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An efficient copper(III) catalyst in the four electron reduction of molecular oxygen by L-ascorbic acid

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ABSTRACT

The anionic $[Cu^{III}(LH_{-4})]^-$ complex (L = 8,17-dioxa-1,2,5,6,10,11,14,15-octaaza-tricyclo[13.3.1] eicosane-3,4,12,13-tetrone) is one of the rare copper(III) compounds, which exhibits high stability in basic aqueous solution. This copper(III) compound catalyses the reduction of molecular oxygen by L-ascorbic acid at pH 7.97, in Tris–HCl buffer solution at the ionic strength of 0.1 M (NaCl). Stoichiometry of the reaction (2:1.07 for L-ascorbic acid:dioxygen) indicates formation of water and dehydroascorbic acid as primary products. Based on detailed kinetic measurements, the rate equation $-d[AscH^-]/dt = k_{obs}[AscH^-]^{0.5}[Cu^{III}(LH_{-4})][O_2]^{0.5}$ was obtained. The proposed mechanism includes a fast redox pre-equilibrium between the copper(III) centre and its reduced, copper(II) form, induced by the presence of L-ascorbate. The equilibrium constant K_1' at pH 8 ($8.78 \pm 3.11 \times 10^{-3}$) and k values for the forward and backward reactions ($1.18 \pm 0.68 \times 10^6$ and $1.46 \pm 0.82 \times 10^8$ M^{-2} s^{-1}, respectively) were determined by stopped-flow technique, following the decrease in absorbance of the copper(III) form at 550 nm. In the presence of molecular oxygen, re-oxidation of the copper(II) form of the catalyst takes place, based on cyclic voltammetry (CV) measurements. The decrease of the Cu(II) → Cu(III) oxidation and the subsequent increase of the Cu(III) → Cu(II) reduction current peaks in the CV spectrum, when argon is exchanged to dioxygen atmosphere, indicate a relatively fast oxidation rate for $[Cu^{II}(LH_{-4})]^{2-}$. The determined $\Delta S^{\ddagger}$ (-41 ± 2 $Jmol^{-1}$ K^{-1}) for the catalytic reaction indicate an associative mechanism for the formation of the catalytically active copper–oxygen species that will react with the L-ascorbate to yield dehydroascorbate as product.

1. Introduction

Direct reaction between a closed electron shell bearing organic substrate and the triplet ground state of dioxygen is normally spin-forbidden. Therefore, electron flow from the substrate to the dioxygen molecule is often assisted by the active site of metalloenzymes, which lower the activation energy need of the oxidation. When dioxygen is used by the enzyme as the ultimate electron acceptor molecule, reactive oxo-, peroxo-, or oxyl-intermediates may form, which then will decompose to hydrogen peroxide, or water as side products by gaining electrons from the substrate molecule which itself will degrade to the oxidized product and restore the reduced form of the enzymatic active site. In case of iron-containing enzymes, like the diiron-containing soluble methane monooxygenase (sMMO) [1] or the heme- [2], or nonheme [3] oxygen-activating iron enzymes, it is well established that the catalytic cycle proceeds via iron(IV) transient species. Also, for the copper-containing peptidylglycine α-amidating monooxygenase (PHM) [4] and dopamine β-monooxygenase (DβM) [5], intermediates formulated as Cu(II)–OOH and Cu(II)–$O^{\bullet -}$/Cu(III)=O have been proposed. Hamilton et al. suggested the possible role of copper(III) in monocopper enzymes, such as galactose oxidase [6], however, this oxidation state is generally considered to be inaccessible in biological systems. Trivalent complexes of copper, on the other hand, with bis(biureth)- [7], bis(oxamide)- [7], peptide- [8], dithiocarbamate- [9], are well characterized, and extensive studies on electron transfer reactions of copper(III) peptide complexes [10] and copper(III) imine–oxime complexes [11] have been carried out. High-valent copper complexes occur in biomimetic chemistry [12], and oxidizing ability of copper(II)–OOH and dicopper(II)–OOH [13] species is widely studied. Other macrocyclic [14] and diglycylethylenediamine copper(III) complexes [15] have been reported to oxidize ascorbic acid. No example can be found, where copper(III) shows catalytic activity in oxidation reactions against a substrate,

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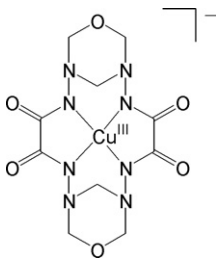

Scheme 1. Structure of the catalyst $[Cu^{III}(LH_{-4})]^-$.

although the presence of copper(III) ion is assumed in some cases [16]. It can be presumed, that a stable copper(III) complex with the appropriate ligand environment can possibly act as a catalyst that cycles between the copper(III)/copper(II) states.

Template synthesis of remarkably stable square-planar copper(III) complexes based on hydrazide and hydrazide/oxime ligands have been reported [17]. Here we present one of these complexes, $[Cu^{III}(LH_{-4})]^-$(L = 8,17-dioxa-1,2,5,6,10,11,14,15-octaazatricyclo[13.3.1]eicosane-3,4,12,13-tetrone, Scheme 1) [17a] as a catalyst in the oxidation reaction of L-ascorbate anion by molecular oxygen.

