COOXIDATION REACTIONS OF ASCORBIC ACID

Eva SCHWERTNEROVÁ, Dana M.WAGNEROVÁ and Josef VEPŘEK-ŠIŠKA

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

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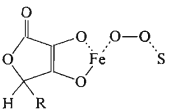
Catalytic oxidation of salicylic acid by molecular oxygen, induced by the oxidation of ascorbic acid, leads to the formation of a mixture of isomers of dihydroxybenzoic acid. The reaction is catalyzed by a series of metal chelates, especially Fe(II) and Co(II), the most marked catalytic effect having been found in the case of tetrasulphophthalocyanine of cobalt (CoTSP). The kinetics of the oxidation of ascorbic acid in the presence of salicylic acid was followed, and the formation of hydrogen peroxide as reaction intermediate was proved.

Reactions where the oxidation of a substrate by molecular oxygen is contingent upon the presence of a catalyst and a reducing species oxidized simultaneously with the substrate, are referred to as cooxidation reactions. In biological systems, this type of reactions is catalysed by monooxygenases or mixed function oxidases, which catalyse the splitting of the molecule of oxygen and the transfer of the oxygen atom to the substrate[1]. The first and best known chemical system modelling the function of monooxygenases is the so called Udenfriend's system where the complex FeEDTA is the catalyst, ascorbic acid is the reducing agent, and salicylic acid or another aromatic substance is the oxidized or hydroxylated substrate[2].

In the place of ascorbic acid, isoascorbic acid, ninhydrine, alloxane, tetrahydropteridine or pyrimidine, or another reducing agent may be used[3,4]. In some model systems, metal ions in the lower oxidation states, for instance Cu(I), Ti(III), Sn(II), V(II) can serve as reducing agents[5]. Data on the kinetics of cooxidation systems have, so far, been rather scarce.

The function of the reducing agent inducing the oxidation of the substrate rests, according to Hamilton's considerations[4], in the transfer of two electrons to one atom of oxygen in the oxygen molecule. The second oxygen atom is transferred to, and accepts two electrons from the substrate. If the overall four-electron change on the oxygen molecule is a one step process (concerted mechanism), formation of hydrogen peroxide is out of question. The mechanism consisting in the transfer of the oxygen atom from the oxidation agent to the substrate is called the oxene or oxenoid[6] mechanism. It is supported by experiments with labeled oxygen made in some enzymatically catalysed systems[7,8].



(*A*)

(S — substrate)

Udenfriend's system[4] is one of those in which the reaction is supposed to proceed by the oxenoid mechanism. The consideration that cooxidation proceeds in a complex intermediary (*A*) (catalyst, ascorbic acid, oxygen, salicylic acid) is compatible with the statement[9,10] that no hydrogen peroxide is formed in Udenfriend's system.

Other authors[11,12] consider the radicals $OH^{\bullet}$ or $O_2H^{\bullet}$ as the reactive intermediaries, the reducing agent serving only to reduce the oxidized catalyst.

Cooxidation reactions are complex processes yielding several products. To elucidate the reaction mechanism, it would be necessary to follow the concentration changes of several components, which is difficult if not impossible. Very often it is difficult to prove that a cooxidation reaction proceeds at all. It was the aim of the present paper to find criteria suitable for getting evidence of a cooxidation process, to obtain basic kinetic data about one of the best known cooxidation systems (oxygen–ascorbic acid–salicylic acid), and to contribute to the understanding of the role of metal ions in cooxidation processes.

