Note

Possible Formation of Dehydro-L-ascorbic Acid from 2,3-Diketo-L-gulonic Acid in an Aqueous Solution

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The reaction of 2,3-diketo-L-gulonic acid (DKG), which is one of the important intermediate products in the degradation of L-ascorbic acid (ASA) in both food and biological systems, in an aqueous solution was studied. The formation of a small amount of the γ-lactone, dehydro-L-ascorbic acid (DASA), from DKG was observed. This strongly suggests the chemical possibility of a reverse reaction in DASA hydrolysis which has been long believed to be irreversible.

Key words: 2,3-diketo-L-gulonic acid; dehydro-L-ascorbic acid; L-ascorbic acid; lactonization

It is known that ASA is oxidized to DASA, which is irreversibly hydrolyzed to yield DKG. DKG is an important intermediate compound in the oxidative degradation of ASA; therefore, an investigation on the chemical reactivity of DKG is considered important to provide information about the mechanism for degradation of ASA in food and biological systems. L-Lyxonic acid, L-xylonic acid, L-threonic acid and oxalic acid are well known as degradation products of DKG, and at the initial stage of degradation, the formation of two δ-lactones of DKG, the 2,3-enediol form (2,3-DKGL) and 3,4-enediol form (3,4-DKGL) of δ-lactone, has been reported. It has also been reported that the amount of 3,4-DKGL formed from DASA or DKG was about 10% under N₂ gas bubbling in a neutral solution. In this study, the yield of DASA, the γ-lactone of DKG, was determined and compared with those of 2,3-DKGL and 3,4-DKGL formed from DKG. Although the hydrolysis of DASA has been considered to be irreversible, the possible formation of γ-lactone (DASA) from DKG is investigated.

All chemicals were obtained from Wako Pure Chemical Industries, except for dithioerythritol (DTE) from Nacalai Tesque. The potassium salt of DKG, the sodium salt of 2,3-DKGL, and DASA were prepared according to the methods of Kagawa, Tanaka, and Ohmori, respectively. The potassium salt of DKG was purified by dissolving it in a small amount of water and reprecipitating three times with ice ethanol. A 3,4-DKGL solution was prepared by incubating a DKG solution (pH 7, 1/15 M phosphate buffer) at 30°C for 60 min. Adequate sensitivity for the analytical method used to quantify DASA was ensured by measuring as ASA after reducing with DTE. Therefore, the reaction of DKG in an aqueous solution was studied in the presence of DTE. DKG and DTE were dissolved in distilled water to make a final concentration of 25 mM and incubated at 30°C for 60 min. The effect of pH on the yield of DASA was examined by dissolving DKG in aqueous solution at various pH values (adjusting with a solution of HCl or NaOH) to make a final concentration of 50 mM and then keeping at 30°C for 60 min. Subsequently, after the DKG solution had been adjusted to pH 6, the same volume of a 50 mM DTE solution was added, and the reaction mixture was kept at 30°C for 20 min. ASA and the two δ-lactones were determined by a Shimadzu LC-6A liquid chromatograph equipped with an electrochemical detector (BAS LC-4B). HPLC analysis was carried out with an Inertsil ODS-2 column (150 × 4.6 mm id.; GL-Sciences) under the following conditions: mobile phase, 50 mM sodium phosphate buffer (NaH₂PO₄-H₃PO₄, pH 2.3); flow rate, 0.5 ml/min; detection, amperometrically at an applied potential of 500 mV. Figure 1 shows a high-performance liquid chromatogram for ASA, 2,3-DKGL, and 3,4-DKGL.

The yield of DASA formed from DKG was determined and compared with those of the two δ-lactones (Table 1). The yield of ASA was 0.44%, whereas those of 2,3-DKGL and 3,4-DKGL were 1.98% and 0.60%, respectively. The formation of the two δ-lactones, 2,3-DKGL and 3,4-DKGL, from DKG has been reported in the literature, but precise values for yield have not previously been reported. In the presence of a reducing reagent such as DTE, DASA was easily reduced to ASA. Although it is widely believed that the hydrolysis of DASA is irreversible, this result indicates that the formation of the γ-lactone, DASA, from DKG could occur similarly to the formation of the δ-lactones. We also confirmed the formation of ASA from DKG in the presence of Cys, GSH and H₂S, besides DTE. It therefore seems that a small amount of DKG had reversibly changed to ASA in the presence of a reducing reagent.

To confirm the formation of DASA from DKG, the effect of pH value on the yield of DASA was studied and compared with those of the δ-lactones (Table 2). It has been reported that DASA was quite unstable under neu-
Table 1. Formation of Lactones from DKG

<table>
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<th>Yield (%)</th>
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<tr>
<td>ASA</td>
<td>0.44 ± 0.09</td>
</tr>
<tr>
<td>2,3-DKGL</td>
<td>1.98 ± 0.10</td>
</tr>
<tr>
<td>3,4-DKGL</td>
<td>0.60 ± 0.18</td>
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Each value is presented as the mean ± SD (n = 5) and is expressed as a ratio, with the initial amount of DKG regarded as 100%. DKG contained originally a trace amount of ASA, DASA, and δ-DKGLs (ASA 0.02 ± 0.01%; DASA 0.10 ± 0.02%; 2,3-DKGL 0.21 ± 0.02%; 3,4-DKGL 0.32 ± 0.02%). The yield of DASA was calculated by subtracting the initial amounts of ASA and DASA in DKG, and those of δ-DKGLs were calculated by subtracting the initial amounts in a similar way.

Table 2. Effect of pH on the Yields of Lactones from DKG

<table>
<thead>
<tr>
<th>pH of DKG solution</th>
<th>Yield (%)</th>
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<tr>
<td></td>
<td>DASA (detected as ASA) 2,3-DKGL 3,4-DKGL</td>
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<tr>
<td>pH 2</td>
<td>0.46 ± 0.14** 0.88 ± 0.14 0.50 ± 0.03*</td>
</tr>
<tr>
<td>pH 3.5</td>
<td>0.32 ± 0.03 1.22 ± 0.15 0.37 ± 0.07***</td>
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<tr>
<td>pH 6</td>
<td>0.28 ± 0.07 1.20 ± 0.51 0.63 ± 0.04</td>
</tr>
<tr>
<td>pH 7</td>
<td>0.12 ± 0.07*** 1.80 ± 0.29 2.32 ± 0.14***</td>
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Each value is presented as the mean ± SD (n = 8-12) and is expressed as a ratio, with the initial amount of DKG regarded as 100%.

low, because it is considered that the formation of δ-lactones from DKG occurred easily. The formation of DASA from DKG even in the neutral pH region suggests that this reaction could also occur in biological systems; however, in the physiological pH region, this reverse reaction would make only a slight contribution to the reaction of DKG.

These results suggest that the reverse reaction in DASA hydrolysis is possible and that DKG might possess a minor ASA function in food and biological systems (Scheme). This finding might be helpful to understand the complex behavior and roles of ASA in various foods and biological systems.

References
6) Takagi M., Kawajiri A., Nakata, K. and Morita N., Behavior of 3,4-Endiol Form of 2,3-Diketo-Gulono-δ-Lactone Formed from Dehydro-L-Ascorbic Acid in Deoxygenated and Neutral Solution.
Formation of Dehydro-L-ascorbic Acid