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CATALYTIC ACTIVITY OF COPPER BISTHIOSEMICARBAZONATES IN CERTAIN MODEL REACTIONS OF OXIDATION OF BIOLOGICAL SUBSTRATES

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UDC 541.128.34:541.49:546.56:542.943.7

For a number of bisthiosemicarbazones (bis-TSC), high antitumor and antiviral activities have been found [1]. In some cases, the cytotoxic effects of these compounds are increased when they are used in the form of complexes with Cu(II) [1, 2]. Even though these facts are well known, the nature of the biological activity of such compounds still remain unclear.

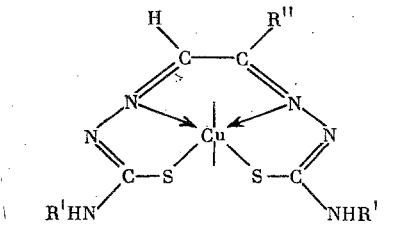
It is suggested in [3] that the antitumor activity of the Cu chelate of 3-ethoxy-2-oxobutyraldehyde bisthiosemicarbazone and its analogs is related to an effect on the synthesis of nucleic acids *in vivo*. According to data on the influence of copper complexes of this type on processes of cell respiration [4], the complex may shunt a certain section of the electrical transport circuit, disrupting the process of oxidative phosphorylation. Most probable, however, is a mechanism in which copper complexes of bis-TSCs inhibit the synthesis of reducing substrates of the respiratory chain — a succinate or HAD·H,* interacting with the thiol groups of the enzymes of the Krebs cycle and disrupting their normal metabolic function.

Evidence in favor of this hypothesis may be found in experimental data on the interaction of Cu complexes of bis-TSCs with thiols, in the course of which the thiol is oxidized to a disulfide and the metal in the complex is reduced to Cu(I) [5].

Under aerobic conditions, in our opinion, this process may proceed in a catalytic regime. Thus, we can assume that the biological activity of bis-TSC complexes is due to their catalytic properties in reactions of cell substrate oxidation.

In order to confirm this hypothesis, we have investigated the catalytic activity of a series of Cu complexes of bis-TSCs in reactions of cysteine and hydroquinone oxidation by molecular oxygen. The first of these compounds is a model of a thiol-containing metabolite; the second is a model of natural coenzyme Q, a central component in the respiratory chain of mitochondria.

As oxidation catalysts we used Cu complexes of bis-TSCs that we have synthesized in [6]:



*Dihydronicotinamideadenine dinucleotide.

A. N. Nesmeyanov Institute of Heteroorganic Compounds, Academy of Sciences of the USSR, Moscow. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 1, pp. 29-33, January, 1985. Original article submitted October 28, 1983.

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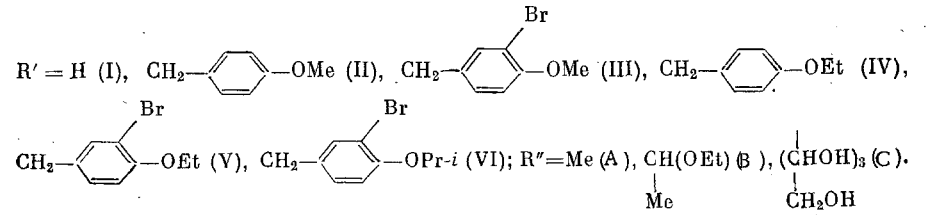

