MECHANISM OF INHIBITED OXIDATION OF ASCORBIC ACID BY OXYGEN*

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A quantitative analysis was made of the influence of inhibitors on the autoxidation of ascorbic acid, catalysed by cuprous ions; constants of partial equilibria and the constant of the rate determining step $k_1 = 24{\cdot}1\ s^{-1}$ were determined. The autoxidation proceeds *via* a reaction mechanism with two substrates of the "ordered" type under formation of three ternary complexes of which, however, only one is reactive. Functional relations following from the proposed mechanism were modelled by a computer and compared with experimental results.

In the course of ascorbic acid autoxidation, cupric ions as well as cupric complexes added to the reaction system as catalysts are reduced to copper(I) (ref.[1]). Inhibition of the autoxidation reaction has so far only been quantitatively treated on the basis of a chain mechanism hypothesis, the inhibition being explained by breaking the reaction chains[2]. We have, however, demonstrated that the autoxidation of ascorbic acid does not proceed *via* a chain mechanism but *via* formation of an intermediary ternary complex[1].

In the present paper, efforts were aimed at a quantitative treatment of the inhibition of the autoxidation reaction on the basis of a ternary complex mechanism. According to this assumption, the inhibition rests in the formation of complexes between the catalyst (Cu^+) and inhibitor which are in an equilibrium with the reactive intermediates *i.e.* the catalyst–substrate complexes. This mode of treatment was originally used for interpreting the inhibition effect in enzyme reactions[3].

EXPERIMENTAL

Ascorbic acid (Index Pharmacorum No 3, Czechoslovakia) was a Farmakon product. The stock solution was kept in darkness under nitrogen and was never older than three days. All other chemicals used were A.R. grade. Chelating agents used for inhibiting the reaction, EDTA, tetraethylenepentamine (TETR), triethylenetetramine (TRIEN), histidine (HIS), bis-(aminoethyl)glycol ether-N,N,N′,N′-tetraacetic acid (Titriplex VI, TIT VI), nitriloatricetic acid (NTA), diethylenetriamine (DIEN), and diethylenepentaminetetraacetic acid (Titriplex V, TIT V) were

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Merck and Koch and Light products. Nitrogen was purged of oxygen by passing it through a solution of chromium(II) chloride in diluted hydrochloric acid.

Equipment and procedure. Reaction conditions and procedure for studying the kinetics of the autoxidation of ascorbic acid were identical to those described previously[1]. The concentration of oxygen was determined by polarography at a constant potential −0·7 V (S.C.E.). The initial oxygen concentration was adjusted by saturating the solutions with oxygen or by bubbling them through with nitrogen. The reaction vessel without free space above the solution eliminated the possibility of changes in oxygen concentration due to establishment of an equilibrium between the liquid and gas phases.

The reaction was followed in a Britton–Robinson buffer medium at pH 9 at 25·0°C and at an analytical concentration of cupric ions added $[M]_t = 9{\cdot}01 \cdot 10^{-6}$M. The concentration of ascorbic acid was varied within $4{\cdot}5 \cdot 10^{-4}$—$4{\cdot}5 \cdot 10^{-3}$M, the concentration of oxygen was $9{\cdot}5 \cdot 10^{-5}$—$5{\cdot}9 \cdot 10^{-4}$M. The dependence of the initial reaction rate v_0 on the ratio [Cu]/[inhibitor] was studied under the same conditions but at a constant concentration of ascorbic acid $4{\cdot}5 \cdot 10^{-3}$M. The concentration of the chelating agent was also constant $9{\cdot}01 \cdot 10^{-6}$M and the concentration of the cupric ions added varied within 0—$2{\cdot}7 \cdot 10^{-5}$M.

