Chemistry of Natural Compounds and Bioorganic Chemistry

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

1. Cobalt and iron phthalocyanine complexes

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Water-soluble Co and Fe phthalocyanines catalyze autooxidation of ascorbic acid and hydroquinone. Co and Fe phthalocyanines with cationic substituents in macroligands and also sulfo- and carboxy-substituted phthalocyanines were found to be the most active. Using the specific radical traps, the formation of active oxygen species ($O_2^{-\cdot}$, $OH^{\cdot}$) in the course of the dark reaction of autooxidation was established.

Key words: phthalocyanine complexes, autooxidation, active oxygen species.

It has been shown previously[1] that Fe and Co complexes with macrocyclic and chelating ligands, such as *o*-phenanthroline, corrin, and bis(salicylidene)ethylenediamine, are effective catalysts of autooxidation of biological substrates, and the formation of active oxygen species (AOS), *viz.*, $O_2^{-\cdot}$, H_2O_2, and $OH^{\cdot}$, in these reactions were established in some cases.[2] The role of AOS is discussed extensively in the literature in relation to biological activities of various types: antitumor, antiviral, and fungicidal.[3]

The search for new catalytic systems for generation of AOS among metal complexes of other types with macrocyclic ligands and the design of biologically active compounds on their basis are of great importance.

Metal phthalocyanines are of particular interest in this aspect. They are well-known as catalysts of oxidation of a wide range of organic and inorganic substrates,[4] and on the other hand, as efficient sensitizers for photodynamical therapy of cancer.[5]

Taking together the published data on autooxidation of phthalocyanines, the effect of reducing agents (hydrazine, hydroxylamine, ascorbic acid, *etc.*) on this process, and the data on catalysis of oxidation reactions and decomposition of peroxides and hydroperoxides by

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phthalocyanines,[4] one can assume that all these reactions involve AOS as the most important intermediates. Moreover, numerous indirect data (see for example, Refs. 6 and 7), which point to the formation of AOS in the reactions catalyzed by phthalocyanines.

Nevertheless, to the best of our knowledge, direct identification of AOS and determination of efficiency of their formation in reactions involving phthalocyanines and molecular oxygen were not carried out previously.

To solve these problems, we have studied:

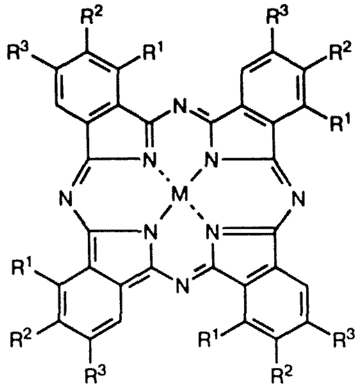
— the autooxidation of ascorbic acid and hydroquinone in the presence of metal phthalocyanines; the effect of radical traps on the rate of autooxidation;

— generation of superoxide anion radical in aqueous solutions of the complexes;

— decomposition of hydrogen peroxide and the formation of hydroxyl radical in the course of this process.

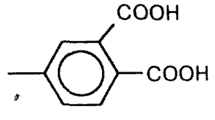
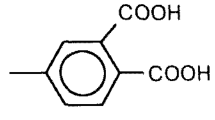
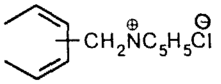
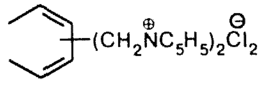
Co and Fe phthalo- and 2,3-naphthalocyanine complexes containing anionic (sulfo and carboxyl) or cationic (pyridiniomethyl and isothiuroniomethyl) groups at the macroligand were studied as catalysts of these reactions (Table 1).

Cobalt, $CoPc(COOH)_8$, and iron, $FePc(COOH)_8$, tetrakis(4,5-dicarboxy)phthalocyanines were prepared by interaction of pyromellitic anhydride with the corresponding metal salts in the presence of urea and catalytic amounts of ammonium molybdate followed by saponification of the resulting imides with alcoholic alkali and transformation of the salts into free acids. Cobalt, $CoPc[C_6H_3(COOH)_2]_4$, and iron,



$FePc[C_6H_3(COOH)_2]_4$, tetrakis-4-(3,4-dicarboxyphenyl)phthalocyanines were synthesized by heating a mixture of 3,3′,4,4′-tetracyanobiphenyl, the metal salt, and ammonium molybdate in benzophenone followed by saponification of the resulting nitriles. Chloromethylation of the corresponding phthalocyanines with *sym*-dichlorodimethyl ether in the presence of $AlCl_3$ and pyridine or triethylamine, analogously to chloromethylation of copper phthalocyanine,[8,9] yielded Co and Fe octakis(chloromethyl)phthalocyanines. Their reaction with pyridine (heating in toluene) gave pyridinium salts $[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ and $[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$. Similarly, pyridinium salt $[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$ was obtained from cobalt