EXPERIMENTAL

Chemicals

Ascorbic acid (Czechoslovak Index Pharmacorum No 3) was a Farmakon product. A stock solution was prepared fresh every three days and kept in darkness under a nitrogen blanket. Dehydroascorbic acid (pure) was Fluka made, salicylic acid (pure) was a product of Lachema, Brno. Dihydroxybenzoic acids were supplied by EGA-Chemie, Kepler & Reif, Parke Davis & Co. Chelating agents – ethylenediaminetetraacetic acid (EDTA), diethylenetriamine (DIEN), triethylenetetramine (TRIEN), tetraethylenepentamine (TETR), nitrilotriacetic acid (NTA), cyclohexane-1,2-diaminetetraacetic acid (Titriplex IV – TIT IV), diethylenetriaminepentaacetic acid (Titriplex V – TIT V), bis(aminoethyl)glycol ether N,N,N′,N′-tetraacetic acid (Titriplex VI – TIT VI), histidine (HIS), and *o*-phenantroline (*o*PHEN) were products of Merck and Koch & Light Laboratories, all A.R. grade. Tetrasulphophthalocyanines Co(II), Fe(III), Mn(III) and VO^{2+} were prepared by a procedure described previously[13]. The Co complex of 5-sulphosalicylaldehyde–ethylenediimine (CoSSAD) was prepared at the Institute of Complex Catalysis, Academy of Sciences, Rostock, German Democratic Republic.

EXPERIMENT AND PROCEDURE

Absorption spectra of ascorbic, salicylic, dihydroxybenzoic, and dehydroascorbic acids were registered by a Specord spectrophotometer UV–VIS (Zeiss, Jena).

Yields of dihydroxybenzoic acids formed by the oxidation of salicylic acid were determined by spectrophotometry after extraction with chlorophorm and ether. The organic phase was evaporated and the residue dissolved in 0·1M-HCl; the concentration of the mixture of dihydroxybenzoic acids was then determined at $\bar{\nu} = 31 \cdot 10^3\ cm^{-1}$ (ref.[14]). To verify the reliability of the above separation, blank determinations were made with only one component of the reaction mixture *i.e.* one isomer of dihydroxybenzoic acid present at a time. When studying the effectivity of the catalysts, the concentration of dihydroxybenzoic acid was determined in two parallel samples: one containing the catalyst under investigation and the other containing Fe(II) EDTA (*i.e.* the Udenfriend's system). Both samples were bubbled through with oxygen for 20 min.

No increase in the conversion of salicylic acid was attained by prolonged bubbling (up to 240 min). The composition of the solution was: phosphate buffer pH 7·0, ascorbic acid $6{\cdot}0 \cdot 10^{-3}$M, salicylic acid $2{\cdot}4 \cdot 10^{-3}$M, catalyst $5{\cdot}2 \cdot 10^{-4}$M.

Kinetics of the oxidation of ascorbic acid was followed in systems catalysed by Fe(II) EDTA at pH 7 (Udenfriend's system) and by CoSTP at pH 9. A thermostatted reaction vessel (20°C) was provided with a worm pump ensuring the circulation of the solution between the reaction vessel and the spectrophotometer cuvette and the stirring and saturation of the solution with air. The concentration of dissolved oxygen during the experiment was constant: $2{\cdot}6 \cdot 10^{-4}$M. The thickness of the cuvette was 2 cm. The decrease in the concentration of ascorbic acid was followed spectrophotometrically at a constant wave number $\bar{\nu} = 37{\cdot}5 \cdot 10^3$ cm^{-1}. The initial concentration of ascorbic acid was $4{\cdot}0 \cdot 10^{-5}$M, the concentration of salicylic acid was varied in the range $4 \cdot 10^{-6}$—$4 \cdot 10^{-4}$M; the concentration of the catalyst was $5 \cdot 10^{-6}$M.

Concentration of hydrogen peroxide in the reaction mixture was determined polarographically using a Radelkis OH 102 (Hungary). The reaction system — a solution containing Fe(II) EDTA or CoTSP as catalysts — was bubbled through with oxygen directly in the polarographic vessel; after 15, 30, or 60 min, the reaction was stopped by adding sulphuric acid, oxygen expelled by bubbling through nitrogen, and the peroxide wave with $E_{1/2} = -1{\cdot}5$ V (S.C.E.) was registered. The concentration of hydrogen peroxide was determined by the method of standard additions.

