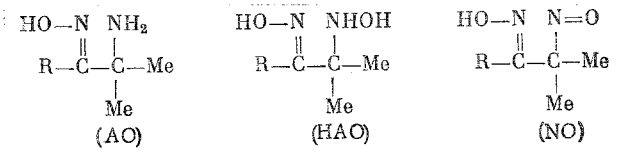
COMPLEXES OF COBALT(III) WITH DERIVATIVES OF OXIMES AS CATALYSTS OF ELECTRON TRANSFER FROM THE COMPONENTS OF THE RESPIRATORY CHAIN

G. N. Novodarova, L. L. Kiseleva, M. Yu. Tuvin, A. I. Stetsenko, L. P. Sharova, and M. E. Vol'pin

UDC 541.128:541.49:546.73:547.288.4:542.943.7:547.9

Complexes of transition metals exhibit elevated physiologic activity [1], but the mechanisms of action of the complexes with the components of the living cell are still unknown in many cases. The capacity of transition metal complexes for reacting with intracellular electron transport systems in respiration [2] and photosynthesis [3] was recently demonstrated. Active systems were found among complexes of cobalt with chelating ligands in particular; these complexes can activate oxygen and catalyze the oxidation of the components of the respiratory chain: nicoatinamide-adenine dinucleotide (NADH) and ubiquinone, in model chemical reactions [4]. In continuing the search for new compounds capable of acting on the energetic processes of the cell, chelate complexes of cobalt(III) with derivatives of oxims were investigated: with α-aminooximes (AO), α-hydroxylaminooximes (HAO), and α-nitrosooximes (NO)



Some complexes of cobalt(III) with AO are described in [5, 6], and synthesis of complexes of cobalt with HAO and the x-ray diffraction study of their structure were also recently described [7, 8].

The catalytic properties of complexes of Co(III) with oxime derivatives of these types in reactions of oxidation of models of natural coenzyme Q_{10} (ubiquinone) such as hydroquinones Q_1 and Q_9 were investigated in the present study. In addition, the effect of these complexes on the oxidative activity of the mitochondria was also studied.

EXPERIMENTAL

The complexes of Co(III) with AO and HAO were synthesized according to [5-7]. Hydroquinone was purified by two recrystallizations from acetone, Q_1 was obtained from Cerak (West Berlin), and Q_9 was supplied by E. A. Obol'nikova ("Vitamin" Scientific and Industrial Association). Absolute ethanol, distilled N,N'-dimethylacetamide (DMAA), and reference isooctane were used as the solvents. The initial rate of absorption of oxygen by the reaction mixture, determined volumetrically, served as the measure of the catalytic activity of the complexes in reactions of oxidation of hydroquinone. The reactions were conducted in a DMAA–absolute ethanol mixture (1:5) at 30°C with intense mixing in an atmosphere of O_2. The concentrations of hydroquinone and the cobalt complex were, respectively 10^{-1} and 10^{-3} M. The catalytic activity of the complexes in the oxidation of Q_1 and Q_9 were determined spectrophotometrically by the increase in the optical density corresponding to formation of quinone (λ 275 nm). The reactions were conducted in an isooctane–ethanol mixture (1:1) at ~20°C in air. The concentrations of the substrates and the complex were, respectively, 10^{-4} and 10^{-6} M. Absorption of oxygen by the complexes in the absence of substrates was determined in DMAA with a 10^{-2} M concentration of the complexes at 30°C and with intense stirring in an atmosphere of O_2.

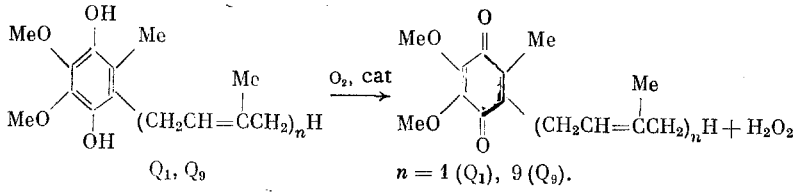
A. N. Nesmeyanov Institute of Heteroorganic Compounds, Academy of Sciences of the USSR, Moscow. Chemical-Pharmaceutical Institute, Leningrad. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 10, pp. 2185-2191, October, 1984. Original article submitted April 25, 1983.