Table 1. Co and Fe complexes with ligands of phthalocyanine series

Complex	M	R¹	R²	R³
$CoPc(COONa)_8$	Co	H	COONa	COONa
$FePc(COONa)_8$	Fe	H	COONa	COONa
$CoPc(SO_3Na)_2$*	Co			
$FePc(SO_3Na)_2$*	Fe			
$CoPc[C_6H_3(COOH)_2]_4$	Co	H		H
$FePc[C_6H_3(COOH)_2]_4$	Fe	H		H
$[CoPc(CH_2N^+C_5H_5)_4]Cl^-{}_4$*	Co			
$[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$*	Co			
$[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$	Co	H		
$[CoNc(CH_2N^+C_5H_5)_8]Cl^-{}_8$	Co	H		
$[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$*	Fe			
$[CoPc\{CH_2\overset{+}{S}C(NH_2)_2\}_8]Cl^-{}_8$*	Co			
$CoPc(SO_3H)_2$*	Co	H	H	H

* Positions of the substituents were not established.

tetrakis(chloromethyl)-2,3-naphthalocyanine. The reaction of cobalt octakis(chloromethyl)phthalocyanine with thiourea in the presence of H_3BO_3 yielded isothiouronium salt of cobalt octakis(chloromethyl)phthalocyanine $[CoPc\{CH_2SC(NH_2)_2{}^+\}_8]Cl^-{}_8$. Cobalt, $CoPc(SO_3H)_2$, and iron, $FePc(SO_3H)_2$, disulfophthalocyanines were synthesized by sulfation of unsubstituted phthalocyanines with chlorosulfonic acid at 100—105 °C. Sodium salts of all the aforementioned sulfo- and carboxyl-substituted phthalocyanines were prepared by treatment of aqueous solutions of the acids with a slight excess of alkali followed by concentration of the solutions to dryness.

Autooxidation of ascorbic acid (AH_2) and hydroquinone (Hq). Autooxidation of AH_2 and Hq in the presence of phthalocyanines can be presented schematically by reaction (1).

$$AH_2 + O_2 \xrightarrow{[M]} A + H_2O_2 \qquad (1)$$

The autooxidation was studied by spectrophotometry. The catalytic activitity of the complexes was characterized by the α value [$\alpha = (\Delta D/D) \cdot 100$ %], which reflects the conversion of the substrate for a definite time.

The results obtained show that under these conditions Fe and Co complexes with the cationic substituents in the ligand are the most efficient oxidation catalysts (Table 2), and pyridinium salts $[CoNc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ and $[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ are the most active among them.

The catalytic activity of the $[FePc(CH_2N^+C_5H_5)_8]Cl_8^-$ complex decreases threefold already 6 min after the beginning of the reaction. Special spectral measurements demonstrate that this is associated with the destruction of the complex itself during autooxidation.

Table 2. Oxidation of ascorbic acid (AH_2) and hydroquinone (Hq) in the presence of metal phthalocyanine complexes

Complex	$\alpha^* = (\Delta D/D)100$ %			
	AH_2		Hq	
	H_2O	DMSO	H_2O	DMSO
$[CoNc(CH_2N^+C_5H_5)_8]Cl^-{}_8$	51.5	—	5.2	—
$[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$	35.8	41.2	8.6	15.6
$[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$	10.5	—	1.0	—
$CoPc(SO_3H)_2$	8.8	2.2	0.3	0.6
$[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$	3.9	59.2	0.8	2.8
$FePc(COONa)_8$	1.6	**	0.5	**
$FePc(SO_3Na)_2$	1.4	44.3	—	3.6
$FePc[C_6H_3(COOH)_2]_4$	1.2	8.7	0.4	1.1
$[CoPc(CH_2N^+C_5H_5)_4]Cl^-{}_4$	0.8	13.2	0.6	0.7
$CoPc[C_6H_3(COOH)_2]_4$	0.1	0.7	0.2	0
$CoPc(COONa)_8$	0	**	0.3	**
$CoPc(SO_3Na)_2$	—	2.8	—	0
$[CoPc\{CH_2SC(NH_2)_2{}^+\}_8]Cl^-{}_8$	—	5.2	0.1	0.1

* Calculated for 1 min. ** Complexes are insoluble in DMSO.

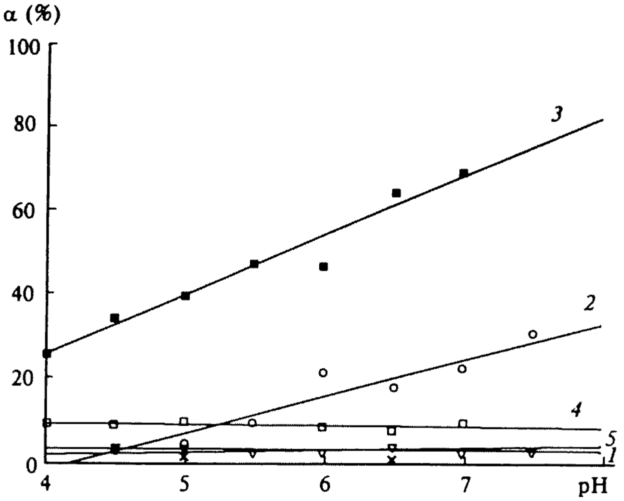


Fig. 1. pH-Dependence of autooxidation of ascorbic acid in the absence (*1*) and in the presence of complexes $[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ (*2*), $[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ (*3*), $FePc(SO_3Na)_2$ (*4*), $CoPc(COONa)_8$ (*5*).

