ACTA CHEMICA SCANDINAVICA 3 (1949) 844–849

# Oxidation of Ascorbic Acid in the Presence of Copper

JORMA ERKAMA

*Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland*

Numerous investigations of the effect of copper on the oxidation of ascorbic acid[1–12] have revealed *inter alia* that hydrogen peroxide is formed as an oxidation product, simultaneously as the copper reduced to univalent cuprous ion is reoxidized, through the action of air, to bivalent cupric. The amount of oxygen used has also been studied and was shown to vary in copper-catalyzed oxidation from 0.5 to 1 mol $O_2$ for each ascorbic acid molecule[5,7,8]. This variation in amount of oxygen used is explained by an intermediate reaction product, hydrogen peroxide, being changed by further reactions. Should the hydrogen peroxide decompose completely, as for instance in the presence of catalase, the oxygen amount used is only one O-atom; on the other hand, should the hydrogen peroxide remain entirely as such in the solution the oxygen amount used is 2 atoms. Schümmer[4] and Mystkowski[5] reported, that hydrogen peroxide markedly accelerates the oxidation of ascorbic acid. Also Dekker and Dickinson[3] stressed this fact and assumed that in the initial phase of the reaction mechanism the ascorbate oxidizes slowly, through the agency of cupric ions, to an ion akin to semiquinone, which in the subsequent phase immediately and rapidly oxidizes, through the action of air, to dehydro-ascorbic acid.

Some observations made while investigating the oxidation of ascorbic acid near neutral reaction and in comparatively high copper concentrations promted the study reported here. An interesting fact was then observed, namely, that the consumption of oxygen was exceedingly small during complete oxidation of ascorbic acid, while red cuprous oxide was simultaneously precipitated. As the precipitation of cuprous oxide, obviously, played a decisive role in the reaction mechanism the present work was initiated with the view to clarify this phenomenon. The fact that precipitation of copper in conjunction with oxidation of ascorbic acid has hitherto passed unnoticed may chiefly be due to the fact that most of the kinetic investigations concerning

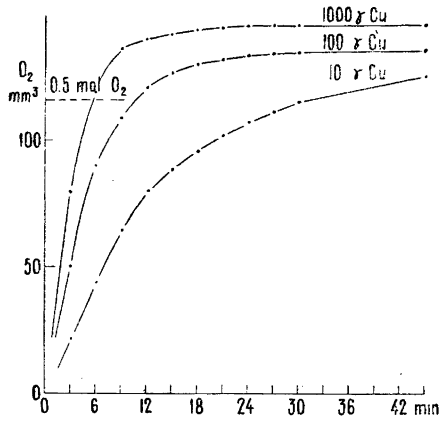


*Fig. 1. Effect of copper concentration on the amount of oxygen used in the oxidation of ascorbic acid in acetate buffer of pH 4.20.*

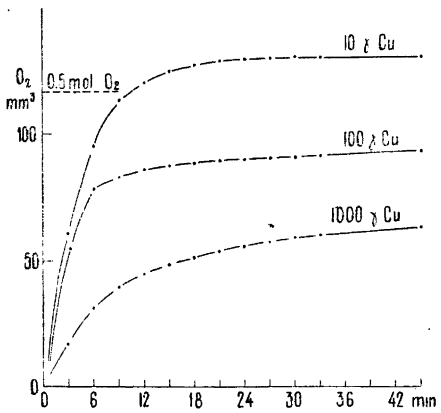


*Fig. 2. Effect of copper concentration on the amount of oxygen used in the oxidation of ascorbic acid in acetate buffer pH 5.95.*

ascorbic acid oxidation were carried out in relatively acid solutions, and moreover in the presence of comparatively low concentrations of copper. Mystkowski [6] is probably the only one who mentions precipitation of oxidulated copper. He, however, found no strict parallel between the amount of ascorbic acid oxidized and $Cu_2O$ formed.

## EXPERIMENTAL

Figs. 1 and 2 show the amounts of oxygen used as measured with the Warburg manometer in experiments wherein 1.84 mg *l*-ascorbic acid were oxidized in the presence of varying concentrations of $CuSO_4$ in a 0.1 *N* acetate buffer at 30° C and at two different pH-levels, at 4.20 and 5.95 respectively. Shaking rate 100 per minute, fluid volume 5 ml, ascorbic acid put in side arm and added after temperature equilibration and zero readings taken.

It is obvious that in the two experiments the effects of copper on the amount of oxygen used are in opposition to each other. On employing 10 $\gamma$ of copper, the oxygen amount used in both experiments is approximately the same, *i. e.* about 0.55 moles. In copper concentrations higher than this the oxygen amount used increased at pH-level 4.20 but decreased perceptibly at pH-level 5.95, sinking to about 0.28 moles on employing 1000 $\gamma$ copper. Despite this fact the ascorbic acid oxidized completely as indicated by titration at the end of the experiment.

