DETECTION OF A COMPLEX INTERMEDIATE IN THE OXIDATION OF ASCORBIC ACID BY THE COPPER(II) ION

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Summary A complex intermediate of the copper(II) ion with the ascorbate anion was detected by using a rapid scanning spectrophotometer with a stopped-flow apparatus. The intermediate was confirmed to be a copper(II) complex ion by electron paramagnetic resonance measurements. The dependence of the absorbance of the complex on the concentrations of reactants and on the pH of the reaction solutions indicates that the complex is Cu(II)Ha+ where Ha is the ascorbate anion ligand. The absorption maximum, the molar absorption coefficient, and the formation constant of the complex are 410 nm, 33 ± 1 M⁻¹ cm⁻¹ at 420nm, and 42 ± 6 M⁻¹, respectively. The values are comparable to those in the other metal complexes of the ascorbate anion.

Copper(II) ion catalyzes the autoxidation of L-ascorbic acid (1-5). Kinetic studies of the reaction suggested the formation of a complex intermediate between the copper(II) ion and the monodissociated acid (4, 5). HAYAKAWA et al. (6) and SHTAMN et al. (7) clarified that the L-ascorbic acid is oxidized by the copper (II) ion even in the absence of an oxygen molecule, and they proposed a mechanism containing the formation of the complex intermediate. As the ascorbate oxidase contains the copper(II) ion, the detection of the complex intermediate of the ascorbic acid with the copper(II) ion may be significant in this connection.

The present study attempts to clarify the situation for the copper(II) ion system by using a degassed solution to lower the oxygen content, with particular attention being paid to the detection of any transient intermediates.

EXPERIMENTAL

Reagents. The L-ascorbic acid used in this investigation was of guaranteed reagent grade from Tokyo Kasei. As the recrystallized L-ascorbic acid gave the
same results as those of the non-purified L-ascorbic acid, the L-ascorbic acid was used without further purification. The copper(II) sulfate was purified by recrystallizing it twice. The other materials were of commercial reagent grade and were used without further purification. The water used was distilled twice with potassium permanganate in an all glass apparatus. The concentration of the stock copper(II) ion solution was determined by means of iodometry. The L-ascorbic acid solutions of known concentrations were made by accurately weighing out the required amount and dissolving the L-ascorbic acid quickly in acetate buffer or phosphate buffer solutions which were bubbled with nitrogen gas for 10 min before the preparation of the solutions. Fresh solutions were prepared prior to each measurement. The buffer solutions were prepared from sodium acetate and acetic acid above pH 3.5 and from potassium phosphate and sulfuric acid at pH 3. Sodium sulfate was used to adjust the ionic strength.

Measurements. The visible spectra of the reaction mixtures were recorded by using a Union RA-1300 rapid scanning spectrophotometer which has a mixing apparatus for the reactant solutions. The dead time of the apparatus was about 1 msec, and the optical path-length of the observation quartz tube cell was 2 mm. The mixing apparatus could be controlled with a thermostat to within 0.5°C. The absorbance of an intermediate was measured by the stopped-flow technique using the same instrument. pH was measured with a Hitachi-Horiba F-5 pH meter. The electron paramagnetic resonance (EPR) signal was recorded by the rapid quenching technique (8).

Prior to use, all solutions were bubbled with a nitrogen gas for 10 min in order to reduce the concentration of the dissolved oxygen.

RESULTS AND DISCUSSION

The visible and EPR spectra of the intermediate

The mixed solution of ca. 0.01 M copper(II) sulfate and L-ascorbic acid changed quickly to brown after mixing near pH 5 and an yellow precipitate was formed after a while. The precipitate was converted to reddish brown copper(I) oxide after several hours and the supernatant liquid became colorless. Figure 1 shows the spectra of the mixture at different times before the formation of the yellow precipitate. The absorption band near 400 nm was ascribed to the brown intermediate because both reactants, CuSO₄, and L-ascorbic acid, have no band near 400 nm (broken line in Fig. 1). This band was also observed in the solution containing no dissolved oxygen.

The EPR signals of the frozen samples of the brown compound and of the yellow compound were measured at 77 K. The yellow compound had no signal so that it was concluded to be a dehydroascorbic acid-copper(I) complex. Figure 2 shows the EPR signals of the brown compound and a frozen sample of the copper (II) aqueous solution. The signal of the brown compound (A in Fig. 2) was sharp,
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Fig. 1. The absorption spectra of reaction mixture at different times: 1. 0 ms, 2. 100 ms, 3. 400 ms; 25 mM CuSO₄ and 20 mM NaHA. A: 25 mM CuSO₄, B: 20 mM NaHA.

Fig. 2. EPR spectra of the frozen samples of the reaction mixture (A) and of CuSO₄ aqueous solution (B) at 77 K.

while the signal of the aquo-copper(II) ion (B in Fig. 2) was broad. The anisotropic signal A had a characteristic property of the copper(II) complex ion.

The ascorbate radical has been detected by previous workers (9–13). Larger-crantz observed the signal of the ascorbate radical for several hours at pH 6.6–9.6 (11) and Yamazaki and Piette observed that the radical has a lifetime of several
seconds in the oxidation of L-ascorbic acid by the peroxidase (10). On the basis of these, OGATA et al. proposed a mechanism involving the formation of a free ascorbate radical in the autoxidation of L-ascorbic acid catalyzed by the copper (II) ion (4). If the free ascorbate radical had been produced in our system, it may have been detected by means of a stopped-flow technique or a rapid quenching technique. No signal of the radical, however, was detected in the present study. This is probably due to the high reactivity of the copper(II) ion with the radical compound and does not necessarily reflect its absence.