As can be seen from Table 2, the complexes predissolved in DMSO are substantially more active in the autooxidation of ascorbic acid or hydroquinone in aqueous buffer solutions than the same complexes dissolved in water. It is of note that the addition of the same amount of DMSO to the aqueous solution of complex does not give this effect. Apparently, predissolution of these complexes in DMSO decreases substantially the aggregation characteristic of complexes with this structure due to axial coordination of DMSO. Simultaneously, the effective concentration of the active catalytic metal centers increases, and the autooxidation of substrates is accelerated.

The study of the effect of pH of the medium (pH from 4 to 8) on the catalytic properties of some complexes with cationic and anionic substituents at the macroligand has shown that the rate of autooxidation of ascorbic acid in the presence of complexes with cationic substituents is substantially higher and increases slowly with an increase in pH (Fig. 1). In the case of complexes with anionic substituents, the rate of autooxidation is small and it did not change in the pH range studied. Apparently, this reflects the importance of electrostatic interactions between the ascorbic acid monoanion (pK_a = 4.17) and the ionic complexes during autooxidation.

Formation of superoxide anion radical. In aqueous solutions of the complexes studied, the formation of superoxide anion radical ($O_2{}^{-\cdot}$) was monitored by the reduction of Nitro Blue Tetrazolium NBT^{2+} following its displacement from the coordination sphere of a metal with the CN^- ion (reaction (2)).

$$[M^{2+}] + O_2 \longrightarrow [M^{2+} \cdot O_2] \xrightarrow{KCN} [M^{3+}] + O_2^{\dot{-}} \qquad (2)$$

Table 3. Determination of $O_2^{-\cdot}$ from the reduction of NBT^{2+} in aqueous solutions of metal phthalocyanine complexes

Complex	$[O_2^{-\cdot}] \cdot 10^6$/mol
$CoPc(SO_3H)_2$	2.0
$FePc[C_6H_3(COOH)_2]_4$	1.6
$CoPc[C_6H_3(COOH)_2]_4$	1.3
$[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$	1.2
$FePc(SO_3Na)_2$	1.1
$CoPc(COONa)_8$	0.7
$[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$	0
$[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$	0
$FePc(COONa)_8$	0

It is known that in aqueous solutions, the reactions, which involve $O_2^{-\cdot}$, result in the reduction of NBT^{2+} into diformazan.[10]

The spectral data show (Table 3) that the addition of KCN to aqueous solutions of the most complexes results in the reduction of Nitro Blue Tetrazolium (NBT^{2+}). They also indicate the presence of $O_2^{-\cdot}$ in the coordination sphere of a metal in complexes $CoPc(SO_3H)_2$, $CoPc[C_6H_3(COOH)_2]_4$, $FePc(SO_3Na)_2$, $CoPc(COONa)_8$, and $FePc[C_6H_3(COOH)_2]_4$ in amounts comparable to the concentration of these complexes. However, in the case of cobalt complexes $[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ and $[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$ active in oxidation of ascorbic acid, and also of complex $FePc(COONa)_8$, the superoxide anion radical could not be detected by this method.

Formation of hydroxyl radical in the decomposition of hydrogen peroxide in the presence of metal complexes can occur according to the Fenton reaction (reaction (3)).

$$[M^{2+}] + H_2O_2 \longrightarrow {}^{\cdot}OH + OH^- + [M^{3+}] \qquad (3)$$

To determine the $OH^{\cdot}$ radical, we make use of the known fact[11] that 2-deoxyribose can undergo oxidative fragmentation under the action of the hydroxyl radical, to give, *inter alia*, malonaldehyde (MDA). The resulting fragments react with thiobarbituric acid (TBA) on heating giving colored products (λ_{max} 450 and 532 nm). Absorption at 450 nm is possibly related to the formation of an adduct of one molecule of TBA with a molecule of MDA.[12]

As can be seen from the data obtained (Table 4), hydroxyl radicals arise in the system containing phthalocyanine, hydrogen peroxide, and ascorbic acid, which is confirmed by the formation of TBA-reactive products. In the case of predissolution of the complexes in DMSO, which, as was mentioned above, enhances the autooxidation of AH_2, the increase in the yield of TBA-reactive adducts was also observed, which is an indirect indication of the involvement of the same catalytic centers in catalytic oxidation of ascorbic acid and in decomposition of hydrogen peroxide (reactions (1) and (3)).

Table 4. Determination of hydroxide radicals by oxidation of 2-deoxyribose

Complex	H_2O		DMSO	
	A_{450}	A_{532}	A_{450}	A_{532}
$FePc(SO_3Na)_2$	0.19	0.08	0.29	0.07
$[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$	0.16	0.03	0.43	0.03
$FePc(COONa)_8$	0.08	0.05	*	*
$CoPc(COONa)_8$	0.08	0.04	*	*
$CoPc(SO_3Na)_2$	—	—	0.29	0.17
$FePc[C_6H_3(COOH)_2]_4$	**	**	0.19	0.13
$CoPc[C_6H_3(COOH)_2]_4$	**	**	0.11	0.07
$CoPc(SO_3H)_2$	—	—	0.08	0.01

* Complexes are insoluble in DMSO. ** Complexes are insoluble in water.

