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Transition Metal Complexes as Catalysts in Biochemical Systems. Interaction with Electron Transfer Processes

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It was suggested that synthetic metal complexes can be used as catalysts for regulation of certain processes in living cells. The complexes have been selected on the basis of their catalytic activity in model chemical reactions such as autooxidation of NADH, Q_4H_2 and cytochrome c. Cobalt(II) complexes with 1,2,3,7,8,12,13,17,18,19- decamethyloctadehydrocorrin, N,N'-bis-(salyciliden)ethylenediamine, o-phenanthroline and other chelates proved to be active catalysts. A possibility of creating catalytic processes competing with the enzymatic ones has been tested experimentally by examples of this complex interaction with the mitochondrial respiratory chain and with the photosynthetic electron transfer system of purple bacteria. It has been shown that some of the above-mentioned chemical catalysts of the respiratory chain components autooxidation can be integrated in electron transport at subcellular level and can carry out catalytic electron transfer from coenzyme Q to oxygen in mitochondria. Such a process competes with the enzymatic one and is comparable with it in rate. Cobalt(II) tris-o-phenanthroline perchlorate was shown to interact with photosynthetic electron transfer system of purple bacteria, stimulating membrane energization in chromatophores.

Introduction

It is generally accepted that the activity and selectivity of enzymes are much superior to those of synthetic chemical catalysts. However it is not the common thing for all cases. The last data of Wilke [1] show that some chemical homogeneous catalytic processes, for instance the dimerisation of propylene, have a turn-over-number (the number of converted substrate molecules per active site and second) so large, that it is comparable with those of fast enzymes, *e.g.* catalase. We suppose the modern coordination chemistry and homogeneous catalysis to open the possibilities to create some abiotic transition metal catalysts capable to take part effectively in metabolism of some biological substrates in the living cell. This could open new ways to regulate some biochemical processes. Respiratory chain components may serve as appropriate objects in the investigation of such a problem. The cell respiration is a fundamental living process, being the source of energy for living organisms. The main part of the energy is formed in the respiratory chain, as a result of the step-wise oxidation of organic substrates and is utilized for formation of ATP. In the course of the respiratory chain electrons are gradually transferred from substrates to oxygen through the chain of components:

$$\text{substrate} \xrightarrow{\bar{e}} \text{NADH} \rightarrow \text{FAD} \rightarrow \text{coenzyme Q} \rightarrow$$

$$\rightarrow \text{cyt } b \rightarrow \text{cyt } c \rightarrow \text{cyt } a_1, a_3 \rightarrow O_2$$

We have attempted to catalyse the electron transfer from some components of the cellular respiratory chain directly to the molecular oxygen, making a bypass for enzymatic process by means of a transition metal complex. Up to now a large number of organic compounds are known to be active in such electron transfer [2]. As far as we know there were no definite ideas on the application of transition metal complexes in such systems. Up to date there are few examples of application of transition metal complexes in mitochondria investigation [3, 4].

The photoinduced electron transfer in the chromatophore membrane was the other object of the transition metal complex action.

Transition metals complexes with conjugated ligands, capable of oxygen activation and fast electron transfer, could be active catalysts of such processes.

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Experimental

Reagents

NADH (see Abbreviations) (basic compound) content ⩾80%) was from Reanal (Hungary); DHNA was prepared according to [5]; Q_4H_2 was prepared by reduction of the corresponding ubiquinon with $NaBH_4$ in aqueous ethanol [6] with a subsequent extraction by isooctane; cytochrome c from horse heart of 'Sigma' type III (basic compound content 98%) was reduced with $Na_2S_2O_4$. A mixture of 67% oxidized and 33% reduced cytochrome c was used in order to avoid an excess of the reductant; this mixture is characterized by a α- to β-band absorption ratio of 1:1,3 as compared to the 1:2 ratio observed for the completely reduced cytochrome c.

Cobalt complexes with salen and other transition metal salen complexes were synthesized as described in [7], cobalt complexes with a corrine ligand related to the group of vitamin B_{12} and organocobalamines as well as perchlorates of dmodc complexes of cobalt and nickel were obtained according to the known methods [8, 9]. Complexes of Co, Ni, Fe, Cu, VO and Mn with ethp were prepared from the chlorides of the corresponding metals and ethioporphyrin II as in [10]. The chloride of Fe^{III} dp IX was obtained as described previously [11]; so-called heme c was isolated according to [12]; cobalt and copper complexes with phen and also with substituted o-phenanthrolines (X-phen) were prepared according to the following methods: $[Co(phen)_3](ClO_4)_2 \cdot 2H_2O$, $[Co(phen)_3](ClO_4)_3 \cdot 2H_2O$, $[Co(X\text{-}phen)_3](ClO_4)_3 \cdot 2H_2O$, $[Cu(phen)_2](ClO_4)_2$, $[Cu(X\text{-}phen)_2](ClO_4)_2$ [13]; $[Co(phen)_3]Cl_3 \cdot 7H_2O$, $[Co(phen)_3]Cl_2 \cdot 7H_2O$, $[Co(X\text{-}phen)_3]Cl_2 \cdot 7H_2O$: [14]; $[Co(phen)_2Cl_2] \cdot 3.5H_2O$: [15]; $[Cu(phen)]Cl_2$ and $[Cu(X\text{-}phen)]Cl_2$: [16].

The following commercial preparations were employed: o-phenanthroline and 5,6-dimethyl-o-phenanthroline (Chemapol), 4,7-diphenyl-o-phenanthroline (Reachim). 5-Nitro-o-phenanthroline was prepared by nitration of o-phenanthroline with the mixture of 18% oleum and 'steaming' HNO_3 (d = 1.4) under the temperature not higher than 120° for 2 hours. 5-Nitro-o-phenanthroline was precipitated on neutralisation (pH 7) of the reaction mixture by 40% NaOH and was purified by recrystallisation from water, yield 48%, m.p. 198° [17].

