Transition-metal complexes as catalysts of active oxygen species formation in autooxidation reactions

2.* Oxidative degradation of nucleic acids by cobalt and iron phthalocyanine complexes

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Co- and Fe-phthalocyanine complexes with cationic and anionic substituents in the macroligand, which are effective in autooxidation of ascorbic acid in aqueous solutions, are studied in the reaction of oxidative cleavage of nucleic acids. It was shown for the first time that water-soluble, metal-phthalocyanine complexes can cleave nucleic acids in the reaction of dark conjugated autooxidation of ascorbic acid. Fe-octakis(pyridiniomethyl)phthalocyanine chloride is the most efficient in oxidative degradation of RNA target, and Co- and Fe-phthalocyanine cationic complexes are the most efficient in oxidative degradation of DNAs. Using the radical traps, it was shown that the oxidative degradation of nucleic acids is caused by OH-radicals.

Key words: phthalocyanine complexes of Co and Fe, oxidative degradation of nucleic acids.

The interest in studies of oxidative cleavage of nucleic acids (NAs) by transition-metal complexes (MC) arise mainly in the search for new antitumor preparations and development of new systems for solution of some problems of molecular engineering. Recently, the nuclease activity of a series of transition metal complexes, primarily, phenanthroline complexes of copper,[2] porphyrin complexes of iron and manganese,[3] and EDTA complexes of iron has been revealed.[4] Previously, we have demonstrated the cleavage of DNA- and RNA-targets under the action of both free corrin complexes of cobalt[5,6] and complexes covalently bonded with oligodeoxyribonucleotides.[7] The nuclease activity of these metal complexes results from their ability to generate OH-radicals in conjugated oxidation of an appropriate reducing agent, *e.g.*, ascorbic acid (AA).

In the present work, the cleavage of NA-targets under the action of Co and Fe complexes with macrocyclic ligands, *viz.*, phthalocyanine (Pc) and naphthalocyanine (Nc) is studied. Phthalocyanines, like corrins, belong to tetrapyrrole macrocycles, they are characterized by high stability and are known as catalysts in reactions involving molecular oxygen in organic media.[8] It has been shown[1] that a series of water-soluble Co and Fe phthalocyanine complexes catalyse efficiently the autooxidation of ascorbic acid (AA), in which the active oxygen species (AOS) are involved. It was established in the case of oxidation of 2-deoxyribose that some metal complexes decompose hydrogen peroxide affording the OH radical. These data make it possible to consider metal phthalocyanine complexes as promising systems for the cleavage of NAs.

DNA of plasmids pUC19 and 5S RNAs of *Escherichia coli* (*E.coli*) and *Thermus thermophila* (*Th. thermophila*) ribosomes were used as NA-targets. They are characterized by different spatial structures: DNA of plasmid pUC19 is a double-stranded, circular superhelix (3264 bp), while 5S RNAs of *E.coli* (120 nucleotides) and *Th. thermophila* (122 nucleotides) contain both single-stranded and double-stranded regions.

The ability of metal complexes to cleave RNA was assayed by carrying out the reaction under conditions of conjugated autooxidation of ascorbic acid at 37 °C for 30 min (pH 7.5), the ratios MC : AA and MC : NA were both 1 : 10. The analysis of cleavage products in the case of ^{32}P-5S RNA was carried out in 12 % polyacrylamide gel (PAAG) followed by autoradiography. It was found that several Co- and Fe-phthalocyanine complexes can induce efficient, nonspecific cleavage of RNA, affording a random set of cleavage products. The efficiencies of the cleavage of RNA-targets with Co and Fe

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complexes with phthalocyanine ligands in a *N*-ethylmorpholine buffer (50 mmol L^{-1}, pH 7.76) in the presence of AA are given below.