The kinetics of decomposition of hydrogen peroxide by some phthalocyanine complexes was studied by differential pulse polarography.

Iron sulfophthalocyanine, $FePc(SO_3Na)_2$, was found to be the most active in decomposition of hydrogen peroxide (Table 5). $[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ and sodium salts of Co and Fe carboxyl-substituted phthalocyanines exhibit significant activity. The fact that the activity of certain complexes in decomposition of hydrogen peroxide correlates with their activity in oxidation of 2-deoxyribose is evidence of the formation of the hydroxyl radical in decomposition of H_2O_2 under the action of these complexes.

To confirm the possibility of autooxidation of ascorbic acid with the active oxygen species, we have studied the influence of the enzymes, catalase (*Cat*) and superoxide dismutase (*SOD*), on the rate of this reaction. The experiment was carried out using the most active complexes, $[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$, $FePc(SO_3Na)_2$, and $[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$. As can be seen from Table 6, in some cases, superoxide dismutase and, to a greater extent, catalase inhibit catalytic autooxidation of ascorbic acid, which can indicate the intermediate formation of $O_2^{-\cdot}$ and hydrogen peroxide in this reaction. The strongest inhibition was found in the presence of the most catalytically active complex $[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$: the addition of *SOD* decreased the degree of conversion of substrate from 57 % to 42 %, and the addition of catalase decreased it to

Table 5. The initial rates of decomposition of hydrogen peroxide with some phthalocyanine complexes

Complex	$\upsilon \cdot 10^5$/mol L^{-1} min^{-1}
$FePc(SO_3Na)_2$	9.80
$[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$	7.80
$FePc(COONa)_8$	7.60
$CoPc(COONa)_8$	5.60
$CoPc(SO_3H)_2$	2.00
$[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$	0.50

Table 6. Effect of superoxide dismutase (*SOD*) and catalase (*Cat*) on the catalytic properties of complexes $[\text{CoPc}(\text{CH}_2\text{N}^+\text{C}_5\text{H}_5)_8]\text{Cl}^-{}_8$, $[\text{FePc}(\text{CH}_2\text{N}^+\text{C}_5\text{H}_5)_8]\text{Cl}^-{}_8$, and $\text{FePc}(\text{SO}_3\text{Na})_2$

Complex	$\alpha^* = (\Delta D/D)\cdot 100$ %			
	Control	*SOD*	*Cat*	*SOD* + + *Cat*
AH_2	5.3	5.9	2.5	—
$[\text{CoPc}(\text{CH}_2\text{N}^+\text{C}_5\text{H}_5)_8]\text{Cl}^-{}_8$	21.6	20.4	16.6	—
$[\text{FePc}(\text{CH}_2\text{N}^+\text{C}_5\text{H}_5)_8]\text{Cl}^-{}_8$	57.4	42.3	9.7	4.3
$\text{FePc}(\text{SO}_3\text{Na})_2$	12.2	9.0	0.5	0.5

* Calculated for the initial 3 min.

10 %. In the case of the $\text{FePc}(\text{SO}_3\text{Na})_2$ complex, addition of *SOD* affects the catalytic activity only insignificantly, while catalase inhibited autooxidation of ascorbic acid almost completely. Independently of the structure of the complex, the simultaneous addition of catalase and superoxide dismutase increases the inhibition of autooxidation only slightly and is comparable with the effect of catalase alone.

This effect of catalase on autooxidation of ascorbic acid in the presence of iron phthalocyanine complexes confirms the formation of hydrogen peroxide in this reaction, which, in turn, can decompose efficiently to generate hydroxyl radicals as was exemplified by oxidation of 2-deoxyribose in the presence of the $\text{FePc}(\text{SO}_3\text{Na})_2$ complex. This is in favor of involvement of the OH radicals in the catalytic systems of autooxidation of ascorbic acid and is in accord with literature data[13] on ascorbic acid as the ambident reagent, which is capable of binding oxygen radicals and reducing metal ions.

Complex $[\text{CoPc}(\text{CH}_2\text{N}^+\text{C}_5\text{H}_5)_8]\text{Cl}^-{}_8$ was found to be significantly less sensitive to the action of catalase and superoxide dismutase (see Table 6). This fact shows the substantial differences in behavior of the Co and Fe complexes. For example, as was mentioned above (see Table 3), in the presence of cobalt complex $[\text{CoPc}(\text{CH}_2\text{N}^+\text{C}_5\text{H}_5)_8]\text{Cl}^-{}_8$, one cannot detect oxygen following its displacement from the coordination sphere of the metal in the form of superoxide anion radical, in contrast to the aforementioned iron complexes $[\text{FePc}(\text{CH}_2\text{N}^+\text{C}_5\text{H}_5)_8]\text{Cl}^-{}_8$ and $\text{FePc}(\text{SO}_3\text{Na})_2$. Apparently, in the case of cobalt complexes, hydrogen peroxide is not formed as a free intermediate of oxidation, which is in accord with the known data on the difference in the mechanisms of the Fenton reaction in the presence of Co and Fe complexes.[14]

Taken together, the results obtained show that the system containing water-soluble cobalt (or iron) phthalocyanine and ascorbic acid can be regarded as an efficient source of active oxygen species, such as the superoxide anion radical and the hydroxyl radical.

