# CATALYTIC EFFECT OF COPPER IONS AND CHELATES ON THE OXIDATION OF ASCORBIC ACID*

E.SCHWERTNEROVÁ, D.M.WAGNEROVÁ and J.VEPŘEK - ŠIŠKA

*Institute of Inorganic Chemistry,*
*Czechoslovak Academy of Sciences, 160 00 Prague 6*

Received September 9th, 1975

The kinetics of the autoxidation of ascorbic acid, catalysed by copper ions, obeys the Michaelis–Menten law even in the presence of an inhibitor. As a consequence, the reaction proceeds by the mechanism of a ternary complex. The reaction catalyst proper is cuprous, not cupric ions, the reoxidation of cuprous ions by oxygen being slow in comparison with the overall rate of ascorbic acid autoxidation. An addition of chelating agents of the polyamine type inhibits the autoxidation of ascorbic acid; the inhibition effect, however, is less marked than as would correspond to the stability of the respective cupric chelates.

L-Ascorbic acid is oxidized by molecular oxygen to dehydroascorbic acid. Aside from the copper containing metal enzyme ascorbate-oxidase, the reaction is catalysed by some of the transition metals, for instance copper, iron, and vanadium, both free or bound in complex compounds[1].

For the non-enzymatic oxidation of ascorbic acid, two different mechanisms for the catalysis by free and complex ions were proposed by Khan and Martell[1-3]. The difference rests in the rate determining step, the reaction order, and the products of the oxygen reduction. Jameson and Blackburn[4] assume the autoxidation of ascorbic acid to proceed under formation of a tervalent copper complex. In a slow reaction, the complex liberates a radical of the arcorbic acid HA$^{\bullet}$ which is further oxidized to dehydroascorbic acid. Hatano and Kato[5] found that ascorbic acid reduced cupric ions in a one-electron process, forming a univalent copper chelate with dehydroascorbic acid. Reoxidation of cuprous ions by oxygen and formation of an ascorbate radical in various phases of the mechanism is taken into account in almost all the papers quoted above.

Efforts to identify the ascorbate radical by EPR spectroscopy did not lead to an unanimous result[6]. Yamazaki and coworkers[6], however, report that they did obtain a proof of the radical, and that they were also able to study the kinetics of its formation. Bielski[7] observed the optical absorption spectrum of the ascorbate radical and determined the rate of its desintegration.

The variances in kinetic data regarding the autoxidation of ascorbic acid are

* Part II in the series Oxidation by Molecular Oxygen; Part I: Z. Naturforsch. *29b*, 689 (1974).

to a great extent due to the fact that the reaction is trace catalysed[8] and, consequently, the catalyst is difficult to define. In the present study, the investigations were aimed in the first place at the mechanism of catalysis by copper ions as they are catalytically more active than ions of other transition elements.

## EXPERIMENTAL

*Chemicals.* Ascorbic acid (Index Pharmacorum No 3, Czechoslovakia), was a Farmakon product. Stock solution, approximately $5 . 10^{-2}$M was freshly prepared every three days; it was kept in a black bottle without access of light and bubbled through with a tiny stream of nitrogen. Chelating agents, EDTA, tetraethylenepentamine (TETR), triethylenetetramine (TRIEN), histidine (HIS), bis-(aminoethyl)glycolether NNN'N'-tetraacetic acid (Titriplex VI, TIT VI), nitrilotriacetic acid (NTA), diethylenetriamine (DIEN), diethylenepentaminetetraacetic acid (Titriplex V, TIT V), were products of Merck and Koch-Light, other chemicals were of A.R. grade. Nitrogen was purged of oxygen by passing it through a set of Drechsel bottles containing chromium(II) chloride.

*Equipment and procedure.* The concentration of oxygen during the reaction was followed by polarography at a constant potential of −0·7 V (S.C.E.) using a polarograph LP 55 and an EZ2 recorder. The initial concentration of oxygen (about $2·6 . 10^{-4}$M) was given by its solubility at normal atmospheric conditions. Calibration was made with 0·1M and 1M solutions of KCl with $1 . 10^{-5}$M methylene blue added to suppress the oxygen maximum. The reactions proceeded in a Britton–Robinson buffer medium. The concentration of the copper ions added was within $4·5 . 10^{-6}$—$2·6 . 10^{-5}$M, the concentration of the chelating agent was $9·01 . 10^{-6}$M.

