

Redox Behaviour of Some Superoxide Dismutase-Mimetic Copper(II) Complexes of *N*-Salicylideneaminoalkanoate Type with Diazoles

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Eight copper(II) complexes with a tridentate Schiff base and diazoles such as pyrazole, imidazole, and 3,5-dimethylpyrazole have been investigated voltammetrically at a hanging mercury drop electrode in phosphate buffer of pH 7.4. The complexes undergo a reversible one-electron reduction followed by the dissociation of the copper(I) compounds at a slow scan rate. The half-wave potential and rate constant data are reported and discussed with a superoxide dismutase-like activity of the complexes.

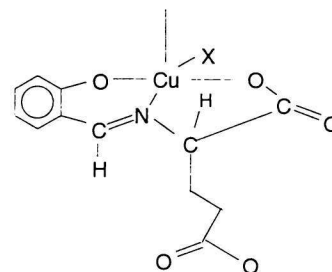
The dismutation of the toxic superoxide anion radical ($O_2^{\cdot-}$) to oxygen (O_2) and hydrogen peroxide (H_2O_2) by SOD is connected with a cyclic change of the oxidation state of the metallic atom (*e.g.* Cu) in the active site of metalloenzyme [1, 2].

The coordination compounds of copper with the tridentate *N*-salicylideneaminoalkanoate ligands exhibit a significant superoxide dismutase (SOD)-like activity [3–5].

In general, the SOD-like activity depends on the structural and redox properties of model coordination compounds. In our previous investigation [6] we have shown a complex character of the redox behaviour of the Schiff base type coordination compounds containing an additional pseudohalide ligand. The voltammetric picture is a function of pH and it indicates a partial decomposition of Cu(I) complexes.

The present paper deals with a new group of Cu(II) complexes with the tridentate Schiff base derived from glutamic acid and the additional heterocyclic *N*-donor ligand of the diazole type X (X = pyrazole, imidazole or 3,5-dimethylpyrazole) (Formula 1).

In the solid state these diazole-(*N*-salicylidene-glutamato)copper(II) complexes adopt a square pyramidal structure containing approximately transplanar chromophore $[CuN_2O_2]$ [7]. The apical position of tetragonal pyramid may be occupied by the donor atom of adjacent complex molecule (crystal structure contacts). In aqueous solutions weak apical bonds are evidently interrupted and replaced by



A schematic representation of (*N*-salicylidene-glutamato)copper(II) complex.

Formula 1

weakly coordinated H_2O molecules [7]. This way these complexes can serve as simple models of the Cu,Zn-SOD active site, which contains transplanar chromophore $[CuN_2]$ [8].

The determination of the redox potentials of the Cu(II) complexes as well as the kinetics of their dissociation after the one-electron reduction at the physiological pH value of 7.4 was the aim of the study.

EXPERIMENTAL

The following Cu(II) complexes were tested: $[Cu(sal-L-glu)(H_2O)_2] \cdot H_2O$ (I), $[Cu(sal-L-glu)pyrazole]$ (II), $[Cu(sal-L-glu)imidazole]$ (III), $[Cu(sal-L-glu)-3,5-dimethylpyrazole]$ (IV), $[Cu(sal-D,L-glu) \cdot 3H_2O]$ (V), $[Cu(sal-D,L-glu)pyrazole]$ (VI), $[Cu(sal-D,L-glu)imidazole]$ (VII), $[Cu(sal-D,L-glu)-3,5-dimethylpyrazole]$ (VIII).

The complex *I* was prepared according to [7], the complexes *II*–*IV* were synthesized according to [9]. The complex *V* was obtained after racemization of complex *I* [10]. The complexes *VI*–*VIII* were synthesized at the temperature $\theta = 60$ – 65 °C by the reaction of the parent compound *V* with corresponding diazole (in methanolic solution and at the mole ratio 1 : 2).

The electrochemical measurements were carried out with polarographic analyzer PA 4 (Laboratorní přístroje, Prague) and EG&G PAR Model 273A (Princeton, NY). The three-electrode arrangement comprises a hanging mercury drop electrode as a working electrode, a large-surface auxiliary platinum electrode, and a saturated calomel reference electrode standardized for the redox couple ferricinium/ferrocene in DMSO ($E_{1/2} = 0.400$ V vs. SCE).

The measurements were performed at $\theta = 22$ °C in argon atmosphere. A solution of the tested complexes

with the concentration $c = 1 \times 10^{-3}$ mol dm⁻³, buffered with phosphate buffer ($c = 5 \times 10^{-2}$ mol dm⁻³) to pH 7.4, contained NaClO₄ ($c = 1 \times 10^{-1}$ mol dm⁻³) as supporting electrolyte.