Experimental

Cobalt tetrakis-4-(3,4-dicarboxyphenyl)phthalocyanine $\text{CoPc}[\text{C}_6\text{H}_3(\text{COOH})_2]_4$. A mixture of 3,3',4,4'-tetracyanobiphenyl* (3.3 g, 13 mmol), anhydrous CoCl_2 (0.61 g, 5 mmol), $(\text{NH}_4)_2\text{MoO}_4$ (0.33 g, 2 mmol), pyridine (0.5 mL, 6 mmol), and benzophenone (5 g, 27 mmol) was stirred at 275—278 °C for 2 h and cooled. The melt was ground, treated with hot water, filtered off, and dried. Resulting cobalt tetrakis(dicyanophenyl)phthalocyanine (4.7 g, 6 mmol) was refluxed for 7 h in 50 % alcoholic KOH (1 : 10, w/w), the dense suspension was diluted with 2 volumes of ethanol, filtered while hot, and the precipitate was washed with ethanol and chloroform on the filter and dried. The dry residue was dissolved in water, acidified with conc. HCl, and the precipitate was washed on the filter with 5 % HCl yielding 2.52 g (63 %) of the complex. Found (%): C, 63.01; H, 2.42; N, 9.68. $\text{C}_{64}\text{H}_{32}\text{CoN}_8\text{O}_{16}$. Calculated (%): C, 62.59; H, 2.63; N, 9.13. UV (alcoholic solution of triethylamine), λ_{max}/nm: 333, 680.

Iron tetrakis-4-(3,4-dicarboxyphenyl)phthalocyanine $\text{FePc}[\text{C}_6\text{H}_3(\text{COOH})_2]_4$ was prepared analogously using FeBr_2 (under He, at 263—265 °C), yield 50 %. UV (alcoholic solution of triethylamine), λ_{max}/nm: 320, 620 sh, 681.

Iron tetrakis(4,5-dicarboxy)phthalocyanine $\text{FePc}(\text{COOH})_8$.[16] A mixture of pyromellitic anhydride (10.9 g, 0.05 mmol), urea (30 g, 0.05 mmol), anhydrous Na_2SO_4 (20 g, 0.141 mmol), FeBr_2 (2.7 g, 12.5 mmol), and $(\text{NH}_4)_2\text{MoO}_4$ (0.5 g, 2.5 mmol) was stirred at 200—205 °C for 3 h. The melt was cooled, ground, and refluxed with water (*ca.* 1 L). The suspension was filtered, the precipitate was washed on the filter successively with hot water, 5 % HCl, and again with water and dried. The resulting imide was refluxed for 6 h with 50 % alcoholic KOH (60 mL), the mixture was diluted with ethanol, the suspension was filtered, and the precipitate was washed with ethanol until the filtrate became colorless and dried. The dry residue was dissolved in water, the solution was filtered, and acidified with conc. HCl to pH 1. The precipitate formed was filtered off, washed on the filter with 5 % HCl, and dried. The product was additionally purified by continuous extraction of impurities with hot MeOH and then with acetone. The yield of the complex was 5.83 g (43 %). Found (%): C, 50.85; H, 1.99; N, 11.67. $\text{C}_{40}\text{H}_{16}\text{FeN}_8\text{O}_{16}$. Calculated (%): C, 51.24; H, 1.83; N, 11.96.

Sodium salt of iron tetrakis(4,5-dicarboxy)phthalocyanine $\text{FePc}(\text{COONa})_8$. Iron tetrakis(4,5-dicarboxy)phthalocyanine (3.4 g, 3.7 mmol) was dissolved in distilled water (1.5 L) containing NaOH (1.18 g, 30 mmol), the precipitate was filtered off, the filtrate was concentrated to dryness on a boiling water bath and dried additionally at 110—115 °C to constant weight. The yield is quantitative (4.10 g).

Sodium salt of cobalt tetrakis(4,5-dicarboxy)phthalocyanine $\text{CoPc}(\text{COONa})_8$ was prepared analogously from pyromellitic anhydride, urea, and CoCl_2 (0.075 : 0.75 : 0.019) in the presence of Na_2SO_4 and $(\text{NH}_4)_2\text{MoO}_4$ in a yield of 42.5 %.