Composition of MC	Mean value of efficiency of RNA cleavage (%)
$[FePc(CH_2N^+C_5H_5)_8]Cl^-_8$ (**1**)	86
$CoPc(SO_3Na)_2$ (**2**)	82
$FePc(SO_3Na)_2$ (**3**)	78
$FePc(COONa)_8$ (**4**)	74
$CoPc(SO_3H)_2$ (**5**)	70
$CoNc(C_6H_4SO_3Na)_4$ (**6**)	64
$[CoPc(CH_2N^+C_5H_5)_8]Cl^-_8$ (**7**)	35
$[CoPc(CH_2\overset{+}{S}C(NH_2)_2)_8]Cl^-_8$ (**8**)	27
$CoPc(COONa)_8$ (**9**)	22
$[CoNc(CH_2N^+C_5H_5)_8]Cl^-_8$ (**10**)	16
$[CoPc\{SO_2N(CH_2COOH)C_6H_4OH\}_3]$ (**11**)	16

The accuracy of determination of the efficiency of cleavage was ±3—5%. The cationic Fe complex, $[FePc(CH_2N^+C_5H_5)_8]Cl^-_8$, possesses the highest nuclease activity, it also exhibited the highest acivity in autooxidation of AA.[1]

In the search for optimal cleavage conditions, the dependence of the degree of RNA cleavage on the concentration of AA was revealed. Hence, it was found for the $[FePc(SO_3Na)_2]$ complex (Fig. 1) that the maximum cleavage was observed at the concentration of AA from 2 to 5 mmol L^{-1}; at the higher concentrations, the efficiency of cleavage increases insignificantly, therefore, these concentrations were used in subsequent experiments.

In addition, it was found that the degree of RNA cleavage depends substantially on the nature and concentration of the buffer used for the reaction (Table 1) (the pH values for the buffers were from 7.5 to 7.76). An efficient cleavage of RNA was observed in buffer solutions of *N*-ethylmorpholine or potassium phosphate. In the case of Tris-HCl and HEPES buffers, the degree of RNA cleavage is lower. This fact is in accord with the literature data that Tris-HCl and HEPES are radical traps themselves.[9] The efficiency of RNA cleavage increases when the concentrations of buffers decrease. The comparison of rates of hydrolysis of 5S RNA with metal complexes **1**, **3**, **4**, and **5** (see above) demonstrated that in the presence of complex **1**, $[FePc(CH_2N^+C_5H_5)_8]Cl^-_8$, the cleavage of RNA is practically complete in 10 min. In the presence of other complexes, the maximum cleavage was achieved in 30 min (Fig. 2).

Table 1. Effect of the nature and concentration of buffer on the efficiency of cleavage of 5S RNA with iron phthalocyanine complex $[FePc(CH_2N^+C_5H_5)_8]Cl^-_8$

Buffer	Mean value of efficiency of 5S RNA cleavage (%) at various concentrations of buffers (mol L^{-1})			
	0.5	0.25	0.1	0.05
Tris-HCl (pH 7.53)	32	28	49	50
HEPES (pH 7.5)	5	38	68	76
Potassium phosphate (pH 7.51)	23	29	36	82
N-Ethylmorpholine (pH 7.76)	23	50	77	89

Note. The efficiency of cleavage is determined with an accuracy of ±3—5 %.

In the case of plasmid pUC19, the analysis of cleavage products was carried out in agarose gel followed by their visualization with ethidium bromide. The complete cleavage of DNA of the plasmid pUC19 (Fig. 3) was observed only for metal complexes containing cationic substituents in the macroligand (Co and Fe octakis(pyridiniomethyl)- and octakis(isothiuroniomethyl)phthalocyanine chlorides, **1**, **7**, **8**, and **10**). Such a selectivity apparently reflects an additional contribution of electrostatic forces in the interaction of cationic complexes with the negatively charged carbohydrate-phosphate DNA backbone, which results in their spatial

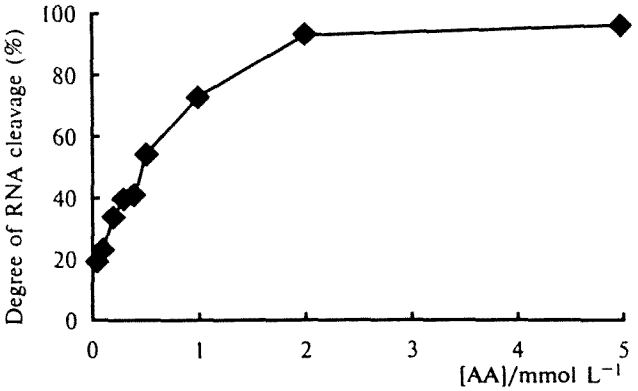

Fig. 1. The degree of cleavage of 5S RNA of *E.coli* ribosomes with metal complex $FePc(SO_3Na)_2$ (concentration 10^{-4} mol L^{-1}) *vs.* concentration of AA (37 °C, 30 min).

