Kinetic Analysis of the Oxidation of Ascorbic Acid in an Open Reactor with Gas Bubbling

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An open reactor with gas bubbling with various oxygen partial pressure was applied to investigate the role of oxygen in the oxidation of ascorbic acid (AA). The reaction kinetics was described well by the zero-th order mechanism and the rate constant was exactly proportional to the oxygen partial pressure but no effect of initial AA concentration was observed. The reaction rate increased with an increase in pH and decreased by the addition of EDTA as a chelating agent to trace metals suggesting the involvement of metals in the AA oxidation process. The temperature dependence of the kinetics was measured from 0 to 70°C and the activation energy was obtained to be 62.1 kJ/mol. On the contrary, the AA oxidation was described by the 1-st order kinetics in a closed reactor system because of the limiting oxygen supply. From these results, the aerobic AA oxidation scheme was estimated with the reaction between molecular oxygen and AA as a limiting step.

Keywords: kinetic analysis, oxidation of ascorbic acid, oxygen partial pressure, zero-th order kinetics

1. Introduction

As ascorbic acid (AA) is an important food ingredient, its stability in processing and storage has attracted many researchers interest. Therefore, many kinetic analyses for the stability of AA have been carried out [1] but still there is controversy and no established theory is available. Metal ion and metal chelate catalyzed oxidation of AA was investigated and the catalytic contribution of cupric and ferric chelate was clarified but no effect of oxygen partial pressure was reported [2].

Wilson et al. [3] comprehensively carried out a kinetic study of the oxidation process of AA in solution using an isothermal microcalorimeter. They found that the oxidation reaction of AA was described by the 1-st order kinetics with respect to the oxygen concentration. The reaction rate was found to increase with pH, oxygen concentration and with coexistence of metals.

As for nonthermal processing, high pulsed electric field treatment was applied to orange-carrot juice [4] and orange juice-milk beverage [5]. Degradation process of AA in the processing was described by the zero-th order kinetics in the former while the 1-st order kinetics was applied in the latter. Sonication was applied to orange juice and the 1-st order kinetics was applied [6].

In the long time storage, AA degradation has been described by the 1-st order kinetics for orange-carrot juice [4] and blood orange juice [7] while the zero-th order kinetics was applied for orange juice-milk beverage [5] and orange, grape, and pomegranate juices, and sour cherry nectar [8]. Among these investigations, many researchers pointed out the important role of oxygen [3, 9, 10] and the use of oxygen scavenger material was proved to be effective for the preservation of AA [10].

In the present paper, we apply an open reactor with gas bubbling with the oxygen partial pressure controlled from 0 to 100 % to investigate the role of oxygen in AA oxidation process directly.

2. Experimental

2.1 Materials and sample preparation

Ascorbic acid sodium salt was purchased from Nacalai Tesque (Kyoto). Acetonitrile, acetic acid, disodium dihydrogen ethylenediamine tetraacetic dehydrate (EDTA-2Na), and ethylene tetraacetic acid tetrasodium salt tetrahydrate (EDTA-4Na) were obtained from Kanto Kagaku (Tokyo). All other reagents used were of reagent grade. Gas containing 0 (N₂), 5, 20 (air), 50, and 100% oxygen gas in a cylinder was supplied by Uno Sanso (Fukui).

2.2 Open reactor with gas bubbling

To a 100 mL three neck flask kept in a water bath at a controlled temperature (0 to 70°C), 100 mL of ascorbic acid (AA) sodium salt at various concentrations (0.1 to 0.5 g/L) dissolved in a buffer solution (pH: 4–8) was added and gas containing a certain oxygen level was introduced and was mildly bubbled through a teflon tube to start the reaction. The AA solution was sampled at intervals to analyze AA. A buffer with 1/15 M KH_2PO_4 –1/15 M Na_2HPO_4 was used for pH 6 and 8, and 0.05 M potassium hydrogen citrate – 0.1 N NaOH was used for pH 4.

2.3 Closed reactor system

To 100 mL of 0.15 g/L ascorbic acid (AA) sodium salt dissolved in Sorensen buffer at pH8.0 (1/15 M KH_2PO_4 – 1/15 M Na_2HPO_4), air was bubbled for 5 min to saturate oxygen. Then, the solution was poured into 8 mL glass tubes with minimum head space and sealed with screw caps. Tubes were kept in a heat block (Dry Thermo Unit DTU-1C, Taitech Corporation, Saitama) at a controlled temperature to start the reaction and were taken out at intervals to analyze AA.