Cobalt octakis(chloromethyl)phthalocyanine $\text{CoPc}(\text{CH}_2\text{Cl})_8$. Triethylamine (6 mL, 44 mmol) was added dropwise with stirring to anhydrous AlCl_3 (15 g, 112 mmol). The mixture was cooled to *ca.* 20 °C and $\text{ClCH}_2\text{OCH}_2\text{Cl}$ (9 mL, 105 mmol) was added dropwise. The mixture was heated to 65 °C, cobalt phthalocyanine (0.75 g, 1.3 mmol) was added. The mixture

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* Prepared analogously to pyromellitonitrile[15] by dehydration of the corresponding tetraamide.

was heated at 95 °C for 2 h, after cooling to *ca.* 20 °C, it was poured into a mixture of ice (100 g) and conc. HCl (10 mL). The resulting suspension was filtered, the precipitate was washed on the filter successively with 5 % HCl, water (to neutral reaction), ethanol, and acetone and dried on air. The precipitate was treated with $CHCl_3$. The filtrate was concentrated to dryness and a mixture of products of chloromethylation was obtained (0.39 g, 30 %), from which cobalt octakis(chloromethyl)phthalocyanine (0.2 g, 15 %) was isolated by column chromatography on Al_2O_3 ($CHCl_3$ as the eluent). Found (%): Cl, 29.22. $C_{40}H_{24}Cl_8CoN_8$. Calculated (%): Cl, 29.56. UV (chlorobenzene), λ_{max}/nm (log ε): 678 (5.00), 646 (4.59), 614 (3.49), 334 (3.69).

Cobalt octakis(pyridiniomethyl)phthalocyanine $[CoPc(CH_2N^+C_5H_5)_8]Cl^-{}_8$. *A.* A suspension of cobalt octakis(chloromethyl)phthalocyanine (0.15 g, 0.16 mmol) in pyridine (13 mL, 160 mmol) was refluxed with stirring for 1 h; after cooling to *ca.* 20 °C, the precipitate was filtered off, washed with acetone on the filter and dissolved in water (20 mL). The solution was filtered again, the filtrate was concentrated *in vacuo* to dryness. The residue was treated with ethanol, the suspension was filtered, and the precipitate was dried on air yielding 0.24 g of the complex (95 %). Found (%): N, 13.75; Cl, 18.40. $C_{80}H_{64}Cl_8CoN_{16}$. Calculated (%): N, 14.08; Cl, 17.82. UV (water), λ_{max}/nm (relative intensity): 675, 610 sh, 340 (2.00 : 0.85 : 1.00).

B. Pyridine (1.6 mL, 20.2 mmol) was added to a suspension of cobalt octakis(chloromethyl)phthalocyanine (1.2 g, 1.28 mmol) in dry toluene (75 mL) and the mixture was stirred at 90—95 °C for 7 h and cooled, the precipitate was filtered off, washed on the filter with toluene, and dried. The product was dissolved in water (100 mL), the solution was filtered, conc. HCl (0.3 mL) was added and the solution was concentrated on a boiling water bath to dryness. The residue was ground, stirred with acetone (20 mL), filtered off, and dried. The yield of pyridinium salt was 1.91 g (96.5 %). Found (%): C, 60.14; H, 3.65; N, 13.82; Cl, 18.33. $C_{80}H_{64}Cl_8CoN_{16}$. Calculated (%): C, 60.35; H, 4.05; N, 14.08; Cl, 17.82.

Analogously, pyridinium salt $[FePc(CH_2N^+C_5H_5)_8]Cl^-{}_8$ was prepared from iron octakis(chloromethyl)phthalocyanine in a yield of 95.5 %. Found (%): C, 59.72; H, 3.85; N, 13.76; Cl, 17.95. $C_{80}H_{64}Cl_8FeN_{16}$. Calculated (%): C, 60.47; H, 4.06; N, 14.10; Cl, 17.85. UV (water), λ_{max}/nm (relative intensity): 689, 605 sh, 335 (1.15 : 0.75 : 1.00).

Cobalt tetrakis(pyridiniomethyl)phthalocyanine $[CoPc(CH_2N^+C_5H_5)_4]Cl^-{}_4$. A mixture of cobalt tetrakis(4-chloromethyl)phthalocyanine (1.54 g, 2 mmol) prepared by chloromethylation of cobalt phthalocyanine with paraform in chlorosulfonic acid in the presence of NaCl,[17] toluene (80 mL), and pyridine (2.5 mL, 2.4 g, 30 mmol) was stirred at 90—92 °C for 7 h, the precipitate was filtered off, washed with toluene, dried, and then dissolved in distilled water (100 mL). The resulting solution was filtered and the filtrate was concentrated on a boiling water bath. The residue was dried yielding 2.15 g (99.5 %) of pyridinium salt. Found (%): N, 15.12; Cl, 13.88. $C_{56}H_{40}Cl_4CoN_{12}$. Calculated (%): N, 15.54; Cl, 13.11.