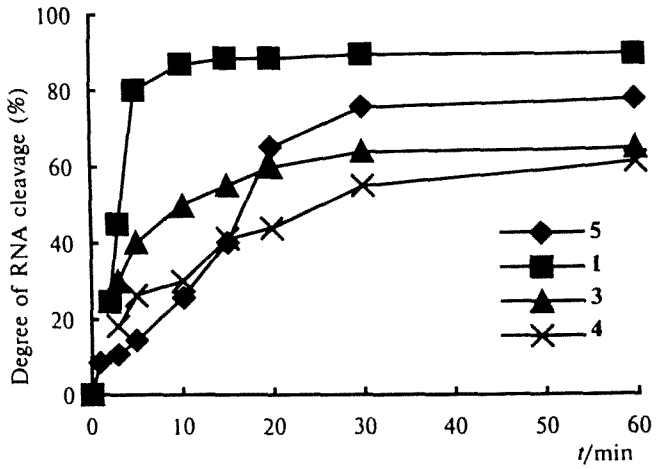

Fig. 2. The time dependence of degree of cleavage of 5S RNA of *E.coli* ribosomes with metal complexes **1**, **3**, **4**, and **5**. For reaction conditions see Experimental.

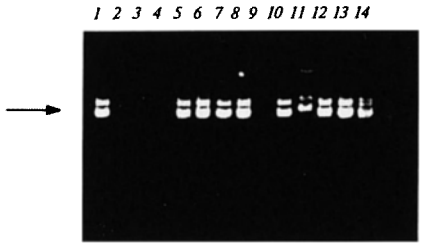

Fig. 3. Electrophoretic separation (agarose gel) of cleavage products of DNA-plasmid pUC19 in the presence of AA ($5 \cdot 10^{-2}$ mol L^{-1}) and metal complexes **1**—**11**: plasmid pUC19 under conditions of the reaction (*1*); complexes **7** (*2, 9*), **8** (*3*), **10** (*4*), **2** (*5*), **3** (*6, 10*), **5** (*7*), **6** (*8*), **1** (*11*), **4** (*12*), and **9** (*13*); plasmid pUC19 (control) (*14*). The position of the native DNA-plasmid pUC19 is marked by the arrow. The upper band corresponds to the relaxed form of the plasmid and the lower band corresponds to the superhelix form of DNA.

proximity. The radicals formed in the immediate vicinity to the NA-target can attack it more efficiently.

The effect of radical traps on the efficiency of the cleavage of NAs was studied to prove that the cleavage of NAs in the presence of MC involves active oxygen radicals. The following compounds were used as radical traps: *p*-nitrosodimethylaniline (PNDA) (trap of hydroxyl-radical) and enzymes catalase (whose substrate is H_2O_2) and superoxide dismutase (*SOD*) (whose substrate is superoxide anion-radical). It should be noted that *SOD* and catalase are insensitive to the radicals that are not present in the bulk.

In the case of 5S RNA, the addition of catalase and PNDA into the reaction mixture decreases the efficiency of the NA-target cleavage with complex **1** by 15—20 % on average (Fig. 4), whereas the addition of *SOD* does not affect the efficiency of cleavage. This fact can indicate that OH$^{\cdot}$ is the reactive species, which causes oxidative destruction of RNA. However, the introduction of radical traps does not practically affect the cleavage of DNA of plasmid pUC19 with complex **1**.

This difference in effects of radical traps on the efficiency of cleavage of RNA and DNA can reflect structural features of NA-targets, which determine their interaction with metal complexes. The interaction of MC with the "loose" spatial structure of RNA can be weaker than with the regular structure of DNA, and the radicals formed can escape the coordination sphere of the metal atom into the bulk, where they react with the traps. The interaction of MC with the regular structure of DNA is rather strong, thus, the AOS generated, apparently, do not escape the solution, but react directly with the DNA chain.