The reaction was studied in a thermostated (25·0°C) polarographic cell with a side branch containing mercury. The cell was sealed with a silicone rubber stopper. The free space over the solution was removed by raising the level of the solution directly under the stopper with the aid of the mercury in the side branch. Reaction components or nitrogen were introduced into the solution by syringes.

The concentration of hydrogen peroxide formed during the reaction was determined by polarography. For determining the consumption of hydrogen peroxide by a subsequent reaction, hydrogen peroxide was added to the reaction mixture previously freed of oxygen, and its concentration was measured at constant potential −1·6 V (S.C.E.) for 30 min. The concentration of ascorbic acid was determined from its anodic wave with the half-wave potential $E_{1/2} = -0·49$ V (1N mercurous sulphate electrode) in a Britton–Robinson buffer medium at pH 8·82 (ref.[9]).

For measuring the absorption spectra of the solution, spectrophotometer Pye-Unicam SP 800B was employed. The reaction of cupric ions or cupric chelates with ascorbic acid took place in a thermostated vessel (25°C) provided with a worm expeller to ensure circulation of the solution between the reaction vessel and the flow-through cell of the spectrophotometer[10]. The thickness of the cell was 1 cm. A solution of cupric sulphate was added to the solution of ascorbic acid ($5·0 . 10^{-2}$M) neutralised with sodium hydroxide to the 1st degree; the resulting concentration of copper was $3·0 . 10^{-3}$M. For experiments with cupric chelates, solid ascorbic acid was added to the solution of the same concentration of copper as above and of a ratio [Cu]/[chelating agent] = 1. In the cases where no absorbance change was observed in an acid solution, the solution was additionally made alkaline by adding sodium hydroxide. Before mixing, the reaction components were purged of oxygen by a stream of purified nitrogen which was also used as a protecting atmosphere above the solution level. The absorbance of the solution of free cupric ions was followed at 800 nm wave length, that of the $Cu^{2+}$-TRIEN complex within 595—620 nm. The

absorption spectrum of ascorbic acid is to a large extent pH dependent; in an acid medium, the maximum of the undissociated acid lies at 245 nm, in an alkaline medium containing mostly monoanions of the acid, at 265 nm.

Kinetic calculations were made on a calculator Hewlett–Packard 9830 A equipped with a plotter 9862 A. The initial reaction rates $v_0$ were determined with the aid of a standard program for polynomial regression. Through the experimental points of the dependence oxygen concentration–time a polynomial was led; the curve was best fitted with a polynomial of the 4th degree. The value of its first derivative in $t = 0$ *i.e.* the coefficient of the linear term of the polynomial, gives the initial reaction rate $v_0$.

The linearisation of the Michaelis-Menten relationship and the calculation of the kinetic constants was made using a rearranged standard program for linear regression. For each set of data of the initial concentration of ascorbic acid [A] and the corresponding initial reaction rates $v_0$, the methods of Lineweaver–Burke (variables $1/v_0$ and $1/[A]$) and of Eadie (variables $v_0/[A]$ and $v_0$) and of Wilkinson (variables $[A]/v_0$ and [A]) ref.[11]) were used. Constants $V$ and $K$ contained in Table I are mean values of the three procedures.

## RESULTS AND DISCUSSION

*Reaction kinetics.* Oxidation of ascorbic acid by molecular oxygen catalysed by copper ions was followed in a Britton–Robinson buffer medium. After a sufficiently long time, the stoichiometry of the reaction approaches the ratio 0·5 mol $O_2$ for 1 mol of ascorbic acid which corresponds to reduction of oxygen all the way to water. At first, the dependence of the initial reaction rate of the autoxidation on pH was followed in the presence of EDTA and DIEN. In all experiments, the concentration of the substrate was held constant $4{\cdot}5 \cdot 10^{-3}$M and in excess of the concentration of copper ions. The initial reaction rate was determined from the dependence of the oxygen concentration on time by the method described above. The dependence of the initial reaction rate on pH passes through a maximum at pH 9 (Fig. 1). Ascorbic acid is a bivalent acid, its dissociation constants being $K_1 = 9{\cdot}16 \cdot 10^{-5}$ and $K_2 = 4{\cdot}57 \cdot 10^{-12}$ (ref.[2]); consequently, undissociated acid $H_2A$, its monoanion $HA^-$ and dianion $A^{2-}$ are present in the solution. On the basis of a material balance and of the dissociation constants, concentrations of individual dissociation forms were calculated at pH 9 and at the analytical concentration of ascorbic acid $1 \cdot 10^{-3}$M. At pH 9 when the reaction rate passes through a maximum, 99·9% of the ascorbic acid are in the form of monoanions; the concentration of the undissociated acid is of the order of $10^{-8}$M and the concentration of the dianion of the order of $10^{-6}$M. This is in agreement with previous findings, namely that the monoanion of ascorbic acid is the most reactive one with respect to oxygen[2]. Further experiments were made at pH 9.