0568-5230/84/3310-1993$08.50

Rat liver mitochondria were obtained by using the standard method of removal [9]. The concentration of protein in the mitochondria was determined with biuret reagent [10]. The rate of absorption of O_2 by the mitochondria was measured polarographically with a sealed platinum electrode [11]. The measurement medium (MM) contained saccharose (0.15 M), KCl (0.075 M), KH_2PO_4 (5 mM), $MgCl_2$ (2 mM), pH 7.4. The oxidation substrate, succinate (10 mM) and rotenone (2 μM) were placed in the MM before the measurement began, and the reaction was initiated by introduction of the mitochondria. ADP (200 μM) was added to the cell to determine the rate of phosphorylation of the mitochondria, 2,4-dinitrophenol (DNP) (100 μM) was used as the interrupter of respiration, and the concentration of mitochondrial protein in the polarigraphic cell was usually 2 mg.

DISCUSSION OF RESULTS

It was previously shown that transition metal complexes which catalyze the oxidation of quinoid substrates in model chemical reactions can in many cases be involved in electron transport processes in intact mitochondria [2]. The most active systems were found among Co(II) complexes, particularly in a series of chelate complexes of Co(II) with fatty and octadehydrocorrin ligands. However, the related complexes of Co(II) with dimethylglyoxime were inactive. In continuing the search for catalytic systems which model the oxidase functions of the mitochondria, the group of chelate complexes of Co(III) with oxime derivatives: AO, HAO, and NO, was investigated. The catalytic systems were selected in chemical reactions which model the ubiquinone:oxidase activity of the living cell on the example of oxidation of synthetic substrates: hydroquinone and its derivatives Q_1 and Q_9



Q_1, Q_9 $\quad n = 1\ (Q_1),\ 9\ (Q_9).$

Four basic types of complexes were studied: (I): complexes with AO (ligand L) of the composition $[Co(L_2-H)X_2]$; (II): complexes with HAO (ligand L') of the composition $[Co(L'-H)_2X]$; (III): complexes with HAO (L') of the composition $[Co(L'-H)\cdot(L'-2H)]$; (IV): complexes with NO (ligand L") of the composition $[Co(L_2"-H)X_2]$. According to the x-ray diffraction data [8], the type (I) complex $[Co(L_2-H)(NO_2)_2]$ is a hexadentate coordination compound with cis oxime groups bound by an intramolecular hydrogen bond and axial NO_2 ions. It was found that HAO is oxidized during complexing due to dehydrogenation at the N—H bond; all four nitrogen atoms in the complex are in a state with sp^2 hybridization. The absence of νNH in the IR spectrum of the complex confirms the above. The type (II) complex, $[Co(L'-H)_2Cl]$, has a pseudo-octahedral structure with a Cl...Co—Cl bridge bond and trans oxime groups [8].

Type (III) complexes also contain dehydrogenated HAO, which was shown by the x-ray diffraction study of the $[Co(L'-H)\cdot(L'-2H)Py]$ complex.* This complex is a pentadentate coordination compound in the form of a tetragonal pyramid with oxime groups in the cis position. The structure of the $[Co(L'-H)(L'-2H)]$ complex was not studied, but it is possible to assume that it is a tetradentate coordination compound. Determination of the molecular weight by ebullioscopy in acetone showed that the complex is a monomer (molecular weight: found 442; calculated 444).

Type (IV) complexes contain a coordinated neutral molecule of nitrosooxime (L") and its anion $(L"-H)^-$. All of the complexes studied are diamagnetic, which confirms the presence of Co(III) in them. The catalytic activity of these complexes is reported in Table 1. Only the cobalt complexes were active, while similar Cu(II) and Ni(II) compounds were inactive.

Table 1 shows that the activity of these groups of complexes is very different. Complexes with type AO ligands did not exhibit important catalytic activity in the oxidation of all three substrates studied. These are coordination-saturated complexes with a ligand containing an inert amino group. In cases where the complexes with the AO ligand exhibited weak

*The study was conducted by Yu. A. Simonov.