Calculations were made on a calculator Hewlett-Packard 9830 A equipped with a plotter 9862 A. Determinations of the initial reaction rate and linearisation of the Michaelis–Menten law were made by the same procedure as that described in the previous paper[1]. Linear extrapolation of the dependences between the experimental constants and the concentrations of the chelating agent and oxygen were made with the aid of a standard program for linear regression. For the probable reaction mechanism, an equation was derived describing the dependence of the initial reaction rate v_0 on the ratio [Cu]/[inhibitor]. The equations were introduced into the program in the form of a multiline function and plotted by using a standard program for calculation of a general function of one variable $X = [M]_t/[L]_t$, (L_t being the analytical concentration of the chelating agent). When modelling the functional course of the curves, estimated values of the equilibrium constants were introduced and selected by trial and error. For final verification of the validity of the mechanism, constants determined from kinetic measurements were inserted, and the final curve was compared with the curve v_0 *vs* $[M]_t/[L]_t$ obtained by experiment.

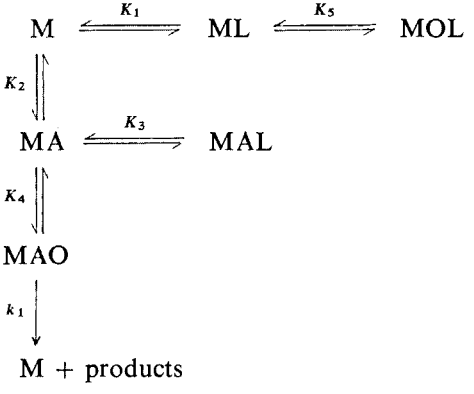
RESULTS AND DISCUSSION

Kinetics of uninhibited reaction. The kinetics of catalysed autoxidation of ascorbic acid was studied by the method of initial reaction rates in dependence on the concentration of both substrates, ascorbic acid and oxygen. The dependence of the initial reaction rate v_0 on the concentration of ascorbic acid for four initial oxygen concentrations obeys the Michaelis–Menten law in the entire range studied. By linearising the Michaelis–Menten relation in the simple form

$$v_0 = VK[A]/(1 + K[A]) \qquad (1)$$

values for V and K were obtained for the individual initial oxygen concentrations. V is the limiting reaction rate for high substrate concentrations, K is the equilibrium Michaelis constant, and [A] is the initial concentration of ascorbic acid (Table I).

To get at the physical meaning of these experimental constants and the mode of calculation of the actual equilibrium constants and of the rate constant, considerations with respect to the reaction mechanism have first to be made. The validity of the Michaelis–Menten law throughout the entire region of substrate concentrations and in the presence of inhibitors[1] gives evidence that the reaction proceeds by the mechanism of the ternary complex. The rate determining reaction in this case consists in the breakdown of the ternary complex of the catalyst with the two mutually reacting substrates. As was demonstrated in the previous paper[1], the reaction is catalysed by cuprous ions, and copper remains in the reduced state (+1) during the entire reaction cycle. This implies that the ternary complex will probably be formed by only one reaction path, *viz.* by bonding oxygen to a binary cuprous complex of ascorbic acid. The binary adduct of the free cuprous ion with molecular oxygen cannot be stable to the extent as to be considered as an intermediate which would give rise to a ternary complex with ascorbic acid. Since there was no irreversible reaction observed between the chelating agent and one of the substrates or the catalyst (*e.g.* oxidation or reduction) the inhibition is evidently reversible and rests in the formation of complexes of the catalyst with the inhibitor. Considering these conditions, a reaction mechanism follows implying that partial equilibria are sufficiently mobile as compared to the ternary complex breakdown.



(A)

(M Cu^+, A ascorbic acid, O oxygen, L inhibitor).