To compare the oxidation rates a test was made: employing 1000 $\gamma$ of copper under the above given conditions the ascorbic acid oxidized was determined by titrating samples taken at 3 minute intervals. Figure 3 shows that ascorbic acid oxidized very rapidly at pH-level 5.95. At the same time the red precipitate formed during the very first minutes of the reaction. At pH 4.20 the oxidation rate was markedly slower.

The decrease in the amount of oxygen used is coupled with the cuprous oxide precipitated during the reaction. Besides the case in which maximum copper concentration (1000 $\gamma$ Cu) was employed a red turbidity was also observed at pH 5.95 when 100 $\gamma$ of copper were used. In examining the effect of pH on the precipitation of cuprous oxide under the above conditions (0.01 mmol ascorbic acid, 0.016 mmol $CuSO_4$ in 5 ml 0.1 $N$ acetate buffer) it was found that precipitation began at a pH-level above 4.5. In phosphate buffer having acid reaction no red precipitate could be detected, but the copper phosphate precipitated in neutral or alkaline reactions turned yellow after the addition of ascorbic acid.

The addition of protein prevented cuprous oxide from precipitating and increased the total amount of oxygen used (Fig. 4). The effect of ovalbumin is similar to that of casein, and cooked and uncooked vegetable proteins. Aspartic acid and glutamine also prevent the precipitation of cuprous oxide.

## DISCUSSION

The kinetic investigations concerning the oxidation of ascorbic acid indicate that in neutral and acid reactions the monovalent ion of ascorbic acid is coupled with the absorption of oxygen [3,9,10]. The fact that the oxidation rate is independent of ascorbic acid concentration indicates that decomposition of the copper ascorbate complex (CuA) to an intermediate product of semiquinon-like structure seems to be the rate-determining step. Dekker and Dickinson[3] assumed the following reaction series to take place:

$$Cu^{++} + H_2A \rightleftharpoons CuA + 2H^+ \quad (1)$$

$$CuA \rightarrow Cu^+ + \text{Intermediate products} \quad (2)$$

$$\text{Intermediate products} \rightarrow \text{Dehydroascorbic acid} \quad (3)$$

$B^-$ denoting the intermediate its aerobic decomposition is as follows:

$$B^- + O_2 + H^+ \rightarrow D + HO_2 \quad (4)$$

$$2\ HO_2 \rightarrow H_2O_2 + O_2 \quad (5)$$

or the anaerobic decomposition:

$$B^- + Cu^{++} \rightarrow D + Cu^+ \quad (6)$$

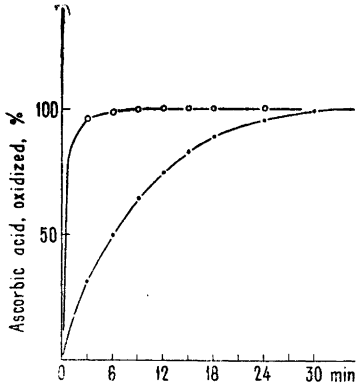


*Fig. 3. The oxidation of ascorbic acid in acetate buffer. Systems contained in 50 ml: 36 ml acetate buffer; 18.4 mg ascorbic acid; 39.3 mg Cu $SO_4 \cdot 5\ H_2O$.*

●——● *pH 4.20*
○——○ *pH 5.95*

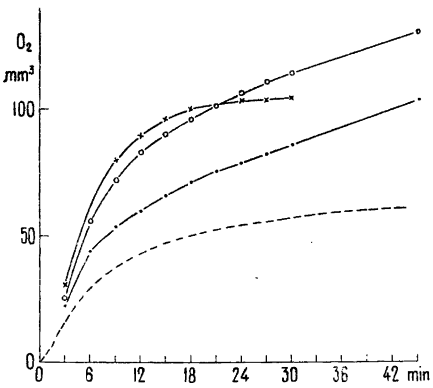


*Fig. 4. Effect of different casein concentrations on the oxygen uptake of ascorbic acid in acetate buffer of pH 5.95 and with 1000 γ of copper.*

– – – – *without protein*
●——● *3 mg casein*
○——○ *10 mg casein*
×——× *50 mg casein*

The cuprous ion formed re-oxidizes to cupric ion:

$$Cu^{+} + O_2 + H^{+} \rightarrow Cu^{++} + HO_2 \quad (7)$$
$$2\ HO_2 \rightarrow H_2O_2 + O_2 \quad (8)$$

In the reaction series given above the oxygen amount used is 1 mol per molecule of ascorbic acid oxidized. If, however, the oxidation of cuprous ions is prevented, for instance by its precipitating from the solution as cuprous oxide, the oxygen amount used decreases in aerobic oxidation to 0.5 mol and in anaerobic oxidation to zero.