TABLE 1. Catalytic Activity of Complexes in Model Reactions of Oxidation of Quinoid Substrates (v_{st} = mole/liter•min)

Type of complex	No. by order	Complex	R	Oxidation substrate: hydroquinone $v \cdot 10^5$	Q_1, $v \cdot 10^7$	Q_8, $v \cdot 10^7$
(I)	1	$[Co(L_2-H)(CH_3COO)_2]$	Et	0	0	1,2
	2	$[Co(L_2-H)(Br_2)]$	Me	1,4	0	1,2
	3	$[Co(L_2-H)(NO_2)_2]$	Me	0	0	0
	4	$[Co(L_2-H)(SCN)_2]$	Me	0	0	0
	5	$[Co(L_2-H)I_2]$	Et	5,2	0	0
	6	$[Co(L_2-H)Cl_2]$	Et	3,0	2,0	8,6
	7	$[Co(L_2-H)Br_2]$	Et	9,8	1,1	3,9
(II)	1	$[Co(L'-H)_2Cl]$	Ph	6,6	0,8	0,4
	2	$[Co(L'-H)_2I]$	Ph	2,8	0,9	3,9
	3	$[Co(L'-H)_2Br]$	Ph	3,4	3,3	12,5
	4	$[Co(L'-H)_2NO_3]$	Ph	7,0	1,7	5,5
	5	$[Co(L'-H)_2OH]$	Ph	0	0	0
	6	$[Co(L'-H)_2NO_3]$	Me	28,0	1,7	9,0
	7	$[Co(L'-H)_2I]$	Me	6,5	0,9	3,9
	8	$[Co(L'-H)_2Cl]$	Me	23,0	1,7	3,1
	9	$[Co(L'-H)_2Cl]$	H	3,3	0	1,6
	10	$[Co(L'-H)_2Br]$	Me	5,5	1,7	9,0
(III)	1	[Co(L'–H)(L'–2H)Im]*	Ph	22,0	4,5	12,1
	2	[Co(L'–H)(L'–2H)]	Ph	30,0	3,3	9,0
	3	[Co(L'–H)(L'–2H)Py]*	Ph	27,0	6,4	15,6
	4	[Co(L'–H)(L'–2H)]	Me	46,0	4,7	13,6
	5	[Co(L'–H)(L'–2H)Py]*	Me	79,0	5,6	14,4
	6	[Co(L'–H)(L'–2H)4-APy)]*	Ph	84,0	5,6	2,3
(IV)	1	$[Co(L_2''-H)Cl_2]$	Ph	0	0	0
	2	$[Co(L_2''-H)Br_2]$	Ph	0	0	0
	3	$[Co(L_2''-H)Br_2]$	Me	0	2,2	5,5

*Im: imidazole; Py: pyridine; 4-APy: 4-aminopyridine.

TABLE 2. Effect of the Complexes on the Functional Parameters of Mitochondria ($5 \cdot 10^{-5}$ mole/liter concentration of complexes)

Type of complex	No. by order	Complex	R	Activation of respiration, % of control	Oxidative phosphorylation (RC)*	Integrity of membrane, % activation by DNP†
	1	No complex (control)		100	5,4	100
(I)	1	$[Co(L_2-H)(CH_3COO)_2]$	Et	147	5,3	100
	2	$[Co(L_2-H)Br_2]$	Me	100	3,7	90
	3	$[Co(L_2-H)(NO_2)_2]$	Me	120	5,4	100
	4	$[Co(L_2-H)(SCN)_2]$	Me	100	3,3	90
	5	$[Co(L_2-H)I_2]$	Et	113	4,0	100
	6	$[Co(L_2-H)Cl_2]$	Et	106	4,0	100
	7	$[Co(L_2-H)Br_2]$	Et	106	4,5	100
(II)	1	$[Co(L'-H)_2Cl]$	Ph	287	0	0
	2	$[Co(L'-H)_2I]$	Ph	233	0	0
	3	$[Co(L'-H)_2Br]$	Ph	433	0	0
	4	$[Co(L'-H)_2NO_3]$	Ph	100	3,6	87
	5	$[Co(L'-H)_2OH]$	Ph	180	2,0	36
	6	$[Co(L'-H)_2NO_3]$	Me	247	0	0
	7	$[Co(L'-H)_2I]$	Me	387	0	0
	8	$[Co(L'-H)_2Cl]$	Me	153	0	40
	9	$[Co(L'-H)_2Cl]$	H	100	5,4	100
	10	$[Co(L'-H)_2Br]$	Me	193	0	0
(III)	1	[Co(L'–H)(L'–2H)Im]‡	Ph	180	0	0
	2	[Co(L'–H)(L'–2H)]	Ph	573	0	0
	3	[Co(L'–H)(L'–2H)Py]‡	Ph	126	0	5
	4	[Co(L'–H)(L'–2H)] ‡	Me	147	4	76
	5	[Co(L'–H)(L'–2H)Py] ‡	Me	233	0	0
	6	[Co(L'–H)(L'–2H)4-APy]‡	Ph	500	0	0
(IV)	1	$[Co(L_2''-H)Cl_2]$	Ph	500	0	0
	2	$[Co(L_2''-H)Br_2]$	Ph	367	0	0
	3	$[Co(L_2''-H)Br_2]$	Me	233	0	23