The influence of chelating agents on the kinetics of the reaction was studied using chemicals quoted in the experimental part. The dependence of $v_0$ on the ratio [Cu] : : [chelating agent] ($[M]_t/[L]_t$) was followed between values 0–3; the chelate concentration was kept constant in the entire range and amounted to $9{\cdot}01 \cdot 10^{-6}$M;

the analytical concentration of the added copper ions varied. The substrate was added in excess, its concentration being $4{\cdot}5 \cdot 10^{-3}$M.

In all cases, the reaction rate increases monotonously with the ratio $[M]_t/[L]_t$. The dependence of the reaction rate on the ratio $[M]_t/[L]_t$ is of dual type as is evident from Figs 2 and 3; according to the initial course the curves may be described as linear and concave. The curvature at higher values of the ratio $[M]_t/[L]_t$ is probably due to hydrolysis of free metal ions and has, therefore, no intimate relation to the inhibition mechanism. Considering the shape of the dependence, the chelating agents used fall into two groups: A linear course is exhibited by EDTA, TRIEN, HIS, TIT VI, a concave course was observed in the case of TETR, NTA, DIEN, and TIT V. In all chelating agents used, the inhibition effect on the reaction rate is rather small compared to high stability constants of the corresponding cupric complexes which lie within $10^{10}-10^{24}$. The initial reaction rate measured without an addition of copper ions but in the presence of a chelating agent (ratio $[M]_t/[L]_t = 0$) *i.e.* under conditions when the catalytic effect of trace impurities should be suppressed, is mostly low but still measurable. The reaction rate in a system free of both the catalyst and the chelating agent is higher and varies within $3{\cdot}6 \cdot 10^{-7}-5{\cdot}8 \cdot 10^{-6}$ $Ms^{-1}$.

From each group of the chelating agents one was selected for a detailed determination of the reaction kinetics: EDTA for representing the linear dependences, and TETR for representing the concave ones. The dependence of the initial reaction

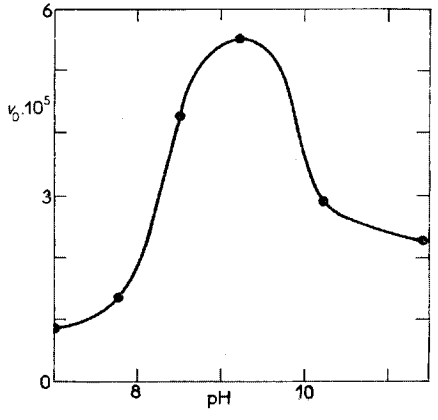



FIG. 1

Dependence of Initial Reaction Rate $v_0$ on pH

$4{\cdot}5 \cdot 10^{-3}$M ascorbic acid; $9{\cdot}01 \cdot 10^{-6}$M-$CuSO_4$; $2{\cdot}82 \cdot 10^{-4}$M-$O_2$; $9{\cdot}01 \cdot 10^{-6}$M-EDTA; Britton–Robinson buffer.

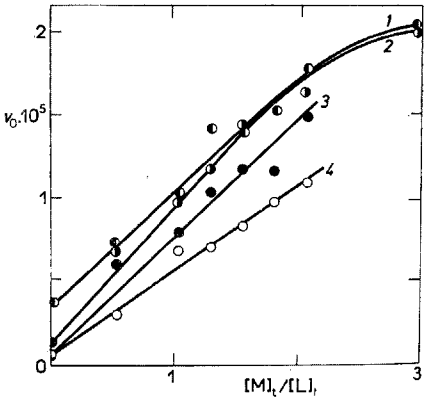



FIG. 2

Dependence of Initial Reaction Rate $v_0$ on Ratio [Cu]/[chelating agent]

