Biomimetic Oxidation of Glutathione by Synthetic Analogues of the Active Centres of 'Blue' Copper Proteins

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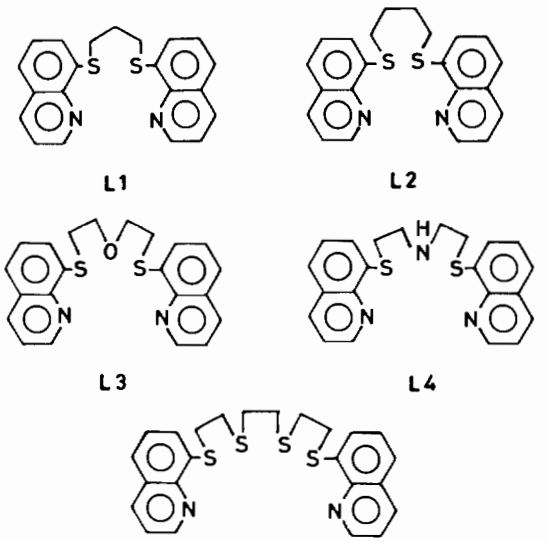
Abstract

The model process of oxidation of reduced glutathione through chelate copper complexes has been studied, the former being structural analogues of the active centres of 'blue' copper proteins. Glutathione forms the relatively stable intermediate $CuLSG^+$ with copper complexes in acetonitrile. The intramolecular electron transfer S(glutathione)→Cu(II) is the rate-determining step of the substrate oxidation. On the basis of rate constant (k_{obs}) values as well as activation energy ($E^{\ddagger}$) values, we have concluded that there is a possibility of functional modelling of active centres of type 1 Cu by copper complexes with thioaza ligands.

Introduction

Among the tasks of bioinorganic chemistry, structural and functional modelling of the properties of biocomplexes takes special position. Recently, considerable progress has been achieved in obtaining synthetic analogues of active centres of 'blue' copper proteins [1–5] which fulfil the functions of biooxidants as well as electron carriers in the organism [6]. The synthesized models have been studied in detail by electron as well as ESR spectroscopy, and have been characterized electrochemically as well as magnetochemically [1–5]. However, a study of these complexes as functional models has not been made.

The authors have attempted to model biooxidation properties of active centres of 'blue' copper proteins on the example of the oxidation of glutathione (of reduced *p*-glutamylcysteinylglycine, GSH). Glutathione is the most widely spread reducing agent *in vivo* containing the thiol group. In blood plasma glutathione does not react directly with oxygen; it can be oxidized both by enzymatic and non-enzymatic methods; in the second case 8 to 30% of this tripeptide is subject to oxidation [7]. The non-enzymatic oxidation is catalyzed by trace amounts of copper(II) [7]. Because of this as a whole it is probable that the process of glutathione oxidation *in vivo* involves participation of 'blue' copper proteins. The synthetic analogues of the active centres of the proteins used here are copper(II) complexes with the thioaza ligands L1 to L5.



Experimental

The copper(II) complex compounds were prepared according to procedures described in the literature [4, 5]. The reduced glutathione used was obtained from Serva (G.D.R.). The solvent used was acetonitrile because the copper(II) complexes studied are not subject to spontaneous redox reactions and destruction in this solvent. The reaction kinetics were studied spectrophotometrically with a Specord UV–Vis apparatus (Zeiss, Jena). Anaerobic experiments were made in measuring cells for inert medium in an argon atmosphere.

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TABLE I. Electronic Parameters of Complexes $CuL(ClO_4)_2$ and their Adducts with Glutathione in the Region 600–800 nm in Acetonitrile

Complex	λ_{max} (nm) (ϵ (M^{-1} cm^{-1}))	
	Original complex[a]	Adduct[b]
$CuL1(ClO_4)_2$	630 (300)	650 (600)
$CuL2(ClO_4)_2$	640 (330)	720 (440)
$CuL3(ClO_4)_2$	720 (440)	780 (460)
$CuL4(ClO_4)_2$	630 (290)	650 (365)
$CuL5(ClO_4)_2$	630 (200)	650 (384)

[a]According to ref. 4. [b]ϵ calculated from kinetic data (see text).

Results and Discussion

The addition of glutathione to the solution of CuL^{2+} complexes (L = L1–L5) in acetonitrile leads to considerable change in the electronic spectra of these complexes. The most characteristic CuL^{2+} absorption band, occurring within the interval 630 to 720 nm [4], is shifted to longer wavelengths and the intensity increases (Table I). The increase in absorbance at λ_{max} occurs practically immediately, *i.e.* during solution mixing. The value of the absorbance extrapolated to time $t_0 = 0$ s is determined only by the concentration of the reagent which is in shortage. In such a case, as with the data for glutathione oxidation by tetraaza copper(II) complexes [8], the assumption of the formation of a long-living intermediate CuL^{2+} with glutathione is logical.

