Biomimetic Reduction of Copper(II) Tetrathia Macrocyclic Complexes

V. V. PAVLISHCHUK, P. E. STRIZHAK, K. B. YATSIMIRSKII
Institute of Physical Chemistry, Ukrainian Academy of Sciences, 252 028 Kiev (U.S.S.R.)
and J. LABUDA*
Department of Analytical Chemistry, Slovak Technical University, 812 37 Bratislava (Czechoslovakia)
(Received July 10, 1989)

Abstract

The reduction of copper(II) tetrathia macrocyclic complexes by glutathione (GSH) in acetonitrile was studied. The formation of the intermediate {Cu(II)-L(SG)}$^+$ was confirmed. The rate is first order in the intermediate concentration. The possibility of a stabilized copper(I)L–thiyl radical system is discussed.

Introduction

The formation of a Cu(II)–mercapto bond, the conditions of its stabilization and the pathways of its redox decomposition are of great interest due to the presence of such a bond in the 'blue' copper proteins. Moreover, the mercapto-amino acid cysteine is the biosubstrate for ceruloplasmine containing also the copper of type I [1]. Relating to this the study of the effect of the copper(II) ligand environment on the kinetics of oxidation of biologically important thiols appears to be interesting.

At present the kinetics of reduction of copper(II) complexes with cyclic and non-cyclic amines [2–4] as well as with thiaaza ligands [5] by cysteine, penicillamine or glutathione has been studied. This paper deals with the reduction kinetics of copper(II) complexes with the polythia macrocyclic ligands L1–L3 by glutathione (GSH) (Fig. 1).

Experimental

The $CuL(ClO_4)_2$ copper complexes were prepared as described elsewhere [6]. Reduced glutathione (Sigma-Aldrich) was used as received; the concentration of its solutions was determined iodometrically. All measurements were made in acetonitrile (Laborchemie Apolda).

The reaction kinetics was studied spectrophotometrically with a Specord UV–Vis apparatus (Zeiss, Jena). The measuring cell was thermostated (to 1 °C) by an external water bath. Anaerobic experiments were made in a cell for inert medium in an argon atmosphere. In the kinetic runs the decrease in absorbance at λ_{max} corresponding to the adducts of copper(II) complexes with glutathione was monitored.

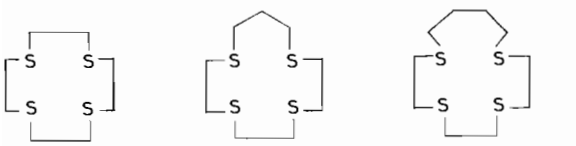

Fig. 1. Polythia macrocyclic ligands.

*Author to whom correspondence should be addressed.

Results

The addition of glutathione to CuL^{2+} leads to a shift of λ_{max} of the characteristic absorption band in the region 600–700 nm (Table 1) and to an increase in absorbance. These changes take place practically immediately. Then the absorbance decreases, either totally (in the inert atmosphere) or partly (to *c.* 90% under anaerobic conditions).

These observations, analogous to those in refs. 2 and 5, we assigned to the formation of a rather stable intermediate of the copper(II) complex with GSH during the reaction. The spectrophotometric titration has shown that the reactants ratio in the intermediate is CuL^{2+}:GSH = 1:1. The formation of the intermediate is accompanied by a jump-like change of solution acidity determined pH-metrically.

TABLE 1. Electronic spectra parameters of $CuL(ClO_4)_2$ and their adducts with glutathione in acetonitrile

Complex	λ_{max} (nm) (ϵ (M^{-1} cm^{-1}))	
	CuL^{2+}	{CuL(SG)}$^+$
$CuL1^{2+}$	605(2000)	676(2200)
$CuL2^{2+}$	654(1950)	619(1900)
$CuL3^{2+}$	599(570)	628(710)

0020-1693/90/$3.50

These data may be attributed to an intermediate of composition $\{Cu(II)L(SG)\}^+$.

$$CuL^{2+} + GSH \rightleftharpoons \{Cu(II)L(SG)^+\} + H^+ \quad (1)$$

The decrease in absorbance of the reaction mixture is caused by electron transfer in the inner coordination sphere from the mercapto group to Cu(II).

The kinetic data analysis by the initial rate method proves that the degradation of the intermediate may be described by the first-order kinetic equation

$$-\frac{d[\{Cu(II)L(SG)^+\}]}{dt} = k_{obs}[\{Cu(II)L(SG)^+\}] \quad (2)$$

The absorbance dependence versus time obtained under aerobic and anaerobic conditions at the same initial concentrations of reagents are nearly the same up to 75–80% of intermediate decay. This makes it possible to determine the rate constant of the intermediate redox degradation without the oxygen-free technique. The integral form of eqn. (2), i.e. the dependence of $\ln A$ versus time, was linear for at least two half-times of reaction and its slope represents the value of k_{obs} (Table 2). The linear dependence $\ln k_{obs}$ versus $1/T$ in the temperature interval 20–60 °C allowed us to determine the activation energy E_a of the decomposition process of the intermediate (Table 3).