2.4 Assay of ascorbic acid

A 20 μL sample was directly injected into a HPLC system (PU-980 HPLC pump, UV-970, Jasco, Tokyo) with a column (Inertsil-NH₂, GL Sciences, Tokyo) eluted by acetonitrile: H₂O: acetic acid=87:11:2 at a flow rate of 2 mL/min detected at 243 nm. The LC chromatogram was processed by a software (Chromato-Pro, Runtime Instrument, Kanagawa).

3. Results and Discussion

3.1 Kinetic analysis of AA oxidation in open reactor with gas bubbling

3.1.1 Kinetics analysis

Figure 1A and 1B show the degradation kinetics of AA with a bubbling by air at 70°C with pH 6.0 described by the zero-th order (Eq.(1)) and the 1-st order kinetics (Eq.(2)), respectively.

$$C_A / C_{A0} = 1 - (k_0 / C_{A0}) t \quad (1)$$

$$C_A / C_{A0} = \exp(-k_1 t) \quad (2)$$

where C_A is AA concentration at a time t , C_{A0} is that at the initial state, and k_0 and k_1 , respectively, are the rate constants in the zero-th order and the 1-st order kinetics. It is evident that the zero-th order kinetics fits exper-

Fig. 1 Kinetic analysis of AA oxidation at 70°C with air bubbling at pH 6.0 described by the zero-th order (A) and the 1-st order kinetics (B). Initial AA concentration was 0.15 g/L.

imental data much better in the present case.

3.1.2 Effect of AA concentration

Effect of AA concentration on AA stability is shown in Fig. 2. Apparently, no effect of AA concentration is observed. In the present case, saturated oxygen concentration is 0.187 mM while the lowest AA concentration in the experiment was 0.505 mM so that AA concentration cannot limit the initial process. The similar situation frequently occurs in the preservation of fruits juices, which contain much higher AA concentration [11].

Fig. 2 Effect of initial AA concentration on the oxidation rate of AA at 50°C with air bubbling at pH 8.0.

3.1.3 Effect of oxygen partial pressure

Effect of oxygen partial pressure on AA oxidation rate is shown in Fig. 3. The reaction rate was strongly affected by O_2 concentration. From the slope of this plot, the zero-th order kinetic parameter, k_0/C_{A0} was obtained. This parameter was plotted to oxygen concentration as shown in Fig. 4. An excellent linear relationship was observed, which shows that the oxygen supply is the limiting step in AA oxidation.

3.1.4 Effect of pH

Figure 5 shows the effect of pH on AA oxidation rate at

Fig. 3 Effect of oxygen partial pressure on AA oxidation rate at 50°C at pH 8.0 with bubbling gas with various O_2 partial pressure. Initial AA concentration was 0.15 g/L.

Fig. 4 Effect of oxygen partial pressure on the zero-th order rate constant, k_0 , for AA oxidation at 50°C, pH 8.0. Initial AA concentration was 0.15 g/L.

Fig. 5 Effect of pH on AA oxidation rate at 70°C with air bubbling. Initial AA concentration was 0.15g/L.

70°C with air bubbling. The reaction rate increase markedly with an increase in pH. In fruits juice, pH is mostly around 4.

3.1.5 Effect of trace metal

At the initial step of the AA oxidation, an important role of trace metals have been reported [2,3]. Therefore, the effect of EDTA addition was carried out to chelate trace metals. As shown in Fig. 6, with addition of EDTA, the AA oxidation rate decreased showing the contribution of trace metals.

3.1.6 Effect of temperature

Figure 7 shows the effect of temperature from 0 to 70°C on the zero-th order rate constant of AA oxidation in the form of Arrhenius plot. A good linear correlation was obtained and from this plot, the activation energy for AA oxidation reaction was obtained to be 62.1 kJ/mol, which is a typical value as a chemical process.

3.2 Kinetic analysis of AA oxidation in closed reactor

Figure 8 shows the kinetic analysis of AA oxidation in closed reactor. Air was introduced to the reaction mixture and bubbled for 5 min at the beginning then sealed to start the reaction at each temperature. In this case,

Fig. 6 Effect of EDTA addition on AA oxidation rate at 50°C with air bubbling at pH 8.0. Initial AA concentration was 0.15g/L.

