

Effects of Fe(III) and Cu(II) Ions on the Autoxidation of L-Ascorbic Acid

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Abstract

The autoxidation reaction of L-ascorbic acid (AsA), and, particularly, the oxidation rates of AsA in the presence of Fe(III) or Cu(II) ions were determined in water and methanol. UV spectral measurement (at 265 nm) and HPLC were used to determine the remaining amounts of AsA in water and methanol, respectively. It was found that, in the presence of metal ions, the autoxidation rate of AsA was significantly affected by the kinds of solvents used, and also by the kinds of metal ions present. Moreover, the first-order rate constants for the oxidation of non-dissociated AsA compared with dissociated-AsA were investigated. It was confirmed that the oxidation of AsA was more accelerated in the dissociated form of AsA than in the non-dissociated form of AsA in either with Fe(III) or Cu(II). It was also found that the Cu(II) at a concentration of 0.1 μM had a more significant effect on the first-order rate constants for the autoxidation of AsA than Fe(III) at 5 μM .

Key words: L-ascorbic acid, autoxidation, Fe(III), Cu(II)

INTRODUCTION

AsA is an important antioxidant in food biological systems. In general, autoxidation should be defined as the apparently uncatalyzed oxidation of a substance exposed to oxygen. However, since it is unlikely that the direct reaction of dioxygen with usual biomolecules occurs at significant rates, a catalyst, such as transition metal, is needed to enhance autoxidation rates (1,2). It has long been believed that AsA is oxidized to form monodehydro-AsA (MDAsA) resulting in dehydro-L-ascorbic acid (DAsA) and AsA by disproportionation. Further degradation through hydrolysis of DAsA to diketogulonic acid (DKG) results in an irreversible degradation reaction of AsA, and further oxidation of DKG to threonic acid (THA) and oxalic acid (OxA) (1). But the autoxidation mechanism, the mechanism of the reaction of AsA with an oxygen molecule, has not been fully clarified. This oxidation process is known to be accelerated by heavy metal ions (2-4), particularly iron and copper. Iron and copper catalyze various reactions in biological systems such as hydroxyl radical production by superoxide-generating systems (5), lipid peroxidation (6,7), as well as the autoxidation of AsA (2). The oxidation of AsA by metal ions has been the subject of many investigations. A study of the metal ions catalyzed oxidation of AsA was carried out by Weissberger et al. (8,9), Scaife (10), Khan (11,12), and more recently by Buettner (13,14). However, the autoxidation reaction of AsA in the presence of heavy metal ions has not been fully clarified. The concentrations of Fe(III) and Cu(II) used mainly in this study were 0.1 μM ~5 μM ; these concentrations have never been reported on before. The previous studies used concentrations of metal ions about 10

times higher than in this study. Moreover, it was reported that dissociated AsA was more reactive than the non-dissociated AsA in the autoxidation reaction pathway of AsA in the absence of metal ions.

Therefore, in this study autoxidation of non-dissociated AsA (AsA) and dissociated AsA (sodium-L-ascorbate: AsA-Na) were examined. Specifically, the oxidation rates of AsA in the presence of Fe(III) and Cu(II) ions were investigated in water and methanol. AsA is a well-known water-soluble vitamin. Consequently, this study examined AsA in water as well as methanol. Because methanol has a hydrophilic group as well as a hydrophobic group, methanol was used. The effects of Fe(III) and Cu(II) ions on the autoxidation of AsA in water were compared with that in methanol.

MATERIALS AND METHODS

AsA, sodium-L-ascorbate (AsA-Na), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were obtained from Waco Pure Chemical Industries, Ltd.. AsA was further purified by recrystallization. Ultra-refined water with an electrical resistance of 18 $\text{M}\Omega \cdot \text{cm}$ was used in this experiment. Commercial methanol was used.

Autoxidation reaction of AsA

AsA was dissolved in water or methanol at a concentration of 50 μM . The concentrations of Fe(III) and Cu(II) used in this study were 0.1 μM ~5 μM , respectively. The oxidation reaction was carried out by introducing oxygen gas to the AsA solution at a flow rate of 200 mL/min at room temperature. The effects of Fe(III) or Cu(II), and dissociation of AsA on the oxidation rates of AsA in water and methanol were examined.