*1* TIT VI; *2* EDTA; *3* TRIEN; *4* HIS; $4{\cdot}5 \cdot 10^{-3}$M ascorbic acid; $2{\cdot}82 \cdot 10^{-4}$M-$O_2$; $9{\cdot}01 \cdot 10^{-6}$M chelating agent; Britton–Robinson buffer pH 9.

rate $v_0$ on the initial ascorbic acid concentration was followed for two concentrations of either of the chelating agents and, moreover, a system free of chelates, *i.e.* with free copper ions present. In all cases, the experimental dependences (Fig. 4) obeyed the simple Michaelis–Menten relation

$$v_0 = \frac{VK[\mathrm{A}]}{1 + K[\mathrm{A}]}, \qquad (1)$$

where $K$ is the Michaelis constant, $[\mathrm{A}]$ the initial substrate concentration, and $V$ the limiting reaction rate at a high substrate concentration obeying $V = k[\mathrm{M}]_t$; ($[\mathrm{M}]_t$ is the analytical concentration of the catalyst.) The initial concentration of oxygen was constant during all experiments and is, therefore, included in the formal constants $V$ and $K$. The validity of the Michaelis–Menten law was verified previously by the linearisation methods described before, and the constants given in Table I were determined. The addition of a chelating agent not only does not affect the validity of the Michaelis–Menten law but influences only slightly the value of the Michaelis–Menten constant characteristic of the stability of the active intermediate. Since the limiting reaction rates $V$ in systems containing chelating agents are lower than those in systems with free copper ions, it is evident that a chelating agent acts as inhibitor;

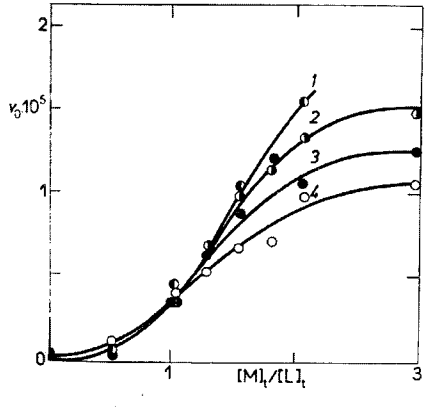



FIG. 3

Dependence of Initial Reaction Rate $v_0$ on Ratio [Cu]/[chelating agent]

*1* TIT V; *2* DIEN; *3* TETR; *4* NTA; $4{\cdot}5 \cdot 10^{-3}$M ascorbic acid; $2{\cdot}82 \cdot 10^{-4}$M-$O_2$; $9{\cdot}01 \cdot 10^{-6}$M chelating agent; Britton–Robinson buffer pH 9.

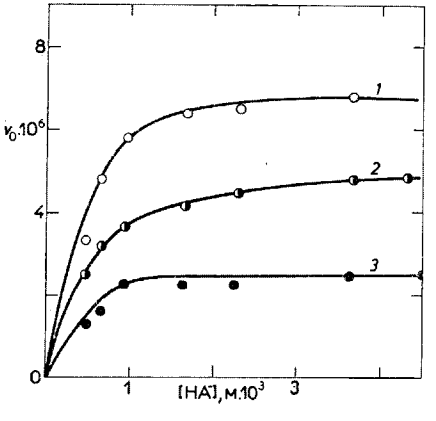



FIG. 4

Dependence of Initial Reaction Rate $v_0$ on Initial Concentration of Ascorbic Acid; Michaelis–Menten Law

*1* Cu, without inhibitor; *2* [Cu]/[EDTA] = = 1; *3* [Cu]/[TETR] = 1; $9{\cdot}01 \cdot 10^{-6}$M-$CuSO_4$; $2{\cdot}82 \cdot 10^{-4}$M-$O_2$; Britton–Robinson buffer pH 9; 25·0°C.

there is no question of a specific chelate catalysis where the catalytic activity of a metal chelate would be higher than that of a metal ion.

In all cases and irrespective of the presence of chelating agents, hydrogen peroxide was found after all oxygen had been consumed by the autoxidation of ascorbic acid. The concentration of the peroxide was $1 \cdot 10^{-5} - 1 \cdot 10^{-4}$M. If there was still unreacted ascorbic acid present in the mixture at the time of measurement, a marked decrease in hydrogen peroxide concentration was observed as it is probably consumed in a subsequent reaction – oxidation of the ascorbic acid.