Kinetic calculations were made on a desk-top calculator Hewlett-Packard 9830 A equipped with a plotter 9862 A. The determination of the initial reaction rates was made by a method described previously[15] on the basis of fitting the experimental curve with a suitable polynomial. Actual concentrations of ascorbic and salicylic acids were calculated by our own programme for linear two-component analysis of the spectra.

RESULTS AND DISCUSSION

Catalysis by Complex Fe(II)EDTA

The study was started with reproducing experiments with hydroxylation of salicylic acid in Udenfriend's system (with Fe(II) EDTA as catalyst). According to information obtained by spectrophotometry after extraction, the only product of hydroxylation is 2,5-dihydroxybenzoic (gentisinic) acid[2].

From absorption spectra of the individual isomers of dihydroxybenzoic acid which can theoretically be formed by hydroxylation of salicylic acid it is, however, evident that the wave numbers of the characteristic absorption bands differ only very little (Fig. 1). Table I gives the values of molar extinction coefficients for the second and third absorption bands. It will be noted that the absorption spectra do not permit quantitative determination of the ratio of the isomers of dihydroxybenzoic acid in the reaction products to be made.

The determination of the yields of dihydroxybenzoic acids is further biased by different distribution coefficients of the isomers. It was found by blank experiments after extraction of the individual components of the reaction mixture that 30% of the initial quantity of 2,3-dihydroxybenzoic acid (DHB), 80% of 2,4-DHB, and

50% of 2,6-DHB acid is extracted under the conditions described. The separation of ascorbic acid from the reaction products is not reliable. The presence of ascorbic acid in the sample processed for spectrophotometric determination can distort the spectrum at wave numbers $\bar{\nu} < 36 \cdot 10^3$ cm^{-1}.

Since the shapes and positions of the maxima in the spectrum of the reaction products change with the reaction conditions, it can be assumed that a mixture of isomers is formed. It seems that isomers with the higher wave numbers of the second maximum and the lower wave numbers of the third maximum are more frequent, which would correspond to 2,3- and 2,5-DHB acids. This is in agreement with the data reported by Grinstead[11] on a somewhat different reaction system, after separation of the products by paper chromatography.

TABLE I

Molar Extinction Coefficients of Isomers of Dihydroxybenzoic Acid in 0·1M-HCl

Dihydroxybenzoic acid	II. absorption band		III. absorption band	
	$\bar{\nu}$ / cm^{-1}	ε / l mol^{-1} cm^{-1}	$\bar{\nu}$ / cm^{-1}	ε / l mol^{-1} cm^{-1}
2,3-	40·5	$5{\cdot}78 \cdot 10^3$	31·6	$3{\cdot}12 \cdot 10^3$
2,4-	38·9	$1{\cdot}08 \cdot 10^4$	33·9	$4{\cdot}10 \cdot 10^3$
2,5-	42·0	$6{\cdot}50 \cdot 10^3$	30·16	$4{\cdot}18 \cdot 10^3$
2,6-	39·8	$7{\cdot}23 \cdot 10^3$	33·4	$3{\cdot}03 \cdot 10^3$

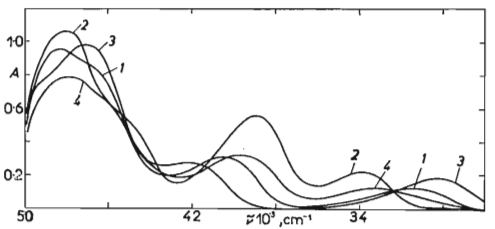


FIG. 1

Absorption Spectra of the Isomers of Dihydroxybenzoic Acid (DHB)

0·1M-HCl; [DHB] $4{\cdot}0 \cdot 10^{-5}$M; cell thickness 2 cm. *1* 2,3-DHB; *2* 2,4-DHB; *3* 2,5-DHB; *4* 2,6-DHB.