RESULTS AND DISCUSSION

The cyclic voltammograms of the complexes exhibit a pair of peaks (labeled C1/A1 in Fig. 1) corresponding to the one-electron reduction and subsequent oxidation of the original complex particles. For some complexes these voltammograms are shown in Fig. 1 (solid lines). The peak potential values (E_p) depend on the potential scan rate: with the decreasing scan rate the E_{pc1} values become more positive and the E_{pa1} values more negative. At the scan rate of $\nu = 500$ mV s⁻¹, the differences of the peak potentials, ΔE_p , lie within the range of 50 up to 100 mV and the anodic to cathodic peak current ratio is near to one. It indicates a quasi-reversible redox process localized on the central copper atom. The half-wave potential values were calculated as the arithmetic mean of E_{pc1} and E_{pa1} , and are summarized in Table 1. The $E_{1/2}$ values were found to be independent of the scan rate over the range of $\nu = 20$ – 500 mV s⁻¹

Table 1. Voltammetric Data (for the Scan Rate of $\nu = 100$ mV s⁻¹) and SOD-Like Activity of Copper(II) Complexes [Cu(sal-Y-glu)X]

Optical isomer Y	Ligand X	E_{pc1}	E_{pa1}	$E_{1/2}$	E_{pa2}	IC ₅₀
L	H ₂ O, <i>I</i>	-0.190	-0.140	-0.165	0.030	2.38±0.06
	Pyrazole, <i>II</i>	-0.185	-0.130	-0.158	0.070	1.50±0.02
	Imidazole, <i>III</i>	-0.208	-0.142	-0.175	0.065	1.31±0.01
	3,5-Dimpyr, <i>IV</i>	-0.185	-0.150	-0.168	0.050	2.02±0.04
D,L	H ₂ O, <i>V</i>	-0.187	-0.134	-0.161	0.030	2.77±0.06
	Pyrazole, <i>VI</i>	-0.192	-0.127	-0.160	0.050	2.12±0.04
	Imidazole, <i>VII</i>	-0.193	-0.142	-0.168	0.060	1.87±0.04
	3,5-Dimpyr, <i>VIII</i>	-0.200	-0.155	-0.178	0.070	2.71±0.08

The potential values are in V vs. SCE and IC₅₀ values are in μmol dm⁻³.

The cyclic voltammograms obtained at slower scan rate ($\nu = 20$ – 200 mV s⁻¹) contain at the reverse scan another anodic peak, A2, at $E_{pa2} = 0.030$ – 0.070 V which is the result of the stripping process of Cu⁰. With the decreasing scan rate, the first anodic counterpeak A1 diminishes and the current of the stripping peak A2 becomes higher (Fig. 2). This picture is quite common to copper complexes and indicates a partial decomposition of the copper(II) compounds. It can be explained according to the ECE mechanism: The electrochemical reduction of the Cu(II) complex at E_{pc1} (step E₁) is followed by the chemical step C — the decomposition of the electrogenerated Cu(I) complex under the formation of Cu⁺_{aq} which is electroactive at the potential more positive than E_{pc1} . Thus, for the thermodynamic

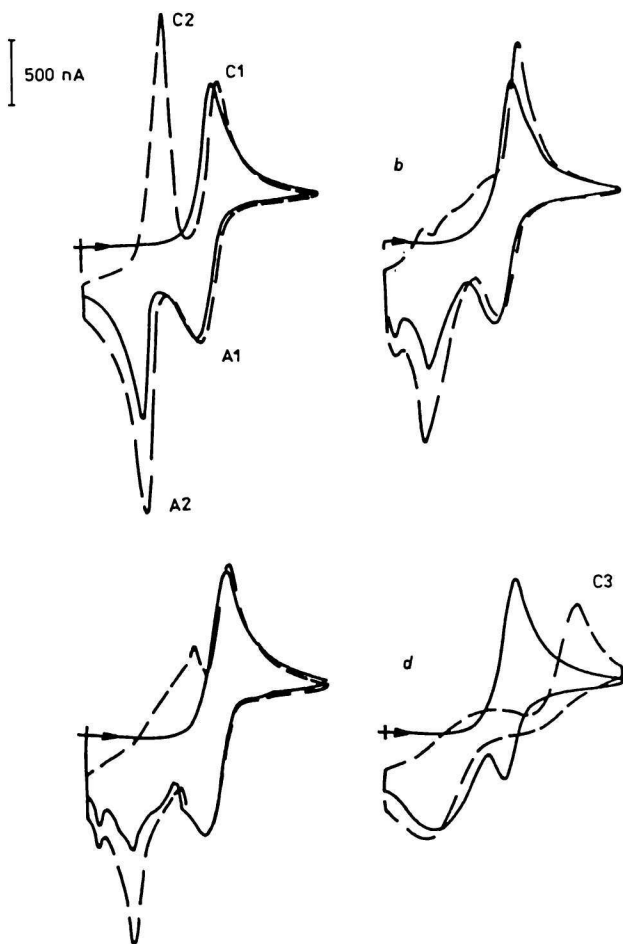
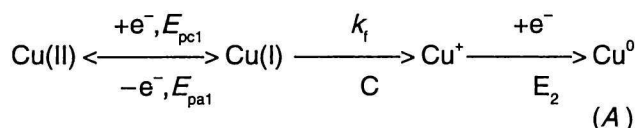


