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# The Improvement of Endoglucanase Characteristics After Compression by Cell Press

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## ABSTRACT

Effects of pressure up to $1.5 \times 10^8$ pascal on the structure and function of endoglucanase was studied. When the enzyme was pressurized at $1.5 \times 10^8$ pascal for 1 h at 20°C, the enzyme remained its activity on release of pressure, but its pH-activity and temperature-activity curves are different from that of the native enzyme and the half-life at 50°C was elongated about onefold. $\alpha$-helex content of the enzyme and the number of tryptophan residue on the surface of enzyme molecules were increased. After the enzyme was compressed at $4.5 \times 10^7$ pascal for 9 h, the enzyme remained its activity, whereas its structure was nonreversibly changed.

**Index Entries:** Endoglucanase; pressure; conformation; activity.

## INTRODUCTION

High pressure affects the structure and function of enzymes in a complex way. Recently interest in research and application of biological and biochemical phenomenon caused by high pressure has increased (*1,2*). The work previously described the changes on secondary bond formation between the enzyme and solvent, the effect of pressure on the reaction rate catalyzed by the enzyme (*3*), the change of substrate specificity (*4*).

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However these experiments mostly utilized sophisticated equipment for determination of the changes in activity and conformation during the course of the experiments. We wish to describe a simple pressure generation system (cell press) to examine the effects of pressure on the structure and function of endoglucanase. We did not concern ourselves with the change in enzyme properties accompanying the pressure course, but to the effects after pressure release from the enzyme. Some new enzymatic properties were generated—the temperature-activity curve and pH-activity curve of enzyme were changed, the half—life was elongated. Furthermore some structural information was obtained. The conformation changes of the pressure treated enzyme are rather stable. The improvement of pressurized enzyme properties was closely related to the conformational changes.

## MATERIALS AND METHODS

### Materials

Endoglucanase from Cellulase "ONOZUKA R-10" (Yakult Honsha Co. Ltd., Japan) was purified by Sephadex G-75, DEAE Sephadex A-50, and Superose 12 (fast protein liquid chromatography) column chromatography. The product showed one band as determined by polyacrylamide electrophoresis with a mol wt of 41,000 (*5*); Carboxymethylcellulose was purchased from Shanghai Chemical Reagent Co. All other reagents are of A-grade purity.

### Methods

#### *Pressure Generation System*

The pressure equipment used was a cell press made in the United States (French Co. Ltd.). A pressure vessel was a cylinder, 6 cm in diameter and 13 cm in height with a hole of 1.0 cm diameter at the center. The hole was filled with enzyme solution.

#### *The Preparation of Pressurized Enzyme*

The enzyme dissolved in 0.1*M* acetate buffer, pH 4.8, was placed into the pressure vessel. Air in the pressure vessel was removed by pushing the piston slowly. Then the enzyme solution held at a defined pressure for period of time at room temperature. The pressurized enzyme was obtained upon release of pressure. The temperature of the enzyme solution determined before and after application of pressure did not change.

#### *Enzyme Assays*

Enzyme activity was determined by dinitrosalicylic acid method (*6*). Carboxymethylcellulose, as substrate, was hydrolyzed by the enzyme at 50°C, pH 4.8, 0.1*M* acetate buffer for 30 min.

### *Spectra Analysis*

Spectra studies of the enzyme was carried out after the release of pressure. UV spectra was determined using a Hitachi 557 double wavelength, double beam spectrophotometer. Circle dicroism was recorded by a Hitachi JA-500C CD spectrometer.

### *The Determination of Half-Life of Enzyme*

The enzyme solution was incubated at 50°C, then the activity calculated. The half-life was calculated according to the following formula:

$$K = \{ 2.31\ g\ [(\text{activity})_t / (\text{activity})_o] \} / t; \qquad T_{1/2} = 0.693 / K$$

### *The Determination of Tryptophan Residues*

Determination of tryptophan residues carried out according to the method of Spande (*7*). *N*-bromosuccinimide as modifier was added to the enzyme solution, the decrease in absorbance at 280 nm was determined and the tryptophan residue content was calculated.

## RESULTS

### The Effect of Pressure on the Activity of Enzyme

Under the following conditions: (a) enzyme was pressurized under $4.5 \times 10^7$ pascal for different times; (b) enzyme was pressurized for 1 h at different pressure, the pressurized enzyme maintained its original activity.