Cobalt octakis(isothiouroniomethyl)phthalocyanine $[CoPc\{CH_2SC(NH_2)_2{}^+\}_8]Cl^-{}_8$. A mixture of cobalt octakis(chloromethyl)phthalocyanine (1.2 g, 1.28 mmol), thiourea (0.95 g, 12.5 mmol), and H_3BO_3 (0.77 g, 12.5 mmol) in water (12 mL) was stirred at 85—90 °C for 1 h, cooled, and diluted with water (200 mL). The precipitate was filtered off and washed with water until the filtrate became colorless. Mother liquors were concentrated to dryness on a boiling water bath. The residue was ground, stirred with ethanol, the suspension was filtered, and the precipitate was washed on the filter with ethanol several times and dried. The yield of isothiouronium salt was 1.91 g (97.5 %). Found (%): C, 36.20; H, 3.38; N, 21.12; Cl, 18.29; S, 15.58. $C_{48}H_{56}Cl_8CoN_{24}S_8$. Calculated (%): C, 36.77; H, 3.61; N, 21.45; Cl, 18.09; S, 16.36. UV (water), λ_{max}/nm (relative intensity): 679, 640, 340 (1.55 : 0.95 : 1.00).

Cobalt octakis(chloromethyl)-2,3-naphthalocyanine $CoNc(CH_2Cl)_8$. Triethylamine (4.4 mL, 32 mmol) was added dropwise with stirring to anhydrous $AlCl_3$ (11 g, 82 mmol), the mixture was cooled to *ca.* 20 °C, and $ClCH_2OCH_2Cl$ (6.6 mL, 77 mmol) was added. The mixture was heated to 65 °C, cobalt 2,3-naphthalocyanine[18] (0.55 g, 0.7 mmol) was added, the temperature was increased to 95 °C and the mixture was kept at this temperature for 2 h. After cooling to *ca.* 20 °C, the reaction mixture was poured into a mixture of ice (100 g) and conc. HCl (10 mL). The resulting suspension was filtered, the precipitate was washed on the filter successively with 5 % HCl, water (to neutral reaction), ethanol, and acetone, dried on air, and treated with $CHCl_3$. The octakis(chloromethyl) derivative was isolated from the filtrate by column chromatography on silica gel ($CHCl_3$—EtOH, 200 : 1). The yield was 0.28 g (33.7 %). Found (%): Cl, 24.35. $C_{56}H_{32}Cl_8CoN_8$. Calculated (%): Cl, 24.46. UV (quinoline), λ_{max}/nm (relative intensity): 760, 690 (1.18 : 1.00).

Cobalt octakis(pyridiniomethyl)-2,3-naphthalocyanine $[CoNc(CH_2N^+C_5H_5)_8]Cl^-{}_8$. A mixture of cobalt octakis(chloromethyl)-2,3-naphthalocyanine (0.12 g, 0.10 mmol) and pyridine (10 mL, 124 mmol) was refluxed with stirring for 1 h. After cooling to *ca.* 20 °C, the reaction mixture was filtered, the precipitate was washed with acetone on the filter and then dissolved in water (5 mL). Acetone (250 mL) was added to the filtrate, the resulting fine precipitate was filtered off, washed with acetone, and dried on air. The yield of the complex was 0.12 g (65 %). Found (%): N, 12.03; Cl, 15.62. $C_{96}H_{72}Cl_8CoN_{16}$. Calculated (%): N, 12.50; Cl, 15.85. UV (water), λ_{max}/nm (log ε): 750 (4.97), 668 (4.38), 332 (4.87).

Cobalt tetrakis(chloromethyl)-2,3-naphthalocyanine $CoNc(CH_2Cl)_4$ and its pyridinium salt $[CoNc(CH_2N^+C_5H_5)_4]Cl^-{}_4$. Chloromethylation of cobalt 2,3-naphthalocyanine with a mixture of paraform and chlorosulfonic acid analogously to the procedure for copper phthalocyanine[9] yielded cobalt tetrakis(chloromethyl)-2,3-naphthalocyanine in a yield of 76 %. Found (%): Cl, 14.87. $C_{52}H_{28}Cl_4CoN_8$. Calculated (%): Cl, 14.73. Pyridinium salt was prepared in a yield of 95 % by the reaction of cobalt tetrakis(chloromethyl)-2,3-naphthalocyanine with pyridine in toluene at 90—95 °C analogously to the preparation of pyridinium salt of cobalt octakis(chloromethyl)phthalocyanine. Found (%): N, 13.25; Cl, 11.15. $C_{72}H_{48}C_{14}CoN_{12}$. Calculated (%): N, 13.13; Cl, 11.08.

Cobalt octakis(isothiouroniomethyl)-2,3-naphthalocyanine $[CoNc\{CH_2SC(NH_2)_2{}^+\}_8]Cl^-{}_8$. A mixture of cobalt octakis(chloromethyl)-2,3-naphthalocyanine (0.12 g, 0.10 mmol) and thiourea (1 g, 13.9 mmol) in water (25 mL) was refluxed for 1 h. The hot reaction mixture was filtered, and the filtrate was poured into acetone (200 mL). The precipitate formed was filtered off, washed with ethanol and acetone, and dried. The yield of isothiouronium salt was 0.11 g (60 %). Found (%): S, 14.15. $C_{64}H_{64}Cl_8CoN_{24}S_8$. Calculated (%): S, 14.48. UV (water), λ_{max}/nm (log ε): 714 (4.61).