Since the binary adduct is formed with one substrate (MA) only, the supposed mechanism is a variation of the reaction with two substrates of the "ordered" type. There are, in fact, three ternary complexes resulting in the reaction, but only one of them is reactive from the viewpoint of the reaction studied. The influence of potential side- or consecutive reactions of ternary complexes containing inhibitor is eliminated by using the method of initial reaction rates. The breakdown of the active ternary

complex MAO being the rate determining reaction, the initial reaction rate follows from the equation

$$v_0 = k_1[\mathrm{MAO}] . \tag{2}$$

Solving first the reaction in the absence if an inhibitor, *i.e.* the reaction given by the left-hand column of the scheme, the analytical concentration of copper will clearly be

$$[\mathrm{M}]_t = [\mathrm{M}] + [\mathrm{MA}] + [\mathrm{MAO}] . \tag{3}$$

Expressing the concentration of the individual components by equilibrium constants $K_2 = [\mathrm{MA}]/[\mathrm{M}]\,[\mathrm{A}]$, $K_4 = [\mathrm{MAO}]/[\mathrm{MA}]\,[\mathrm{O_2}]$ and inserting into (*2*), we obtain

$$v_0 = V_1K_2K_4[\mathrm{A}]\,[\mathrm{O_2}]/(1 + K_2[\mathrm{A}] + K_2K_4[\mathrm{A}]\,[\mathrm{O_2}]) , \tag{4}$$

which expresses the kinetic implications of the mechanism supposed, and which is in fact another form of the Michaelis–Menten law for the given mechanism. V_1 is the limiting reaction rate of an uninhibited reaction for which holds $V_1 = k_1[\mathrm{M}]_t$.

From the right-hand sides of equations (*4*) and (*1*) whose validity has just been experimentally confirmed, terms for the dependence of experimental constants V and K on the concentration of oxygen (Table I) are obtained by comparing the coefficients for equal powers of variable [A].

$$1/V = 1/V_1K_4[\mathrm{O_2}] + 1/V_1 , \tag{5}$$

$$K = K_2 + K_2K_4[\mathrm{O_2}] . \tag{6}$$

TABLE I

Equilibrium Constants and Limiting Reaction Rates of Uninhibited Reactions

pH 9, 25·0°C [Cu] = $9{\cdot}01 \cdot 10^{-6}$M.

O_2	V_a, Ms^{-1}	K^b
$9{\cdot}54 \cdot 10^{-5}$	$3{\cdot}58 \cdot 10^{-6}$	$1{\cdot}20 \cdot 10^{3}$
$1{\cdot}83 \cdot 10^{-4}$	$7{\cdot}20 \cdot 10^{-6}$	$1{\cdot}32 \cdot 10^{3}$
$2{\cdot}82 \cdot 10^{-4}$	$1{\cdot}57 \cdot 10^{-5}$	$1{\cdot}36 \cdot 10^{3}$
$5{\cdot}92 \cdot 10^{-4}$	$1{\cdot}90 \cdot 10^{-5}$	$2{\cdot}06 \cdot 10^{3}$

[a] Limiting reaction rate, [b] Michaelis constant.

The equations are solved graphically by plotting $1/V$ *vs* $1/[O_2]$ and K *vs* $[O_2]$. The slope and intercepts stand for constants K_2, K_4, and V_1, and/or k_1. From linear dependences obtained by plotting the experimental points, the rate constant of the rate determining step and the equilibrium constants were obtained by a linear regression program: $k_1 = 24{\cdot}1\ s^{-1}$, $K_2 = 9{\cdot}75 \cdot 10^2\ l\ mol^{-1}$, and $K_4 = 1{\cdot}78 \cdot 10^2\ l\ mol^{-1}$. K_2 is the stability constant of the assumed cuprous ascorbate, K_4 is the consecutive stability constant of the ternary complex.

