9. Hohlneicher, G. and Gutmann, M., *Int. J. Quantum Chem. 29*, 1291 (1986).
10. McWeeny, R., *Rev. Mod. Phys. 32,* 335 (1960).
11. Salem, L., *Nouv. J. Chim. 2,* 559 (1978).
12. Ponec, R. and Strnad, M., *Collect. Czech. Chem. Commun. 55,* 896 (1990).
13. Ponec, R. and Strnad, M. *Int. J. Quantum Chem., 42*, 501 (1992).
14. Ponec, R. and Strnad, M., *Collect. Czech. Chem. Commun. 58,* 1751 (1993).
15. Ponec, R., *Collect. Czech. Chem. Commun. 50,* 1121 (1985).
16. McWeeny, R. and Kutzelnigg, W., *Int. J. Quantum Chem. 2,* 187 (1968).
17. Hashimoto, K., *Int. J. Quantum Chem. 21*, 861 (1982).
18. Ponec, R. and Strnad, M., *J. Phys. Org. Chem. 5,* 764 (1992).
19. Kutzelnigg, W., *Top. Curr. Chem. 41*, 31 (1973).
20. Ponec, R., *Collect. Czech. Chem. Commun. 52*, 555 (1987).
21. Ponec,R. and Strnad, M., *Collect. Czech. Chem. Commun. 55,* 2583 (1990).
22. Dewar, M. J. S., *J. Am. Chem. Soc. 106*, 209 (1984).

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Kinetics of the Reduction of Tetraaza Macrocyclic Complex $Cu(TAAB)^{2+}$ by Glutathione

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The reduction of a copper(II) complex with the macrocyclic ligand tetrabenzo[*b,f,j,n*]-[1,5,9,13]-tetraazacyclohexadecine by glutathione in aqueous solution has been investigated spectrophotometrically in both anaerobic and aerobic conditions. The reaction rate is first-order with respect to the concentration of reactants. According to the values of activation parameters and the course of spectral change during the reaction, an outer-sphere redox mechanism has been suggested. The copper complex initiates the catalytic oxidation of glutathione by dioxygen. The results are compared with the catalytic effect of $Cu(TAAB)^{2+}$ at the oxidation of ascorbic acid and hydroxylamine.

In connection with modelling the natural redox processes under the participation of copper-containing proteins, a systematic attention is paid to the kinetic study of the reduction of copper(II) coordination compounds by different reducing agents. Synthetic macrocyclic complexes are widely used in the investigations because of an excellent possibility of systematic changing the ligand structure and, consequently, the physicochemical properties and chemical reactivity of the Cu(II) redox centre.

The ligand TAAB (tetrabenzo[*b,f,j,n*]-[1,5,9,13]-tetraazacyclohexadecine, Fig. 1) is characterized by rather flexible geometry around the copper atom and a highly delocalized electronic structure which lead to an easy and reversible Cu(II) to Cu(I) reduction [1]. In our previous studies the reduction of $Cu(TAAB)^{2+}$ by ascorbic acid [2] and hydroxylamine [3] was investigated. An influence of the substrate concentration and pH on the reaction mechanism was observed. This complex acts as a catalyst at the radical oxidation of ascorbic acid by dioxygen in weak acidic medium [4]. However, in neutral and weak alkaline solutions the catalytic effect is lowered due to the deprotonation of $Cu(TAAB)^{2+}$ as well as $HO_2^{\bullet}$ radical [1].

Among reducing agents utilized in biomimetic studies the sulfur-containing bioreductant glutathione (GSH) is commonly used. The formation of Cu(II)—SG adducts was shown at the reduction of copper(II) tetrathia as well as diazadithia macrocyclic complexes [5, 6]. Such intermediates might be very useful for modelling the function of active centres of "blue" copper proteins. We were, therefore, interested in extending the previous investigations to the system $Cu(TAAB)^{2+}$ and GSH in order to determine the kinetic parameters of the redox reaction, to propose the type of reagents interaction and to examine a possibility of the catalytic effect of copper complex on the autooxidation of GSH. The results are presented which allow us to describe in more detail the reactivity of both the $Cu(TAAB)^{2+}$ complex and glutathione.

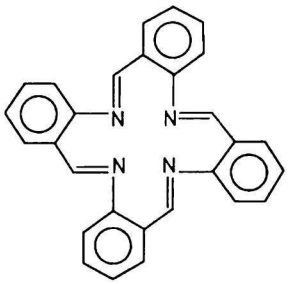

Fig. 1. The macrocyclic ligand TAAB.