Catalysis by Other Complexes

In other experiments, the catalytic effect of various complexes on cooxidation reactions was studied. The complexes employed were of two types: ferrous chelates of the polyamine type and ring chelates forming mostly adducts with molecular oxygen. The results are compiled in Table II. The catalytic effect is characterised by the yield of dihydroxybenzoic acids expressed in per cent, the yield in Udenfriend's system at pH 7 and otherwise equal conditions standing for 100%. A catalytic effect comparable to that of FeEDTA was found only with FeTIT IV, FeNTA at pH 7, and with CoTSP at pH 11 (pH regions where FeEDTA and CoTSP have their maximum catalytic activities differ a great deal. For this reason, the activities of the two catalysts are compared at a pH corresponding to the maximum catalytic activity of each). Selke and Krause[16] ascribed the low activity of CoSSAD as catalyst for hydroxylation to the oxidation of the ligand by the hydrogen peroxide formed.

Fig. 2 correlates the values of standard potentials of the catalysing complexes ($Fe(II)L \rightleftharpoons Fe(III)L + e^-$), calculated from the stability constants of the oxidized and reduced forms of the complex[17], respectively, to the yields of the cooxidation reaction. In spite of the fact that stability constants are available for only a part of the complexes, it is evident from the figure that a region of standard potentials exists where the catalytic properties of the complexes reach their maximum. Catalysts with potentials above or below this range exhibit a lower catalytic effect.

TABLE II

Yields of Cooxidation of Salicylic Acid with Various Catalysts

Phosphate buffer pH 7, ascorbic acid $6 \cdot 10^{-3}$ M, salicylic acid $2{\cdot}4 \cdot 10^{-3}$ M, catalyst $5 \cdot 10^{-4}$ M.

Catalyst	Yield %	Catalyst	Yield %
Fe EDTA	100	CoTSP[a]	98
Fe TIT IV	99	CoTSP[b]	84
Fe NTA	94	CoTSP[c]	24
Fe HIS	65	MnTSP	81
Fe TIT V	39	FeTSP	74
Fe TIT VI	33	VOTSP	60
CuEDTA	20	CoSSAD	0
Fe TRIEN	17		
Fe TETR	15		
Fe^{2+}aq	12		
Fe *o*PHEN	5		
Fe DIEN	0		

[a] pH 11·06, [b] pH 9·0, [c] pH 6·75.

If hydrazine, hydroxylamine, or thiosemicarbazide are substituted for ascorbic acid, oxidation of salicylic acid does not take place. On the scale of the redox potentials, these species are able to take over the function of ascorbic acid in the cooxidation system; equally as in the case of ascorbic acid, their autoxidation is catalysed by CoTSP. In the cooxidation system, ascorbic acid can be replaced by some reducing agents only since neither the reducing properties nor the interaction with the catalyst are a sufficient condition.

In the course of the cooxidation reaction proceeding in the system catalysed by FeEDTA and CoTSP, formation of hydrogen peroxide was proved polarographically. The dependence of the concentration of hydrogen peroxide on time passes through a maximum which points to its participation in subsequent reactions. In the system catalysed by CoTSP the maximum concentration of hydrogen peroxide corresponds to 40–60% of the initial concentration of oxygen, in the system of Udenfriend to 30–45%. The concentration of the resulting hydrogen peroxide is therefore sufficient to permit a reliable proof of its presence. These results are at variance with literary data[9,10] stating that no hydrogen peroxide is formed in Udenfriend's system. It is, however, possible that the statements were based on experiments made in the final reaction stages of the reaction, with hydrogen peroxide already consumed.

Kinetics of Cooxidation Reactions

In kinetic experiments, the consumption of ascorbic acid in the cooxidation system was followed. The course of the reaction in the presence of FeEDTA, FeTIT IV, FeNTA, and CoTSP as catalysts is depicted in Fig. 3. It is evident that the reaction catalysed by CoTSP proceeds at a much higher rate than that catalysed by other complexes; at pH 11, the reaction rate is almost two orders of magnitude higher than that in Udenfriend's system at pH 7. The dependence of the initial reaction rate on the initial concentration of the substrate (*i.e.* salicylic acid) was followed in the system catalysed by CoTSP at pH 9. As expected, the reaction rate decreases