2. Experimental

Materials. L-Ascorbic acid and the salts that were used for buffer preparation and for cyclic voltammetry were commercial products of reagent grade and were used without any further purification. Double distilled water was used as solvent. The $K[Cu^{III}(LH_{-4})]$ catalyst has been synthesized as it was reported earlier for $LiK[Cu(LH_{-4})]_2{\cdot}10H_2O$ [17a] except that instead of $LiOH{\cdot}H_2O$ and KOH, only KOH was used during the synthesis.

Instruments. Electronic absorption spectra were recorded on a Beckman DU 650 spectrophotometer connected to a temperature controller (±0.1 °C accuracy). The pH of solutions was measured with a digital pH meter (Schott). Cyclic voltammograms (CVs) of the complex and L-ascorbate solutions were obtained in water containing 0.1 M $NaClO_4$ as supporting electrolyte at 298 K. Hanging mercury drop electrode (HMDE) was used as a working electrode, 3 M calomel electrode (CE) and a platinum wire used as a reference and counter electrode, respectively. Scan rates ranging from 100 to 800 mV s^{-1} and a potential range from +0.200 to −1.250 V were applied.

Kinetic measurements. Reactions were performed in 1 cm pathlength quartz cuvettes, in 3 mL solutions. The partial orders for the reactants and the catalyst were determined under pseudo-first-order conditions, with systematically varying the initial concentrations of $AscH^-$, $[Cu^{III}(LH_{-4})]^-$, or dioxygen at 298 K, while the initial concentration of the other two reactants and the pH were maintained constant. Stirring rate had no effect on the reaction rates. The concentration of $[Cu^{III}(LH_{-4})]^-$was essentially unchanged before and after the reactions, based on its 550 nm absorption band intensity. Air-saturated and buffered (50 mM Tris–HCl) aqueous solutions were used, the pH was set to 7.97 and the ionic strength was fixed at 100 mM with NaCl. The reaction rates and k_{obs} values were calculated by integration method (up to 75% conv.). For the determination of the activation parameters, temperature was varied between 288 and 308 K. Dioxygen concentration was calculated based on empirical formula [18] using Eq. (1), for $t < 30$ °C and Eq. (2) for 30 °C < t < 50 °C, respectively.

$$\mathrm{DO} = \frac{0.678(P-p)}{35+t} \quad (1)$$

$$\mathrm{DO} = \frac{0.827(P-p)}{49+t} \quad (2)$$

where P is the barometric pressure, p is the water vapour pressure, t is the temperature in °C and DO is the concentration of dissolved dioxygen in mg L^{-1}. Concentration of the molecular oxygen in solution was changed by mixing different volumes of air- and nitrogen-saturated buffer solutions in a sealed cuvette. Thus only the initial rates (up to 10–15% conversion) could be used for further calculations to determine the partial order for dioxygen.

3. Results and discussion

Reaction of L-ascorbic acid with dioxygen in the presence of $[Cu^{III}(LH_{-4})]^-$. Catalytic reactions were carried out in buffered aqueous solutions at an optimum pH 8 for the following reasons. At pH ≤ 7 the catalyst decomposes within a few cycles, whereas above pH 8, the ascorbic acid (pK_a values are 4.17 and 11.6) is present both in the monoanionic ($AscH^-$) and in some extent, the dianionic (Asc^{2-}) form, that may alter the observed mechanism. At pH 8, on the other hand, these complications do not occur, and we can assume a dominant reaction mechanism between the copper(III) catalyst and the $AscH^-$ anion.

In order to determine the stoichiometry of the catalytic reaction, dioxygen uptake has been measured gas-volumetrically. During the oxidation of L-ascorbic acid to dehydroascorbic acid (Asc) half equivalent of dioxygen was consumed compared to the transformed L-ascorbic acid (see Fig. S1). Based on this, the two-electron oxidation of an ascorbate molecule results in the four-electron reduction of a dioxygen molecule according to Eq. (3) similarly to the native L-ascorbate oxidase, a blue multicopper oxidase enzyme [19].

$$2AscH^- + O_2 \xrightarrow{[Cu^{III}(LH_{-4})]^-} 2Asc + 2OH^- \quad (3)$$

To be noted, that the observed excess of O_2 consumption (apart from the ~5% error of the measurement) can be ascribed to the oxidation of ascorbate catalysed by some unidentified impurity in the solutions (*vide infra*). Since the stoichiometry of the L-ascorbic acid oxidation catalysed by various metal ions is 1:1 with respect to L-ascorbic acid and dioxygen [20], such reactions will cause some increase in the observed dioxygen consumption.

Kinetic measurements. The decrease in $AscH^-$ concentration was followed by UV–vis spectroscopy measuring the intensity of the band ascribed to $AscH^-$ at 265 nm (ε = 14,360 M^{-1} cm^{-1}). Among the applied conditions that are summarized in Table 1, the L-ascorbic acid is present predominantly as $AscH^-$ [21]. During calculations, only the monoanionic form has been considered to interact with the catalyst. Fig. 1 illustrates the spectral changes during a typical oxidation reaction.