EXPERIMENTAL

$Cu(TAAB)(NO_3)_2$ was synthesized as described previously [7]. Fresh solutions of glutathione (Sigma, Aldrich) were prepared immediately prior to the experiment. The Britton—Robinson buffer solutions were prepared from anal. grade chemicals in deionized water which was distilled from potassium permanganate in an all-glass apparatus. Dioxygen was removed from the solutions by bubbling argon for 15 min.

After solutions of $Cu(TAAB)^{2+}$ were placed in a 5 cm path length quartz cell, provided with openings for filling and bubbling, they were deoxygenated as described above. The solution was brought to the constant temperature and finally, the deoxygenated solution of glutathione was added by using a precision gastight syringe.

The reaction was monitored at the $Cu(TAAB)^+$ maximum absorption wavelength (λ_{max} = 660 nm, ε = 5200 cm^{-1} mol^{-1} dm^3 [8]) at which the $Cu(TAAB)^{2+}$ absorption is only low and can be neglected. The spectrometer Specord M 40 (Zeiss, Jena) was employed. The temperature was controlled by a standard water-jacketed cell holder in the spectrometer.

Changes in the concentration of dioxygen were monitored voltammetrically at – 0.5 V *vs.* SCE. The PA 4 polarographic analyzer (Laboratorní přístroje, Prague) was used with the electrochemical cell closed from the atmosphere. A platinum electrode served as the indicating electrode.

RESULTS

The addition of glutathione to the solution of $Cu(TAAB)^{2+}$ in the inert atmosphere causes a gradual increase in the absorbance at λ_{max} = 660 nm which represents the characteristic absorption band of the Cu(I) complex with the ligand TAAB. No shift of this absorption band was detected. After 0.5—1.5 h the precipitate of $Cu(TAAB)^+$ was observed in the reaction mixture. The reaction stoichiometry was determined by monitoring the spectrophotometric titration of $Cu(TAAB)^{2+}$ with glutathione. It has been found that 1 mol of GSH is consumed for the one-electron reduction of 1 mol of Cu(II) complex under anaerobic conditions.

The rate of the reaction was studied as a function of the concentration ($c(Cu(TAAB)^{2+})$ = 3.6 × 10^{-5}—5 × 10^{-4} mol dm^{-3}, c(GSH) = 5 × 10^{-5}—4.5 × 10^{-3} mol dm^{-3}) and pH (4.65—6.15). The ionic strength was kept constant at 0.1 mol dm^{-3} by the addition of KNO_3. First order of the reaction with respect to both $Cu(TAAB)^{2+}$ and GSH concentrations has been proved using the method of initial reaction rate. The reaction kinetics can be expressed by the rate equation

$$\frac{dc\left(Cu(TAAB)^{+}\right)}{dt} = k_{obs}c\left(Cu(TAAB)^{2+}\right)c(GSH) \quad (1)$$

The kinetic curves $c(Cu(TAAB)^+)$ *vs.* time linearized according to the usual equation for the second-order reaction were linear up to at least 70 % completion, thus confirming the first-order dependence on both reagents. This makes it possible to determine the observed rate constants which are given in Table 1.

In the region of high GSH concentration (c > 5 × 10^{-4} mol dm^{-3}) the saturation effect was observed. For the reaction rate, v, the following expression has been obtained

$$v = \frac{kKc(GSH)}{1+Kc(GSH)}c\left(Cu(TAAB)^{2+}\right) \quad (2)$$

where k = (2.0 ± 0.3) × 10^{-3} s^{-1} and K = (1.0 ± 0.2) × 10^4 mol^{-1} dm^3 at pH = 5.65 and θ = 25.0 °C.

The second-order rate constant, k_{obs}, increases with the decreasing acidity of the solution (Table 1). For the region of pH from 4.65 to 6.15 the plot of k_{obs} *vs.* $1/c(H^+)$ is a straight line with the intercept

$$k_{obs} = k_a + k_b\frac{1}{c\left(H^+\right)} \quad (3)$$

where k_a = (0.4 ± 0.2) mol^{-1} dm^3 s^{-1} and k_b = (5.4 ± 0.4) × 10^{-6} s^{-1} (r = 0.9992).

The temperature study of the reaction (Table 1) at pH 4.65 where the acid-independent path (k_a) is predominant, yielded the activation parameters $\Delta H^{\neq}$ = (42 ± 4) kJ mol^{-1} and $\Delta S^{\neq}$ = (– 110 ± 10) J $(K\ mol)^{-1}$. At the evaluation of the pH and temperature dependences of k_{obs}, the constant error of the k_{obs} values has been considered as the experimental error of k_{obs} for pH 4.65 and θ = 25.0 °C which was significantly lower than that of other k_{obs} values and, therefore, at the small number of data the result was determined by this error value.