The decomposition of hydrogen peroxide formed in the reaction has not been considered in the reaction series above. This would complicate the reaction considerably as copper is capable of decomposing hydrogen peroxide both catalatically and peroxidatively. Dekker and Dickinson assumed cupric ion together with hydrogen peroxide to catalyze the oxidation of the ascorbate ion so that the hydrogen peroxide itself does not decompose but accumalates in the solution:

$$Cu^{++} + H_2O_2 \rightarrow CuH_2O_2^{++} \quad (9)$$
$$CuH_2O_2^{++} + A^{--} \rightarrow Cu^{+} + H_2O_2 + B^{-} \quad (10)$$

Should hydrogen peroxide react quantitatively according to reactions (9) and (10) it would not change the oxygen amount used although the reaction rate would be accelerated. Obviously the catalatic or peroxidative decomposition of hydrogen peroxide would, on the other hand, decrease the oxygen amount used. It is probable that both in the catalatic and in the peroxidative reactions the copper peroxide functions as an active oxygen donator (*cf.* Krause [13]). This compound, however, forms only during the precipitation of copper hydroxide. The cupric ion does not decompose hydrogen peroxide.

The solubility product of copper hydroxide is $3.9 \times 10^{-19}$ (Näsänen [14]) wherefore in the concentration employed it should precipitate at pH-levels below 6. The acetate buffer, however, prevents it from precipitating. Consequently it is probable that under the conditions employed the hydrogen peroxide does not notably partake in the reactions. The small oxygen amount used on employing 1000 $\gamma$ of copper may, consequently, be due primarily to the precipitation of cuprous oxide.

In comparing the curves depicting oxygen amounts used with those for ascorbic acid oxidation (Figs. 2 and 3) it is seen that oxygen is consumed even after all ascorbic acid has been oxidized in the presence of 1000 $\gamma$ copper. This fact indicates that dehydroascorbic acid is formed chiefly anaerobically, and that oxygen is used solely for the oxidation of cuprous ions. According to Dekker and Dickinson the aerobic reaction (4) is, however, markedly faster than the anaerobic reaction (6) probably because of the instability of perhydroxyl, $HO_2$, in acid solutions. Obviously the precipitation of cuprous oxide greatly accelerates the anaerobic reaction by shifting the equilibrium thereof to the side of dehydroascorbic acid.

Should the reaction mixture contain compounds such as amino acids or proteins, which form complex linkages with copper, the precipitation of cuprous oxide is prevented. A natural result of this is an increase in the oxygen amount used, as shown in Figure 4. The intermediate $B^-$ decomposes aerobically and also the cuprous ion re-oxidizes to cupric ion. The oxidation of cuprous ion may, according to Mapson [11], also take place with hydrogen peroxide. Mapson is of the opinion that the reaction

$$2\ Cu^+ + 2H^+ + H_2O_2 \rightarrow 2Cu^{++} + 2H_2O$$

is the most natural explanation for the decomposition of hydrogen peroxide.

## SUMMARY

The effect of copper on the oxidation of ascorbic acid is studied. It is shown that in acetate buffer the ascorbic acid causes precipitation of cuprous

oxide in the presence of copper salt concentrations of approximately 0.001 *M* or more. As a result of this phenomenon the oxygen amount used in ascorbic acid oxidation is found to decrease so that considerably less than one atom is required for each molecule of ascorbic acid. This evidence promted some kinetic investigations.

It is probable that the oxidation of ascorbic acid in copper catalysis is an anaerobic process, and the accelerating effect which oxygen has on the reaction is based mainly on the reoxidation of the cuprous ion.

The proteins and some amino acids prevent cuprous oxide from precipitating and the oxygen amount used from decreasing.

The writer is indebted to students N. Petterson and B. Rehell for their valuable assistance with the experiments.

## REFERENCES

1. Barron, E. S. G., De Meio, R. H., and Klemperer, F. *J. Biol. Chem.* **112** (1936) **625.**
2. Lyman, C. M., Schulze, M. O., and King, C. G. *J. Biol. Chem.* **118** (1937) **757.**
3. Dekker, A. O., and Dickinson, R. G. *J. Am. Chem. Soc.* **62** (1940) **2165.**
4. Schümmer, H. *Biochem. Z.* **304** (1940) **1.**
5. Hand, D. B., and Greisen, E. C. *J. Am. Chem. Soc.* **64** (1942) **358.**
6. Mystkowski, E. M. *Biochem. J.* **36** (1942) **494.**
7. Steinman, H. G., and Dawson, C. R. *J. Am. Chem. Soc.* **64** (1942) **1212.**
8. Petersen, R. W., and Walton, J. H. *J. Am. Chem. Soc.* **65** (1943) **1212.**
9. Weissenberger, A., LuValle, J. E., and Thomas, D. S. *J. Am. Chem. Soc.* **65** (1943) **1934.**
10. Weissenberger, A., and LuValle, J. E. *J. Am. Chem. Soc.* **66** (1944) **700.**
11. Mapson, L. W. *Biochem. J.* **39** (1945) **228.**
12. Dodds, M. L. *Arch. Biochem.* **18** (1948) **51.**
13. Krause, A. *Ber.* **71B** (1938) **1229.**
14. Näsänen, R. *Ann. Acad. Sci. Fennicae* Ser. A. Tom **59** (1943) no. **7.**

Received May 21, 1949.