5-amino-o-phenanthroline was obtained by reducing 5-nitro-o-phenanthroline with $SnCl_2$ in HCl conc. The mixture was heated on the water bath, with the following decomposition by 40% NaOH at cooling (0°). The yield 25%, m.p. 202.

Catalytic Activity Assay

The autooxidation of NADH and DHN was carried out in aqueous or aqueous methanol solutions at 25 °C in the air, and was monitored on a Specord UV-VIS spectrophotometer; NADH absorption was measured at 340 nm (ϵ_{340} = 5080 in MeOH, ϵ_{340} = 5020 in aqueous solution). DHNA absorption was determined at 365 nm (ϵ_{365} = 4600 in MeOH). The initial rates of NADH and DHNA oxidation were taken as a measure of the catalyst activity, the extent of substrate conversion never exceeding 25%. Q_4H_2 oxidation was carried out at the isooctane–water interphase (pH = 6.8) under shaking (60 per minute) at 25 °C in the air. An extent of Q_4H_2 oxidation was evaluated from the absorption difference at 275 nm assuming $(\epsilon_{ox} - \epsilon_{red})_{275}$ = 12250 [6]. The catalyst activities were estimated from the mean rates of the absorption increase at 275 nm during the first 5 min.

The reaction of the reduced cytochrome c oxidation was carried out in aqueous solutions (pH = 6.0) at 25 °C in the air. The stability of pH was strictly controlled in the course of all of the experiments. Oxidation of cytochrome c was monitored spectrophotometrically by absorption at 550 nm (ϵ_{550} = 2250). The activity of catalysts was calculated from the initial rates of cytochrome c oxidation during the first 5 min.

The dissolved oxygen concentration changes in the NADH catalytic autooxidation study were monitored polarographically by means of selective oxygen electrode covered with a permeamble membrane as described in [18].

Biochemical Experiments

Rat liver mitochondria were isolated according to [19] in the medium containing 0.25 *M* sucrose and 1 m*M* EDTA, pH 7.4. Submitochondrial particles (the Keilin–Hartree preparation) were prepared from beef heart according to [20] by extraction of the minced tissue with 0.1 *M* phosphate buffer pH = 7.4 with a subsequent differential centrifugation. The CoQ-deficient Keilin–Hartree particles were prepared by a 5 times extraction of a lyophylized preparation with pentane [21]. After the last extraction the preparation was dried in a rotary evaporator and suspended in 0.25 *M* sucrose at 40–50 mg protein/ml.

Plant mitochondria were isolated from a homogenate of 4-days pea roots in a medium containing 0.3 *M* sucrose, 0.005 *M* EDTA, 0.006 *M* $MgCl_2$, 1% albumin, 0.04 *M* cystein, 0.13 *M* KH_2PO_4 pH = 7.4 according to [22].

Protein concentration was measured by the biuret method [23], using bovine serum albumin as a standard.

Oxygen consumption was measured polarographically with a rotating unprotected platinum electrode.

Transition metal complexes were dissolved in the distilled 2% aqueous methanol. Rothenone,

antimycin A, thenoyltrifluoroacetone and CoQ_6 were used as solutions in twice distilled ethanol.

Photosynthesizing purple bacteria *Rhodopseudomonas sphaeroides* strain 1760-I, were grown and fractionated as described [24]. RC particles were obtained from LDAO-treated chromatophores [25]. Isolation and repeated purification were performed as described [26]. RC were eluted from the column with the Na-phosphate buffer gradient (0.12–0.15 *M*) pH = 7.5, containing also 0.05% LDAO. The purity of the preparations obtained was controlled spectrophotometrically and by means of electrophoresis.

Photoinduced changes of in absorption (ΔA) were monitored by a single beam differential spectrophotometer with time resolution 0.5 or 1 ms. The photoinduced changes for bacteriochlorophyll were measured at 870 nm, for carotenoids at 530 nm.

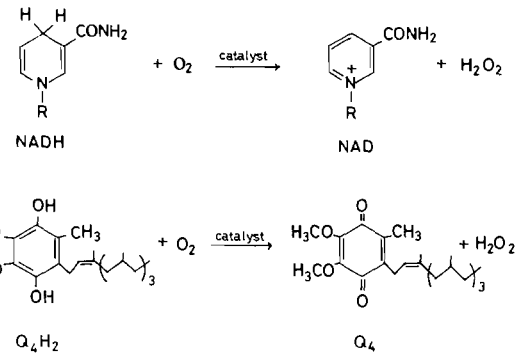
TABLE I. Catalytic Activity of the Transition Metal Complexes in NADH Autooxidation (10^{-4} *M* NADH, 10^{-5} *M* complex, 10^{-2} *M* O_2, MeOH, 25 °C).