Determination of the remaining amount of AsA

The amount of remaining AsA in the reaction mixture in water was determined by using a Shimadzu double-beam UV-180 spectrophotometer. It was estimated by the absorbance of the reaction solution at 265 nm, the wavelength of the absorption maximum of AsA in water. And the amount of remaining AsA in the reaction mixture in methanol was determined by using HPLC under the following conditions: column, Lichrosorb NH₂; column size, 4.6 mm (i.d.) × 150 mm; mobile phase, acetonitrile-water-acetic acid (50:20:2, v/v); apparatus, Shimadzu LC-10AD; flow rate, 1.4 mL/min; detector, electrochemical Detector (BAS LC-4B), potential, 500 mV vs. Ag / AgCl; injection volume, 20 μL.

Calculation method of first order rate constants

First-order rate constants were obtained from plots of $\log [a/(a-x)]$ vs. times 2.303 as the initial concentration of AsA approximate the value of the slope (a). The value of x means the concentration of the product at each time (11).

Statistical analysis

Significant differences between two groups were evaluated by Student's *t*-test.

RESULTS AND DISCUSSION

Effect of Fe(III) on the autoxidation of AsA

The oxidation of non-dissociated AsA (AsA) compared with dissociated-AsA (AsA-Na) were investigated. Fig. 1-(a), (b)

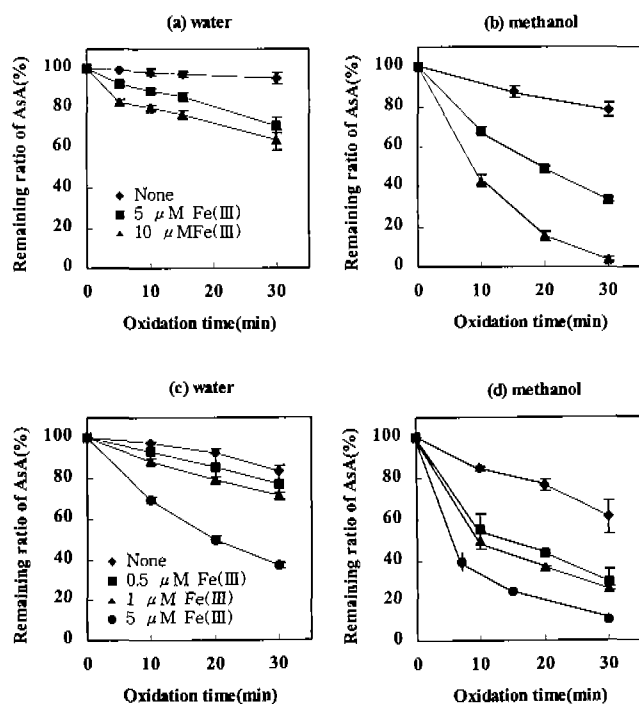


Fig. 1. Degradation of AsA in the presence of various concentrations of Fe(III). Data represent mean \pm SD ($n=5$) and are expressed as ratio with the initial amounts of AsA regarded as 100%. AsA [(a),(b)] and AsA-Na [(c),(d)] concentrations: 50 μM; reaction solution: water, methanol; reaction temperature: 25°C

shows the effect of Fe(III) ion on AsA autoxidation. In water, after oxidation for 30 min, the remaining amount of AsA in the presence of 5 μM Fe(III), and in the absence of Fe(III) was about 71.2%, and 94.8%, respectively. This indicates that the oxidation of AsA was more accelerated in the presence of Fe(III) than in the absence of metal ions. On the other hand, after oxidation for 30 min in methanol, the remaining amount of AsA in the presence of 5 μM Fe(III) was about 2.6 times lower than it was in the absence of Fe(III). Moreover, in the presence of 10 μM Fe(III), AsA was almost completely oxidized. The results show that AsA is readily oxidized in the presence of Fe(III) in methanol rather than in water because the concentration of oxygen was much higher in the methanol than in water. It was supposed that the concentration of oxygen in each solution were saturated because oxygen gas was bubbled into both the solutions. These results reflect the oxygen solubility of each solution. In general, oxygen solubility of organic solutions has not been fully clarified yet. But it was confirmed that the oxygen solubility was about 10 times higher in methanol rather than in water. The oxidation of AsA is greatly accelerated with the increasing Fe(III) both in the water and methanol.