Through spectrophotometric titration at stable CuL^{2+} initial concentration and at various initial concentrations of glutathione the molar ratio of reagents in the adduct has been found to be 1:1. The binding of the deprotonized as well as the protonized form of glutathione corresponds to this stoichiometry:

$$CuL^{2+} + GSH \overset{K_{-H^+}}{\rightleftharpoons} CuLSG^+ + H^+ \quad (1)$$

$$CuL^{2+} + GSH \overset{K}{\rightleftharpoons} CuL(GSH)^{2+} \quad (2)$$

Because the copper(II) ions express strong inclination towards the formation of a mercaptide bond, reaction (1) is considered to be more probable. From the fact that the value of the absorbance at time t_0 is determined exclusively by the concentration of the reagent which is in shortage, it follows that the K_{-H^+} value is relatively high.

The shift of the absorption band as well as the increase in the extinction coefficient ϵ can be ascribed to the formation of a new Cu(II)–S bond with considerable charge transfer $n(S) \rightarrow d_{x^2-y^2}(Cu\text{-}II)$. The intensity of this band decreases to zero at times and the electronic spectra gain the shape characteristic for the CuL^+ complexes (L = L1–L5) [4]. Through spectrophotometric titration it has been found that 1 mole of glutathione is consumed for the reduction of 1 mole of CuL^{2+}. The measurements made under anaerobic conditions offered the same stoichiometry as in the presence of oxygen.

TABLE II. Rate Constant k_{obs} Obtained in Experiments with Different $CuL1(ClO_4)_2$ and Glutathione (GSH) Concentrations at 25 °C

$10^4 \times c(CuL1^{2+})$ (M)	$10^4 \times c(GSH)$ (M)	$10^3 \times k_{obs}$ (s^{-1})
1.25	2.50	1.1
2.50	2.50	0.9
5.00	2.50	0.9
2.50	0.50	0.8
0.50	250.0	0.8

TABLE III. Kinetic Data for Reduction of Complexes CuL^{2+} by Glutathione and Half Wave Redox Potential of Complexes

Complex	$10^4 \times k_{obs}$ (s^{-1})	$E^{\ddagger}$ (kcal mol^{-1})	$E_{1/2}$ (V) *vs.* SCE[a]
$CuL1^{2+}$	9.0	8.6	0.40
$CuL2^{2+}$	7.7	6.1	0.66
$CuL3^{2+}$	6.4	6.6	0.44
$CuL4^{2+}$	5.0	6.8	0.48
$CuL5^{2+}$	4.5	32.5	0.53

[a]According to ref. 4.

On the basis of the experiments which have been made, the reaction studied can be described by the equation:

$$2CuL^{2+} + 2GSH \longrightarrow 2CuL^+ + GSSG \quad (3)$$

Assuming that the intramolecular electron transfer S(glutathione) → Cu(II) in the intermediate is the rate-determining step of the reaction, the kinetic equation of the unimolecular reaction is valid for the process studied:

$$-\frac{d[CuLSG^+]}{dt} = k_{obs}[CuLSG^+]$$

In such a case it is possible to obtain the value of the absorbance A_0 at λ_{max} of the considered intermediate by extrapolation of the dependence $\ln A_t = -k_{obs}t$ to time t_0; from the former the extinction coefficient of the corresponding intermediate can be obtained. The ϵ values determined in this way are given in Table I. Rate constants (k_{obs}) (Tables II and III) as well as the ϵ coefficients have been determined at different ratios of initial concentrations

of CuL^{2+} and glutathione. The values of both parameters are independent of the reagent concentration. The linearity of $\ln A_t$ dependence on t is preserved during 80–90% of the time of decomposition of the intermediate. These facts confirm that the kinetic model used does not contradict the experimental data.

From the $\ln k_{obs}$ dependences on temperature for the interval 20 to 60 °C, the values of the activation energy $E^{\ddagger}$ of the intramolecular electron transfer in the intermediate $CuLSR^{+}$ have been calculated. The temperature dependence of $\ln k_{obs}$ is linear, which shows the stability of the reaction mechanism within the given temperature interval. The values k_{obs} and $E^{\ddagger}$ do not show correlation with those of the potential of the reduction of $CuL1^{2+}$ to $CuL5^{2+}$ determined in ref. 4.

Attention has been paid to the considerable difference in $E^{\ddagger}$ value in the case of the complex $CuL5^{2+}$. The main difference in $CuL5^{2+}$ from the other complexes studied lies in the number of sulphur atoms in the ligand which can be involved in coordination. In the CuL5 complex, considerable transfer of electron density from sulphur donor atoms exists by which the electron transfer S(glutathione) → Cu(II) is made more difficult and which leads to the increase in the $E^{\ddagger}$ value.

The obtained data thus allow one to consider the CuL^{2+} complexes studied not only as structural but also as functional models of active centres of 'blue' copper proteins.

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