Kinetics of the inhibited reaction. The overall mechanism of an inhibited reaction was solved by essentially the same procedure as before. The material balance of the overall copper concentration is in this case

$$[M]_t = [M] + [MA] + [ML] + [MAL] + [MLO] + [MAO]. \qquad (7)$$

If we now express the concentrations of the individual components by equilibrium constants $K_1 = [ML]/[M][L]$, $K_3 = [MAL]/[MA][L]$, $K_5 = [MOL]/[ML] \cdot [O_2]$, and insert into equation (*L*) we obtain, after rearrangement, the following expression for the initial reaction rate of an inhibited reaction

$$v_0 = \frac{V_1K_2K_4[A][O_2]}{1 + K_1[L] + K_1K_5[L][O_2] + K_2[A] + K_2K_3[L][A] + K_2K_4[A][O_2]}. \qquad (8)$$

Comparing coefficients of equal powers of variable [A] at the right-hand sides of equations (*1*) and (*8*) and rearranging to a form suitable for graphical solution we obtain

$$1/V^i = (1 + K_4[O_2] + K_3[L])/V_1K_4[O_2], \qquad (9)$$

$$1/K^iV^i = L + (K_1 + K_1K_5[O_2])[L]/V_1K_2K_4[O_2], \qquad (10)$$

where V^i and K^i are experimental constants of the inhibited reaction. For $[L] = 0$ *i.e.* in the absence of inhibitors, equations (*9*) and (*10*) take the form of equations (*5*) and (*6*) obtained previously.

The slope of the dependence $1/V^i$ and the concentration of the inhibitor [L] at a constant initial oxygen concentration is $K_3/(V_1K_4[O_2])$. The stability constant K_3 of the mixed complex MAL may be determined from this expression, V_1 and K_4 being known from the solution of the equation for the uninhibited reaction, and the initial concentration of oxygen $[O_2]$ being also known. Similarly, it is possible to determine the slope $(K_1 + K_1K_5[O_2]/V_1K_2K_4[O_2])$ from the dependence $1/K^iV^i$ *vs* $\cdot [L]$ and further the value of $K_1/(1 + K_5[O_2])$, the value of K_2 being also known.

An alternative of the graphical solution of equations (9) and (10) consists in plotting $1/V^i$ and $1/K^iV^i$ on the reciprocal of the initial oxygen concentration $1/[O_2]$ at a constant inhibitor concentration. This alternative solution, however, requires another set of experimental data. From the slope $(1 + K_3[L])/V_1K_4$ of the dependence $1/V^i$ on $1/[O_2]$, constant K_3 may be calculated. The remaining stability constants K_1 and K_5 belonging to the binary complex with inhibitor ML and to mixed complex MOL may be obtained from expressions for the slope $(1 + K_1[L])/V_1K_2K_4$ and the intercept $K_1K_5[L]/V_1K_2K_4$ of the dependence $1/K^iV^i$ on $1/[O_2]$.

In the previous paper[1], values of experimental constants for an inhibited reaction were reported; the constants were determined for constant concentration of oxygen ($2{\cdot}82 \cdot 10^{-4}$M) and for varying concentrations of inhibitors EDTA and TETR. Constants K_3 and values of expressions $K_1(1 + K_5[O_2])$ were calculated for both inhibitors from the dependences $1/V^i$ and $1/K^iV^i$ on the concentration of the inhibitor using the method described above (Table II). An approximate determination of constants K_1 and K_5 will be dealt with later. A solution of the reaction mechanism (*A*) has thus been obtained, and the rate constant of the rate determining step and the constants of partial equilibria were calculated from kinetic data depicting the dependence of the reaction rate on the concentration of the two substrates and the inhibitor. The calculations do not distinguish between the analytical and actual concentrations