Also the effect of the autoxidation reaction of the dissociated form of AsA (AsA-Na) in the presence of Fe(III) was investigated. Fig. 1-(c), (d) shows that in the presence of 5 μM Fe(III), after oxidation of AsA-Na for 30 min in water, the remaining amount of AsA is obtained was 37%. The remaining amount of AsA was approximately 3.6 times lower than in the absence of metal ions. In methanol, after oxidation for 30 min, the remaining amount of AsA was approximately 2.6 times lower than in the absence of metal ions.

According to the result, the autoxidation reaction of AsA-Na is accelerated in the presence of Fe(III) both in water and methanol, as with AsA. Both in water and methanol, the oxidation of AsA was more accelerated with the dissociated form of AsA than with the non-dissociated form of AsA. The oxidation of AsA-Na in the presence of Fe(III) was more unstable in methanol than water because the concentration of oxygen was much higher in methanol than in water.

It has been reported that the catalytic activity of cupric ions is more than that of ferric ions toward the oxidation of the ascorbate anion at the height acidity (11). The pH of AsA and AsA-Na in water was measured at 4.5 and 6.7, respectively in this study. The oxidation rate of AsA in the presence of Cu(II) compared with Fe(III) was also determined.

Effect of Cu(II) on the autoxidation of AsA

Fig. 2-(a), (b) shows the effect of Cu(II) ion on AsA autoxidation. The remaining amount of AsA in the presence of 5 μM Cu(II) after oxidation for 10 min in the water, was almost 0%. It appeared that the oxidation of AsA was accelerated in the presence of Cu(II), after oxidation for 10 min in the water. These results are consistent with the finding of Buettner (13) who reported that Cu(II) is approximately

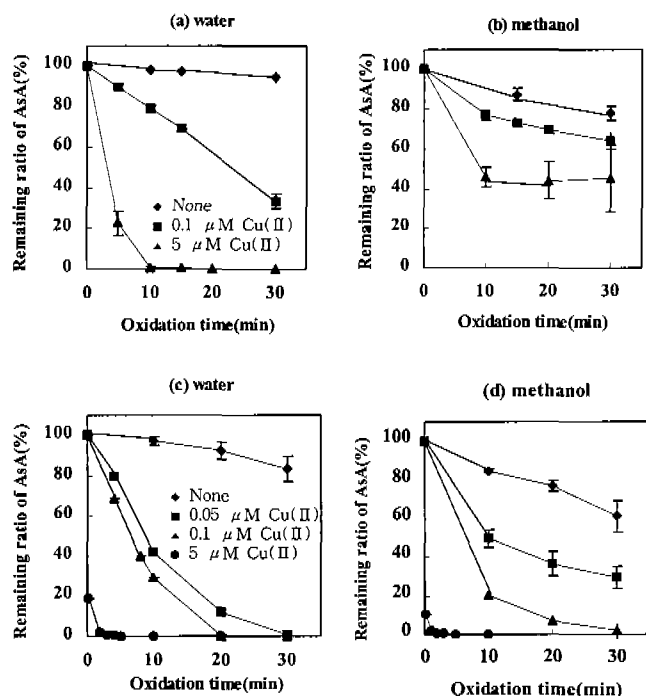


Fig. 2. Degradation of AsA in the presence of various concentrations of Cu(II). Data represent mean \pm SD ($n=5$) and are expressed as ratio with the initial amounts of AsA regarded as 100%. AsA [(a),(b)] and AsA-Na [(c),(d)] concentrations: 50 μ M; reaction solution: water, methanol; reaction temperature: 25°C

80 times more efficient a catalyst for ascorbate oxidation than Fe(III) in phosphate buffers. However, the AsA in the methanol remained stable after oxidation for 10 min. It is suggested that the oxidation of AsA in the presence of Cu(II) is stimulated only in a short time. The results indicated that the effect of Fe(III) and Cu(II) ions on AsA autoxidation differs. The oxidation rate of AsA is faster in water than in methanol in the presence of Cu(II). It was found that the catalytic effects of Cu(II) were more rapid than Fe(III) in water, which agreed with the results of the earlier study (11), but had a reverse effects in methanol. Thus, in the presence of metal ions the autoxidation rate of AsA was significantly affected by the kinds of solvents and metal ions introduced.