The initial catalyst concentration can be determined from its absorption band at 550 nm (ε = 7480 M^{-1} cm^{-1}), before the addition of $AscH^-$ (Fig. 1, solid line). This absorption can be assigned as an $N \rightarrow Cu^{3+}$ charge-transfer band as it was reported earlier [17a]. (Note, that in contrast with a typical kinetic experiment, concentration of the catalyst was increased to illustrate the spectral changes at 550 nm in Fig. 1. This way, the catalyst/dioxygen ratio is higher, than in a normal kinetic assay, where the dioxygen concentration is at least two orders of magnitude higher, than the catalyst concentration that is enough to sustain the pseudo first-order conditions.) Upon addition of the substrate (Fig. 1, dotted line) the 550 nm band slightly decreases and a new band at 265 nm from the $AscH^-$ can be seen. After more than 90% of the $AscH^-$ is transformed, the original spectrum is almost completely restored (Fig. 1, dashed line). These spectral changes are attributed to the partial reduction of copper(III) to copper(II) by the added ascorbate. We obtained more information about this first equilibrium step using stopped-flow technique with the exclusion of dioxygen, as it will be presented later. Electrochemical experiments (CV) also

Table 1
Kinetic data for the copper(III)-catalysed reaction of L-ascorbate and dioxygen at pH 8.

Exp.	Temp. (°C)	$[AscH^-]$ (10^{-5} M)	$[Cu^{III}(LH.4)^-]$ (10^{-6} M)	$[O_2]$ (10^{-4} M)	$-d[AscH^-]/dt$ (10^{-9} M s^{-1})	V_0 (10^{-9} M s^{-1})	k_{obs} (M^{-1} s^{-1})
1	25	3.42	2.39	2.60	9.02 ± 0.10		40.02 ± 0.44
2	25	4.86	2.39	2.60	10.93 ± 0.08		40.68 ± 0.31
3	25	7.92	2.39	2.60	15.06 ± 0.17		43.91 ± 0.50
4	25	9.30	2.39	2.60	15.53 ± 0.08	17.03 ± 0.47	41.79 ± 0.20
5	25	10.56	2.39	2.60	17.31 ± 0.15		43.71 ± 0.39
6	25	13.18	2.39	2.60	18.78 ± 0.20		42.45 ± 0.46
7	25	16.78	2.39	2.60	20.40 ± 0.22		40.86 ± 0.44
8	25	21.27	2.39	2.60	22.99 ± 0.21		40.90 ± 0.37
9	25	11.22	1.44	2.60	11.38 ± 0.14		46.27 ± 0.57
10	25	10.77	2.39	2.60	17.31 ± 0.15		43.28 ± 0.39
11	25	10.89	3.82	2.60	27.76 ± 0.49		43.18 ± 0.76
12	25	10.95	4.78	2.60	33.24 ± 0.64		41.21 ± 0.79
13	25	10.84	5.74	2.60	40.97 ± 0.69		42.52 ± 0.71
14	25	10.65	6.69	2.60	45.47 ± 0.76		40.86 ± 0.68
							42.26 ± 1.64[a]
15	25	8.36	2.39	0.33		5.86 ± 0.12	
16	25	8.54	2.39	0.43		7.92 ± 0.32	
17	25	8.45	2.39	0.61		8.34 ± 0.21	
18	25	8.69	2.39	0.90		11.90 ± 0.36	
19	25	9.34	2.39	1.30		13.25 ± 0.53	
20	25	9.64	2.39	1.45		13.99 ± 0.28	
21	25	9.78	2.39	2.00		15.45 ± 0.32	
22	15	11.67	4.78	3.19	18.54 ± 0.30		20.10 ± 0.33
23	20	10.70	4.78	2.86	26.70 ± 0.41		31.93 ± 0.50
24	30	10.97	4.78	2.37	50.49 ± 1.01		65.51 ± 1.36
25	35	10.31	4.78	2.21	62.74 ± 1.13		86.95 ± 1.57

[a] Mean value of the kinetic constant k_{obs} and its standard deviation $\sigma(k_{obs})$ were calculated as $k_{obs} = (\Sigma_i \omega k_i / \Sigma_i \omega_i)$ and $\sigma(k_{obs}) = \Sigma_i \omega_i (k_i - k_{obs})^2 / (n-1) \Sigma_i \omega_i)^{1/2}$, where $\omega_i = 1/\sigma_i^2$.

support a fast reduction of copper(III) to copper(II) by ascorbate (*vide infra*).

The time sequence of a typical catalytic reaction can be seen in Fig. 2. Plots of $2[AscH^-]^{0.5}$ *vs.* time can be fitted with a straight line under pseudo first-order conditions (see Fig. 2 for example) in agreement with a half-order dependence with respect to the substrate. The pseudo first-order rate constant, k' can be calculated from the slopes of these fitted lines for the individual experiments performed at various initial substrate concentrations. The reaction rate can be given according to Eq. (4) for each individual experiment (Table 1, experiments 1–8):

$$\frac{-d[AscH^-]}{dt} = k'[AscH^-]^{0.5} \quad (4)$$

where $k' = k_{obs}[Cu^{III}(LH_{-4})^-]^m[O_2]^n$.