TABLE II

Equilibrium Constants and Character of Inhibition

Inhibitor	$K_1(1 + K_5 \cdot [O_2])$	K_1 / 1 mol^{-1}	K_3 / 1 mol^{-1}	K_5 / 1 mol^{-1}	K_3/K_5
		Linear course of the curve			
EDTA[a]	$1{\cdot}15 \cdot 10^5$	$1{\cdot}9 \cdot 10^4$	$5{\cdot}30 \cdot 10^4$	$2 \cdot 10^4$	2·7
TRIEN[b]	10^5	10^5	10^4	10^4	10^{-1}
HIS[a]	—	10^5	10^5	10^3	1
TIT VI[b]	—	10^4	10^3	10^4-10^5	10^{-1}
		Concave course of the curve			
TETR[a]	$3{\cdot}11 \cdot 10^6$	$5{\cdot}2 \cdot 10^5$	$1{\cdot}21 \cdot 10^6$	$2 \cdot 10^4$	2·4
NTA[a]	—	10^6	10^6	10^3-10^4	1
DIEN[c]	—	10^6	10^7	10^5	10^1
TIT V[c]	—	10^6	10^7	10^5	10^1

[a] Noncompetitive; [b] mixed competitive; [c] mixed anticompetitive.

of the substrate since they are both in a sufficient surplus with respect to the catalyst. Otherwise the system is not open to solution[3].

An equal simplification in expressing the concentration of the inhibitor is permissible due to a low stability of the complexes with inhibitors.

Modelling functional relations. On the basis of the expected reaction mechanism, a relation was derived for the dependence v_0 on the ratio [Cu]/[inhibitor]. The aim of the calculation was to verify the mechanism, and to explain quantitatively the dual type of the experimental curves obtained in systems with different inhibitors. In the previous paper[1], it was found that the dependence of v_0 on $[M]_t/[L]_t$ is in the presence of inhibitors EDTA, TRIEN, HIS, and TIT VI linear whereas it is concave in the systems containing TETR, NTA, DIEN, and TIT V.

As in the case of deriving the kinetic equation, the calculation was based on basic equations (*2*) and (*7*); moreover, material balance for the inhibitor concentration was taken into account

$$[L]_t = [L] + [ML] + [MAL] + [MOL], \qquad (11)$$

since the ratio $[M]_t/[L]_t$ acquires values between 0 and 3 and the inhibitor is not always in excess. Expressing the concentration of the individual components by equilibrium constants, inserting (*7*) and (*11*) into (*2*), and rearranging, we obtain

$$v_5 = k_1\{[-(E + \sqrt{(E^2 + 4DK_2K_4^2[A][O_2]X)}]/2D\}, \qquad (12)$$

where

$$D = \{(K_1 + K_2K_3[A] + K_1K_5[O_2])(1/K_2[A] + 1 + K_4[O_2])\}/[L]_t \qquad (13)$$

$$E = K_4[O_2](1 + K_2[A] + K_2K_4[A][O_2])/[L]_t + \\ + K_1 + K_2K_3[A] + K_1K_2[O_2] - (K_1 + K_2K_3[A] + K_1K_5[O_2])X \qquad (14)$$

and

$$X = [M]_t/[L]_t.$$

Dependence v_0 *vs* X given by equation (*12*) was plotted on the computer with the aid of a program for the course of a general function of one variable. Experimental constants of the uninhibited reactions k_1, K_2, and K_4 were introduced into the program; values for constants K_3 and $K_1(1 + K_5[O_2])$ were known for the reaction inhibited with EDTA and TETR. Constant K_5 belonging to the mixed complex MOL is not known but in view of the composition of the complex a value close to that of constant K_4 characterising the stability of the ternary complex MAO may be

expected. Values of the order of $10^3K_4 - 0{\cdot}1K_4$ were, therefore, inserted for K_5 and selection made by trial and error. Further increases or decreases in the values of K_5 have no influence on the course of the curve. The course of the curves computed for varying values of K_5 was compared with experiment, and for systems containing EDTA and TETR is presented in Figs 1 and 2. The agreement between calculation and experiment is fair; the fact that experimental values of v_0 for $[M]_t/[L]_t \approx$ ≈ 3 are below the calculated ones is probably due to hydrolysis of the free metal ion. The curve fitting best the experimental values served for determining constants K_1 and K_5 given in Table II.