No.	Catalyst	$V \times 10^7$ *M* min^{-1}
1	Without catalyst	0
2	Mn(salen)	0.8
3	Ni(salen)	0.5
4	Cu(salen)	1.3
5	Co(salen)	8.0
6	$Cu(acac)_2$	0.4
7	$VO(acac)_2$	0.9
8	$Co(acac)_2$	3.5
9	$Cu(DH)_2$	0.2
10	$[PyCo(DH)_2]Cl$	4.0
11	$[PyRh(DH)_2]Cl$	0.5
12	$[(NH)_2Co(acacen)]Cl$	0.8

Results and Discussion

Chemical Systems

At the first stage of this work we undertook a search of homogeneous catalysts which would stimulate the autooxidation of certain respiratory chain components such as NADH, ubiquinol (a synthetic model compound Q_4H_2 was used) and ferrocytochrome c by the air in aqueous or methanolic media at pH 7 and 25 °C (in order to approach physiological conditions as close as possible):

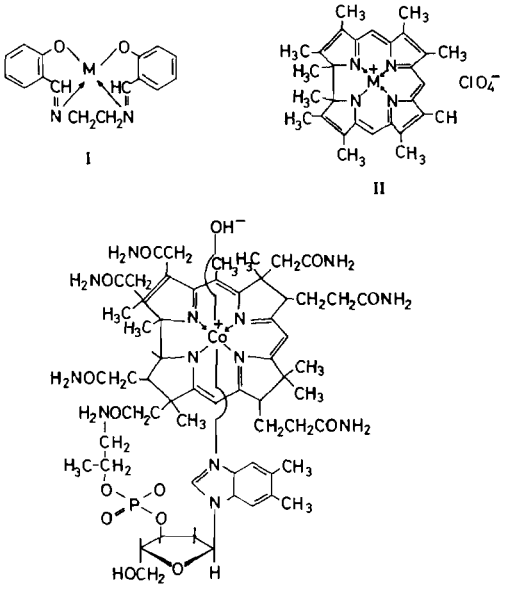


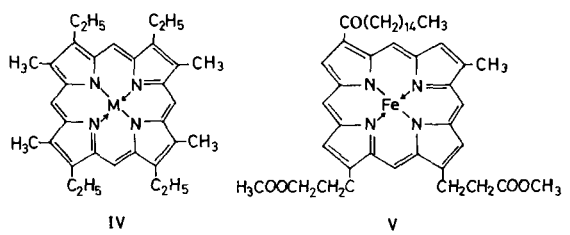
$$\text{Ferrocytochrome } c\,(Fe^{II}) + O_2 \xrightarrow{\text{cat.}} \text{Ferricytochrome } c\,(Fe^{III})$$

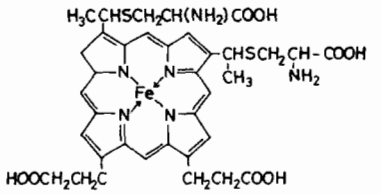
The direct oxidation of NADH by molecular oxygen is known to be rather slow probably due to the direct two-electron transfer to non-excited oxygen molecule being forbidden [27].

It could be supposed that transition metal complexes capable of changing the spin state of O_2 molecule upon coordination should stimulate NADH autooxidation. Indeed, we have found that various complexes of vanadium, chromium, manganese, iron, cobalt, copper *etc.* catalyse autooxidation of NADH in aqueous or alcoholic solutions at room temperature. Thus, catechole complexes of copper and vanadium or σ-phenanthroline complexes of cobalt and copper as well as some cobaltous complexes with chelating ligands proved to be active catalysts [28–32].

The catalytic action of the following types of complexes was studied in more detail:







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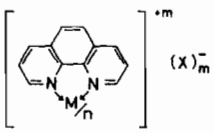
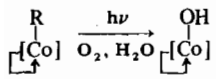


VII

TABLE II. Catalytic Activity of Co and Ni(dmodc) Complexes and of Vitamin B_{12} Group Complexes in NADH Autooxidation (10^{-4} *M* NADH, 10^{-5} *M* complex, 2×10^{-4} *M* O_2, H_2O (pH 6.8), 25 °C).

No.	Catalyst	$V \times 10^7$ *M* min^{-1}
1.	Without catalyst	0.1
2.	[Co(dmodc)] (ClO_4)	27.8
3.	[PyCo(dmodc)] (ClO_4)	10.2
4.	[Ni(dmodc)] (ClO_4)	0.1
5.	Aquacobalamine (vit B_{12a})	3.4
6.	Cyancobalamine	0.1[a]
7.	Methylcobalamine	0.1[a]
8.	Adenosylcobalamine	0.1[a]
9.	Aquacobinamide	2.5
10.	Cyancobinamide	0.1[a]
11.	Adenosinecobinamide	0.1[a]

[a]The experiments were carried out in the darkness in order to prevent the decomposition according to the scheme:



R = CN, CH_3, adenosyl.

(1) transition metal complexes with Schiff bases (I); (2) corrine (III) and octadehydrocorrine complexes (II); (3) porphyrine complexes (IV–VI) and (4) o-phenanthroline complexes (VII).

The first group comprises plane chelate complexes of transition metals with Schiff bases as ligands some of which can coordinate and activate oxygen [29]. Among these complexes that of Co(II)–Co(salen) was the most active (Table 1).

Another group comprises cobalt complexes with corrin ligand which belong to the group of vitamin B_{12} and organocobalamines and of cobalt and nickel complexes with dmodc [30]. Among those complexes Co(dmodc) was extremely active (Table II).

In order to elucidate the mechanism of NADH catalytic autooxidation we have studied the kinetics of this reaction in the presence of Co(salen) and Co(dmodc). In both cases the reaction was found to be close to first-order with catalyst and 0.5-order with NADH. The catalytic reaction can be described as following:

$$[Co^{II}] + NADH + O_2 \overset{K}{\rightleftharpoons} [Co^{II}] \cdot NADH \cdot O_2$$

$$[Co^{II}] \cdot NADH \cdot O_2 \xrightarrow{k_1} [Co^{II}] + NAD^+ + HO_2^-$$

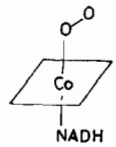
where the reaction rate is expressed by an equation

$$W = \frac{k_1 K [NADH] [O_2] [Co]}{1 + K [NADH] [O_2]}$$

The data obtained indicate the step of oxygen coordination to be essential for catalytic activity of cobalt complexes. Thus, the complex of nickel with the same ligand which is unable to coordinate oxygen lacked the catalytic activity (Tables I, II). The addition of imidazole and pyridine which compete in coordination to cobalt were found to impede the reaction markedly.