Further evidence on the half-order for the substrate is seen in Fig. 3, where the reaction rates of the individual experiments (Table 1, experiments 1–8) calculated according to Eq. (4) are plotted against the square root of the initial concentrations of L-ascorbate. The linear dependence clearly indicates a partial order of one half in the substrate.

Runs were performed at various initial catalyst concentrations (Table 1, experiments 9–14). Reaction rates show a linear dependence on copper(III) concentration as can be seen in Fig. 4, indicating first-order in the catalyst ($m = 1$). Linear fit to data points crosses the ordinate axis above zero that can be interpreted as a contribution from the autoxidation reaction of the L-ascorbate to the overall reaction rate. Addition of EDTA efficiently inhibits the oxidation of $AscH^-$ in the absence of $[Cu^{III}(LH_{-4})]^-$, as it was found earlier for similar systems [22]. In fact, this "background" reaction

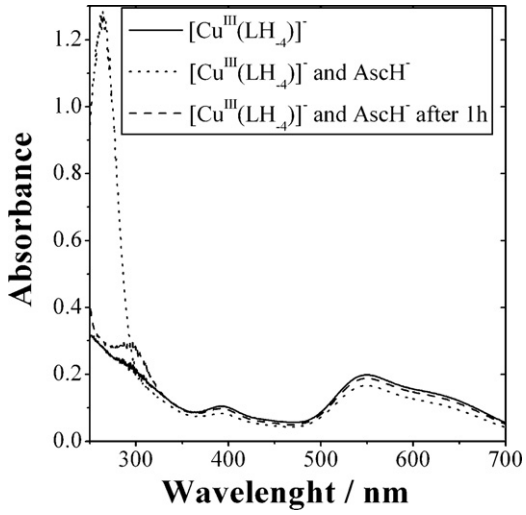

Fig. 1. Changes in copper(III) concentration in the presence of L-ascorbate and dioxygen in water (pH 7.97, $\mu = 0.1$ M with NaCl, 298 K, $[Cu^{III}(LH_{-4})^-]_i = 2.65 \times 10^{-5}$ M, $[AscH^-]_i = 8.92 \times 10^{-5}$ M and $[O_2] = 2.6 \times 10^{-4}$ M).

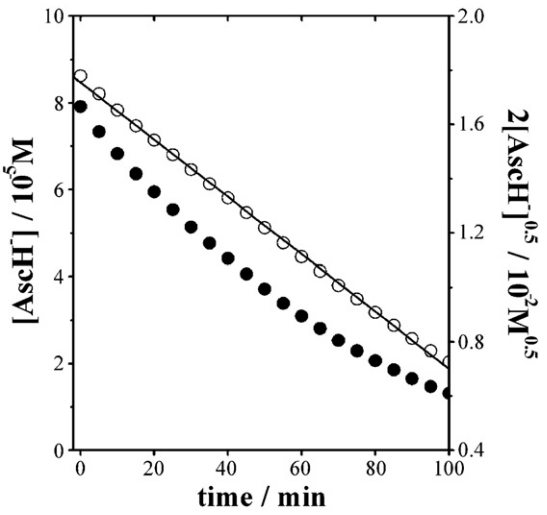

Fig. 2. Plot of L-ascorbate concentration and its square root as a function of time (pH 7.97, $\mu = 0.1$ M, 298 K, $[Cu^{III}(LH_{-4})^-] = 2.39 \times 10^{-6}$ M, $[AscH^-]_i = 7.92 \times 10^{-5}$ M and $[O_2] = 2.6 \times 10^{-4}$ M, see Table 1, exp. 3).

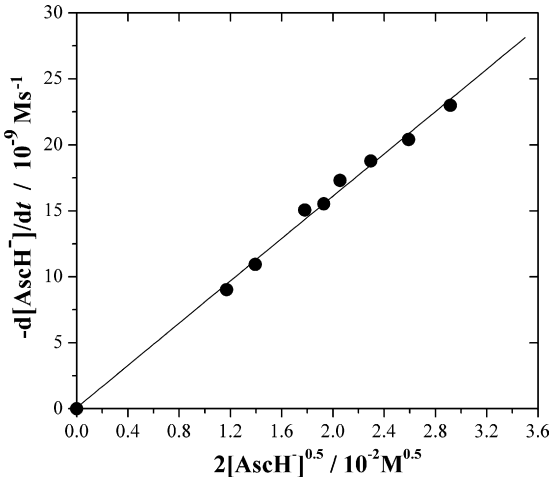

Fig. 3. Reaction rate as a function of square root of the initial L-ascorbate concentration (pH 7.97, μ = 0.1 M, 298 K, [AscH$^-$] is varied between 3.42–21.27 × 10^{-5} M, [Cu^{III}(LH$_{-4}$)$^-$] = 2.39 × 10^{-6} M and [O$_2$] = 2.6 × 10^{-4} M, see Table 1, exp. 1–8).

is a catalytic oxidation caused by the metal ion content of the water used.