For systems with other inhibitors where no detailed kinetic studies were made, constants K_3 and $K_1(1 + K_5[O_2])$ are not known. In these cases, the procedure consisted in inserting into equation (*12*) values of constants for the uninhibited reactions k_1, K_2, and K_4 which are common to all systems; for unknown constants K_1, K_3, and K_5, arbitrary combinations of probable values were matched and dependences of v_5 on $[M]_t/[L]_t$ were modelled using the above described program. The influence of the individual constants manifests itself on the shape of the curves. For instance, for low values of K_1 the course is linear; as the value of K_1 increases, the concave curvature of the curve appears and increases. At a constant value of K_1,

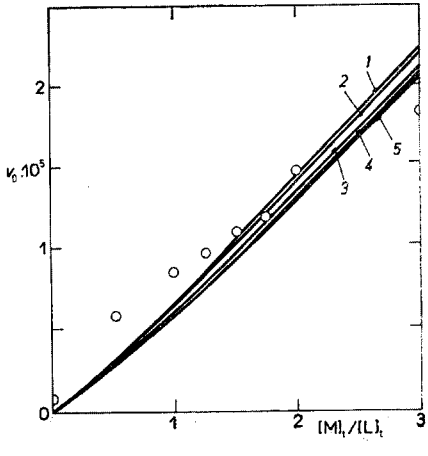


FIG. 1

Dependence of Initial Reaction Rate v_0 on $[M]_t/[L]_t$; Comparison of Computed Curves with Experimental Values

EDTA; $[Cu] = 9{\cdot}01 \cdot 10^{-6}$M; $[O_2] = 2{\cdot}82 \cdot 10^{-4}$M; ascorbic acid $5{\cdot}0 \cdot 10^{-3}$M; *1* $K_5 = 10^3K_4$, *2* $K_5 = 10^2K_4$, *3* $K_5 = 10K_4$, *4* $K_5 = K_4$, *5* $K_5 = 0{\cdot}1K_4$.

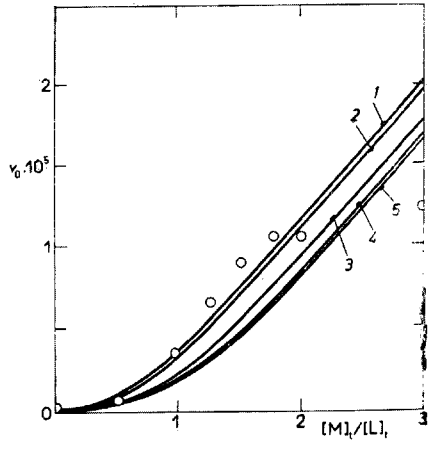


FIG. 2

Dependence of Initial Reaction Rate v_0 on $[M]_t/[L]_t$; Comparison of Computed with Experimental Values

TETR; $[Cu] = 9{\cdot}01 \cdot 10^{-6}$M; $[O_2] = 2{\cdot}82 \cdot 10^{-4}$M; ascorbic acid $5{\cdot}0 \cdot 10^{-2}$M; *1* $K_5 = 10^3K_4$, *2* $K_5 = 10^2K_4$, *3* $K_5 = 10K_4$, *4* $K_5 = K_4$, *5* $K_5 = 0{\cdot}1K_4$.

the curvature increases with an increase in ratio K_3/K_1. A similar, only less marked influence, is induced by an increase in ratio K_4/K_5. Since the modelled function responds very sensitively to a change in the constants, especially K_1 and K_3, the number of possible combinations is limited. By comparing the modelled curves with experimental points it is thus possible to find the best combination of constants for each inhibitor. The constants may in most cases be estimated within one order of magnitude accuracy. A comparison of the best modelled curves with experimental points for representative inhibitors TRIEN, HIS, NTA, and DIEN is given in Figs 3 and 4. The estimated values of the constants are compiled in Table II.