*See text.
†DNP was added after the complex.
‡See Table 1 for arbitrary symbols.

catalytic activity, it rapidly decreased in time until the reaction totally stopped. Complexes of Co(III) with HAO exhibited significantly higher activity. The activity of these complexes was a function of the nature of the acid ligand and increased in the order $I^- < Br^- < Cl^- < NO_3^-$. It was shown that a bathochromic shift of the charge transfer band in the direction from NO_3^- to I^- is observed in these complexes, which could indicate weakening of the bond of the acid ligand with the central atom.

Type (III) complexes, formally tetradentate coordination compounds, and their adducts, organic bases, were most active. It was observed that the effect of the substituent R on the catalytic activity was insignificant in all groups of complexes, but complexes with R = Me were usually more active than complexes with R = Ph. Complexes of Co(III) with nitrosooximes [type (IV)] exhibited no catalytic activity. The change in the lipophilic nature of the substrate in going from hydroquinone to Q_9 did not significantly alter the catalytic activity of the complexes with respect to these substrates.

It was hypothesized that the catalytic activity of the complexes in the reactions studied was due to their capacity to coordinate oxygen. Absorption of oxygen by solutions of type (I)-(IV) complexes in the absence of oxidizable substrates was investigated to test this hypothesis. It was found that type (I) and (IV) complexes did not absorb oxygen, while type (II) and (III) complexes absorbed oxygen very intensely. These data are in agreement with the well-known facts on bonding of oxygen in coordination-unsaturated complexes of cobalt with oxime ligand, primarily with the dimethylglyoximate ligand [12]. In the presence of complexes of this type, the catalytic reaction can take place according to an activation mechanism which includes the formation of a substrate—catalyst—oxygen ternary complex [4].

The next stage of the study consisted of investigating the capacity of this group of complexes for reacting with the mitochondrial electron transport system. Since ubiquinone is the key component of this system, it could be hypothesized that complexes exhibiting ubiquinone oxidase activity in model chemical reactions would stimulate the oxidative activity of the mitochondria, i.e., respiration.

The data on the effect of type (I)-(IV) complexes on respiration and other functional parameters of intact mitochondria are reported in Table 2. It was found that some of the complexes of types (II), (III), and (IV) caused marked activation of respiration, while type (I) complexes were totally ineffective: the rate of respiration did not vary within a wide range of concentrations of these complexes (5, 50, or 100 μM). In the case of complexes of types (II), (III), and (IV), the greatest activation of respiration was observed with a 50 μM concentration of the complex, and a further increase in the concentration usually resulted in inhibition of respiration.

There could be two causes of the effect of activation of mitochondrial respiration under the effect of type (II) and (III) complexes: The catalytic activity of these complexes in oxidation of mitochondrial ubiquinone, i.e., the capacity to bypass the respiratory chain, or the effect of interruption of respiration when oxidation of the substrates stopped being related to phosphorylation of ADP and storage of energy in the form of ATP. In addition, the complexes could have an aspecific effect on the mitochondrial membrane, causing structural alterations and inhibiting membrane-bound enzymes.

The effect of the complexes on phosphorylation was judged by the respiratory control (RC) value determined by the ratio of the respiration rates on addition and subsequent depletion of ADP. The RE values determined in the presence of the complexes (see Table 2) indicate that only the type (I) complexes with low catalytic activity altered respiratory control very little (RC = 5.4); in the presence of the other types of complexes, the RC was usually equal to zero. This means that most of the complexes of types (II), (III), and (IV) significantly perturb processes of oxidative phosphorylation in the mitochondrial membrane. A comparison of the isostructural complexes showed that an increase in the hydrophobicity of R (going from R = H to R = Me and Ph) usually increased the interrupting effect.