Performing the catalytic reactions at different dioxygen pressures (Table 1, experiments 15–21) the initial reaction rates show linear dependence on the square root of the initial dioxygen concentrations (Fig. S2). This strongly indicates half-order dependence with respect to dioxygen (n = 0.5). Based on our results, the overall rate law can be written at constant pH, temperature and ionic strength according to Eq. (5):

$$\frac{-\mathrm{d}[\mathrm{AscH}^-]}{\mathrm{d}t} = k_{obs}[\mathrm{AscH}^-]^{0.5}[\mathrm{Cu^{III}(LH_{-4})}^-][\mathrm{O_2}]^{0.5} \quad (5)$$

Temperature dependence of the kinetic constant k_{obs} was measured in order to determine the activation parameters. The $\Delta H^{\ddagger}$ calculated from Eyring's equation is 50.3 ± 2.2 kJ mol^{-1}, and the $\Delta S^{\ddagger}$ is −41 ± 2 Jmol^{-1} K^{-1} at 298 K. Summary of the rate constants and other parameters for the individual assays are given in Table 1.

Stopped-flow (SF) kinetics. In nitrogen-saturated, buffered solutions the copper(III)/copper(II) equilibrium according to Eq. (6) can be measured in the presence of different concentrations of AscH$^-$.

$$2[\mathrm{Cu^{III}(LH_{-4})}]^- + \mathrm{AscH}^- + \mathrm{OH}^- \overset{K_1}{\rightleftharpoons} 2[\mathrm{Cu^{II}(LH_{-4})}]^{2-} + \mathrm{Asc} + \mathrm{H_2O} \quad (6)$$

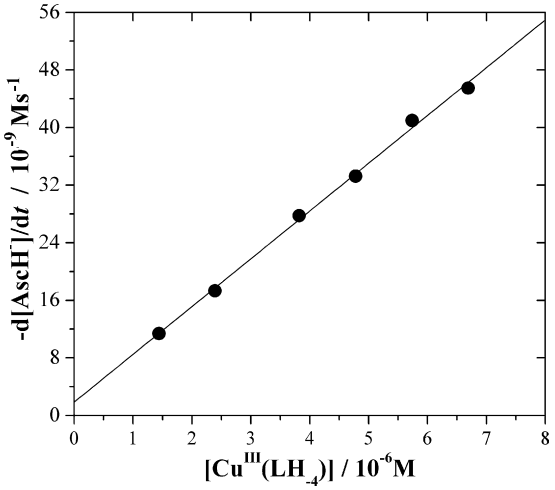

Fig. 4. Plot of the reaction rate as a function of the initial catalyst concentration (at pH 7.97, μ = 0.1 M, 298 K, [AscH$^-$] = 10.65–11.22 × 10^{-5} M, [Cu^{III}(LH$_{-4}$)$^-$] = 1.44–6.69 × 10^{-6} M and [O$_2$] = 2.6 × 10^{-4} M, see Table 1, exp. 9–14).

Rapid mixing of various amounts of ascorbate solutions to a solution of the catalyst and concomitantly following the decrease in the 550 nm absorption band allows us to determine the equilibrium constant. The K_1 can be transformed to K_1' according to Eq. (7) among the applied reaction conditions.

$$K_1' = \frac{K_1[\mathrm{H_2O}]}{[\mathrm{OH}^-]} \quad (7)$$

The rate constant for the forward reaction (k_1') can be obtained directly from the initial rate for copper(III) decay in the presence of 1–50 equivalent of AscH$^-$. Typical SF curves are shown in Fig. S3. The values for the apparent rate constants and equilibrium constants are summarized in Table S1. The extent of the reduction of copper(III) to copper(II) is small as it can be seen from the value of the equilibrium constant at pH 8 (8.78 ± 3.11 × 10^{-3}).

Cyclic voltammetry measurements. It was previously reported [17a] that the complex undergoes a reversible one-electron reduction according to Eq. (8) in aqueous solution.

$$[\mathrm{Cu^{III}(LH_{-4})}]^- + \mathrm{e} \overset{E_{1/2}}{\rightleftharpoons} [\mathrm{Cu^{II}(LH_{-4})}]^{2-} \quad (8)$$

In our system the $E_{1/2}$ was −0.09 V *vs.* a 3 M calomel electrode at a 200 mV s^{-1} scan rate (Fig. 5, solid line spectrum). The peak currents ($|i_a|$ = 0.855 μA and i_c = 0.866 μA, respectively) show the full reversibility of this redox couple among the experimental conditions. When the solution is exposed to air, the picture changes significantly (Fig. 5, dashed line spectrum).