### The Effect of Pressure on the Structure of Enzyme

Under the same conditions as above a, b, CD spectra were recorded (Figs. 1 and 2). When pressure is defined, elliticity at 208 nm increased in direct ratio with pressure time. When time is defined, elliticity at 208 nm also increased with the increase in pressure. Because $\alpha_H = ([\theta]_{208} + 4000)/(-2800)$, the $\alpha$-helix content of the pressurized enzyme was increased.

### Some Enzyme Characteristics of Pressurized Enzyme ($1.5 \times 10^8$ pascal, 1 h)

#### *The Stability of the Enzyme*

After the pressure treatment, the activity and UV absorbance of enzyme at 280 nm were observed daily. When enzyme was stored at 4°C, there is not obvious change of activity and absorbance at 280 nm within 8 d (after that time the enzyme became cold deactivation and some white precipitation appeared in solution).

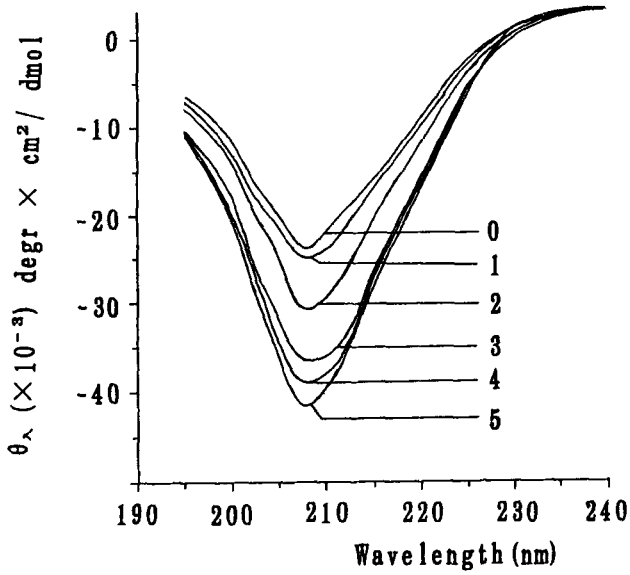


Fig. 1. Effect of pressure time on CD spectra of endoglucanase. Pressure is $4.5 \times 10^7$ pascal. (0) 0; (1) 10 min; (2) 20 min; (3) 1 h; (4) 4 h; (5) 9 h.

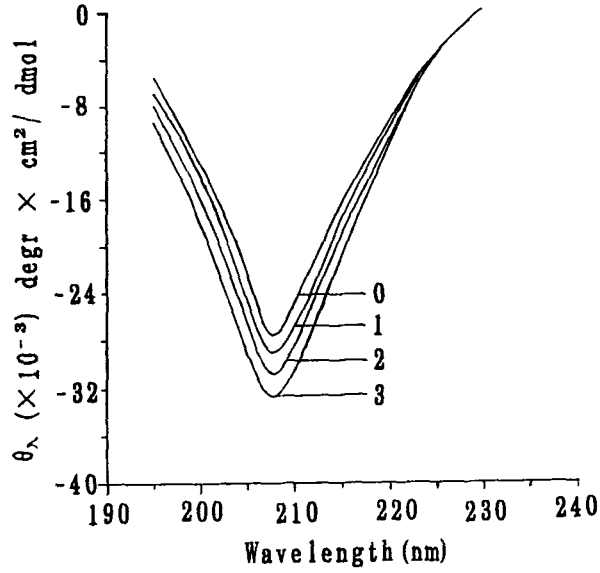


Fig. 2. Effect of pressure on CD spectra of endoglucanase. Pressure time is 1 h. (0) o; (1) $6 \times 10^7$ pascal; (2) $1.2 \times 10^8$ pascal; (3) $1.5 \times 10^8$ pascal.

### *Temperature-Activity Curve*

The optimal temperature for the pressurized enzyme was at 60–70°C. The optimum temperature for the native enzyme was at 60°C. It is noteworthy that within low temperature about 30–40°C, the pressurized enzyme had higher activity than native enzyme (Fig. 3).