Fig. 7 Effect of temperature on AA oxidation rate at pH 8.0 with air bubbling. Initial AA concentration was 0.15g/L.

Fig. 8 Kinetic analysis of AA oxidation in closed reactor system at pH 8.0. To the reaction mixture air was introduced and bubbled at the beginning for 5 min then sealed. Initial AA concentration was 0.15g/L.

the 1-st order kinetics was applicable because oxygen is consumed stoichiometrically in the AA oxidation process as follows [3].

$$d[O_2]/dt = k_1[O_2] \quad (3)$$

In the closed reactor system, $[O_2]$ decreased with time because it was consumed so that the 1-st order kinetics were observed while $[O_2]$ was constant in the open reactor system so that the zero-th order kinetics were observed. From the temperature dependency of k_1 in Fig. 8, the activation energy was obtained from the Arrhenius plot (data not shown) to be 51.2 kJ/mol, which is not different from the former case with the open reactor. This correspondence between the two shows that the same chemical reaction proceeds irrespective to openness or closedness of the reactor.

3.3 Scheme of AA oxidation in aerobic conditions

From the above results, the AA oxidation scheme is shown in Fig. 9, which has been basically proposed by Wilson et al. [3] from the kinetic analysis of AA oxidation by the closed reactor system using an isothermal microcalorimeter. In the present case, we applied open reactor system with gas bubbling to control the oxygen partial pressure directly and obtained a similar scheme. Effect of trace metal works at the initial step to pull-out hydrogen molecule from reduced AA to form monoanion ascorbic acid. Effect of pH will be also related to this step. Then, molecular oxygen attacks monoanion ascorbic acid to form ascorbate radical anion which spontaneously turn to dehydroascorbic acid. The dehydroascorbic acid can be reduced by mild reducing agent to reduced AA so this reportedly works as vitamin C. However, dehydroascorbic acid is not readily absorbed across the intestinal mucosa [9] so that active vitamin C would be mainly

Fig. 9 Reaction scheme of AA oxidation in aerobic condition.

reduced AA. Dehydroascorbic acid also changes to diketogulonic acid, which has no vitamin C activity anymore.

In the above scheme of AA oxidation, the limiting step as a whole is the reaction between molecular oxygen and monoanion ascorbic acid. When oxygen is sufficiently available like in the present open reactor system, the reaction proceeds in the zero-th order kinetics while oxygen supply is limited like the closed reactor system, the AA oxidation proceeds in the 1-st order kinetics. In the long time preservation of food, the situation will be the same. In some reports, 1-st order kinetics have been applied but in others zero-order kinetics were employed. In the latter case, however, the oxygen supply into the package might have some role in the preservation.

4. Conclusions

An open reactor with gas bubbling with various oxygen partial pressure was effective to investigate the role of oxygen directly in AA oxidation process. The reaction kinetics were described well by the zero-th order mechanism and the rate constant was exactly proportional to the oxygen partial pressure. The reaction rate increased with an increase in pH and decreased by the addition of EDTA as a chelating agent to trace metals suggesting the involvement of metals in the process. With change in temperature from 0 to 70°C, the activation energy was obtained to be 62.1 kJ/mol. On the contrary, the reaction kinetics were described by the 1-st order kinetics in a closed reactor system because of the limiting oxygen supply. From these results, keeping out oxygen and low temperature operation are effective to preserve AA in the processing and storage.

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ガス通気開放型反応器を用いたアスコルビン酸酸化反応の 速度論的解析

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酸素分圧を種々に変化させたガスを用いた通気開放型反応器を用いてアスコルビン酸 (AA) 酸化反応の速度論的解析を行った。反応速度は零次反応で記述でき、零次反応速度定数は酸素分圧に完全に比例したが、初期 AA 濃度には全く影響されなかった。反応速度は pH の上昇に伴い上昇、また、EDTA の添加により減少し、このことは、AA 酸化反応における微量金属の関与を窺

わせた。温度を 0℃ から 70℃ まで変化させて活性化エネルギーを求めた結果、62.1 kJ/mol となった。一方、ガス通気をしない閉鎖型反応器においては反応速度は一次反応であった。以上の結果に基づいて、AA と分子状酸素との反応を律速段階とする好気条件での AA 酸化反応機構を推定した。