The current peak for the reduction step increases, due to the compensational enhancement of the anodic current resulting from the fast re-oxidation of copper(II) by dioxygen. The oxidation peak almost totally disappears due to the same effect, and another oxidation peak appears at higher potentials (0.18 V, see Fig. 5, dashed line), possibly originating from a copper(III)-dioxygen adduct, that can be proposed as an intermediate in the catalytic process (*vide infra*). Addition of AscH$^-$ to the solution in a 1:10 ratio restores the original peaks (Fig. 5, dotted line), whereas the peak at ∼0.18 V is missing, indicating that the copper(III)-dioxygen adduct rapidly reacts with the added AscH$^-$.

Proposed mechanism. A reaction mechanism that fits the kinetic, SF kinetic, UV–vis spectroscopic and CV data is shown in Scheme 2.

As it was shown in Fig. 1, the initial concentration of copper(III) is decreased by the added ascorbate. The K_1' constant of the cop-

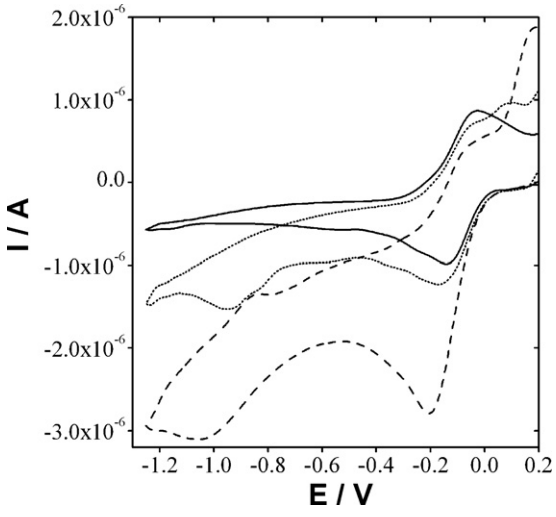

Fig. 5. CV spectra of [Cu^{III}(LH$_{-4}$)]$^-$ under argon (solid line), in the presence of air (dashed line) and in the presence of 10 equivalents of L-ascorbate and air (dotted line), in 0.1 M NaClO$_4$ solution against 3 M KCl calomel electrode with 200 mV s^{-1} scan rate, T = 298 K, [AscH$^-$] = 10 × 10^{-3} M, [Cu^{III}(LH$_{-4}$)$^-$] = 10^{-3} M, and [O$_2$] = 2.6 × 10^{-4} M).

$$2[Cu^{III}(LH_{-4})]^{-} + AscH^{-} + OH^{-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 2[Cu^{II}(LH_{-4})]^{2-} + Asc + H_2O$$

$$2[Cu^{II}(LH_{-4})]^{2-} + O_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [(LH_{-4})Cu^{III}(O_2)Cu^{III}(LH_{-4})]^{4-}$$

$$[(LH_{-4})Cu^{III}(O_2)Cu^{III}(LH_{-4})]^{4-} \xrightarrow[\text{r.d.s.}]{k_3} 2[(LH_{-4})Cu^{III}(O)]^{2-}$$

$$2[(LH_{-4})Cu^{III}(O)]^{2-} + AscH^{-} + H_2O \longrightarrow 2[Cu^{III}(LH_{-4})]^{-} + Asc + 3OH^{-}$$

Scheme 2. Suggested mechanism for the copper(III)-catalysed reaction of L-ascorbate and dioxygen at pH 8.

per(III)/copper(II) equilibrium in the presence of various amounts of ascorbate was calculated from the SF results under nitrogen atmosphere. When the solution is exposed to air, the absorption band at 550 nm grows quickly, i.e. copper(II) is oxidized to copper(III) and after all the ascorbate has reacted the absorbance approaches to its original value. Re-oxidation of copper(II) to copper(III) is supported by the results from the cyclic voltammetry experiments, too.

Based on the observed rate law, among catalytic conditions, one molecule of substrate and dioxygen contributes to the formation of two catalytically active species, each of which contains one copper. This species then can react with the substrate in a fast step to produce Asc and thus closing the catalytic cycle. We believe that the catalytic cycle starts with two pre-equilibria in which formally a peroxodicopper(III) intermediate is formed which then can dissociate to mononuclear copper(III)-oxyl radical anions in the rate-limiting step (k_3). These species are proposed on the analogy of peroxodicopper(II) complexes that are well known in the literature [12] and the negative activation entropy for the reaction ($-41 \pm 2\,J mol^{-1}\,K^{-1}$ at 298 K) is in accordance with an associative mechanism for the dioxygen activation. Since copper(III) intermediates like the ones proposed in this mechanism have never been experimentally described, their presence is hypothetic. Applying steady-state treatment for this mechanism, where $d[(LH_{-4})Cu^{III}(O_2)Cu^{III}(LH_{-4})^{4-}]/dt = 0$, Eq. (9) will describe the rate of product formation and the following rate equation (10) can be obtained:

$$\frac{-d[AscH^-]}{dt} = \{k_3[(LH_{-4})Cu^{III}(O_2)Cu^{III}(LH_{-4})^{4-}]\}^{0.5} \quad (9)$$

$$\frac{-d[AscH^-]}{dt} = \sqrt{\frac{k_2k_3}{k_{-2}+k_3} \times [O_2] \times \frac{k_1[Cu^{III}(LH_{-4})^-]^2[AscH^-][OH^-] + k_{-2}[(LH_{-4})Cu^{III}(O_2)Cu^{III}(LH_{-4})^{4-}]}{k_{-1}[Asc][H_2O] + k_2[O_2]}} \quad (10)$$

