Figure 2. The changes in the fluorescence spectra under high pressure were observed at over 300 MPa, but the spectra were reversible depending on decompression. The $<\nu>$ values were also reversible. In other words, hysteresis in the recovery of the fluorescence parameters was apparent. Thus it suggested that high-pressure-induced structural changes in $\alpha$-AI were reversible.

Changes in the fourth derivative UV-spectroscopy are shown in Figure 3. The changes in the tertiary structure detected by fluorescence spectra and by fourth derivative UV-spectroscopy under high pressure were indicated at over 300 MPa, but the tertiary structure was reversible depending on decompression.

Changes in the secondary structure of $\alpha$-AI are shown in Figure 4. It suggested that the effects of a high-pressure treatment on changes in the secondary structure of $\alpha$-AI were little. The IgE-specific binding activities of pressurized $\alpha$-AI to sera from allergic patients against wheat are shown in Figure 5. The IgE-specific binding activities significantly decreased over 200 MPa.

The present results suggest that the effects of a high-pressure treatment on the secondary and tertiary structure of $\alpha$-AI were not apparent. However, our studies indicated that the IgE-specific binding activities of pressurized $\alpha$-AI to serum from allergic patients against wheat were decreased by high-pressure treatment.

It is known that the pressure-induced elimination of allergenicity is related to the tertiary structural changes of allergen molecules [4]-[6]. In our studies, measurement of the fluorescence spectra, center of spectral mass under and analysis of fourth derivative UV-spectroscopy under high pressure measured tyrosine residues as probe. Therefore, it may possible that pressure-induced modification of $\alpha$-AI’s tertiary structure was confirmed at the parts which do not include tyrosine residue. This study is suspected that the epitopes of $\alpha$-AI do not contain tyrosine residues, and the decrease of IgE-specific

![Figure 1. Fluorescence spectra of $\alpha$-AI under high pressure.](image1)

![Figure 2. Pressure dependence of the center of spectral mass of $\alpha$-AI.](image2)

![Figure 3. Fourth derivation UV-spectroscopy of $\alpha$-AI under high pressure.](image3)
binding activities is probably caused by the tertiary structural changes of these parts of α-AI.

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