EXPERIMENTAL

The kinetics of cysteine oxidation were studied spectrophotometrically according to the decrease in absorption of the monoanion in the UV region (39,000-43,000 cm^{-1}) as the substrate concentration was varied ($1 \cdot 10^{-4}$ to $4 \cdot 10^{-4}$ M) and as the catalyst concentration was varied ($5 \cdot 10^{-7}$ to $5 \cdot 10^{-6}$ M). The reaction was performed in 0.1 M borate buffer at pH 9.2 (25°). The pH dependence of the oxidation rate was investigated in a series of 0.1 M borate–phosphate buffer solutions in the pH interval 7.5-10. The dependence of the reaction rate on the O_2 concentration in the gas phase (20-100% by volume) was studied volumetrically according to the O_2 absorption. The same technique was used to investigate the oxidation of hydroquinone in a mixture of N,N'-dimethylacetamide and ethanol (1:5). The hydroquinone concentration was 10^{-1} M, the catalyst concentration 10^{-3} M, p_{O_2} = 30 cm H_2O. The presence of H_2O_2 in the reaction medium was determined by procedures given in [7]. The quantity of water was determined by coulometric titration, using the Fischer method.

DISCUSSION OF RESULTS

The reaction of copper bisthiosemicarbazonate Cu(II)L with a thiol under anaerobic conditions proceeds in accordance with the scheme [5]

$$Cu(II)L + 2RSH \rightarrow Cu(I)RS + LH_2 + {}^1/_2RSSR$$

where L is a doubly deprotonated ligand.

In the presence of molecular O_2, the catalytic cycle may be closed through oxidation of the thiol complex of Cu(I) and regeneration of the original compound Cu(II)L. The overall scheme of such a catalytic process is expressed by the equation

$$2RSH + {}^1/_2O_2 \xrightarrow{Cu(II)L} RSSR + H_2O$$

In Table 1 we present the experimental results obtained in our investigation of the catalytic activity of copper complexes of bis-TSCs in the oxidation of cysteine (RSH). The data on the initial reaction rates show that most of these compounds increase the rate of substrate oxidation; however, even the most active of these compounds are lower in catalytic activity than Cu(II) hydrated ions ($v_0 = 165 \cdot 10^{-7}$ mole/liter·min). The ligands themselves do not manifest any catalytic activity. Co(II) complexes that were tested for comparison give practically no increase in the rate of cysteine oxidation. Modifications of the bis-TSC structure have significant effects on the reaction rate. The greatest effect is observed when the radical R'' is varied. Thus, for all of the substituted bis-TSCs, the rate increases when the change is made from methylglyoxal derivatives (series A compounds) to butyraldehyde and glycozone derivatives (series B and C, respectively). For the unsubstituted bis-TSCs (R = H), such a relationship is not observed; however, in comparison with the substituted analogs, an increase in activity is evident in series A and B. For the substituted bis-TSCs, we were unable to find any unambiguous relationship between the catalytic activity and the nature of the substituent R. These results indicate that the reaction rate is a complex function of several parameters, such as the polarity of the molecule, steric effects of the substituents, thermodynamic stability of the chelate, etc.

In the example of the Cu chelate of 3-ethoxy-2-oxobutyraldehyde bisthiosemicarbazone, we investigated the catalytic oxidation of cysteine in more detail. The initial oxidation rates were determined in relation to the concentrations of substrate and catalyst, the O_2 partial pressure, and the pH of the medium. According to the results obtained in these studies, the overall kinetic equation of the reaction can be written as follows:

TABLE 1. Catalytic Activity of Cu Complexes of Bis-TSC in Oxidation of Cysteine and Hydroquinone by Molecular O_2*

R″		(I)	(II)	(III)	(IV)	(V)	(VI)
A	a	20,0	0	0,7		0	14,2
	b	2,4	1,7	4,4		2,5	0
B	a	43,5	13,8	0,7	20,0	10,0	
	b	2,4	0	0	3,6	0	
C	a	20,5	61,0	50,0	55,6	41,0	
	b	0	2,0	2,3	7,2	2,5	

*Initial oxidation rates: a) cysteine, $v_0 \cdot 10^7$; b) hydroquinone, $v_0 \cdot 10^5$ moles/liter·min.