Fig. 1. Cyclic voltammograms for the reduction of the complexes [Cu(sal-L-glu)(H₂O)₂]·H₂O (a), [Cu(sal-L-glu)pyrazole] (b), [Cu(sal-L-glu)imidazole] (c), and [Cu(sal-L-glu)-3,5-dimethylpyrazole] (d) ($c = 1 \times 10^{-3}$ mol dm⁻³) in phosphate buffer ($c = 5 \times 10^{-2}$ mol dm⁻³) of pH 7.4, ionic strength $I = 1 \times 10^{-1}$ mol dm⁻³ with NaClO₄, hanging mercury drop electrode, initial potential of 0.2 V vs. SCE, scan rate $\nu = 100$ mV s⁻¹, $\theta = 22$ °C. The first scan — solid line, the second scan — dashed line.

reasons the liberation of Cu^+ is immediately followed by its reduction to Cu metal (step E_2)



Hence, the voltammetric behaviour of the complexes under study confirmed that the complexes of copper in the oxidation state Cu(I) are unstable and persist in solution only for a period of voltammetric time scale.

The follow-up reaction increases the observed height of the peak A1 which contains currents from the reduction of the original Cu(II) complex as well as Cu^+ . As a consequence of this the peak current ratio i_{pa1}/i_{pc1} decreases with the scan rate up to about 0.4 for $\nu = 20 \text{ mV s}^{-1}$

The number of electrons (z) exchanged at different scan rates (Table 2) was calculated using the equation

$$\frac{i_{pc1}}{\nu^{1/2} z^{3/2}} = \frac{i_{pc1}(\text{rev})}{(\nu(\text{rev}))^{1/2} (z(\text{rev}))^{3/2}} \quad (1)$$

Table 2. Total Number of Electrons (z) Exchanged at the Reduction of Copper(II) Complexes [Cu(sal-Y-glu)X] at the Different Potential Scan Rate ($\nu = 20$ to 200 mV s^{-1}) and the Voltammetrically Determined Rate Constants (k_f) of the Follow-Up Decomposition Reaction

Optical isomer Y	Ligand X	$\nu / (\text{mV s}^{-1})$				k_f / s^{-1}
		200	100	50	20	
z						
L	H ₂ O, I	1.25	1.28	1.28	1.31	2.1
	Pyrazole, II	1.25	1.32	1.36	1.41	2.6
	Imidazole, III	1.25	1.32	1.34	1.42	2.7
	3,5-Dimpyr, IV	1.27	1.33	1.34	–	2.3
D,L	H ₂ O, V	1.26	1.31	1.31	1.34	2.5
	Pyrazole, VI	1.24	1.35	1.39	1.47	3.2
	Imidazole, VII	1.25	1.33	2.39	1.39	3.0
	3,5-Dimpyr, VIII	1.25	1.30	–	–	2.4

where rev denotes the data obtained for reversible process (in our case for $\nu = 500 \text{ mV s}^{-1}$ and $z = 1$). The enhancement of the cathodic peak current over its one-electron height was used to calculate the rate constant of the Cu(I) dissociation (k_f) from the equation

$$\frac{i_p'}{i_p'(\text{rev})} = \frac{0.400 + (k_f/a)}{0.396 + 0.470(k_f/a)} \quad (2)$$

where i_p' is the peak current normalized for scan, $i_{pc1}/\nu^{1/2}$, and $a = zF\nu/RT$ [11]. The $i_{pc1}(\text{rev})$ values were measured at the scan rate of $\nu = 500 \text{ mV s}^{-1}$, where the i_{pa1}/i_{pc1} ratio was equal to one. The rate constants k_f are given in Table 2. Their values indicate a similarity of the original Cu(II) complexes also from the kinetic point of view.

