Short Communication

Enhancement of Activities of Cellulases under High Hydrostatic Pressure

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Numerous studies on the potential for use of high-pressure treatment in food science and technology have recently been reported.1–6) High-pressure treatment of foods causes denaturation of proteins,7,8) gelatinization of starch,9) and death of microorganisms.7,8) However, reports describing enzymatic reactions under high hydrostatic pressure are few in number. Although human beings do not often have to take changes in pressure into account, pressure is a thermodynamic parameter of enzyme-catalyzed reaction just like temperature. After application of high pressure to enzymes, Asaka and Hayashi10) reported that polyphenoloxidase in pear fruit was activated by such treatment. Prat-Gay et al.11) also reported that the activities of regulatory enzymes in the Benson-Calvin cycle from spinach chloroplasts were stimulated by high-pressure treatment. However, these enzymes were treated with high pressure and then their activities were examined under atmospheric pressure; the enzymatic reactions were not examined under high pressure. Fukuda and Kunugi12–15) studied the dependence on pressure of various kinetic parameters using proteases. Reactions catalyzed by trypsin11) and by carboxypeptidase Y13) were repressed by high pressure, but the activity of thermolysin was markedly stimulated.12) Okamoto et al.16) showed that high pressures could be useful to control enzymatic proteolysis and to selectively digest β-lactoglobulin in bovine milk whey. These results are very interesting and suggest that enzymatic reaction at high pressure is effective and high pressure can be applied to production of valuable materials by enzymatic reaction. Furthermore, it is expected that in digestion of insoluble substrates by enzymes under high pressure, the substrate is damaged by pressure, resulting in easy enzymatic digestion, i.e., in the enzymatic reaction under high pressure using substrates hard to digest, a greater effect of high pressure on digestion of the substrate can be expected.

Therefore, on the basis of this conception, to investigate the effects of high pressure on enzymatic reactions, at the beginning, we examined the reactions catalyzed by Acucelase17–19) (9 cellulase components from Aspergillus aculeatus No. F-5020) under high hydrostatic pressure. The Acucelase preparation was dissolved (2 mg/ml) in 0.1 M acetate buffer (pH 5.0). As substrates, we used microcrystalline cellulose (Avicel SF; mean particle size 6–10 μm; Asahi Kasei Co., Ltd., Tokyo) for measurements of avicelase activity and carboxymethylcellulose (CM-cellulose) (degree of substitution, DS, 0.65; degree of polymerization, DP, 500; Wako Pure Chemical Industries Ltd., Osaka) for measurements of CM-cellulase activity (1% solution in the same buffer). The solution of substrate was incubated at 37°C for 5 min, and then the enzyme solution (125 μl) was mixed with the substrate solution (125 μl) in a polyethylene tube (final volume, 250 μl). The tube was capped and placed in a Teflon tube (volume, 5 ml) that was filled with deionized water kept at 37°C. The Teflon tube was capped and immersed in lamp oil in the sample compartment of a pressure vessel. The apparatus was closed and pressure from 100 to 5000 kg/cm² was applied at 37°C for 10 min (for CM-cellulase activity) or for 100 min (for avicelase activity) with a hand-type oil pressure generator (type KPSB; Hikari Kotsu Co., Hiroshima). After the enzyme solution was mixed with the substrate solution, the time required to reach the desired pressure was 3 min, and from release of pressure to the end of the reaction, 3 min was needed. Accordingly the enzymatic reaction at atmospheric pressure was done at 37°C for 16 min (CM-cellulase activity) and 106 min (avicelase activity). After the pressure had been released, the reducing sugar released in the reaction mixture was measured as glucose by the method of Somogyi and Nelson.21) The amount of the reducing sugar was taken to represent an apparent enzyme activity. The results are shown in Fig. 1. CM-cellulase activity was enhanced and maximum CM-cellulase activity was obtained at 4000 kg/cm² (1.7 times greater than the activity at atmospheric pressure). With respect to the digestion of Avicel, the apparent activity at 3000 kg/cm² was 1.5 times higher than that at atmospheric pressure. Enzymatic reactions were also done under other conditions, as follows. (1) Enzyme solutions were treated with various high pressures at 25°C for 10 min and, after release of pressure, enzymatic reactions were done at 37°C for 10 min (CM-cellulase) and 100 min (avicelase) at

![Fig. 1. Effects of High Hydrostatic Pressure on Acucelase-catalyzed Reactions.](image-url)
Table I. Cellulase-catalyzed Reactions under High Hydrostatic Pressure

<table>
<thead>
<tr>
<th>Cellulase preparations</th>
<th>Relative activitya (%)</th>
<th>Avicelase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM-Cellulase activity</td>
<td>Avicelase activity</td>
<td></td>
</tr>
<tr>
<td>Acucelase</td>
<td>174 (4000 kg/cm²)</td>
<td>154 (3000 kg/cm²)</td>
</tr>
<tr>
<td>Meicelase</td>
<td>144 (4000 kg/cm²)</td>
<td>130 (4000 kg/cm²)</td>
</tr>
<tr>
<td>Cellulase A</td>
<td>115 (4000 kg/cm²)</td>
<td>107 (3000 kg/cm²)</td>
</tr>
<tr>
<td>Cellulase T</td>
<td>184 (2000 kg/cm²)</td>
<td>144 (4000 kg/cm²)</td>
</tr>
</tbody>
</table>

Enzymatic reactions were done at 37°C at various pressures with CM-cellulase and Avicel as substrates.

a Relative activity is expressed as the percentage of that at atmospheric pressure for each cellulolytic activity. Numerals in parentheses indicate pressures at which maximum activities were obtained.

b Avicelase activities of Cellulase A under other high pressures, except for 3000 kg/cm², were repressed (60%—85%).

atmospheric pressure using non-pressure-treated substrate. (2) Substrate solutions were subjected to various pressures at 25°C for 10 min and, after release of pressure, enzymatic reactions were done at 37°C for 10 min (for CM-Cellulase) and for 100 min (for Avicel) at atmospheric pressure using non-pressure-treated enzyme. The CM-cellulase and avicelase activities were not enhanced in either experiment, with the exception that the apparent avicelase activity of the enzyme that had been treated with 3000 kg/cm² for 10 min showed about a 1.6-fold increase over non-pressure-treated enzyme.

Further experiments were done to find whether commercial cellulases are enhanced under high pressure. Cellulases, such as Meicelase (from Trichoderma viride; Meiji Seika Kaisha Ltd., Tokyo), Cellulase T (from Trichoderma viride; Amano Pharmaceutical Co., Ltd., Nagoya) and Cellulase A (from Aspergillus niger; Amano Pharmaceutical Co., Ltd.) were reacted under various high pressures with the same conditions as mentioned above, except for the enzyme concentration (0.5 mg/ml). The results are shown in Table I. Enzymatic reactions under various high pressures with the listed cellulases were generally enhanced, although differences in the extents of increases in activities were observed. These observations may be due in parts to the crude nature of the enzymes in these preparations. Experiments were done under the conditions 1 and 2, mentioned above. Under these conditions, no noticeable enhancement of activities of these enzymes was observed.

References