Cobalt disulfophthalocyanine $CoPc(SO_3H)_2$. A mixture of cobalt phthalocyanine (1.0 g, 1.96 mmol) and chlorosulfonic acid (4 mL, 1.5 mmol) was stirred at 100—105 °C for 3 h and cooled, the solution was poured onto crushed ice (50 g), and a 15 % solution of NaOH was added to pH 7. The mixture was stirred for 4—5 h, acidified with conc. HCl (2—3 mL), and stirred for 0.5 h. The suspension was filtered, the precipitate was washed with 2 % HCl, filtered and dried *in vacuo* at 95—100 °C. The yield of cobalt disulfophthalocyanine was 0.96 g (74 %). Found (%): N, 14.62; S, 8.01. $C_{32}H_{16}CoN_8O_6S_2$. Calculated (%): N, 15.32; S, 8.77.

Sodium salt of cobalt disulfophthalocyanine $CoPc(SO_3Na)_2$. Cobalt disulfophthalocyanine (0.72 g, 1 mmol) was dissolved in distilled water (100 mL) containing NaOH (0.08 g, 2 mmol), the solution was filtered, the filtrate was concentrated to dryness on a boiling water bath and additionally dried to constant weight at 105—110 °C. The yield was quantitative. UV (water), λ_{max}/nm (relative intensity): 663, 600 sh, 333 (1.64 : 0.72 : 1.00).

Iron disulfophthalocyanine $FePc(SO_3H)_2$ was prepared analogously to cobalt disulfophthalocyanine in a yield of 69 %.

Sodium salt of iron disulfophthalocyanine $FePc(SO_3Na)_2$ was prepared analogously to sodium salt of cobalt disulfophthalocyanine in a quantitative yield. UV (water), λ_{max}/nm: 663, 630 sh, 333.

Catalytic properties of metal phthalocyanine complexes were studied by spectrophotometry with a Specord M-400 spectrophotometer in glass cuvettes (1 cm path length).

Autooxidation of ascorbic acid and hydroquinone was studied in a 0.05 *M* phosphate buffer (pH 6.0 and 7.8, respectively). The initial reaction rate was measured by the decrease in absorptions of ascorbic acid (λ 262 nm) or hydroquinone (λ 286 nm) within 1.5—2.5 min after the beginning of the reaction. The final concentrations of reagents in the cuvette were $5 \cdot 10^{-5}$ mol L^{-1} of AH_2 or $5 \cdot 10^{-4}$ mol L^{-1} of Hq and $5 \cdot 10^{-6}$ mol L^{-1} of the complex. To study the effect of DMSO on the rate of oxidation of the substrate, the complex was dissolved in DMSO; the final concentration of DMSO in the cuvette was 0.5 %.

Effect of enzymes *Cat* and *SOD* on the rate of autooxidation of ascorbic acid was studied using the above procedure. The final concentration of *SOD* (Serva) in the cuvette was 25 mg mL^{-1}, *Cat* (Olaine plant of chemical reagents), 50 mg mL^{-1}.

Formation of the superoxide anion in aqueous solutions in the presence of metal complexes was quantitated using the reduction of Nitro Blue Tetrazolium (NBT^{2+}) following its displacement by the CN^- ion. The final concentrations of the reagents (mol L^{-1}) in the cuvette were: $5 \cdot 10^{-6}$ for the complex, $5 \cdot 10^{-5}$ for NBT^{2+}, and $8 \cdot 10^{-4}$ for KCN. The reaction was carried out in a 0.05 *M* phosphate buffer (pH 7.8). The reaction was monitored following the absorption at λ 257 nm (NBT^{2+}).

Formation of the hydroxyl radical in the decomposition of hydrogen peroxide in the presence of phthalocyanines and ascorbic acid was determined by oxidation of 2-deoxyribose using the following procedure. The reaction mixture (2 mL) containing a complex ($5 \cdot 10^{-5}$ mol L^{-1}), ascorbic acid ($5 \cdot 10^{-4}$ mol L^{-1}), 2-deoxyribose (10^{-3} mol L^{-1}), and hydrogen peroxide ($4.4 \cdot 10^{-4}$ mol L^{-1}) in a 0.02 *M* phosphate buffer (pH 7.4) was incubated at 37 °C for 60 min. Then a 1 % solution of 2-thiobarbituric acid in 0.05 *M* NaOH (1 mL) and 2.8 % aqueous trichloroacetic acid (1 mL) were added, the mixture was stirred intensely and heated at 100 °C for 10 min. After cooling, absorptions at λ = 450 and 532 nm were measured.

Kinetics of decomposition of hydrogen peroxide was studied by differential pulse polarography with a PA2 laboratory polarograph (Czechoslovakia) in a thermostatted polarographic cell at 25 °C in a 0.05 *M* phosphate buffer (pH 6.0) flushed with Ar; mercury dropping electrode and silver chloride electrodes were used as measuring and reference electrodes, respectively. To study the kinetics of decomposition of H_2O_2 the height of polarographic wave of H_2O_2 at $E_{1/2}$ = 975 mV was measured at definite intervals. The initial concentrations were 10^{-3} mol L^{-1} for H_2O_2 and 10^{-4} mol L^{-1} for the complex, repectively.

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