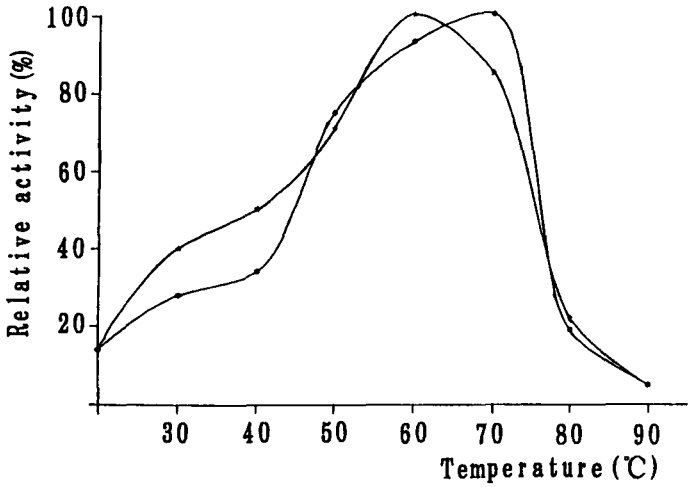


Fig. 3. The temperature-activity curve of endoglucanase. (*) the pressurized enzyme; (●) the native enzyme.

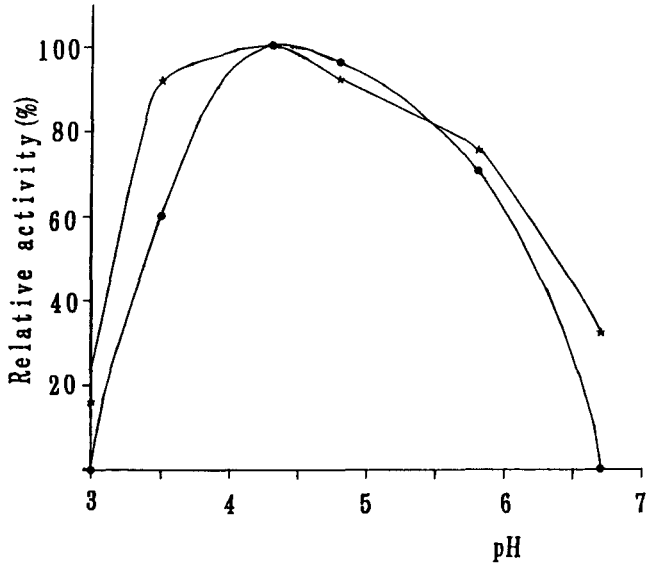


Fig. 4. The pH-activity curve of endoglucanase. (*) the pressurized enzyme; (●) the native enzyme.

### *pH-Activity Curve*

The optimal pH of the pressurized enzyme shifted to the acidic region and the optimal pH range was widened (Fig. 4).

### *The Half-Life of Enzyme at 50°C*

T1/2 for different times (Table 1). The half-life of pressurized endoglucanase was about onefold longer than that of the native enzyme.

Table 1
The Relationship Between Time and Enzyme Half-Life at 50°C[a]

| Time (hr) | 18 | 40 | 64 | 88 |
|---|---|---|---|---|
| T 1/2 of native enzyme (h) | 116 | 118 | 105 | 112 |
| T 1/2 of pressurized enzyme (h) | 199 | 219 | 221 | 213 |

[a]The standard deviation for native and pressurized enzyme are 5.74 and 9.93 respectively.

### *Tryptophan Content on the Surface of Enzyme*

*N*-bromosuccinimide reacted with the native and pressurized enzymes. The relationship between the number of modified trpytophan residues on the surface and remaining activity of enzyme is presented in Fig. 5.

The native and pressurized enzymes included 2 and 3 tryptophan residues, respectively, on the surface of molecule. Both enzymes remained higher in activity when *N*-bromosuccinimide modified the enzyme molecules.

## DISCUSSION

Figures 1 and 2 show that pressure does influence the enzyme conformation and that this change in conformation is stable. The higher the pressure and the longer the action time, the greater the change in enzyme conformation. However, the activity of the pressurized enzyme was retained. These data indicate that the active site of the enzyme has high stability and the conformation change may occur in other parts of the molecule that plays an important role in maintaining the overall enzyme structure. This phenomenon is different than the experiments we carried out with SOD (data not shown). SOD deactivated easily under the same experimental conditions. The main difference between these two enzymes is that SOD is an oligopolymer and endogluconase is a monomer, Jaenick (*1*) have postulated that oligoenzymes are more easily deactivated than monomers. Our experiments confirm this suggestion.

The temperature-activity and pH-activity curves showed that the pressurized endoglucanase has better resistance to the change in environment, especially at lower temperature. The pH region of the pressurized enzyme was broader than for the native enzyme. This may be because of the possibility that the structure of the pressurized enzyme is stronger than that of the native enzyme. The increased pH range and stability may be useful during simultaneous saccharification and fermentation of cellulose to produce alcohol.

