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COBALT-CORRIN COMPLEXES AS CATALYSTS FOR THE OXIDATIVE TRANSFORMATION OF BIOCHEMICAL SUBSTRATES *

G.N. NOVODAROVA and M.E. VOL'PIN

Institute of Organoelement Compounds, Academy of Science of the USSR, Moscow, P.O. Box 117813, USSR

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Various aspects of the catalytic action of Co-corrin complexes upon biochemical substrates are considered. In particular, the role of hydrophobicity of metal complex ligands in the catalytic oxidation of quinols is clarified, and the possibility is discussed of using Co-corrin complexes as catalytic generators of active oxygen radicals targetted for various cell targets especially DNA.

1. Introduction

Our point of departure is the supposition that synthetic metal complex catalysts could be active not only in chemical but also in biochemical systems and living cells. Their use as living-cell synthetic catalysts could open up the possibility for the creation of new types of physiologically active compounds with desirable catalytic properties.

We have hitherto demonstrated the ability of some Co-complex with organic ligands to provide a catalytic process of "by-passing" electron transfer from quinol carriers in biological redox chains of respiration and photosynthesis [1].

Cobalt complexes with corrin-ligand-related B_{12} series have attracted considerable interest. They were the active catalysts in the autooxidation of the important biological substrates NADH, quinols.

Insofar as the quinolic carriers are the electron donors for the Co-corrin catalysts in biochemical systems are concerned, it is important to study the essential factors for quinolic substrate oxidation catalysis. Another key aspect of Co-corrin catalysis is the study of the active species produced in the oxidation of biological substrates. We have already demonstrated the formation of hydrogen peroxide in Co-corrin catalysed autooxidation of NADH and its decomposition under Co(II)-corrin complex action [2].

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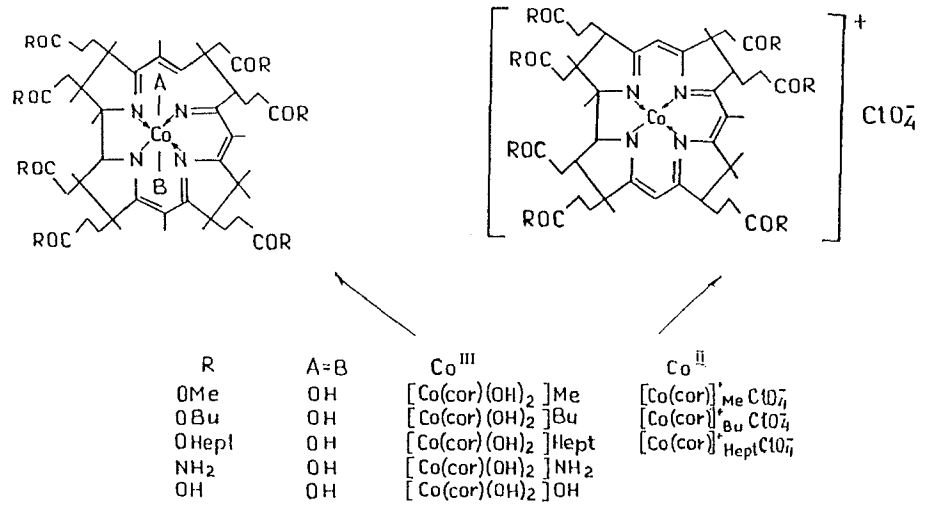


R	A=B	Co^{III}	Co^{II}
OMe	OH	$[Co(cor)(OH)_2]Me$	$[Co(cor)]^{+}_{Me}ClO_4^-$
OBu	OH	$[Co(cor)(OH)_2]Bu$	$[Co(cor)]^{+}_{Bu}ClO_4^-$
OHept	OH	$[Co(cor)(OH)_2]Hept$	$[Co(cor)]^{+}_{Hept}ClO_4^-$
NH_2	OH	$[Co(cor)(OH)_2]NH_2$	
OH	OH	$[Co(cor)(OH)_2]OH$	

Fig. 1. Structural formulae of Co-corrin complexes.

This study constitutes an attempt to identify some radical products of Co-corrin-catalysed autooxidation and to use the active oxygen species for oxidative cleavage of DNA molecules.