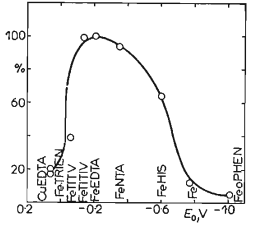


FIG. 2
Cooxidation Yield *vs* Standard Potential of the Catalyst

as the concentration of the substrate goes up. By plotting the initial reaction rate v_0 against the reciprocal of the initial concentration of salicylic acid (Fig. 4), a linear dependence is obtained

$$v_0 = V + a/[S], \qquad (I)$$

where V is the initial reaction rate at a surplus concentration of the substrate. It is the lowest reaction rate which is no longer affected by further increase in the substrate concentration. The slope a is a measure of the influence of the substrate on the reaction rate. In cases where the substrate does not undergo cooxidation nor has an inhibiting effect on the primary reaction as for instance in the system catalysed by CoSSAD, the reaction rate is independent of the substrate concentration, $a = 0$. The values of the limiting reaction rate V and the slope a are $V = 1{\cdot}6 \cdot 10^{-7}$ mol s^{-1} $a = 1{\cdot}11 \cdot 10^{-12}$, and $V = 9 \cdot 10^{-8}$ mol s^{-1}, $a = 4{\cdot}3 \cdot 10^{-13}$ when using salicylic acid and acetanilide, respectively, as substrates.

Mechanism

Experiments with different catalysts revealed that many chelates have catalytic properties. The most effective catalyst in the system studied was CoTSP which gave the same yields as those obtained in Udenfriend's system, the reaction rate being however by two orders of magnitude higher.

The characteristic dependence of the yield of the cooxidation reaction on the standard potential of the catalyst (Fig. 2) shows that the maximum catalytic effect was found in complexes having their standard potentials in a rather narrow range from $-0{\cdot}1$ to $-0{\cdot}5$ V. Catalytically active are consequently ferrous complexes with moderately reducing properties; in contrast, weak or strong reducing properties of the complex cause a decrease in the catalytic activity. This fact agrees with the idea that it is the mixed complex of the catalyst with oxygen and other reaction

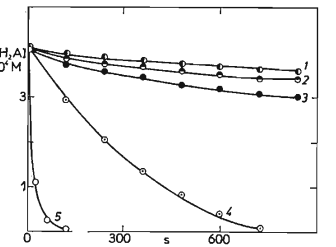


FIG. 3

Dependence of the Concentration of Ascorbic Acid on Time During the Cooxidation Reaction

Initial concentrations: Ascorbic acid $4{\cdot}0 \cdot 10^{-5}$M; salicylic acid $4{\cdot}0 \cdot 10^{-5}$M. Catalyst $5 \cdot 10^{-6}$M; oxygen $2{\cdot}6 \cdot 10^{-4}$M; pH 7. Catalyst: *1* FeNTA; *2* FeTIT IV; *3* FeEDTA (Udenfriend's system); *4* CoTSP (pH 9); *5* CoTSP (pH 11).

components that is the active intermediate of the reaction. In adducts of metal complexes with oxygen, a too high or too low value of the redox potential of the complex ensues an irreversible reduction of oxygen to hydrogen peroxide or a destabilisation of the oxygen–metal bond. If the function of the catalyst consisted only in the generation of free radicals, the yield of the cooxidation reaction should, starting from a threshold value, be independent of the standard potential of the catalyst, and – as a consequence – the dependence could not pass through an extreme. Evidently, the catalytic activity of the complex is not a function of the redox potential alone. More probably, the possibility is manifested to reach a suitable distribution of the electron density in the mixed ligand, *i.e.* a distribution corresponding to the optimum stability and reactivity.

The fact that hydrogen peroxide is formed in the reaction in a rather high concentration is very important for considerations of the reaction mechanism. The presence of hydrogen peroxide is considered as incompatible with the mechanism of a four-electron transfer in one single step[4]. The slow decrease in the concentration of hydrogen peroxide demonstrates that hydrogen peroxide is consumed in subsequent steps – during the oxidation of ascorbic acid and probably also during cooxidation. Salicylic acid itself is not oxidized by hydrogen peroxide.