TABLE 2. First-order rate constants, k_{obs}, of $\{CuL(GS)\}^+$ adduct degradation in acetonitrile at 25 °C

Complex	Initial concentration (M)		$10^4 \times k_{obs}$
	$10^4 \times c_0$ (CuL^{2+})	$10^4 \times c_0$ (GSH)	(s^{-1})
$CuL1^{2+}$	0.4	1.4	2.5
	0.7	1.4	2.0
	2.3	1.4	2.0
	2.3	2.6	2.5
	2.3	5.2	2.2
	2.3	25.0	2.7
$CuL2^{2+}$	0.4	1.4	1.7
	0.8	1.4	1.5
	2.0	1.4	1.9
	2.0	2.5	1.3
	2.0	5.2	1.6
	2.0	25.0	1.4
$CuL3^{2+}$	5.8	1.4	16
	5.8	2.6	15
	5.8	5.2	17
	5.8	25.0	17

TABLE 3. Kinetic parameters of $\{CuL(SG)\}^+$ adduct degradation in acetonitrile at 25 °C

Adduct	$10^4 \times k_{obs}$ (s^{-1})	E_a (kcal/mol)
$\{CuL1(SG)\}^+$	2.3 ± 0.2	6.4 ± 1.0
$\{CuL2(SG)\}^+$	1.6 ± 0.1	12.2 ± 1.2
$\{CuL3(SG)\}^+$	16 ± 2	5.2 ± 0.7

Discussion

The interaction of glutathione with copper(II) polythia macrocyclic complexes leads to a shift of the absorption band $\lambda_{max} \sim 600$ nm and to a low increase in absorbance. The nature of this absorption band in the initial tetrathia complexes is connected most probably with the overlap of charge transfer π (thiaether S) → 3d(Cu(II)) and of d–d transition [6, 7]. The shift of the absorption band is connected with the adduct formation and with the presence of the new charge transfer π (GS^-) → 3d(Cu(II)L) in it [2, 5]. The data given above as well as those in the literature [2, 5] allow us to consider the adduct as $\{Cu(II)L(SG)\}^+$.

There are several possibilities of redox degradation of the copper(II)–thiol intermediates [2, 5, 8] which are analysed in detail in ref. 2.

(i) Innermolecular redox process with inner-sphere electron transfer

$$\{Cu(II)L(SR)\}^+ \longrightarrow \{Cu(I)L(\dot{S}R)\}^+ \quad (3)$$

The formed cation–radical adduct may be stable or undergo further degradation including the redox one. In the latter case the rate of $\{Cu(II)L(SG)\}^+$ degradation is described by the first-order kinetic equation against the intermediate concentration.

(ii) The intermediate itself acts as an oxidant of the mercapto substrate

$$\{Cu(II)L(SR)\}^+ + RSH \xrightarrow{slow} \{Cu(I)L(RSSR)^-\} + H^+ \quad (4)$$

$$\{Cu(I)L(RSSR)^-\} + \{Cu(II)L(SR)\}^+ \xrightarrow{quick} \{Cu(I)L(RSSR)\}^+ + \{Cu(I)L(SR)\} \quad (5)$$

The rate of such a process is described by the second-order kinetic equation

$$-\frac{d[\{CuL(SR)^+\}]}{dt} = k[\{(CuL(SR)\}^+][RSH] \quad (6)$$

(iii) The formation of the di-μ-mercapto complex and the two-electron reduction of copper(II) centres to copper(I). In this case the intensive absorption band of the intermediate in the electron spectrum is not observed and the decomposition rate is described by the second-order kinetic equation with respect to the intermediate concentration and does not de-

pend on the concentration of the bioreductant. According to ref. 2 there exists the sterically advantageous di-μ-mercapto bridge $\{LCu(II)-SR\}_2$ in the activated complex which should also be possible for glutathione.

(iv) In the case of the sterically hindered equatorial ligand the formation of the activated complex with more than one substrate molecule in contrast to case (iii) is impossible. The rate determining step will be

$$2\{Cu(II)L(SR)\}^+ + RSH \xrightarrow{slow} \{Cu(I)L(RS\text{-}SR)\}^+ + \{Cu(I)L(SR)\} + H^+ \quad (7)$$

The degradation rate of the intermediate is proportional to the second order of the intermediate concentration and to the first order of RSH.