It must be mentioned here that replacement of NADH by its model compound DHNA in autooxidation reaction in the presence of the same complex catalysts and under the same conditions resulted in a considerable (2.5-fold) stimulation of the reaction. To our opinion, a slower NADH oxidation is due to adenosine fragment which probably competes with the dihydropyridine one for the coordination place at the Co atom.

All the above mentioned factors together with the kinetic measurements enable us to suggest the so-called 'inner-sphere' mechanism with coordination of both NADH and O_2 to 5- and 6 positions of Co complex and with two-electron transfer from NADH to O_2 through Co atom without the change of Co oxidation state:



It is in good agreement with the observed loss of catalytic activity for the complexes, containing firmly attached ligands in the axial positions (compare Table II, NN 6–8 and NN 10, 11).

The NADH oxidation in the presence of Co(salen) and Co(dmodc) was found to slow down significantly with time. A set of experiments indicated that H_2O_2 produced in the course of the reaction is involved in Co(II) complex catalyst oxidation. The resulting Co(III) complex is catalytically inactive. Addition of catalase or Ni-complex with monoethanolamine destroying H_2O_2 abolished the inhibition [18] (Fig. 1). In general Co(III) complexes are much less active, which can be due to their lower ability to coordinate and activate molecular oxygen. Thus, a number of Co(III) complexes belonging to a group of vitamin B_{12} had revealed

TABLE III. Catalytic Activity of Some Metalloporphyrins in NADH Autooxidation (10^{-4} *M* NADH, 10^{-5} complex, 2×10^{-4} *M* O_2 in H_2O (pH 6.8), 10^{-2} *M* O_2 in MeOH, 25 °C).

No.	Catalyst	V x 10^7 *M* min^{-1}	No.	Catalyst	V x 10^7 *M* min^{-1}
1.	Cu /ethp II[a]	0.1	7.	Fe^{III} dp IX	0.1
2.	Mn /ethp II[a]	0.1	8.	Fe^{II} dp IX[b]	12.0
3.	Ni /ethp II[a]	0.1	9.	Heme *c*(Fe^{III})[c]	0.1
4.	VO /ethp II[a]	0.1	10.	Heme *c*(Fe^{II})[b,c]	18.0
5.	Co /ethp II[a]	0.1	11.	Cytochrome *c*(Fe^{III})	0.1
6.	Fe^{III}/ethp II[a]	0.1	12.	Cytochrome *c*(Fe^{II})[b]	0.1

[a]In MeOH. [b]10^{-5} $NaBH_4$ is added. [c]pH 9.0.

TABLE IV. Catalytic Activity of Copper and Cobalt Complexes in Autooxidation of Some Components of the Respiratory Chain (10^{-4} *M* NADH, 10^{-4} *M* Q_4H_2, 10^{-4} cytochorme *c*, 10^{-5} *M* complex, 2×10^{-4} *M* O_2).

No.	Complex catalyst	V x 10^7 *M* min^{-1}		
		NADH[a]	Q_4H_2[c]	cyt. c
1.	Without catalyst	0.1	1.0	0.2
2.	$[Co(phen)_3](ClO_4)_3$	0.1	33.0	95.0
3.	$[Co(phen)_3](ClO_4)_2$	7.5	50.0	20.6
4.	$[Co(phen)_2Cl_2]$	87.0	40.0	90.0
5.	$[Co(4,7Ph_2\text{-phen})_3]Cl_2$	0	129.0	0
6.	$[Cuphen]Cl_2$	25.0	23.0	80.0
7.	$[Cu(4,7Ph_2\text{-phen})]Cl_2$	20.0	306.0	0
8.	$[Co(dmodc)]ClO_4$	26.0	23.7	–
9.	Co(salen)	8.0[b]	1.6	–
10.	Vitamin B_{12a}	3.4	7.5	–

[a]H_2O. [b]In MeOH. [c]In i-C_8H_{18}–H_2O.

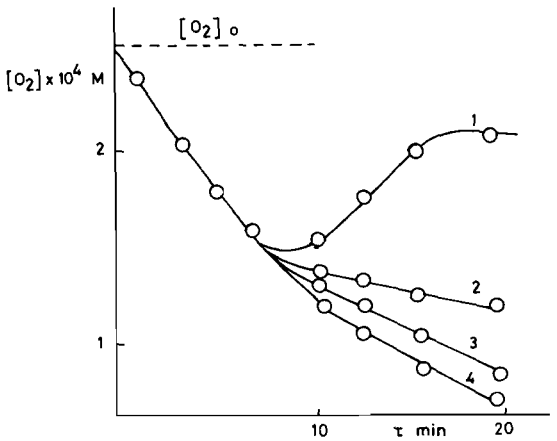


Fig. 1. The inhibition of the Co(dmodc)-catalyzed NADH autooxidation; 1) without any catalase added; 2) 5 mg of catalase added; 3) 10 mg; 4) 50 mg. The reaction mixture contains 10^{-4} *M* NADH, 10^{-5} *M* Co(dmodc), H_2O (pH 6.8), 25 °C. The oxygen consumption is monitored polarographically by means of selective silver electrode.

an activity significantly lower than that of Co(II) complex with related octadehydrocorrin ligand.