Fig. 2-(c), (d) shows the effect of the investigated autoxidation reaction of AsA-Na in the presence of Cu(II). The remaining amount of AsA after oxidation for 5 min was 49% in water and was 42% in methanol in the presence of 0.1

μ M Cu(II). AsA-Na in water and methanol in the presence of Cu(II) were both completely oxidized after 20 min to 30 min. It was confirmed that the oxidation of AsA-Na in the presence of Cu(II) was not very significantly affected by the kinds of solvents which differ from that of the non-dissociated form of AsA. For both AsA and AsA-Na, the catalytic activity of Cu(II) was significantly larger than Fe(III).

As a result, the autoxidation reaction of AsA-Na in the presence of metal ions is accelerated both in water and in methanol as with AsA. The oxidation of AsA-Na was more accelerated than AsA in both water and methanol in the presence of metal ions. The oxidation of AsA-Na in the presence of metal ions was more stable in water than in methanol.

The first-order rate constants for the autoxidation of AsA

Table 1 shows the first-order rate constants for the autoxidation of AsA by molecular oxygen with varying concentration of Fe(III) or Cu(II), present in the catalytic concentrations investigated. In the presence of metal ions, it was found that the first-order rate constant for the autoxidation of AsA was considerably larger with AsA-Na than AsA in water. The pH of both solutions were 6.7 and 4.5, respectively. That is the oxidation of AsA in water is greatly accelerated given an increase in pH. It was also found that Cu(II) ion at the concentration of 0.1 μ M had a very significant affect on the first-order rate constants for the autoxidation of AsA than did Fe(III) at 5 μ M. Compared with Khan and Martell study (11,12), at pH 1.5~3.5, Khan and Martell showed that the first-order rate constants for the autoxidation of AsA was considerably larger with metal ions. Also according to the report by Buettner (13), at pH range 4.5~7.0, the catalytic effect of Cu(II) catalysis was more effective than with Fe(III), results which agree with this study. Thus, in the presence of metal ions, it was found that the first-order rate constants for the autoxidation of AsA was considerably larger with the dissociated form of AsA than with the non-dissociated form of AsA.

SUMMARY

The autoxidation rate of AsA in the presence of metal ions was significantly different between in water and methanol,

Table 1. Rate constants for the metal-ions-catalyzed autoxidation of AsA

	Concentrations of Fe(III)	Rate constants	Concentrations of Cu(II)	Rate constants
AsA	0.01×10^{-5} M	7.2×10^{-5} sec ⁻¹	0.01×10^{-5} M	36.5×10^{-5} sec ⁻¹
	0.5×10^{-5} M	20.0×10^{-5} sec ⁻¹	0.1×10^{-5} M	76.7×10^{-5} sec ⁻¹
	1×10^{-5} M	37.0×10^{-5} sec ⁻¹	0.5×10^{-5} M	482.0×10^{-5} sec ⁻¹
	2×10^{-5} M	78.9×10^{-5} sec ⁻¹		
AsA-Na	0.01×10^{-5} M	10.3×10^{-5} sec ⁻¹	0.005×10^{-5} M	144.2×10^{-5} sec ⁻¹
	0.5×10^{-5} M	61.0×10^{-5} sec ⁻¹	0.01×10^{-5} M	240.8×10^{-5} sec ⁻¹
	1×10^{-5} M	140.0×10^{-5} sec ⁻¹	0.1×10^{-5} M	968.3×10^{-5} sec ⁻¹

and also with Fe(III) and Cu(II) ions present. It was also found that Cu(II) ion at 0.1 μM had a very significant effect on the first-order rate constants for the autoxidation of AsA than did Fe(III) at 5 μM . The first-order constants was confirmed that the oxidation of AsA was more accelerated in dissociated form of AsA than non-dissociated form of AsA in either Fe(III) or Cu(II).

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