At a potential scan rate slower than $\nu = 20 \text{ mV s}^{-1}$, the cathodic peak C1 exhibits a prepeak evidently due to the adsorption of Cu(II) complexes on the electrode sur-

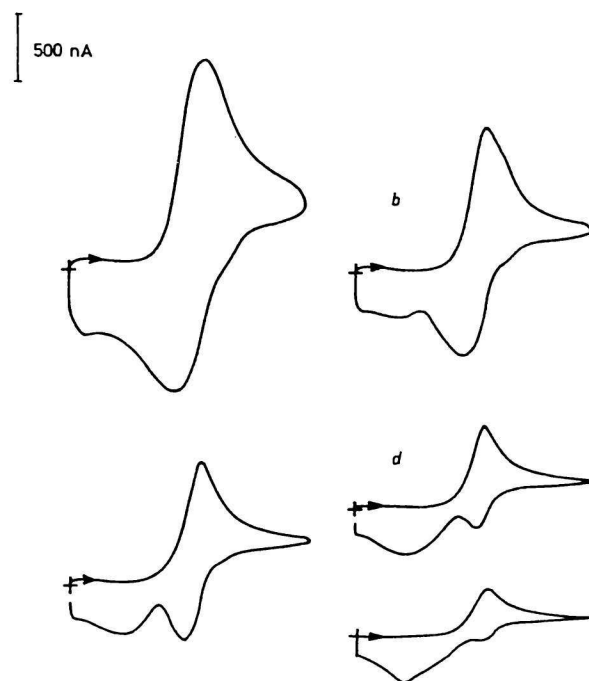


Fig. 2. Cyclic voltammograms for the reduction of the complex [Cu(sal-L-glu)-3,5-dimethylpyrazole] recorded at the scan rate of $\nu = 500 \text{ mV s}^{-1}$ (a), 200 mV s^{-1} (b), 100 mV s^{-1} (c), 50 mV s^{-1} (d), and 20 mV s^{-1} (e). Other conditions as in Fig. 1.

face. At these conditions the small values of the peak potential difference (ΔE_p less than 10 mV) were observed, which are typical for the surface attached depolarizers.

When two successive triangular potentials are applied to the electrode, some new peaks (labeled C2 and C3) occur in the cathodic part of the voltammogram (Fig. 1, dashed lines). These peaks represent counterpeaks to the stripping peak of Cu^0 and they were identified by separate experiments with solutions of copper sulfate and salicylaldehyde, glutamate or diazoles at the mole ratio $n(\text{Cu}) : n(\text{ligand}) = 1 : 1$. The peak C2 with E_p between -0.040 V and -0.130 V corresponds to the reduction of Cu(II) complexes with salicylaldehyde and glutamate (it increases together with the stripping peak height at a repetition of the triangular potential scan).

The signal in the potential region of the original peak C1 or more negative (E_p from -0.370 V to -0.405 V , peak C3) belongs to the reduction of Cu(II) complexes with diazoles formed *in situ* at the electrode.

The voltammetric picture of all complexes under study depends strongly on pH of the solution. This is in accordance with the behaviour of similar Schiff base complexes containing pseudohalide as an additional ligand [6]. All data reported in this paper are valid only for pH 7.4.

All the above-mentioned Cu(II) complexes display a considerable SOD-like activity. This activity has been investigated spectrophotometrically. Superoxide anion radical was generated by enzymatic system of xan-

thine—xanthine oxidase and its amount was determined from the enhancement of absorbance of the INT detector — (3-(4-iodophenyl)-2-(4-nitrophenyl)-5-phenyltetrazolium) chloride [12]. The SOD-like activity of the Cu(II) complexes was characterized by their concentration which causes the 50 % inhibition (IC_{50} value) of the INT reduction by superoxide (Table 1).

A relation between the IC_{50} values and the k_1 rate constants (Table 2) can be seen. This leads to the conclusion that the faster decomposition of copper(II) complexes with pyrazole and imidazole ligands contributes to the increase of their SOD-like activity.

Because the formal redox potential $E^{\circ} = -0.400$ V vs. SCE for the reaction $O_2 + e^- \rightarrow O_2^{\cdot-}$ and $E^{\circ}(\text{pH } 7) = +0.650$ V vs. SCE for the reaction $O_2^{\cdot-} + 2H^+ + e^- \rightarrow H_2O_2$, the model complex must have a redox potential falling between these two values, in order to act as a catalyst of superoxide disproportionation by the given mechanism [1]. The Cu(II)/Cu(I) redox potential for the active centre of Cu,Zn-metalloenzyme is equal to 0.020 V vs. SCE [13].

Although the Cu(II)/Cu(I) potentials of our copper(II) complexes are more negative than that of Cu,Zn-SOD, they fall within the given potential range and react with the superoxide radical. The SOD-like activity has also been observed for bis(*o*-phenanthroline)copper(II) complex [14], the redox potential of which is -0.130 V vs. SCE.

As it results from the measured potential values, the differences in the composition of the investigated Cu(II) complexes influence their redox properties only insignificantly (Table 1). The considerable SOD-like activity of the complexes is evidently connected also with a similarity in the coordination sphere of the central copper atom in these complexes and in the active site of Cu,Zn-SOD. The apical weakly coordinated water molecule could be the place of the reaction with $O_2^{\cdot-}$ as it is in the case of the native enzyme.

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