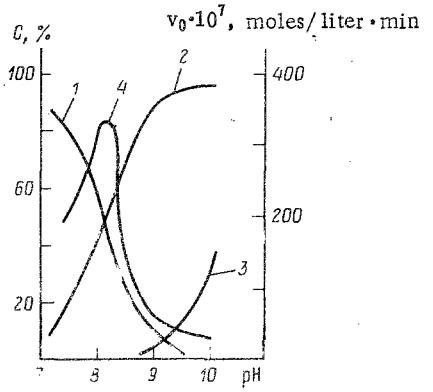

Fig. 1. pH dependence of cysteine oxidation, and curves showing distribution of concentrations of ionic forms of substrate: 1) RSH; 2) RS^-; 3) RS^{2-}; 4) initial oxidation rate.

$$-\frac{d[\mathrm{RSH}]}{dt} = k[\mathrm{Cu(II)L}]_0^1 \cdot [\mathrm{RSH}]_0^{0.5} \cdot [\mathrm{O_2}]^0$$

where d[RSH]/dt is the initial rate of cysteine oxidation; $[Cu(II)L]_0$ and $[RSH]_0$ are the concentrations of the catalyst and substrate at the initial moment of time; $[O_2]$ is the concentration of oxygen in the solution, which is proportional to its partial pressure in the gas phase.

The reaction rate, when the pH is varied over the interval 7.5-10, depends on the concentrations of both the neutral form of the substrate and the monoanion (Fig. 1), the maximum in catalytic activity occurring in the pH region corresponding to acid dissociation of the SH group of cysteine (pK_a = 8.14). This result is in agreement with data reported in [5] on the kinetics of anaerobic oxidation of thiols.

The form of the kinetic equation and the character of the pH dependence indicate a complex mechanism of cysteine oxidation. From an examination of our data and those reported in the literature, we can set forth a hypothesis as to the basic stages of this process.

Initially, the cysteine anion forms with the catalyst a pentacoordinated adduct, which, as a result of an intramolecular redox process and subsequent dissociation, gives a complex of Cu(I) and the product from conversion of the substrate

$$\mathrm{Cu(II)L} + \mathrm{RS^-} \rightleftarrows \mathrm{Cu(II)LRS^-} \xrightarrow{\text{slow}} \mathrm{Cu(I)L^-} + {}^1/_2\mathrm{RSSR}$$

The thermodynamic stability of the complex $Cu(I)L^-$ is comparatively low ($\log \beta$ = 13.1 [5]); and in solution, after preliminary protonation, it is converted to the more stable compound of Cu(I) with cysteine ($\log \beta$ = 17.3 [8])

$$Cu(I)L^- + H^+ \rightleftarrows Cu(I)LH$$
$$Cu(I)LH + RSH \rightleftarrows Cu\ (I)RS + LH_2$$

Along with this, free bis-TSC(LH_2) is formed.

Then the complex Cu(I)RS is subjected to oxidation by molecular oxygen, in accordance with the overall equation

$$Cu(I)RS + {}^1/_4O_2 + RSH \rightarrow Cu(II)(RS)_2 + {}^1/_2H_2O$$

The zero order with respect to oxygen indicates that this reaction proceeds rapidly. The Cu(II) cysteinate that is formed reacts with the free bis-TSC and is converted to the original catalytically active complex Cu(II)L, which is distinguished by a very high thermodynamic stability (log β = 26.57) [9]

$$Cu(II)(RS)_2 + LH_2 \rightleftarrows Cu(II)L + 2RSH$$

Also included in Table 2 are data on the catalytic activity of the Cu complexes of bis-TSCs in the oxidation of hydroquinone by molecular oxygen. In the oxidation of this substrate, in contrast to the oxidation of cysteine, changes in the structure of the ligand do not have any significant effect on the reaction rate.

Upon the addition of hydroquinone to a solution of a bis-TSC Cu complex in N,N'-dimethylacetamide in an Ar atmosphere, no changes in the electronic spectrum of the complex due to reduction of the metal can be observed. Therefore we can eliminate the mechanism that assumes an alternating oxidation and reduction of Cu in complexes with the bis-TSC in the course of the reaction. The catalytic oxidation of hydroquinone in the presence of these complexes is probably accomplished through the formation of an intermediate substrate–catalyst–oxygen ternary complex [10].