Experimental

Co and Fe phthalocyanine complexes were synthesized at the State Scientific Center of the Russian Federation NIOPIK and kindly provided by Prof. E. A. Luk'yanets and Prof. O. L. Kaliya. The structures of the complexes studied were reported previously.[1]

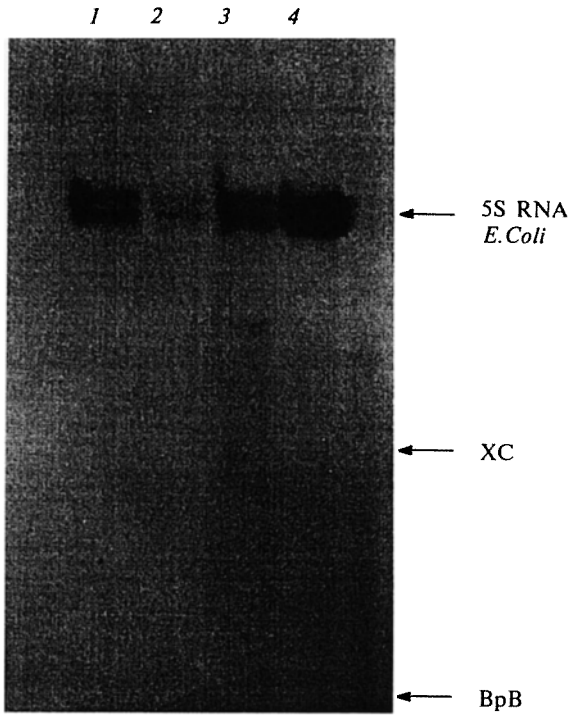

Fig. 4. The effect of radical traps on the efficiency of cleavage of 5S RNA of *E.coli* ribosomes in the presence of AA and complex **1**: total system + *n*-nitrosodimethylaniline ($5 \cdot 10^{-3}$ mol L^{-1}) (*1*), total system + *SOD* (0.1 mg $\cdot$ mL^{-1}) (*2*), total system + catalase (0.2 mg mL^{-1}) (*3*), 5S RNA of *E.coli* ribosomes (control) (*4*). For reaction conditions see Experimental. Positions of markers, *viz.*, Xylene cyanole (XC) and Bromophenol Blue (BpB) are marked by arrows.

5S RNA of *E.coli* and *Th. thermophila* ribosomes were purchased from Sigma. DNA-plasmid pUC19 containing the insert and consisting of 3264 bp, was isolated by ultracentrifugation in a CsCl gradient using a standard procedure.[10]

Hydrolysis of RNA in the presence of metal complexes. A 1 *M* *N*-ethylmorpholine buffer (pH 7.76, 1 μL), a $5 \cdot 10^{-2}$ *M* solution of AA (1 μL), a $1 \cdot 10^{-3}$ *M* solution of a metal complex (1 μL), and water (5 μL) were added to 5'-^{32}P-labeled 5S RNA of *E.coli* (10^4 cpm, OE_{260} 0.1, 2 μL). The reaction mixture was incubated at 37 °C for 30 min, then a solution of transfer RNA (tRNA) (5 mg mL^{-1}, 1 μL) as the coprecipitator, 3 *M* sodium acetate (pH 5.5, 5 μL), and 5 volumes of EtOH were added. The mixture was kept at −20 °C for 18—20 h, centrifuged, the precipitate was dissolved in 80 % aqueous formamide containing Xylene cyanole and Bromophenol Blue dyes. The products of hydrolysis were separated in 12 % PAAG, and their positions were determined by autoradiography. Gel bands corresponding to the initial, noncleaved RNA and its cleavage products were excised and examined with a Delta 300 counter (Tracor Analytic, USA) to determine the efficiency of hydrolysis.