Metalloporphyrins appeared to be inactive in NADH autooxidation (Table III) due to rapid and irreversible oxidation of Co(II) and Fe(II) in protic solvents (Table III). Having used the reduced Fe^{II} dp IX with an excess of a reductant $NaBH_4$ stabilized by lipid environment (so-called 'vesicles') and also reduced heme *c* we succeded in obtaining metalloporphyrin systems with significant catalytic activity [31].

The above mentioned observation justified once more our supposition that the reaction under study is effectively catalyzed only by complexes that contain a transition metal in its lower oxidation state. Nevertheless, cytochrome c in reduced form was inactive as catalyst in NADH autooxidation, probably due to the protein surrounding of the heme preventing coordination of NADH and O_2.

Further on we turned to o-phenanthroline complexes of cobalt and copper. Such complexes are

TABLE V. The rate of Oxygen Consumption (V mca 0/min per I mg of protein) by Rat Liver Mitochondria in the Presence of some Cobalt Complexes and Respiratory Chain Inhibitors. The medium of incubation contains 0.15 *M* sucrose, 0.075 *M* KCl, 0.005 *M* KH_2PO_4 (pH 7.4), 0.0028 *M* $MgCl_2$, 3–4 mg/ml of protein, 0.006 *M* succinate or 0.005 *M* glutamate + 0.005 *M* malate, 10^{-5} *M* complex.

Compound added	Substrate	
	Glutamate + malate	succinate
Without	2.4	2.8
[Co(dmodc)]ClO_4	7.4	17.6
Rotenone	1.4	3.0
Rotenone + [Co(dmodc)]ClO_4	1.4	18.5
Antimycin A	–	0
Antimycin A + [Co(dmodc)]ClO_4	–	17.6
Thenoyltrifluoracetone	–	0
Thenoyltrifluoracetone + [Co(dmodc)]ClO_4	–	0

known to be widely used in redox catalysis [33, 34]. Our data on the catalytic activity of the cobalt and copper o-phenanthroline complexes in NADH, hexahydroubiquinol Q_4H_2 and ferrocytochrome *c* autooxidation are listed in Table IV. The highest activity in NADH oxidation was displayed by $[Co^{II}(phen)_2Cl_2]$. The related cobalt(III) complexes did not show any appreciable activity. This reflects their inability to coordinate molecular oxygen and supports the concept of the essential role of O_2 coordination in the NADH oxidation reaction.

NADH usually is considered to be a hydride donor, while the other two substrates of model oxidation reactions, Q_4H_2 and cytochrome c, can function preferably as one-electron donors. It is therefore pleasurable to suggest that some different mechanism of oxidation of two last substrates is realised, which does not need oxygen coordination by catalyst, but is rather confirmed to sequential oxidation and reduction of the central transition metal atom.

Most of the complexes studied including Co(dmodc) are rather active in Q_4H_2 autooxidation. As regards the oxidation of ferrocytochrome c in the presence of o-phenanthroline complexes the essential feature of the mechanism is, probably, a contact between heme peripheral regions and o-phenanthroline nucleus as had been found for the similar stoichiometric reaction [35]. Introduction of the bulky phenyl substituents into positions 4 and 7 of phenanthroline nucleus markedly reduces the rate of cytochrome *c* oxidation (Table IV).

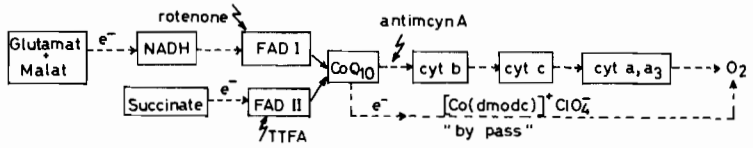
Biochemical Systems

In the next part of the work we attempted to test whether it is possible to use the investigated metal complexes for creating catalytic processes in biological systems. We have chosen (a) the mitochondrial electron transfer chain and (b) the system of photoinduced electron transfer of purple bacterial chromatophores.

The mitochondrial respiratory chain

The compounds found to be the most active in the chemical experiments have been investigated in these studies [36]. The action of some compounds on succinate and glutamate + malate oxidation in rat liver mitochondria is shown in Table V. The addition of Co(dmodc) which is an effective catalyst of NADH and coenzyme Q oxidation was found to stimulate succinate oxidation by the mitochondrial suspension more than 6-fold. In the presence of NADH-dependent substrates (glutamate + malate) there was a 3-fold stimulation of respiration by Co(dmodc) but upon subsequent incubation the rate of respiration returned back to its original level. The corresponding nickel complex Ni(dmodc) being inactive in model chemical systems was no more active with respect to mitochondrial redox systems.

In our further studies we concerned ourselves with the problem of which of the respiratory chain components donates electrons to Co(dmodc) in mitochondrial electron transfer chain. Specific respiratory chain inhibitors were used as a tool. The Co(dmodc) catalysed oxidation of succinate and NADH by intact mitochondria was found to be insensitive to antimycin A which inhibits electron transfer in the cytochrome *b* region of the respiratory chain (Fig. 2). This observation excludes the terminal respiratory chain carriers as possible electron donors in Co(dmodc) catalysis. It has been also shown that mitochondria being osmotically shocked with a subsequent removal of cytochrome *c* by salt extraction did not decrease the Co(dmodc)-dependent succinate oxidase activity of mitochondria. The rate of oxygen consumption by mitochondria oxidizing succinate in