2. Experimental

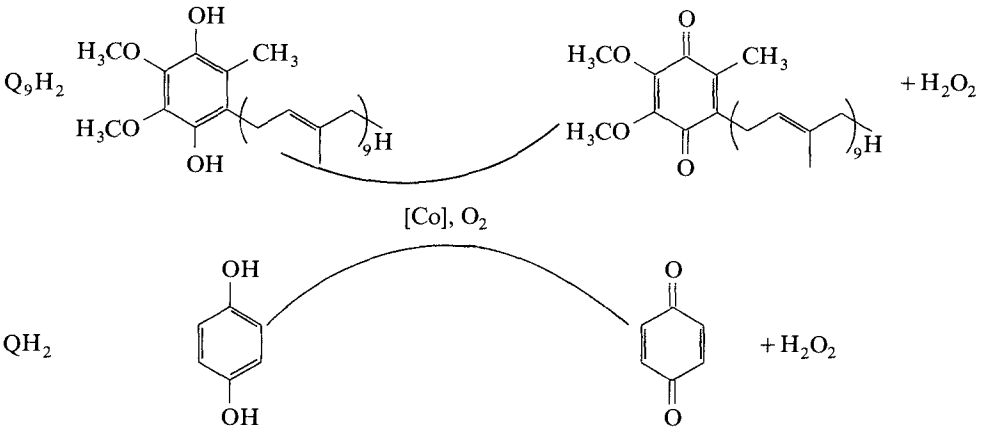
Cobalt-corrin complexes – cobyrinic acid derivatives – were obtained by modification of the natural B_{12} as previously described [3]. These series include Co(III) and Co(II)-complexes and consist of the cobyrinic acid alkyl ethers-hepta-alkyl-cobyrinates (Alk = Me, n-Bu, n-Hept = R), cobyrinic acid amide (R $= NH_2$) and cobyrinic acid itself (R = OH) (fig. 1). All the samples were chromatographically purified.

The catalytic activity of Co-corrin complexes in the autooxidation of quinolic substrates – ubiquinol Q_9H_2 and hydroquinol QH_2 – was measured spectrophotometrically at λ_{275nm} and λ_{290nm} respectively. The catalytic autooxidation of ascorbic acid was performed as in [4]. The hydrogen peroxide was detected by the well-known titanium test [5]; and the formation of OH^0-radicals was demonstrated by EPR-method by trapping with 5,5′-dimethyl-I-pyrrolin-N-oxid (DMPO) [4]. The experiments with DNA were carried out according to the procedure recently reported [6].

3. Results and discussion

We prepared Co-corrin complexes with various hydrophobicity (hydrophobic derivatives of B_{12} series). The most hydrophobic complex of this range was the

heptyl ether of cobyrinic acid, cobyrinic acid and cobinamid were less hydrophobic. We studied the catalytic activity of Co-complexes in the autooxidation of quinols varying in hydrophobicity: quinol Q_9H_2 – nearest analog of natural ubiquinon – and unsubstituted hydroquinol QH_2:



All of Co-corrin complexes demonstrated rather high catalytic activity much greater than that of the starting $B_{12}OH$.

The most hydrophobic complex – heptyl ether cobyrinic acid – was the most active in the hydrophobic quinol Q_9H_2 oxidation; and the most hydrophilic complexes – cobyrinic acid and cobinamid – were the most active in hydrophilic quinol QH_2 oxidation (table 1).

A substantial enhancement of autooxidation is observed in the case of mutual conformity of hydrophobic properties of substrate and catalyst – a so-called "hydrophobic affinity" effect. XPS and UPS data show that the electronic

Table 1
Catalytic activity of cobalt-corrin complexes in autooxidation of quinolic substrates (10^{-4} M Q_9H_2, 10^{-5} M QH_2, 10^{-6} complex)