Comparing our experimental data with the above mentioned schemes we see that the oxidation of GSH by copper(II) polythia macrocyclic complexes takes place as in (i). First-order decay of $\{Cu(II)L(SG)\}^+$ was considered [2] to be atypical except for those cases when the oxidation power of the initial CuL^{2+} allows the formation of metal-stabilized thiyl radicals ($RS^{\cdot}$). However, the copper(II) complexes studied in this paper actually show high positive redox potentials in the region +0.9–1.15 V [9]. Recently [5] we investigated the glutathione reaction with copper(II) complexes with polydentate non-cyclic ligands with the donor set N_xS_y ($x = 2.3$, $y = 2.4$). In the course of this, the $\{Cu(II)N_xS_y(SG)\}^+$ redox decomposition was found to proceed as in (i), too. Since the data of ref. 2 concern only polyamine complexes the first-order decay of the intermediate may obviously be considered to be typical for copper(II) polythia complexes with the satisfactorily high redox potentials [5, 9, 10, 11].

It is still of interest to see how the degradation of the adduct $\{CuL(SR)\}^+$ in amino complexes as in (ii) takes place when the redox potential of $CuL^{2+/+}$ does not allow biosubstrate oxidation. In this case the thiol binding in the Cu(II)–SR intermediate changes the initial complex to that with a mixed coordination sphere which may lead to a considerable positive shift of the redox potential and thus to the increase in the oxidation activity of the complex.

In this work the autoreduction of CuL^{2+} complexes in acetonitrile [9] was neglected for two reasons: (a) all known reactions of intermediate formation (eqn. (1)) take place practically immediately and the equilibrium of these is fully shifted towards 1:1 adduct formation [2, 5], (b) rate constants of autoreduction of polythia macrocyclic complexes are at least 10 times lower [9] than those for glutathione reduction and thus do not bring sufficient error into the determined kinetic parameters.

The comparison of k_{obs} values of the $\{Cu(II)L(SG)\}^+$ redox degradation for complexes with polythia macrocycles and non-cyclic N_xS_y ligands [5] shows that they are approximately of the same order. The values of activation energy, E_a, for $CuL1^{2+}$ and $CuL3^{2+}$ are roughly equal to those for most $Cu(N_xS_y)^{2+}$, only for $CuL2^{2+}$ the E_a is somewhat greater.

Attention is drawn to the irregular change of the activation energy of CuL^{2+} reduction by GSH (Table 3). Such E_a dependence may be explained by the different geometry of the complexes. The most planar chromophore is in the $CuL2^{2+}$ particle. It leads to the poor overlap between the axially bound mercaptide donor sulphur and Cu(II) acceptor orbitals [2]. On the other hand, the stabilization of the Cu(I)L adduct with the thiyl radical may be difficult due to the $CuL2^{2+}$ rigid structure. As a consequence, the activation energy in the case of the complex with a 13-membered ring is increased.

The discussion of the effect of ligand structure and physicochemical properties of the copper(II) complexes on the kinetic parameters of the mercapto adduct degradation is still inadequate due to the lack of data on these reactions as well as on the conditions of stabilization of the Cu(II)–thiol bond.

References

1 V. Albergoni and A. Cassini, *FEBS Lett., 55* (1975) 261.
2 C. H. Anderson and R. A. Holwerda, *J. Inorg. Biochem., 23* (1985) 29.
3 H. K. Balk and R. A. Holwerda, *Inorg. Chem., 22* (1983) 3452.
4 G. D. Stevens and R. A. Holwerda, *Inorg. Chem., 23* (1989) 2777.
5 V. V. Pavlishchuk, J. Labuda and E. Šturdík, *Inorg. Chim. Acta, 151* (1988) 261.
6 V. V. Pavlishchuk, J. Labuda, K. B. Yatsimirskii and P. E. Strizhak, *Proc. 12th Conf. Coordination Chemistry, Bratislava-Smolenice*, Czechoslovakia, 1989, p. 411.
7 N. S. Ferris, W. H. Woodruff, D. B. Rorabacher, T. E. Jones and L. A. Ochrymowycz, *J. Am. Chem. Soc., 100* (1978) 5939.
8 E. Deutsch, M. J. Root and D. Nosco, in A. G. Sykes (ed.), *Advances in Inorganic and Bioinorganic Mechanism*, Vol. 1, Academic Press, New York, 1982, p. 269.
9 V. V. Pavlishchuk, K. B. Yatsimirskii, P. E. Strizhak and J. Labuda, *Inorg. Chim. Acta, 164* (1989) 65.
10 V. V. Pavlishchuk, K. B. Yatsimirskii, P. E. Strizhak and J. Labuda, *Inorg. Chim. Acta, 151* (1988) 133.
11 U. Sakaguchi and A. W. Addison, *J. Chem. Soc., Dalton Trans.*, (1979) 600.