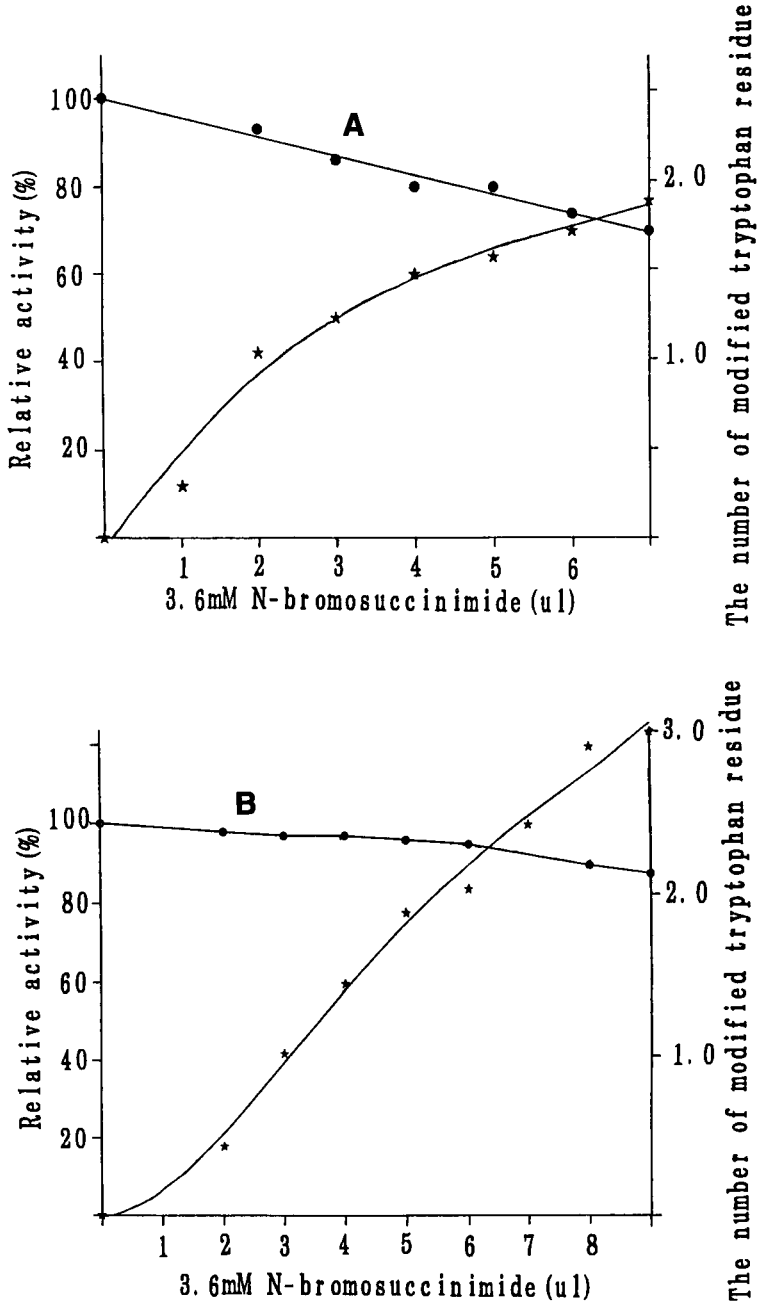


Fig. 5. Modification of tryptophan residues by *N*-bromosuccinimide. (A) the native enzyme; (B) the pressurized enzyme. (*) the number of modified tryptophan residues; (●) the enzyme activity.

The half life of the pressurized enzyme was longer than that of the native enzyme. This may be because of an enzymatic conformation change. The increase in $\alpha$-helix content of enzyme (Fig. 2) promoted the stability of the enzyme.

After the enzyme was treated by pressure, one tryptophan residue previously buried in the interior of the enzyme molecule was located on the surface of the enzyme. The native and pressurized enzyme remained higher activity when *N*-bromosuccinimide modified the enzyme. It was supposed that tryptophan residues were not the necessary residues. The decrease in enzyme activity is owing to partial change in enzyme structure near the active site. Because the structure of the pressurized enzyme is different than the native enzyme, the remaining activity of the pressurized enzyme was higher.

Above all, pressure as a method of modification will improve the properties of the enzyme. It has the characteristics of simplicity, convenience, and easy handling. It is worthwhile to explore this method for use in industry.

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