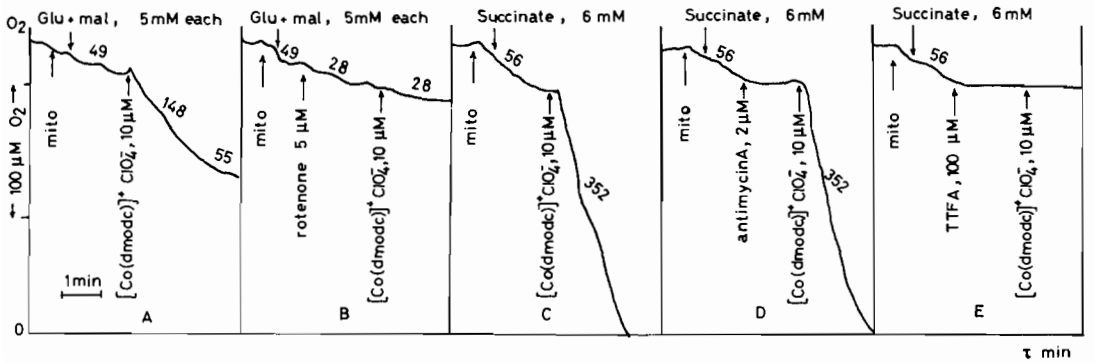


Fig. 2. The effect of Co(dmodc) on substrate oxidation by rat liver mitochondria. The medium of incubation is the same as in Table V. A) Oxidation of 5 m*M* glutamate + 5 m*M* malate; B) the medium was the same as in A, except that the addition of rotenone (5 mcM) precedes the addition of Co(dmodc) (10 mc*M*); C) oxidation of 6 m*M* succinate; D) the medium was the same as in C, except that the addition of antimycin A (2 mc*M*) precedes the addition of Co(dmodc) (10 mc*M*); E) the medium was the same as in C, except that the addition of TTFA (100 mcM) precedes the addition of Co(dmodc) (10 mc*M*).

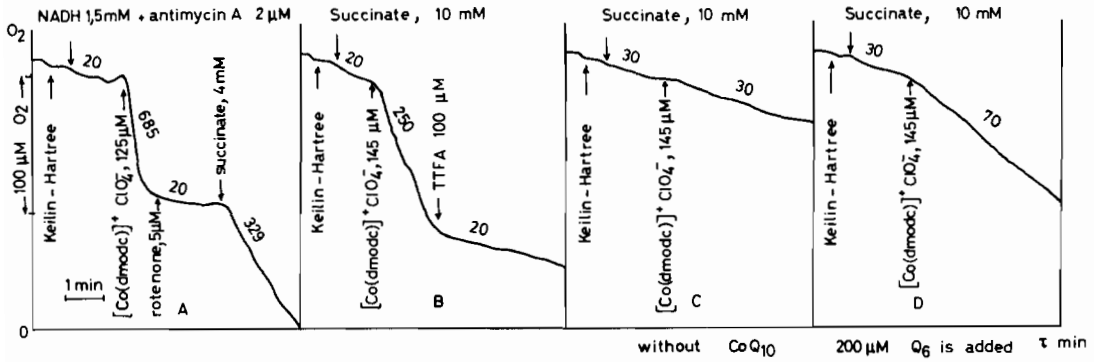


Fig. 3. The effect of Co(dmodc) on substrate oxidation by Keilin–Hartree heart muscle preparation. The medium of incubation contains 0.1 *M* KH_2PO_4 (pH 7.4), 100 mg/ml cytochrome c, 1–2 mg of protein per 1 ml. A) Oxidation of externally added NADH (1.5 m*M*) by Keilin–Hartree preparation preincubated with antimycin A (2 mc*M*); B) oxidation of succinate (10 m*M*) with subsequent addition of TTFA; C) oxidation of succinate (10 m*M*) by CoQ deficient Keilin–Hartree preparation; D) the same as in C with Q_6 (20 mc*M*) added.

the presence of Co(dmodc) was found to be not affected by rotenone which inhibits the reversed electron transfer from succinate to NAD. On the other hand the addition of TTFA which is known to block electron transfer at the succinate dehydrogenase–CoQ step brought about virtually 100% inhibition of respiration in the presence of both succinate and Co(dmodc). Similar results were obtained for the submitochondrial particles (Keilin–Hartree preparation, Fig. 3, A, B) which are devoid of endogenous pyridine nucleotides and do not catalyse the reversed electron transfer and other endergonic functions characteristic of intact mitochondria.

All these data indicate Co(dmodc) to catalyse electron transfer to oxygen from a respiratory chain component localized between flavoproteins and cytochrome b. Coenzyme Q seems to be the most probable electron donor under the conditions studied.

In order to test this possibility we have prepared submitochondrial particles deficient in endogenous CoQ by treatment of the lyophylized Keilin–Hartree preparation with pentane resulted in CoQ extraction.

TABLE VI. Changes of Electron Transfer in RC by Cobalt and Copper o-Phenanthroline Complexes[a]. The medium of incubation contains 4 mc*M* $(BChl)_2$, 0.05% LDAO, 10^{-4} *M* complex, phosphate buffer, pH 7.5, 25 °C).

Cobalt complexes	Relative activity	Copper complexes	Relative activity
$[Co(phen)_3](ClO_4)_2$	0.11	$[Cu(phen)_2](ClO_4)_2$	0.68
$[Co(phen)_3](ClO_4)_3$	0.31	$[Cu(phen)]Cl_2$	1.0
$[Co(phen)_3]Cl_2$	0.36	$[Cu(5\text{-}NO_2\text{-}phen)]Cl_2$	1.0
$[Co(phen)_2]Cl_2$	0.57	$[Cu(4,7\text{-}Ph_2\text{-}phen)]Cl_2$	1.0
$[Co(4,7\text{-}Ph_2\text{-}phen)_3]Cl_2$	0.75	$[Cu(4,7\text{-}Ph_2\text{-}phen)_2](ClO_4)_2$	1.0
$[Co(5\text{-}NO_2\text{-}phen)_3]Cl_2$	1.0	$[Cu(5\text{-}NH_2\text{-}phen)_2](ClO_4)_2$	1.0

[a]The value of relative activity is calculated from the ratio: $A_{870\,exp}/A_{870\,cont}$, taken from $(BChl)_2$ differential absorbance spectra in the presence of complex (exp), and without complex (cont). The smaller the value of the relative activity the greater the effect of complex; when the value is equal to 1.0, it means that the complex has no effect.