N°	Complex	$v \times 10^7$ M min^{-1}	
		Q_9H_2	QH_2
1	$[Co(cor)(OH)_2]_{Me}$	2.6	8.0
2	$[Co(cor)(OH)_2]_{Bu}$	4.3	6.3
3	$[Co(cor)(OH)_2]_{Hept}$	5.6	5.3
4	$[Co(cor)(OH)_2]NH_2$	0	8.7
5	$[Co(cor)(OH)_2]_{OH}$	0	8.3
6	$[Co(cor)]^+_{Me}ClO_4^-$	1.3	8.0
7	$[Co(cor)]^+_{Bu}ClO_4^-$	2.3	7.3
8	$[Co(cor)]^+_{Hept}ClO_4^-$	2.8	6.0
9	$B_{12}OH$	0.4	0.7

influence of ether substituents R in the corrin ligand does not effect the nitrogen and cobalt atoms. Inasmuch as all corrin complexes possess the same Co-coordinational surrounding this result means that the variation of oxidation rates is caused only by specific hydrophobic interaction of a substrate and a catalyst.

The next essential feature of the reaction in question is the formation of hydrogen peroxide, that was observed also in the course of Co-corrin catalysed autooxidation of NADH [2] and ascorbic acid [4]. It is known that similar reactions catalysed by copper- and iron- complexes produce other active oxygen species: superoxide ($O_2^{\cdot-}$) ion and OH^0-radical [7].

The possibility that there might be OH^0-radical generation in the case of Co-corrin complexes was carefully investigated. Such generation was indeed detected in the system: Co(II)-corrin complex-ascorbic acid-oxygen with DMPO-trap by EPR-method [4]. The process taking place may be expressed by the following reaction sequences (where [Co] ≡ Co-complex):

$$[Co]^{3+} + AH_2 + O_2 \rightarrow [Co]^{2+} + 2H^+ + A + O_2^{\cdot-}$$

$$2O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$$

$$[Co]^{2+} + H_2O_2 \rightarrow [Co]^{3+} + OH^- + OH^0,$$

These data led us to the conclusion that such catalytically active Co-corrin complexes could be used as catalytic generators of active oxygen radicals that further may be directed at different cell targets, principally DNA. Recently it has been found that iron and copper complexes, which are well-known catalysts for the production of active oxygen species (superoxide, hydrogen peroxide, OH^0 radical), could result in the non-catalytic cleavage of DNA molecules in the presence of oxygen and reducing agent [7]. It has been suggested that these oxygen species are responsible for cleavage of DNA by some antitumor antibiotics, for example, bleomycine [8].

We have proposed that Co-corrin complexes can also induce catalytic DNA cleavage (that is they possess nuclease activity). And we have investigated the possibility of catalytic DNA-cleavage by Co-corrin complexes in the presence of a reductant and molecular oxygen [6].

We used the plasmid pBR 322 as a DNA-target. It is a double-chain ring superspiralise molecule (form I) containing more than four thousand pairs of nucleodides with a known sequence. Cleavage of one chain resulted in the formation of the relaxed form II. In the case of simultaneous cleavage of both chains the linear form III appeared. These three forms show a different mobility in electrophoresis conditions.

The yield of forms II and III from form I characterises the activity of Co-complexes in DNA-cleavage reaction. We see in table 2 that Co-corrin complexes are very active in catalytic cleavage of DNA. In the absence of the reducing agent – ascorbate – the Co-complexes do not split DNA. We believe, that active Co(II)-complex intercalates in DNA, and hydrogen peroxide forma-

Table 2
Degradation of plasmid pBR 322 DNA in the presence of Co-complexes and the reducing agent. [(Co-complex]-2×10^{-4} M, $[AH_2] = 1.7\times10^{-2}$, 1 h, 37°C, 0,01 M tris-HCl, pH 7.55)

Complex	Forms of DNA, %		
	I	II	III
1 Control	65	35	–
2 $[Co(cor)(OH)_2]_{NH_2}$		100	–
3 $[Co(cor)(OH)_2]_{OH}$		100	–
4 $[Co(cor)(OH)_2]_{Me}$	29	71	–
5 $[Co(cor)]^{+}_{Me}ClO_4^{-}$	45	55	–

tion and decomposition with generation of OH^0-radicals take place extremely close to the molecule-target. We consider this to be a new approach to the problem of specific modification of nucleic acid structures. This approach provides new facilities for the regulation of biochemical processes and should promote the creation of new compounds with high biological activity.

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