Curves v_0 *vs* $[M]_t/[L]_t$ were also modelled for some other alternatives of the inhibition mechanism, for instance on the assumption that only one ternary complex exists, or that the reaction rate is proportional to the concentration of the free metal ion. The functional course of these curves is similar to that for mechanism (A) but the agreement with experiment is poor. The character of inhibition[3] is determined from the values of stability constants of ternary (MAL) and binary (ML) complexes of the catalyst with the inhibitor. According to these values, three types of inhibition are distinguished: non-competitive ($K_3/K_1 = 1$), competitive ($K_3/K_1 \lesssim 10^{-3}$), and anticompetitive ($K_3/K_1 \gtrsim 10^3$). Limiting cases of competitive and anticompetitive inhibition are met in systems where virtually only one of the two complexes exists. In the systems under study, either non-competitive inhibition (both complexes of about the same stability), or a mixed type close to the noncompetitive one was

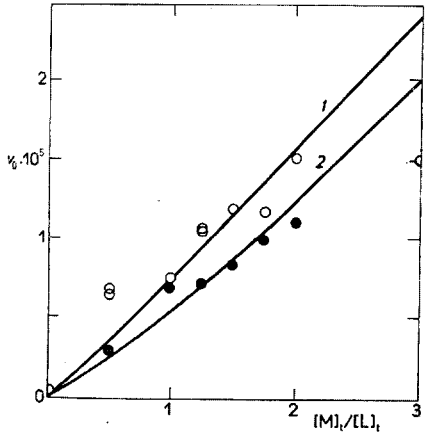


FIG. 3

Comparison of Best Fitting Modelled Curves with Experimental Results; Linear Course $[Cu] = 9{\cdot}01 \cdot 10^{-6}$M; $[O_2] = 2{\cdot}82 \cdot 10^{-4}$ M; ascorbic acid $5{\cdot}0 \cdot 10^{-3}$M; *1* TRIEN, *2* HIS.

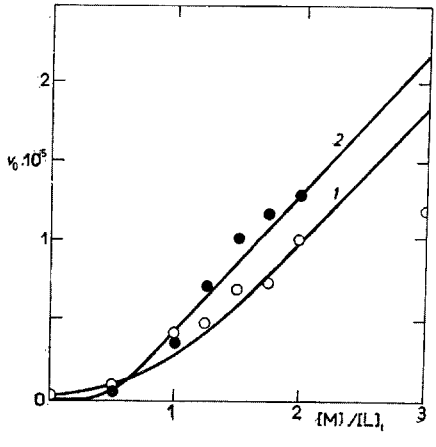


FIG. 4

Comparison of Best Fitting Modelled Curves with Experimental Results; Concave Course $[Cu] = 9{\cdot}01 \cdot 10^{-6}$M $[O_2] = 2{\cdot}82 \cdot 10^{-4}$M; *1* NTA, *2* DIEN.

found. If a mixed type of behaviour is encountered then in the case of inhibitors characterised by a linear dependence of v_0 on $[M]_t/[L]_t$ it is a mixed competitive type ($1 > K_3/K_1 > 10^{-3}$), in the case of inhibitors characterised by concave dependence it is a mixed anticompetitive one ($1 < K_3/K_1 < 10^3$). Since constants K_1 and K_3 were mostly only estimated, the data on the nature of inhibition are only informative.

As was revealed when computing the curves, the dual form of dependences v_0 on $[M]_t/[L]_t$ (linear and concave) is determined by the magnitude of the constants characteristic of the inhibition, especially constant K_1. If $K_1 < 10^5$ the curve is linear, at $K_1 > 10^5$ it is concave; if the value of K_1 exceeds 10^8 a sudden change in the slope at $[M]_t/[L]_t = 1$ appears. The difference is evident from values of K_1 in Table II. The borderline between the two types of the curves depends, of course, on the nature of the catalysts and substrates, *i.e.* on the value of constants K_2 and K_4.