**Formation constant of the intermediate complex**

The stopped-flow curves of the reaction mixtures indicate some absorption at 420 nm just after mixing. This indicates the formation of the complex intermediate during the dead time. The absorbance (D) was essentially constant for several hundreds of msec at the low pH and the low concentrations of the reactants.

When it is assumed that the absorption at 420 nm is assigned to the complex of the copper (II) ion with the ascorbate anion (HA⁻), the CuHa⁺ complex is formed as follows:

\[
\begin{align*}
H_2A & \rightleftharpoons H^+ + HA^- \\
HA^- + Cu^{2+} & \rightleftharpoons CuHa^+ 
\end{align*}
\]

where \( H_2A \) is ascorbic acid. At the initial time of the oxidation of ascorbic acid,

\[
\begin{align*}
a &= [Cu^{2+}] + [CuHa^+] \\
b &= [H_2A] + [HA^-] + [CuHa^+] 
\end{align*}
\]

where \( a \) and \( b \) are the respective total concentrations of copper and ascorbic acid, and \([A^2-]\) is neglected due to the small value of the second dissociation constant of ascorbic acid \((4.57 \times 10^{-12} \text{ M})\) (5). The first dissociation constant of ascorbic acid \( (K_a) \) and the formation constant \( (K) \) of the complex are given by Eqs. 5 and 6, respectively.

\[
\begin{align*}
K_a &= [H^+] [HA^-] / [H_2A] \\
K &= [CuHa^+] / [Cu^{2+}] [HA^-] 
\end{align*}
\]

As the CuHa⁺ complex is the only species which has an absorption at 420 nm in Reactions 1 and 2, the absorbance at 420 nm is given by Eq. 7.

\[
D = \varepsilon l [CuHa^+] \\
= \frac{\varepsilon K a b}{1 + [H^+] / K_a + K a (a + b)}
\]

where \( \varepsilon \) is the molar absorption coefficient at 420 nm and \( l \) is the optical path-length. When \( 1 + K [HA^-] \approx 1 \),

\[
D = \frac{\varepsilon K a b}{1 + [H^+] / K_a (a + b)}
\]
Therefore,

\[
\frac{ab}{D} = \frac{1 + [H^+] / K_n}{\varepsilon l K} + \frac{a + b}{\varepsilon l}
\]

(9)

Figure 3 shows the plot of \(\frac{ab}{D} vs. (a + b)\). The plot is linear at pH 2.75, while the plot curves at the high concentrations of ascorbic acid at pH 4 (● in Fig. 3). This is caused by the considerable concentration of HA\(^-\) at a high pH and a high concentration of ascorbic acid. Table 1 shows the formation constant and the molar absorption coefficient at 420 nm of the complex of the copper(II) ion with HA\(^-\). The value of \(\varepsilon\) at pH 2.75 is in good agreement with that at pH 4 and the value of \(K\) at pH 2.75 is comparable to that at pH 4. These facts support the concept that the

![Figure 3](image)

**Fig. 3.** Determination of \(\varepsilon\) and \(K\); plots of Eq. 3. 1. pH 2.75, 2. pH 3.96.

| Ion   | \(K/M^{-1}\)      | \(\lambda_{\text{max}}/\text{nm}\) | \(\varepsilon_{\text{max}}/\text{M}^{-1}\text{cm}^{-1}\) | Reference |
|-------|-------------------|-----------------------------------|---------------------------------|-----------|
| Cu(II) | 38±4              | 410                               | 33\(^a\)                        | This work |
|       | 46±3              |                                   | 34\(^b\)                        |           |
|       | 37±3              |                                   |                                 |           |
| Fe(III) | 0.55±0.4         | 560                               | 11                               |           |
| Ni(II) |                   | 390                               | 800                              |           |
| Fe(II) |                   | 380                               | 1,325                            |           |
| Co(II) |                   | 390                               | 215                              |           |

\(^a\) The value at 420 nm at pH 2.75. \(^b\) The value at 420 nm at pH 4.
brown intermediate is the complex of the Cu(II) ion with HA\textsuperscript{−}. The disagreement in $K$ is partly caused by the difference in the ionic strength of the solutions (1 M at pH 2.75 and 0.5 M at pH 4).

When the complex intermediate is assumed to be a complex of the copper(II) ion with the ascorbic acid (H\textsubscript{2}A), or with the divalent ascorbate anion (A\textsuperscript{2−}), the dependence of the absorbance on the pH of the solutions does not agree with the present results.

The absorption maximum of the Cu(II)H\textsubscript{a}+ complex ion is comparable to that of the complexes of some non-reductive metal ions with HA\textsuperscript{−} as shown in Table 1\textsuperscript{(14)}. The value of $\varepsilon$ in Cu(II)H\textsubscript{a}+ is comparable to Fe(III)H\textsubscript{a}2+ complex ion \textsuperscript{(15)}. The value of $K$ is comparable to that obtained by the pH measurements of the reaction mixtures in the copper(II) system (5). These results give positive proof of the conclusion that the brown intermediate is the complex of the Cu(II) ion with HA\textsuperscript{−}.

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