As was demonstrated by experiments in the system catalysed by CoTSP, the overall initial reaction rate v_0 is linearly dependent on the reciprocal of the concentration of the substrate (salicylic acid) $1/[S]$ (Fig. 4). This dependence furnishes a criterion for differentiating cooxidation reactions from cases where the substrate is not oxidized although it does inhibit the primary reaction. With reactions proceeding by an associative mechanism, where the inhibition consists in the formation of competing complexes of the substrate (inhibitor) with the catalyst, a linear relation holds for the dependence of the reciprocal of the initial reaction rate $1/v_0$ on the concentration of the substrate $[S]$ (ref.[18]).

In previous studies[15,19] it was demonstrated that the autoxidation of ascorbic acid catalysed by metal phthalocyanines and by other complexes proceeds by the

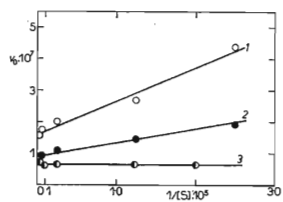
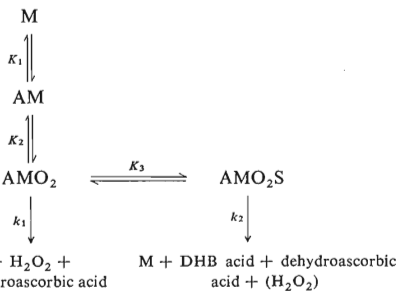


FIG. 4

Initial Reaction Rate *vs* Substrate Concentration

Initial concentration: ascorbic acid $4 \cdot 0 \cdot 10^{-5}$M; catalyst $5 \cdot 0 \cdot 10^{-6}$M; oxygen $2 \cdot 6 \cdot 10^{-4}$M. *1* CoTSP, salicylic acid $4 \cdot 10^{-6}$ to $4 \cdot 10^{-4}$M, pH 9; *2* CoTSP, acetanilide $4 \cdot 10^{-6}$ to $2 \cdot 10^{-4}$M, pH 9; *3* CoSSAD, salicylic acid $4 \cdot 10^{-6}$ $-2 \cdot 10^{-5}$M, pH 7; (cooxidation reaction does not proceed).

mechanism of a ternary complex. The fact that CoTSP catalyses also the cooxidation reaction, and the type of the dependence of the catalytic activity of the complexes on the redox potential suggest that the studied cooxidation reaction can also proceed by the associative mechanism. Thus, a probable mechanism of the reaction may be suggested and its kinetic consequences derived (Scheme 1, where M stands for the catalyst, A for ascorbic acid (reducing agent), and S for salicylic acid (substrate).



SCHEME 1

The reaction scheme is simplified; the formation of other probably unreactive mixed complexes as for instance MO_2, MS, MO_2S, *etc.* is not considered. This however has no influence on the character of the kinetics of the reaction because the method of initial reaction rates is employed in the solution. The initial reaction rate is given by the equation

$$v_0 = v_{01} + v_{02} = k_1[\mathrm{AMO_2}] + k_2[\mathrm{AMO_2S}] . \qquad (2)$$

Expressing the analytical concentration of the catalyst M_0 in the material balance by

$$[\mathrm{M}]_0 = [\mathrm{M}] + [\mathrm{AM}] + [\mathrm{AMO_2}] + [\mathrm{AMO_2S}] \qquad (3)$$

and the actual concentration of the individual components by equilibrium constants $K_1 = [\mathrm{AM}]/[\mathrm{M}][\mathrm{A}]$, $K_2 = [\mathrm{AMO_2}]/[\mathrm{AM}][\mathrm{O_2}]$, and $K_3[\mathrm{AMO_2S}]/[\mathrm{AMO_2}][\mathrm{S}]$, and inserting into (*2*) and rearranging, we obtain

$$v_0 = \frac{(k_1 + k_2[\mathrm{S}])[\mathrm{M}]_0 K_1 K_2 [\mathrm{A}][\mathrm{O_2}]}{1 + K_1[\mathrm{A}] + K_1K_2[\mathrm{A}][\mathrm{O_2}] + K_1K_2K_3[\mathrm{A}][\mathrm{O_2}][\mathrm{S}]} . \qquad (4)$$

