High Pressure Effect on Maillard Reaction

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Reaction velocities of two reactions of the Maillard reaction, the condensation reaction of amino compounds with carbonyl compounds and the successive browning reaction along with melanoidin formation, were measured under 50-500 MPa at 50°C. The condensation reactions of glyceraldehyde with di- or tri-peptides were little affected by the applied hydrostatic pressure, and the reactions of glyceraldehyde, glycolaldehyde, or xylose with amino acids were suppressed very weakly by the pressure. On the contrary, the browning reactions were significantly suppressed by the pressure at 50-200 MPa with the activation volume of 13-27 ml/mol. These results indicate that the high pressure suppresses the browning process rather than the initial condensation process in the total course of the Maillard reaction.

High pressure treatment under ambient temperature is used for processing and preservation of food with the merit of keeping the original flavor, taste, and nutrients.1-5) However, it is often necessary to adopt the pressure treatment at moderately high temperature as described in the report of Taki et al.6) because the high pressure treatment at 40-60°C is effective to inactivate heat-stable spores of Bacillus species. In such combined use of high pressure and high temperature, the pressure effects on possible chemical reactions occurring in food processing are a concern in evaluation the resulting foods. Especially, chemical reactions related to the preparation of foods, such as hydrolysis, Maillard reaction, lipid oxidation, destruction of nutrients, lysinoalanine formation, and so on, should be studied, since there have been few reports concerning high pressure effects on such reactions, though the pressure effects on pure chemical reactions have been studied.7)

In a previous study,8) we found that the acid hydrolysis of various proteins is accelerated under the increased pressure, but that of starches and the other polysaccharides is little or not influenced. Now, we started to study high pressure effects on the Maillard reaction, and describe an interesting observation obtained in an initial study.

The term Maillard reaction refers to a series of complicated reactions, composed of the initial condensation reaction between an amino compound and carbonyl compound, followed by browning reactions including melanoidin formation, and polymerization processes. In this study, we analyze the pressure effects on two reactions separately, that is, the condensation reaction in the initial stage9) and the browning reaction in the advanced stage.

Materials and Methods

Maillard reaction. Carbonyl compounds (50 mM–1 M), such as DL-glyceraldehyde, D-xylose (Nacalai Tesque) and glycolaldehyde (Sigma), were mixed with 0.5 mM–1 M amino compounds, such as alanine amide·HBr (Ala-NH₂), L-His-Gly, or Gly-His-Gly (Sigma) in 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)
Carbonyl compounds were also mixed with \( \beta \)-alanine or L-lysine \( \cdot HCl \) (Nacalai Tesque) at 100 mM NaHCO\(_3\), pH 8.2. These mixtures were kept cooling in an ice bath until the reaction was started. One mM glyceraldehyde was mixed with one mM \( \beta \)-alanine for ESR spectrometry.

The reaction was started by putting a part of the mixture described above into a small plastic bottle and warming it at ambient pressure and 50°C or by pressurizing at 50-500 MPa and 50°C. High pressure was applied to the plastic bottle with a hand-type pressure generator (Hikari Koatsu Co., Type KP5B), using n-hexane as the pressure medium. The temperature was maintained at 50°C for 15–120 min by immersing the pressure vessel in a water bath. After the pressure was released, the samples were taken out and cooled in an ice bath, and the reaction was analyzed.

**Measurement of the reaction.** The extent of condensation reaction was measured by the disappearance of amino groups with 2,4,6-trinitrobenzene sulfonic acid.\(^{10}\) The extent of browning reaction was measured by an increase in the absorbance at 420 nm. The stable free radicals generated with browning in the reaction of \( \beta \)-alanine with glyceraldehyde were measured from the signal with a broad line by ESR spectrometer (Jeol, type JES-RE2X).

The activation volume, \( \Delta V^* \), of the condensation reaction was calculated from the slope of the line given from plots of \( \ln k \) against pressure, where \( k \) is the rate constant obtained. The \( k \) of the condensation reaction was observed as a first-order reaction. The \( k \) of the browning reaction was evaluated from the color development by taking \( A_{420} \) for the first hour as the reaction rate. The \( k \) of free radical generation derived from melanoidin was obtained by plots of \( \ln k \) against pressure.

**Results and Discussion**

**Pressure effects on the condensation reactions**

Condensation reactions of Ala-NH\(_2\), His-Gly, or Gly-His-Gly with glyceraldehyde were not affected by the applied pressure so far as followed by the disappearance of amino group (Fig. 1), but reactions of \( \beta \)-alanine or lysine with glyceraldehyde and of \( \beta \)-alanine with glycolaldehyde were suppressed in a small extent by the applied pressure. These results indicate that the condensation reactions occurring in the initial stage of Maillard reaction are only weakly affected by high pressure.

In general, it is possible to presume the signs of \( \Delta V^* \) in chemical reactions from the mechanistic features; bond formation and ionization contribute to having a negative \( \Delta V^* \), while bond cleavage and neutralization contribute to having a positive \( \Delta V^* \).\(^{7}\) Thus, the acid hydrolysis of proteins accompanying ionization is accelerated by pressurization with a negative \( \Delta V^* \).\(^{8}\) The \( \Delta V^* \) of the condensation reaction described here can be influenced mainly by two opposite factors, namely a C–N bond formation and a neutralization arising from the disappearance of an amino group. The cancellation of the two factors may be a reason why the condensation reaction shows no acceleration by high pressure.