Table 1. Dependences of the Second-Order Rate Constant for the Reduction of $Cu(TAAB)^{2+}$ by Glutathione on pH (at 25.0 °C) and on the Temperature (at pH = 4.65). I = 0.1 mol dm^{-3} (with KNO_3)

pH	4.65	5.15	5.65	6.15
k_{obs}/(mol^{-1} dm^3 s^{-1})	0.50 ± 0.03	1.2 ± 0.3	3.0 ± 0.5	8.0 ± 1.7
θ/°C	25.0	30.0	38.0	50.0
k_{obs}/(mol^{-1} dm^3 s^{-1})	0.50 ± 0.03	0.7 ± 0.1	1.1 ± 0.2	2.0 ± 0.5

Under the aerobic conditions the formation of Cu(I) complex exhibits an induction period. The length of this period is proportional to the initial concentrations of $Cu(TAAB)^{2+}$ and dioxygen and indirectly proportional to the concentrations of H^+ and GSH. Bubbling through the solution by argon during the experiment leads to shortening of the induction period. After dioxygen has been spent, the $Cu(TAAB)^{2+}$ reduction follows a course similar to that in anaerobic media. However, the rate constant of the $Cu(TAAB)^+$ formation is decreased (according to the initial O_2 concentration) when compared to the k_{obs} value obtained in anaerobic conditions.

The amperometric measurement of the O_2 concentration in the course of the reaction in aerobic medium showed a consumption of dioxygen. The initial concentration of O_2 did not change in the absence of $Cu(TAAB)^{2+}$ or GSH in the reaction mixture. The reduced form of the copper complex, $Cu(TAAB)^+$, was irreversibly produced already after about 30—50 % of dioxygen present initially in the solution has reacted on.

The rate of the consumption of dioxygen was a pseudofirst-order with respect to O_2 concentration. The observed rate constant, $k_{obs,d}$, was calculated as the slope of the ln $\{(i - i_\infty)\}$ dependence *vs.* time, where i is the diffusion current of the first step of O_2 reduction and i_∞ is the current measured in the solution saturated by argon. The linearity of this dependence was characterized by the correlation coefficient values from – 0.97 to – 0.99. The effect of the concentration of GSH (5×10^{-4} mol dm^{-3} to 9×10^{-3} mol dm^{-3}) on the rate constant may be expressed as

$$k_{obs,d} = kc(GSH) \qquad (4)$$

where $k = (2.6 \pm 0.5) \times 10^{-2}$ mol^{-1} dm^3 s^{-1} (for $c(Cu(TAAB)^{2+}) = 3.6 \times 10^{-5}$ mol dm^{-3}, pH = 4.65, θ = 25.0 °C and the initial O_2 concentration of 2.4×10^{-4} mol dm^{-3}).

DISCUSSION

The stoichiometry determined for the reaction of $Cu(TAAB)^{2+}$ with glutathione in the anaerobic medium confirmed the usual course of redox changes of the reactants under the action of weak redox agents. The entire reaction can be described as follows

$$2Cu(TAAB)^{2+} + 2GSH \rightarrow 2Cu(TAAB)^+ + \\ + GSSG + 2H^+ \qquad (A)$$

This reaction may proceed by two different ways — an inner-sphere or an outer-sphere mechanism. From the spectral behaviour of the $Cu(TAAB)^{2+}$ and GSH mixture we can conclude that no adduct of the $\{Cu(II)L(GSH)\}^{2+}$ type is formed. Such adducts were detected in the reactions of complexes with the chromophores CuS_4^{2+} [5] and $CuN_2S_2^{2+}$ [6] where the addition of GSH to the Cu(II) complex solution leads immediately to the shift of the characteristic CuL^{2+} absorption band to longer wavelengths and the increase in absorbance. Then, the intramolecular electron transfer S(glutathione) → Cu(II) in the $\{CuL(SG)\}^{2+}$ intermediate occurs and the absorbance slowly decreases [5, 6].

In the case of the reduction of $Cu(TAAB)^{2+}$ by GSH the slow increase in absorbance up to the value given by the total $Cu(TAAB)^+$ concentration was observed. The outer-sphere electron-transfer mechanism is, therefore, more probable. The activation parameters lie also in the region typical for the outer-sphere redox process [9]. For the reaction of $Cu(TAAB)^{2+}$ with ascorbate anion the second-order cross-rate constant, k_{12}, is characterized by the similar values $\Delta H^{\neq}$ = 25.7 kJ mol^{-1} and $\Delta S^{\neq}$ = – 141 J (K $mol)^{-1}$ [2]. Experiments with the high GSH concentration have provided a kinetic evidence of a preceding complexation equilibrium.