For sufficiently great $[S]$ and K_3, the expression may be simplified to

$$v_0 = \frac{k_1[M]_0}{K_3}\frac{1}{[S]} + k_2[M]_0\,, \qquad (5)$$

which corresponds to the empirical linear relation (*1*) where the limiting reaction rate at a high concentration of the substrate is $V = k_2[M]_0$ and the slope $a = k_1[M]_0/K_3$. The limiting reaction rate corresponds to the rate of the secondary reaction (cooxidation) and depends only on the concentration of the catalyst. According to this relation, the values $k_2 = 2 \cdot 10^{-4}\ s^{-1}$ and $3 \cdot 10^{-4}\ s^{-1}$ come out for the reaction rate constant of the secondary reaction during the cooxidation of acetanilide and salicylic acid, respectively. In both cases, the ratio k_1/K_3 is of the order of 10^{-9}. For low concentrations of the substrate the preceding simplification does not hold; the dependence of v_0 on $1/[S]$ is no longer linear and, for $[S] \to 0$ it tends to the value of the reaction rate of the primary reaction alone.

The ternary complex of the catalyst with ascorbic acid and oxygen AMO_2 corresponds to the idea of Hamilton[4] that the oxidizing particle reacts with the substrate. Since however the ternary complex of this type is a reactive intermediary of the autoxidation of ascorbic acid[15], the autoxidation of ascorbic acid will manifest itself as a primary reaction whose product is hydrogen peroxide even in the cooxidation system. The reactive intermediary of the secondary reaction is a mixed ligand complex of all the reaction components which may, in its simplest form, be considered as particle AMO–OS; another possibility is the formation of binuclear reactive complexes of the type AMO–OSM and AMO–OMS. The structure of the reactive complex determines whether cooxidation proceeds *via* an atom or an electron transfer. In the case of an atom transfer the substrate must be bound to the catalyst through an oxygen molecule similarly as in the oxenoid mechanism cited[6]. The complexes AMO–OS and AMO–OSM both satisfy this condition. The reactive complex AMO–OMS permits only the electron transfer to occur. The constant K_3 is characteristic of the stability of the bond of the substrate in the mixed adduct. At a high concentration of the substrate and a sufficiently high K_3, the primary reaction is practically suppressed and only the secondary reaction is of significance. Most authors consider only this limiting case, irrespective of the reaction conditions and of the possibility of a parallel course of both reactions. This consequently leads to an incorrect interpretation of the role of hydrogen peroxide. Hydrogen peroxide found in the reaction mixture may be the product of the primary reaction alone. If the secondary reaction is a multistep process, hydrogen peroxide can also be formed probably as a shortlived intermediary. Only if the primary reaction is suppressed, and if the secondary reaction proceeds in one step, there is no peroxide formed. On the other hand, the absence of hydrogen peroxide is not an unambiguous proof of the oxenoid mechanism since, in the case of a transfer

of four electrons in one step (*i.e.* in the adduct AMO–OMS), no hydrogen peroxide is formed either. Only experiments with labeled oxygen can furnish a reliable criterion of the atom transfer. By this method, the atom transfer was proven in cooxidation reactions catalysed by natural enzymatic systems and in several cases also in model cooxidation systems catalysed by metal complexes[20]. However, in complex systems where there is a possibility of fast isotopic exchanges, this method may not give unambiguous results.

Consequences of the suggested simplified mechanism are in a qualitative agreement with the experimental results. The reaction mechanism explanation proposed allows for explanation of some results obtained by other auhors, *e.g.* of the variances in the results concerning the formation of hydrogen peroxide.

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