When $k_1[Cu^{III}(LH_{-4})^-]^2[AscH^-][OH^-] \gg k_{-2}[(LH_{-4})Cu^{III}(O_2)Cu^{III}(LH_{-4})^{4-}]$ and $k_{-1}[Asc][H_2O] \gg k_2[O_2]$ (this can be presumed based on the SF kinetic results, as K_1 is small, but k_1 and k_{-1} are large), at constant pH and [Asc], Eq. (10) simplifies to Eq. (11), where $C = [OH^-]^{0.5}/\{[Asc][H_2O]\}^{0.5}$:

$$\frac{-d[AscH^-]}{dt} = \sqrt{K_1\frac{k_2k_3}{k_{-2}+k_3}C} \times [AscH^-]^{0.5}[Cu^{III}(LH_{-4})^-][O_2]^{0.5} \quad (11)$$

Eq. (11) satisfies the kinetic data and the deduced kinetic rate law (Eq. (5)), if $k_{obs} = (K_1k_2k_3)^{0.5}/(k_{-2}+k_3)^{0.5}C^{0.5}$. UV–vis spectroscopic results indicates that the [Asc] is constant, since its characteristic absorption band around 300 nm is very low in intensity and does not change significantly over the course of the reaction. This is due to transformation of dehydroascorbic acid to other products that are described in the literature [23]. The relatively high activation enthalpy ($50.3 \pm 2.2\,kJ\,mol^{-1}$ at 298 K) can originate from the repulsion between the negatively charged reacting intermediates.

4. Conclusions

Mechanism of the $2e^-$ oxidation of $AscH^-$ to Asc and the concomitant $4e^-$ reduction of dioxygen to water catalysed by $[Cu^{III}(LH_{-4})]^-$ (Scheme 1) consists of three general stages: (1) activation (reduction) of the metal site by the substrate, (2) reduction of dioxygen by the metal centre to give a reactive copper–oxygen intermediate and (3) quenching the intermediate by the substrate. Although the stoichiometry of the reaction is the same as it was established for the native ascorbate oxidases, our catalytic system cycles through a completely different mechanism compared to the tricopper active site of the native enzymes. In the enzyme the copper(II)/copper(I) redox couple is involved, whereas in our system copper(III) and copper(II) species occur in the catalytic process, due to the ability of the ligand to stabilise copper(III). The O_2 is reduced to water, similarly to several monooxygenase enzymes. A unique feature of the $[Cu^{III}(LH_{-4})]^-$ catalyst is that upon its reduction, the square planar ligand environment is no longer favoured, thus binding an additional ligand at the apical position and the formation of a square pyramidal coordination sphere is likely to take place. This is represented in the suggested mechanism based on different kinetic and spectroscopic methods. Difference in the preferred coordination geometry of the reduced and oxidized metal ion may be a useful activation/de-activation switch in catalytic reactions with copper(III) complexes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcata.2010.10.026.

References

[1] E.G. Kovaleva, M.B. Neibergall, S. Chakrabarty, J.D. Lipscomb, Acc. Chem. Res. 40 (2007) 475–483.
[2] (a) M. Sono, M.P. Roach, E.D. Coulter, J.H. Dawson, Chem. Rev. 96 (1996) 2841–2887;
(b) B. Mennier, S.P. Visser, S. Shaik, Chem. Rev. 104 (2004) 3947–3980;
(c) T.M. Makris, S.G. Sliger, I. Schlichting, Chem. Rev. 105 (2005) 2253–2277.
[3] (a) M.M. Abu-Omar, A. Loaiza, N. Hontzeas, Chem. Rev. 105 (2005) 2227–2252;
(b) M. Costas, M.P. Mehn, M.P. Jensen, L. Que Jr., Chem. Rev. 104 (2004) 939–986.
[4] (a) J.P. Klinman, Chem. Rev. 96 (1996) 2541–2562;
(b) P. Chen, J. Bell, B.A. Eipper, E.I. Solomon, Biochemistry 43 (2004) 5735–5747.
[5] W.A. Francisco, N.J. Blackburn, J.P. Klinman, Biochemistry 42 (2003) 1813–1819.
[6] G.R. Dyrkacz, R.D. Libby, G.A. Hamilton, J. Am. Chem. Soc. 98 (1976) 626–628.
[7] J.J. Staggerda, J.J. Bour, P.J.M.W.L. Birker, Inorg. Chem. 10 (1971) 1202–1205.
[8] (a) D.W. Margerum, K.L. Chellappa, F.P. Bossu, G.L. Burce, J. Am. Chem. Soc. 97 (1975) 6894–6896;
(b) D.W. Margerum, in: T.E. King, H.S. Mason, M. Morrison (Eds.), Oxidases and Related Redox Systems, Pergamon Press, Oxford, 1982, pp. 193–206;
(c) L.L. Diaddario, W.R. Robinson, D.W. Margerum, Inorg. Chem. 22 (1983) 1021–1025;
(d) M.R. McDonald, F.C. Fredericks, D.W. Margerum, Inorg. Chem. 36 (1997) 3119–3124;
(e) S.K. Burke, Y. Xu, D.W. Margerum, Inorg. Chem. 42 (2003) 5807–5817.
[9] (a) P.J. Beurskens, J.A. Cras, J.J. Steggerda, Inorg. Chem. 7 (1968) 810–813;
(b) G. Hogarth, A. Pateman, S.P. Redmond, Inorg. Chim. Acta 306 (2000) 232–236.