The submitochondrial particles thus obtained were practically devoid of the NADH- and succinate oxidase activities. The addition of Co(dmodc) did not affect in this case the rate of oxidation. But the addition of exogenous Q_6 restored the catalytic activity of Co(dmodc) and its sensitivity to inhibition by α-thenoyltrifluoroacetone (Fig. 3, C, D).

Thus in this part of the work we have demonstrated the ability of cobalt octadehydrocorrin complex, which is an active catalyst of a chemical autooxidation, to interfere with biological electron transfer at subcellular level, so that this compound maintains a catalytic process of electron transfer from coenzyme Q to oxygen competing with an enzymatic one and comparable to the latter in rate. It also turns out that Co(dmodc) takes electrons from coenzyme Q as is shown in the scheme in Fig. 2.

It was shown analogously that some o-phenanthroline complexes of cobalt, being the catalysts of chemical autooxidation, manifest their activity also in submitochondrial particles and intact plant mitochondria respiration, probably interacting on the coenzyme Q level [32].

The systems of bacterial photoinduced electron transfer

These systems with cyclic photoinduced electron flow have been explored in considerable detail [37]. They differ from the above discussed mitochondrial electron transfer having oxygen as the terminal electron acceptor. The photoinduced electron transfer can be studied conveniently with the intracellular closed membranous structures which can be isolated from purple bacteria. These particles (chromatophores) retain the photosynthetic electron transfer chain and the energy-coupling system which are involved in the transduction of light energy into the energy of electrochemical ionic gradients. The primary photosynthetic processes are known to occur in the so-called reaction centres (RC) which are molecular complexes of porphyrin pigments with certain quinone containing redox cofactors bound to specific proteins and which are integral constituents of the photosynthetic membranes. These structures are the minimal fragments possessing the specific photochemical activity in the photoinduced electron transfer from bacteriochlorophile to quinone acceptors.

We have studied the effect of cobalt and copper complexes with o-phenanthroline and its derivatives on the photoinduced electron transfer and related functions in RC and chromatophores from purple bacteria [24].

The o-phenanthroline complexes studied with RC have been found to fall into two groups: (i) cobalt complexes which possess an electron donor–acceptor activity and enhance electron transfer in RC and (ii) copper complexes which are not active in electron transport (Table VI).

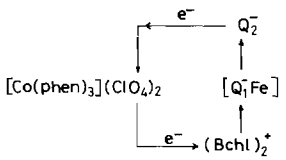
Let us consider the effect observed with an example of the cobalt(II) tris-o-phenanthroline complex perchlorate that 9-fold stimulated the electron transfer in the experiment with RC. In the absence of exogenous cofactors there is only a photoinduced electron exchange between the bacteriochlorophyll dimer $(BChl)_2$ and the quinone acceptors (the primary $[Q_1Fe]$ and the secondary one, Q_2) that occurs in RC. In the actinic continuous saturating light RC are largely in the $(BChl_2)^+[Q_1Fe]Q_2^-$ state. In the presence of the most active cobalt complex $[Co(phen)_3](ClO_4)_2$ (10^{-4} *M*) an induction splash in the $(BChl)_2$ photoinduced kinetics can be observed. A virtually complete oxidation is brought about initially by the actinic illumination, followed by a rapid re-reduction of the pigment. Special experiments with flash photoactivation showed the rate of $(BChl)_2$ re-reduction in the dark to be much higher in the presence of complex as compared to the control. These data can be easily explained since the cobalt complex undergoing consecutive oxida-

TABLE VII. The Level of the Membrane Energization in Chromatophores by Cobalt and Copper o-Phenanthroline Complexes[a]. The medium of incubation contains the suspension of chromatophores with initial optical density 0.6 at 590 nm.

Cobalt complex	Relative activity		Copper complex	Relative activity	
	I	II		I	II
$[Co(phen)_3](ClO_4)_2$	1.58	1.8	$[Cu(4,7\text{-}Ph_2\text{-}phen)]Cl_2$	0.25	0.03
$[Co(phen)_3](ClO_4)_3$	1.42	1.6	$[Cu(4,7\text{-}Ph_2\text{-}phen)_2]ClO_4$	0.5	0.21
$[Co(phen)_2]Cl_2$	1.12	1.35	$[Cu(5,6\text{-}Me\text{-}phen)]Cl_2$	0.54	0.36
$[Co(phen)_3]Cl_2$	1.1	1.3	$[Cu(5\text{-}NH_2phen)_2](ClO)_2$	0.62	0.5
$[Co(5\text{-}NO_2phen)_3]Cl_2$	1.0	1.0	$[Cu(5\text{-}NO_2phen)]Cl_2$	0.78	0.48
$[Co(4,7\text{-}Ph_2phen)_3]Cl_2$	0.2	0.01	$[Cu(phen)_2](ClO_4)_2$	0.80	0.70
			$[Cu(phen)]Cl_2$	0.95	0.76

[a]The value of relative activity is calculated from differantial absorbance spectra: 1) from the ratio $A_{850\,exp}/A_{850\,cont}$ for $(BChl)_2$ and 2) from the ratio $A_{530\,exp}/A_{530\,cont}$ for carotenoides. The greater the value of the relative activity the greater the energizing effect of complex in chromatophores.

tion and reduction can by-pass the segment of the electron transfer chain between the oxidized pigment and the reduced quinones:



The fact that multiple and fully reversible turnovers can be observed in RC in the presence of this complex, confirms the conclusion that the cyclic electron flow is being generated involving this cofactor.