*Absorption spectra.* Spectrophotometric experiments made in connection with the reaction of cupric ions with ascorbic acid in an anaerobic medium revealed that the absorption maximum exhibited by copper(II) sulphate disappeared on addition of the sulphate into the solution of ascorbic acid. Under anaerobic conditions, not even on adding TRIEN to this solution did a bluish-violet cupric chelate with an absorption maximum at 595 nm appear. There is, therefore, no possibility of proving cupric ions in the solution. The yellowish coloration of the solution and/or the yellow precipitate appearing at higher concentrations of the component is probably cuprous ascorbate. The precipitate is insoluble in diluted acids and bases but is dissolved by bubbling oxygen through the solution which thus regains the blue coloration of bivalent copper. Bubbling oxygen through the yellow solution also recovers the absorption maximum appearing at 595 nm in the presence of TRIEN and at 800 nm in the absence of a chelating agent (Fig. 5).

When observing the reaction of cupric chelates with ascorbic acid in an anaerobic medium, a dual type of behaviour was determined. In systems characterised by a concave curve $v_0$ *vs* $[M]_t/[L]_t$, absorption of the cupric chelate disappeared immediately, *i.e.* reduction proceeded even in an acid medium. The other chelates were also reduced quickly but only on making the solution alkaline. If the solution containing

TABLE I

Equilibrium Constants and Limiting Reaction Rates of Autoxidation

| System | $[L]_t$[a] | [Cu] | $K$[b] | $V$[c] $Ms^{-1}$ |
|---|---|---|---|---|
| $[Cu^{2+}]$ | 0 | $9{\cdot}01 \cdot 10^{-6}$ | $1{\cdot}29 \cdot 10^{3}$ | $1{\cdot}57 \cdot 10^{-5}$ |
| [Cu]/[EDTA] = 1 | $8{\cdot}90 \cdot 10^{-6}$ | $8{\cdot}90 \cdot 10^{-6}$ | $2{\cdot}07 \cdot 10^{3}$ | $8{\cdot}94 \cdot 10^{-6}$ |
| [Cu]/[EDTA] = 0·5 | $1{\cdot}77 \cdot 10^{-5}$ | $8{\cdot}85 \cdot 10^{-6}$ | $6{\cdot}26 \cdot 10^{2}$ | $6{\cdot}67 \cdot 10^{-6}$ |
| [Cu]/[TETR] = 1 | $8{\cdot}90 \cdot 10^{-6}$ | $8{\cdot}90 \cdot 10^{-6}$ | $1{\cdot}67 \cdot 10^{3}$ | $5{\cdot}25 \cdot 10^{-6}$ |
| [Cu]/[TETR] = 0·5 | $1{\cdot}77 \cdot 10^{-5}$ | $8{\cdot}85 \cdot 10^{-6}$ | $4{\cdot}08 \cdot 10^{2}$ | $4{\cdot}67 \cdot 10^{-7}$ |

[a] Analytical concentration of chelating agent, [b] Michaelis constant, [c] limiting reaction rate.

a chelating agent was bubbled through with oxygen for about 30 min after the reduction had proceeded, the original absorption maximum characteristic of the cupric chelate reappeared. A parallel experiment, during which the absorption maximum which ascorbic acid gives at 265 nm was followed under equal conditions, demonstrated that at the equal time of introducing oxygen produces full oxidation of the acid; unless the ascorbic acid is removed, there are no cupric ions present in the solution, not even in the presence of oxygen.

*Mechanism.* Unless there is specific chelate catalysis manifested in the system, the inhibition effect should be roughly proportional to the stability of the metal complex with the inhibitor. As was shown by our experiments, the inhibiting effect of chelating agents on the rate of autoxidation of ascorbic acid is much lower than that expected on the basis of stability constants of the respective cupric chelates. Depending on the ratio [Cu]/[chelating agent], the initial reaction rate varies at most within 1·5 order of magnitude (Figs 2, 3), the values $v_0$ being comparable in systems containing different chelates. The results may be explained on the basis of spectrophotometric determinations of the reduction of cupric chelates by ascorbic acid. They suggest that in a sufficient excess of ascorbic acid a major part of bivalent copper will be reduced to a univalent species even in the presence of oxygen unless reoxidation by oxygen proceeds at an exceptionally high rate. In our set-up, at the beginning of the reaction there was a twenty-fold excess of ascorbic acid referred to oxygen concentration, so that there were cuprous ions and cuprous chelates in the reaction mixture. Inhibition therefore rests in the formation of cuprous complexes whose stability is lower than that of the corresponding cupric chelates. The