According to the H^+-dependence of the k_{obs} values, two pathways may be considered for the reaction of $Cu(TAAB)^{2+}$ with GSH. In acidic media the reaction rate does not depend on the H^+ concentration and the original particles of $Cu(TAAB)^{2+}$ and GSH evidently interact at the electron-transfer step (k_a). This acid-independent path was not observed at the reduction of $Cu(TAAB)^{2+}$ by other reducing agents (ascorbic acid [4] and hydroxylammonium chloride [3]) which are stronger acids than GSH (pK_a = 8.75 [10]). The dissociation of GSH is so low that it does not contribute even to the acid-dependent path (k_b) in the weak acidic media. We assume that the $Cu(TAAB)^{2+}$ deprotonized form ($K_2 = 1.4 \times 10^{-7}$ at 25.0 °C [3]) takes place in the reaction within the pH region 5.15 up to 6.15. Thus, the acid-dependent path can be explained by the deprotonation of the complex particle similarly as it was in the case of its reduction by ascorbic acid and hydroxylamine in weak acidic and neutral media (Table 2).

Table 2. Rate Constants for the Acid-Independent (k_a) and Acid-Dependent (k_b) Paths of the Reduction of $Cu(TAAB)^{2+}$ by Different Agents in Anaerobic Conditions and for the Consumption of Dioxygen in Aerobic Conditions at pH 4.65 ($k_{obs,d}$). I = 0.1 mol dm^{-3} (with KNO_3); θ = 25.0 °C

Agent	k_a / $mol^{-1}\ dm^3\ s^{-1}$	k_b / s^{-1}	$k_{obs,d}$ / s^{-1}	Ref.
Glutathione	0.4	5.4×10^{-6}	0.03	This paper
Ascorbic acid	0	5.3×10^{-4}	27	[4]
Hydroxylamine	0	1.0×10^{-7}	–	[3]

In the aerobic conditions the $Cu(TAAB)^{2+}$ complex initiates the oxidation of GSH by dioxygen. In weak acidic medium and at high concentration of GSH, the Cu(II) is completely reduced relatively rapidly (10 to 30 min), whereas the O_2 concentration decreases slowly (1 to 3 h). Similar concentration profiles of the reactants were observed at the redox reaction with hydroxylamine [3]. However, at low pH values a catalytic-like reaction occurs and its course indicates some peculiarities of the macrocyclic complex. As it was shown previously [1, 4], the $Cu(TAAB)^+$ complex cannot be reoxidized by air dioxygen directly and its expressive catalytic effect at the oxidation of ascorbic acid by dioxygen (the kinetic parameters obtained are compared in Table 2) is conditioned by the formation of the protonated superoxide radical, $^{\bullet}HO_2$ (pK_a = 4.69 [11]). The presence of such radical or the effect of traces of soluble $Cu(TAAB)^{2+}$ and/or $Cu(TAAB)^+$ complexes might lead to the catalytic oxidation of thiol group.

REFERENCES

1. Labuda, J., Plaskoň, V., and Pavlishchuk, V. V., *Inorg. Chim. Acta 146,* 13 (1988).
2. Labuda, J. and Šima, J., *Inorg. Chim. Acta 112,* 59 (1986).
3. Labuda, J. and Korgová, E., *Chem. Papers 40,* 301 (1986).
4. Labuda, J., Mocák, J., Hlavačková, E., and Yatsimirskii, K. B., *Chem. Papers 38,* 739 (1984).
5. Pavlishchuk, V. V., Labuda, J., and Šturdík, E., *Inorg. Chim. Acta 151,* 261 (1988).
6. Pavlishchuk, V. V., Strizhak, P. E., Yatsimirskii, K. B., and Labuda, J., *Inorg. Chim. Acta 167,* 47 (1990).
7. Melson, G. A. and Bush, D. H., *J. Am. Chem. Soc. 86,* 4834 (1964).
8. Yatsimirskii, K. B. and Labuda, Ya., *Zh. Neorg. Khim. 25,* 2464 (1980).
9. Basolo, F. and Pearson, R. G., *Mechanism of Inorganic Reactions.* Wiley, New York, 1967.
10. Martin, R. B. and Edsall, J. T., *Bull. Soc. Chim. Biol. 40,* 1763 (1958).
11. Sawyer, T. D. and Valentine, J. S., *Acc. Chem. Res. 14,* 393 (1981).

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