**Pressure effects on browning reaction**

The browning was not observed in the reactions of Ala-NH\(_2\), di- or tri-peptide with glyceraldehyde, but the brownings occurring in the reactions of Lysine or \( \beta \)-alanine with glyceraldehyde, glycolaldehyde, or xylose were greatly suppressed under the increased pressure (Fig. 2). The generation of stable free radicals derived from melanoidin\(^{11,12}\) was suppressed with increasing pressure (Fig. 3).
High Pressure Effect on Maillard Reaction

Fig. 2. Effects of Pressure on Browning Reactions.
Reactions of lysine (---O--) or β-alanine (---Δ-- ) with xylose (each 1 mM), β-alanine with glycolaldehyde (---●--), and lysine (---△--) or β-alanine (---■--) with glycer- aldehyde (each 50 mM) were done in 100 mM NaHCO₃ (pH 8.2) at 50°C for 30-120 min. The reaction rate of brown color development was evaluated as an increase in absorbancy at 420 nm during the first hour.

Fig. 3. Effects of Pressure on Free Radical Formation.
Reaction of β-alanine with glyceraldehyde (each 1 mM) was done at 50°C for 15 min. Reaction rates of brown color development (---O---), and stable free radical formation derived from melanoidin (---●---) were evaluated as an increase in absorbancy at 420 nm, and relative intensity against a manganese marker, respectively.

These suppression effects were extensive up to 200 MPa.

The apparent activation volumes, $\Delta V^{\pm}$, of the condensation and the browning reactions were calculated and shown in Table I. Although two lines were fitted to the observed plots of some browning reactions (Figs. 2 and 3) arbitrarily, the calculation of $\Delta V^{\pm}$ was done tentatively using the plots up to 200 MPa. Data indicate that pressure suppresses the browning reaction more than the condensation reaction of amino groups with carbonyl groups.

It is difficult to explain the suppression of the browning reaction by high pressure in terms of the reaction mechanism, because the reaction processes in the advanced step of Maillard reaction including radical generation, polymerization, and melanoidin formation are not fully elucidated. It may be worthwhile to point out that the diffusion rate of unstable free radicals with hyperfine ESR spectra is greatly reduced under high pressure.

Pressure effects on Maillard reaction

The Maillard reaction as a whole process is suppressed by high pressure greatly: color development of the mixture of β-alanine and

<table>
<thead>
<tr>
<th>Amino compounds</th>
<th>Carbonyl compounds</th>
<th>Apparent $\Delta V^{\pm}$ (ml/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala-NH₂ Glyceraldehyde</td>
<td>0</td>
<td>n.d.</td>
</tr>
<tr>
<td>His-Gly Glyceraldehyde</td>
<td>0</td>
<td>n.d.</td>
</tr>
<tr>
<td>Gly-His-Gly Glyceraldehyde</td>
<td>0</td>
<td>n.d.</td>
</tr>
<tr>
<td>β-Alanine Glyceraldehyde</td>
<td>+8.9^a</td>
<td>+19.2^b</td>
</tr>
<tr>
<td>Lysine Glyceraldehyde</td>
<td>+3.9^a</td>
<td>+12.8^b</td>
</tr>
<tr>
<td>β-Alanine Glycolaldehyde</td>
<td>+4.3^a</td>
<td>+18.0^c</td>
</tr>
<tr>
<td>β-Alanine Xylose</td>
<td>n.d.</td>
<td>+27.0^c</td>
</tr>
<tr>
<td>Lysine Xylose</td>
<td>n.d.</td>
<td>+21.7^c</td>
</tr>
</tbody>
</table>

Apparent $\Delta V^{\pm}$ was obtained under the pressure below

\(^a\) 400 MPa, \(^b\) 500 MPa and \(^c\) 200 MPa, respectively.

\(^d\) Calculated from ESR spectrometry below 200 MPa.

n. d., not determined.

Table I. Apparent $\Delta V^{\pm}$ of Condensation Reactions and Browning Reactions

Reactions of Ala-NH₂, His-Gly or Gly-His-Gly (each 0.5 mM) with glyceraldehyde (50 mM) were done at 50°C in 50 mM HEPES, (pH 8). Reactions of β-alanine or lysine with glyceraldehyde (each 50 mM), β-alanine with glycolaldehyde (each 50 mM), β-alanine or lysine with xylose (each 1 mM), and β-alanine with glyceraldehyde for ESR spectrometry (each 1 mM) were done at 50°C in 100 mM NaHCO₃ (pH 8.2).
glycolaldehyde was only 1/30 at 500 MPa and 50°C, though the condensation reaction proceeded to almost the same extent at the high pressure.

This interesting pressure effect can be an advantage or disadvantage in food processing, because food processing under high pressure could avoid the development of colors and flavors. In addition, the suppression of the browning reaction by high pressure may open new ways in terms of elucidating the detailed mechanism of Maillard reaction related to the preparation of foods and age-associated decline in vital functions.13)

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References