Hydrolysis of DNA-plasmid pUC19 in the presence of metal complexes. A 1 *M* *N*-ethylmorpholine buffer (pH 7.76,

1 μL), a $5 \cdot 10^{-2}$ *M* solution of AA (1 μL), a $1 \cdot 10^{-3}$ *M* solution of a metal complex (1 μL), and water (6 μL) were added to a solution of DNA-plasmid pUC19 in a TE buffer (920 μg μL^{-1}, 1 μL). The reaction mixture was incubated for 30 min at 37 °C, then 1 *M* NaCl (3 μL) and 5 volumes of EtOH were added. The mixture was incubated at *ca.* 20 °C for 10 min and centrifuged, and the products of hydrolysis were separated in 1 % agarose gel and visualized with ethidium bromide.

Study of the effect of the nature and concentration of a buffer solution on the efficiency of RNA cleavage in the presence of metal complexes. 1 *M* solutions (5, 2, 1, and 0.5 μL) of the following buffers: *N*-ethylmorpholine buffer (pH 7.76), Tris-HCl (pH 7.53), HEPES (pH 7.5), and potassium phosphate buffer (pH 7.51) were added to 5′-^{32}P-labeled 5S RNA of *E.coli* (10^4 cpm, OE_{260} 0.1, 1 μL). Then a $5 \cdot 10^{-2}$ *M* solution of AA (1 μL), a $1 \cdot 10^{-3}$ *M* solution of a metal complex (1 μL) and water up to 10 μL were added. The reaction mixture was incubated at 37 °C for 30 min, then tRNA (5 mg $\cdot$ mL^{-1}, 1 μL) was added as the coprecipitator followed by 3 *M* sodium acetate (pH 5.5, 5 μL) and 5 volumes of EtOH. The mixture was incubated at −20 °C for 18—20 h and centrifuged and the precipitate was dissolved in 80 % aqueous formamide containing Xylene cyanole and Bromophenol Blue dyes. The products of hydrolysis were separated in 12 % PAAG and their positions were determined by autoradiography. The efficiency of hydrolysis was determined as described above.

Study of effects of AOS traps on the efficiency of RNA hydrolysis in the presence of $[FePc(CH_2N^+C_5H_5)_8]Cl^-_8$. A 1 *M* *N*-ethylmorpholine buffer (pH 7.76, 1 μL), a $5 \cdot 10^{-2}$ *M* solution of AA (1 μL), a $1 \cdot 10^{-3}$ *M* solution of a metal complex (1 μL), a solution of the corresponding radical trap (catalase: 2 mg mL^{-1}, *SOD*: 1 mg mL^{-1}, PNDA: $5 \cdot 10^{-2}$ mol L^{-1}), (1 μL), and water (4 μL) were added to 5′-^{32}P-labeled 5S RNA of *E.coli* (10^4 cpm, OE_{260} 0.1, 2 μL). The reaction mixture was incubated at 37 °C for 30 min, then tRNA (5 mg mL^{-1}, 1 μL) was added as the coprecipitator followed by 3 *M* sodium acetate (pH 5.5, 5 μL) and 5 volumes of EtOH. The mixture was incubated at −20 °C for 18—20 h and centrifuged and the precipitate was dissolved in 80 % aqueous formamide containing Xylene cyanole and Bromophenol Blue dyes. The products of hydrolysis were separated in 12 % PAAG and their positions were determined by autoradiography. The efficiency of hydrolysis was determined as described above.

Study of effects of AOS traps on the efficiency of hydrolysis of DNA-plasmid pUC19 in the presence of $[FePc(CH_2N^+C_5H_4)_8]Cl^-_8$. A 1 *M* *N*-ethylmorpholine buffer (pH 7.76, 1 μL), a $5 \cdot 10^{-2}$ *M* solution of AA (1 μL), a $1 \cdot 10^{-3}$ *M* solution of a metal complex (1 μL), a solution of the corresponding radical trap (catalase: 2 mg mL^{-1}, *SOD*: 1 mg mL^{-1}, PNDA: $5 \cdot 10^{-2}$ mol L^{-1}, all 1 μL), and water (5 μL) were added to a solution of DNA-plasmid pUC19 (920 mg mL^{-1}, 1 μL) in a TE-buffer. The reaction mixture was incubated at 37 °C for 30 min, then 1 *M* NaCl (3 μL) and 5 volumes of EtOH were added. The mixture was incubated at *ca.* 20 °C for 10 min and centrifuged. The hydrolysis products were separated in 1 % agarose gel and visualized with ethidium bromide.

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