When chelate complexes of Co are used as catalysts for hydroquinone oxidation, H_2O_2 is usually formed [11]. In our case, we could not detect any H_2O_2, even by the use of the extremely sensitive benzidine test [7]. At the same time, we did observe an increase in the percentage content of water in the reaction mixture during the oxidation of hydroquinone by the catalytically active complexes. We have shown experimentally that complexes of Cu with the bis-TSCs do not have any catalytic activity. These data suggest that in the oxidation of hydroquinone by molecular oxygen in the presence of Cu bisthiosemicarbazonates, water is formed.

Thus, the results from these experiments on the catalytic oxidation of models of biological substrates confirm our hypothesis of the possible catalytic nature of the biological activity of Cu complexes of bis-TSCs.

CONCLUSIONS

1. A study has been made of the catalytic activity of a series of copper complexes with bisthiosemicarbazones in reactions of cysteine and hydroquinone oxidation.

2. In the example of the copper complex of 3-ethoxy-2-oxobutyraldehyde bisthiosemicarbazone, a mechanism has been proposed for the catalytic effect of these complexes in the reaction of cysteine oxidation.

3. The previously advanced hypothesis of the possible catalytic nature of the biological activity of copper(II) bisthiosemicarbazonates has been confirmed.

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PATHS OF CONVERSION OF INTERMEDIATE METAL—HYDROPEROXIDE COMPLEX IN THE PROCESS OF CATALYZED DECOMPOSITION OF HYDROPEROXIDE WITH THE PARTICIPATION OF A COPPER CHELATE

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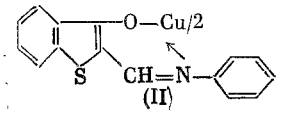
UDC 541.124:541.128:542.92

Variable-valence metal compounds catalyze the oxidation of hydrocarbons, in particular, decomposing hydroperoxides to free radicals. This stage proceeds through the formation and subsequent decomposition of a complex $[M^nL_n \cdot ROOH]$ [1, 2].

The present work has been aimed at obtaining quantitative characteristics of the decomposition of cyclohexenyl hydroperoxide ROOH (I) with the participation of a copper chelate as a catalyst.

EXPERIMENTAL

A study was made of the decomposition of ROOH in the presence of copper(II) bis[(2-phenyliminomethylene)benzo(b)thiophenate-3] in a thermostated cuvette of a Beckmann spectrophotometer, with a considerable excess of the hydroperoxide relative to the catalyst ($[CuL_2] = 2 \cdot 10^{-5}$ M, $[ROOH] = 10^{-1}$ M), in chlorobenzene.



(II) $[CuL_2]$

Under these conditions, the CuL_2 is almost completely bound into a complex with the hydroperoxide $[CuL_2 \cdot ROOH]$ (III) [2]. The decomposition of the hydroperoxide was investigated in the presence of dissolved O_2. The original (II) and the complex (III) have characteristic absorption bands in the visible region due to metal—ligand transitions, respectively at 434 nm ($\log \varepsilon = 4.32$) and 467 nm ($\log \varepsilon = 4.61$). The consumption of (II) and (III) was determined by the decrease in intensities of the absorption bands. Separate experiments were performed with a known rate of formation of free radicals from azobisisobutyronitrile (AIBN). As an acceptor of the free radicals formed by the decomposition of the hydroperoxide or the AIBN, we used the dimer bis(4'-dimethylaminophenyl)-1,2-diphathaloylethane.

DISCUSSION OF RESULTS

The decomposition of ROOH in solutions of CuL_2 is accompanied by a decrease in the absorbance of the solution (Fig. 1a, curves 1-3). At lower temperatures, the kinetic curves

Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheksaya, No. 1, pp. 34-40, January, 1985. Original article submitted August 18, 1983.

0568-5230/85/3401-0027$09.50