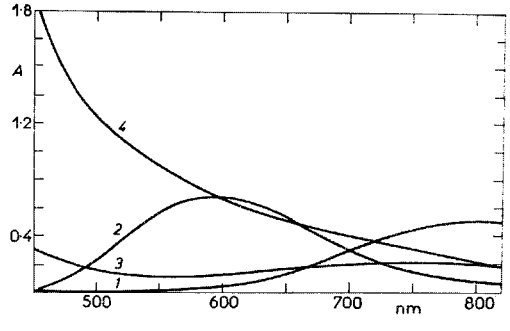



FIG. 5

Changes of Absorption Spectra of Cupric Ions and Chelates on Addition of Ascorbic Acid

*1* 0·1M-$CuSO_4$; *2* [Cu]/[DIEN] = 1; $1{\cdot}0 \cdot 10^{-2}$M-$CuSO_4$; *3* curve *2* on adding ascorbic acid; $5{\cdot}0 \cdot 10^{-2}$M ascorbic acid; *4* [Cu]/[EDTA] = 2; $2{\cdot}0 \cdot 10^{-3}$M-$CuSO_4$; $1{\cdot}0 \cdot 10^{-2}$M ascorbic acid; $1{\cdot}0 \cdot 10^{-2}$M-KOH.

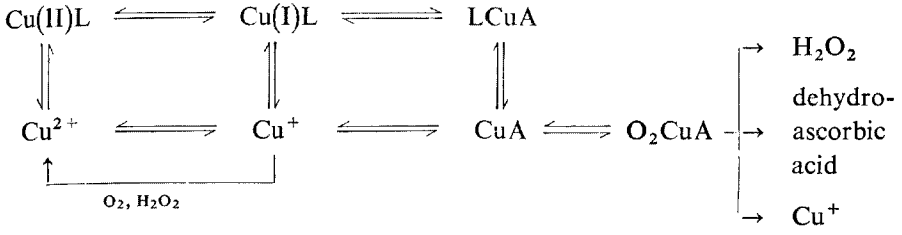
values of stability constants of these cuprous chelates are not available but from analogy with other chelating agents[12], values within $10^5 - 10^{10}$ may be expected. This also induces that not cupric but cuprous ions act as the true catalyst of the reaction, similarly as has already been observed in the case of autoxidation of sulphite[13].

A comparative "non-catalysed" reaction may be represented by a reaction proceeding in the presence of a chelating agent but in the absence of copper ($[M]_t]/[L]_t = 0$). The non-zero rate of the reaction demonstrates that an addition of a chelating agent is not able to cancel out the catalytic effect of traces of metal impurities in whose absence no oxidation of ascorbic acid would take place[8]. The reaction rate in a system without either copper or chelate is higher and, of course, has no physical meaning, depending on the arbitrary presence of catalytic trace impurities in the reaction system.

Considerations on the mechanism of the catalytic autoxidation of ascorbic acid by cupric ions include reoxidation of univalent copper by oxygen as a partial process, with a high rate in comparison with the rate determining reaction[2,3]. The catalytic effect of cupric ions consists, therefore, in that they are the oxidation agent proper, and that oxygen serves only for their recovery. To satisfy the condition of a sufficiently high rate of the partial process, reoxidation of cuprous ions under our experimental conditions would have to be by two orders of magnitude higher than that of the autoxidation found by experiment, *i.e.* $V = 1{\cdot}57 \cdot 10^{-5}\ \mathrm{Ms}^{-1}$. Formally, the rate of the oxidation of cuprous ions by oxygen in an alkaline medium is given by $v_r = k[O_2]\,[Cu^+]$; the rate constant according to Zuberbühler[14] is $k = 3{\cdot}5 \cdot 10^4\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$. Considering the oxygen concentration equal to $2{\cdot}82 \cdot 10^{-4}$M and assuming that the maximum concentration of cuprous ions is equal to their analytical concentration $9{\cdot}01 \cdot 10^{-6}$M, the value $v_r = 8{\cdot}9 \cdot 10^{-5}\ \mathrm{Ms}^{-1}$ comes out for the actual rate of reoxidation; this value is too low to be compatible with the reoxidation of $Cu^+$ as a partial process of the reaction. From the fact that the Michaelis–Menten law is obeyed in both the system containing free metal ions and that containing inhibitors follows that autoxidation of ascorbic acid proceeds in all systems *via* the same mechanism *viz.* the ternary complex mechanism. The rate determining step of the reaction is the breakdown of the ternary complex of the catalyst with both the substrates, ascorbic acid and oxygen.