Rapid oxidation of cobalt complex ($Co^{II} \rightarrow Co^{III}$) by the RC pigment (100 ms) and relatively slow reduction of one ($Co^{III} \rightarrow Co^{II}$) by the quinones (5 s) should displace the steady-state in the functioning RC + cobalt complex system towards accumulation of the $(BChl)_2[Q_1Fe]Q_2^-$ state.

The differential spectra of RC 'light minus dark' also indicate that in the presence of cobalt complex there are the bands of the reduced quinols dominating in the range of semiquinone anion radicals absorption and a long-wavelength shift of the bacteriopheophytin absorption band in the interval characteristic of a local electrostatic field shift in the RC protein upon a photomobilized electron captured by the quinone acceptors.

The ability of the cobalt–phenanthroline complexes to stimulate cyclic electron flow is of particular interest in view of the o-phenanthroline ligand alone being inhibitory to electron transfer from $[Q_1Fe]$ to Q_2 [38]. $CoCl_2 \cdot 6H_2O$ and $NaClO_4$ did not affect the kinetics of the RC photoinduced transitions. Hence the functional effects found should be attributed to the complexes themselves (see Table VI).

Having characterized the behaviour of the complex compounds in the system of functioning RC we evaluate then the action of those compounds on the integral photosynthetic membranes. The cobalt complexes capable of supporting the photoinduced cyclic electron flow from the reduced quinone acceptors to the oxidized pigment were naturally of the primary interest (Table VII).

The addition of complexes to bacterial chromatophores stimulated long-wavelength shifts of the BChl and carotenoid absorption bands, which indicated the protonic electrochemical potential gradient generation in the membrane enhanced.

All the copper compounds shown above to lack donor–acceptor activity with RC system revealed uncoupling action with chromatophores decreasing membrane potential. The mechanism of the copper complexes uncoupling action may be similar to that of other penetrating ions. The copper compounds studied are given in a series according to their decreasing uncoupling activity in Table VI.

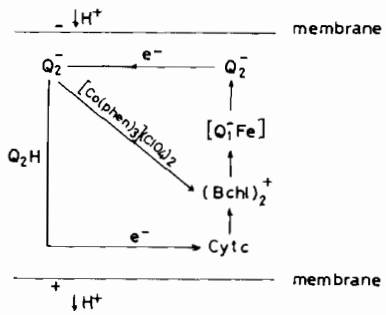
In order to explain such a significant difference between copper and cobaltous o-phenanthroline complexes with respect to their activity in mediating cyclic electron flow in RC and in affecting membrane energization in chromatophores one would consider redox potentials of two groups of complexes. Redox potentials of cobalt complexes (E = 0.3–0.4 V) appear to be just in that narrow range of oxidation–reduction potentials where an 'optimal' catalyst is both an active acceptor and donor with respect to substrate. This enables cobalt o-phenanthroline complexes to operate efficiently in electron transfer between the reduced quinone

(E = 0.10 V) and the oxidized pigment (E = 0.46 V) both in RC and in chromatophores. At the same time this transfer of an electron from the quinones to the oxidized pigment may be hindered in the case of copper o-phenanthroline complexes (E = 0.100–0.120 V).

Obviously, it is also the structure of complexes that should be of importance for their ability to interfere with electron transfer in RC and chromatophores. In particular the low activity of the cobalt complex with 4,7-diphenyl-o-phenanthroline may be explained in terms of an outer sphere mechanism of electron transfer between the o-phenanthroline cobalt complex and $(BChl)_2$. The bulky substituents in o-phenanthroline could hinder a contact of the catalyst molecule peripheral regions with the RC pigment and thus decrease a rate of electron transfer.

It should be emphasized that redox transitions of the (Co^{II}/Co^{III}) complexes are purely electronic and do not involve proton binding or release as occurs with many other redox cofactors (dichlorophenolindophenol, diaminodurene, phenazine methosulfate).

Being lipophylic, cobalt complexes can be incorporated in the electron-transport chain at the inner face of the chromatophore membrane, providing electron transfer from the quinone acceptor to $(BChl)_2$ and enhancement of the electrochemical potential gradient:



Thus, in our opinion, quinoid carriers are the proper electron donors for the complex catalyst in both electron transfer systems – in the mitochondrial respiratory chain and in the chromatophore photoinduced transport.

In conclusion it should be outlined that the data obtained prove a possibility to catalyse the metabolism of biological substrates, including at the level of biological systems, with the use of synthetic metal complexes. In our opinion this approach provides new facilities for regulation of biochemical processes and should promote the revealing of new compounds with high biological activity.

Abbreviations

NADH – reduced nicotinamide adenine dinucleotide
DHNA – N-methyl-4,4′-dehydronicotinamide
Q_4H_2 – hexahydroubiquinol
salen – N,N′-bis-salicyliden-ethylendiamine
acac – acetylacetonate
DH – dimethylglyoxime
acacen – N,N′-bis-acetylacetonethylendiamine
dmodc – 1,2,3,7,8,12,13,17,18,19-decamethyloctadehydrocorrin
phen – o-phenanthroline
ethpII – ethioporphyrin II
dp IX – 2-methyl-4-palmethoil-deiteroporphyrin IX
LDAO – lauryldimethylaminoxide
TTFA – thenoyltrifluoroacetone
RC – reaction centres
BChl – bacteriochlorophyll
TMFD – N,N,N′,N′-tetramethyl-p-phenylenediamine
Q_1, – primary quinone,
Q_2 – secondary quinone

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