Mechanism of inhibited reaction. Kinetic implications of the assumed mechanism are, as has been demonstrated, consistent with all experimental findings. The existence of a mixed complex as an intermediate of the reaction was also concluded by Skov and Vonderschmitt[4] who studied the oxidation of ascorbic acid by hydrogen peroxide and by oxygen under somewhat different conditions, with ferric and cupric complexes of aminopolycarboxylic acids as catalysts. These authors report the validity of the Michaelis–Menten law in all systems and consider a quaternary complex metal–ligand–substrate(1)–substrate(2) (MLAO) as the active intermediate. Since it was proved in our previous paper[1] that copper remains univalent throughout the entire reaction cycle, the existence of a quaternary complex with two polyvalent ligands was not considered probable due to the low stability of cuprous complexes and to the well known tendency of univalent copper towards a low coordination number.

Stability constants reported for cupric complexes of ascorbic acid, as far as they were obtained in a neutral or alkaline medium, seem to belong really to cuprous complexes. The stability constant of cuprous ascorbate found in this study ($\log K_2 = 2{\cdot}99$) is close to the value 2·04 given for cupric ascorbate[4]. Similarly, the constant found[4] for the inhibition of oxidation of ascorbic acid by ethylenediamine $4 \cdot 10^5$ is in agreement with our values of K_1 for the inhibition by DIEN and TRIEN (Table II) although the authors expected a value corresponding to the stability constant of cupric complex with ethylenediamine. On the nature and configuration of cuprous complexes with inhibitors there is not much information available. Univalent copper is slow in forming chelates with typical σ-donors and, besides, many complexes undergo dismutation in an aqueous medium[5].

The reaction mechanism suggested (*A*) assumes the existence of an adduct of a cuprous complex with molecular oxygen MOL even though its existence has not yet been proved. Efforts to synthesize adducts of cuprous complexes with oxygen have not met with success although kinetic evidence of their existence has been reported[5,6]. From empirical rules reported by Zuberbühler[5] for the formation of cuprous ad-

ducts with oxygen, two of them are met in our systems: the ligand should not be redox-active, and the system should possess a low catalytic activity. There was no peroxide decomposition observed in our experiments. Further reactions of the adduct, *i.e.* oxidation of the central atom and formation of hydrogen peroxide, formation of binuclear adduct and/or ligand oxidation could take place only if they proceeded at a higher rate than that of the rate determining step. This would then be manifested by an increase in the initial reaction rate caused by an increase in the concentration of the inhibitor (ligand); this has however not been observed. The effect could easily be mistaken for a specific chelate catalysis which is also manisfested by an increase in the reaction rate occuring with the increase in the concentration of the chelating agent.

In conclusion, it would be of interest to compare the stability constants of all the three ternary complexes. Constants K_4 and K_5 are consecutive stability constants of complexes MAO and MOL (K^{MA}_{MAO} and K^{ML}_{MLO}). The low stability of active ternary complex MAO may be due to the fact that both ligands combine only through oxygen atoms. Univalent copper has a low affinity towards oxygen donors, and besides, the stability of the ternary complex increases, as reported by Sigel[7], if oxygen and nitrogen donors participate in the bond. A certain compensating factor may be seen in the possibility of formation of a π-bond contributing to the stability of the ternary complex. The higher stability of complexes of the MOL type is probably due to the stabilising effect of the inhibitor as a ligand with nitrogen donor. There is apparently a close relation between the stability of the ternary complex and the character of the inhibition. The higher the relative stability of the ternary complex, the more evident is the anticompetitive character of inhibition; a lower stability of the ternary complex results in a tendency towards competitive behaviour. Consequently, a study of inhibition in appropriate reaction systems may yield information on the configuration and reactivity of the ternary complexes whose direct study is often next to impossible.

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