[10] (a) D.W. Margerum, G.D. Owens, Metal Ions in Biological Systems, Vol. 12, Marcel Dekker, New York, 1981, p. 75;
(b) J.M. Anast, D.W. Margerum, Inorg. Chem. 21 (1982) 3494–3501;
(c) W. Levason, M.D. Spicer, Coord. Chem. Rev. 76 (1987) 45–120;
(d) D.W. Margerum, Pure Appl. Chem. 55 (1983) 23–34.
[11] (a) F.M. Al-Sogair, Y. Sulfab, Trans. Met. Chem. 18 (1993) 19–22;
(b) A. Hussein, Y. Sulfab, M. Nasreldin, Inorg. Chem. 28 (1989) 157–160.
[12] E.A. Lewis, W.B. Tolman, Chem. Rev. 104 (2004) 1047–1076.
[13] (a) D. Maiti, A.A. Nerducci Sarjeant, K.D. Karlin, Inorg. Chem. 47 (2008) 8736–8747;
(b) D. Maiti, R. Lucas, A.A. Narducci Sarjeant, K.D. Karlin, J. Am. Chem. Soc. 129 (2007) 6899–6998;
(c) K. Itoh, H. Hayashi, N. Furutachi, T. Matsumoto, S. Nagamoto, T. Tosha, S. Terada, S. Fujinami, M. Suzuki, T. Kitagawa, J. Am. Chem. Soc. 127 (2005) 5212–5223;
(d) S. Thyagarajan, N.N. Murthy, A.A. Narducci Sarjeant, K.D. Karlin, S.E. Rokita, J. Am. Chem. Soc. 128 (2006) 7003–7008.
[14] Y. Yano, S. Takano, Y. Kato, W. Tagaki, J. Chem. Soc. [Perkin II] (1979) 1227–1229.
[15] P. Stevens, J.M. Waldeck, J. Strohl, R. Nakon, J. Am. Chem. Soc. 100 (1978) 3632–3634.
[16] R.F. Jameson, N.J. Blackburn, J. Chem. Soc. Dalton (1976) 1596–1602.
[17] (a) I.O. Fritsky, H. Kozłowski, P.J. Sadler, O.P. Yefetova, J. Świątek-Kozłowska, V.A. Kalibabchuk, T. Głowiak, J. Chem. Soc. Dalton Trans. (1998) 3269–3274;
(b) I.O. Fritsky, H. Kozłowski, O.M. Kanderal, M. Haukka, J. Świątek-Kozłowska, E. Gumienna-Kontecka, F. Meyer, Chem. Commun. (2006) 4125–4127.
[18] Standard Methods for the Examination of Water and Wastewater, 12th ed., American Public Health Association, New York, 1965, pp. 408–410.
[19] (a) A. Messerschmidt, A. Rossi, R. Ladenstein, R. Huber, M. Bolognesi, G. Gatti, A. Marchesini, R. Petruzzelli, A. Finazzi-Agro, J. Mol. Biol. 206 (1989) 513–529;
(b) A. Messerschmidt, R. Ladenstein, R. Huber, M. Bolognesi, L. Avigliano, R. Petruzzelli, A. Rossi, A. Finazzi-Agro, J. Mol. Biol. 224 (1992) 179–205.
[20] (a) P.A. Seib, B.M. Tolbert, Ascorbic Acid: Its Chemistry, Metabolism and Uses, Adv. Chem. Ser. Am. Chem. Soc., Washington, DC, 1982;
(b) M.B. Davies, Polyhedron 11 (1992) 285–321.
[21] R.C. Weast (Ed.), Handbook of Chemistry and Physics, 56th ed., CRC Press, Boca Raton, FL, 1976, p. D150.
[22] (a) A. Rigo, M. Scarpa, E. Argese, P. Ugo, P. Viglino, in: W. Bors, M. Saran, D. Tait (Eds.), Oxygen Radicals Chemistry and Biology, Walter de Gruyter, Berlin and New York, 1984, p. 170;
(b) I.B. Afanas'ev, V.V. Grabovetskii, N.S. Kuprianova, J. Chem. Soc. [Perkin II] (1987) 281–285.
[23] (a) K. Pfeilsticker, F. Marx, M. Bockisch, Carbohydr. Res. 45 (1975) 269–274;
(b) E.K. Koliou, P.V. Ioannou, Carbohydr. Res. 340 (2005) 315–318;
(c) R.C. Kerber, J. Chem. Educ. 85 (2008) 1237–1242.