The reaction of the mitochondria to addition of DNP indicates perturbation of the integrity of the mitochondrial membrane under the effect of the complexes. Stimulation of respiration under the effect of DNP was only observed in the presence of low-activity type (I) complexes, which indicates the preservation of the integrity of the mitochondrial membrane. DNP in the presence of chemically active type (II) and (III) complexes usually did not induce activation of mitochondrial respiration (since the membrane was already damaged by the complexes). Only complexes 4, 5, 8, and 9 (type II) and complex 4 [type (III)] were exceptions; in

their presence, DNP stimulation of respiration was observed. These complexes apparently damage the mitochondrial membrane only insignificantly and weakly inhibit phosphorylation. It was found that the catalytically inactive type (IV) complexes caused strong activation of respiration in the mitochondria and simultaneously inhibited oxidative phosphorylation, i.e., had an interrupting effect.

Inhibitor analysis was used to determine whether the complexes studied had a bypassing effect in the respiratory chain [2]. The capacity of the complexes to take up electrons from the various components of the mitochondrial electron transfer chain, primarily ubiquinone, was tested with inhibitors of respiration (antimycin and rotenone) which selectively block different segments of the respiratory chain. It was found that none of the complexes studied eliminated the antimycin blockade, i.e., exhibited no bypass effect.

The results of the study of the properties of complexes in the mitochondrial respiratory chain thus showed that many complexes of cobalt with HAO and NO [types (II) and (IV)] activate mitochondrial respiratory by a mechanism of interruption of respiration and oxidative phosphorylation. However, these complexes have no bypassing effect and do not transfer electrons from coenzyme Q to oxygen. As a consequence, the catalytic activity determined in model chemical reactions is a necessary but not sufficient condition for the occurrence of catalytic transformations by the complexes in a more complex biochemical system. The actual mechanism of action of each group of complexes is of definite interest and will be the subject of subsequent studies.

CONCLUSIONS

1. The catalytic activity of chelate complexes of Co(III) with derivatives of oximes in oxidation of models of natural coenzyme Q_{10} (ubiquinone) such as hydroquinone, Q_1 and Q_9 was studied; the tetradentate coordination complexes of cobalt(III) with bis(hydroxylamineoxime) and their adducts with organic bases exhibited the highest activity.

2. The capacity of these complexes for reacting with the mitochondrial electron transport system was studied; many of the complexes are effective interruptors of respiration and oxidative phosphorylation in mitochondria.

LITERATURE CITED

1. A. I. Stetsenko, M. A. Presnov, and A. L. Konovalova, Usp. Khim., 50, 665 (1981).
2. Yu. N. Leikin, M. E. Vol'pin, G. N. Novodarova, and E. M. Kolosova, Biokhimiya., 44, 97 (1979).
3. E. P. Lukashev, A. A. Kononenko, N. I. Zakharova, A. B. Rubin, M. E. Vol'pin, G. N. Novodarova, and E. M. Kolosova, Biokhimiya., 45, 237 (1980).
4. M. E. Vol'pin, G. N. Novodarova, E. M. Kolosova, N. V. Guzhova, and A. A. Kononenko, Inorg. Chim. Acta, 50, 21 (1981).
5. R. K. Murman, J. Am. Chem. Soc., 79, 521 (1957).
6. L. P. Sharova, S. A. D'yachenko, A. I. Stetsenko, and V. G. Pogareva, Koordinats. Khim., 8, 984 (1982).
7. S. A. D'yachenko, L. P. Sharova, A. I. Stetsenko, L. B. Volodarskii, V. G. Pogarea, and V. V. Dmitrieva, Koordinats. Khim., 7, 1493 (1981).
8. S. A. D'yachenko, Yu. A. Simonov, A. I. Stetsenko, A. A. Dvorkin, T. N. Malinovskii, and L. B. Volodarskii, Dokl. Akad. Nauk SSSR, 244, 636 (1979).
9. D. Johnson and H. Lardy, in: Method in Enzymology, Vol. 10, Academic Press, New York (1967), p. 94.
10. A. G. Gornall, C. S. Bardawill, and M. M. David, J. Biol. Chem., 177, 751 (1949).
11. B. Chance and G. R. Williams, J. Biol. Chem., 217, 383 (1949).
12. M. J. Carter, L. M. Engelhardt, and F. Basolo, J. Chem. Soc. Chem. Communs., 810 (1973).