Before suggesting a mechanism, let us sum up the results: *1*) Hydrogen peroxide is always formed as a stable intermediate of the reaction. *2*) Cupric ions and chelates are reduced by ascorbic acid to cuprous ions and chelates, respectively. *3*) Reoxidation of cuprous ions by oxygen is not a step of the overall reaction process. *4*) Reaction proceeds by the mechanism of the ternary complex.

Observing these conditions, the following reaction scheme is obtained:




The main reaction path is represented by the middle line of the scheme standing for the reduction, the formation of the binary complex of the presumable cuprous ascorbate, and the ternary complex. The existence of the ternary complex consisting in bonding molecular oxygen to the cuprous complex is supported by the well known fact that natural oxygen carriers containing copper are without exception univalent copper complexes[15]. The breakdown of the ternary complex in the last step consists in either a two-electron transfer within the complex leading directly to products, or else in a one-electron transfer followed probably by the liberation of an ascorbate radical $HA^{\bullet}$ or even a superoxide radical,or $Cu^{2+}$. There has been no possibility so far to decide between these two alternatives. The ascorbate radical forms in a small quantity in the first reaction step during the reduction of cupric ions or complexes. If however no $Cu^{2+}$ is liberated through the breakdown of the ternary complex, this reaction is not a part of the reiterating reaction cycle. Hydrogen peroxide is consumed during the subsequent slower oxidation of ascorbic acid to dehydroascorbic acid. This is confirmed by the eventual stoichiometry of the reaction which approaches the ratio $O_2 : HA^- = 1 : 2$. In the bottom line, the reoxidation of cuprous to cupric ions by oxygen and/or the resulting peroxide is expressed. Even though this reaction cannot be a partial process it can be a side reaction. A quantitative solution of the reaction scheme given will be the subject of a paper to follow.

The essential feature of the described mechanism, which until now has been the best one to satisfy experimental results, is that copper remains formally univalent during the overall reaction cycle, and that its catalytic function rests in acting as electron carrier between the two substrates.

REFERENCES

1. Khan M. M. T., Martell A. E.: J. Amer. Chem. Soc. *90*, 6011 (1968).
2. Kahn M. M. T., Martell A. E.: J. Amer. Chem. Soc. *89*, 4176 (1967).
3. Khan M. M. T., Martell A. E.: J. Amer. Chem. Soc. *89*, 7104 (1967).
4. Jameson R. F., Blackburn N. J.: J. Inorg. Nucl. Chem. *37*, 809 (1975).
5. Hatano A., Kato Y.: J. Vitaminol. (Kyoto) *16*, 99 (1970).

6. Yamazaki I., Mason H. S., Piette L.: J. Biol. Chem. *235*, 2444 (1960).
7. Bielski B. H. J., Comstock D. A., Bowen R. A.: J. Amer. Chem. Soc. *93*, 5624 (1971).
8. Uri N. in the book: *Autoxidation and Antioxidants.* (W. O. Lundberg, Ed.) p. 101. Interscience, New York, London 1961.
9. Šantavý F., Bitter B.: This Journal *15*, 112 (1950).
10. Vepřek-Šiška J., Hasnedl A., Mádlo K.: This Journal *36*, 3096 (1971).
11. Laidler K. J., Bunting P. S.: *The Chemical Kinetics of Enzyme Action*, 2nd Edition, p. 75. Clarendon Press, Oxford 1973.
12. Sillén L. G., Martell A. E.: *Stability Constants of Metal–Ion Complexes.* The Chemical Society, Burlington House, London 1964. Supplement No 1, London 1971.
13. Vepřek-Šiška J., Luňák S.: Z. Naturforsch. *29b*, 689 (1974).
14. Zuberbühler A. D.: Helv. Chim. Acta *53*, 473 (1970).
15. Wilkins R. G. in the book: *Bioinorganic Chemistry* (R. F. Gould, Ed.) Advances in Chemistry series 100, p. 111. American Chemical Society, Washington 1971.