Coordination compounds of cobalt porphyrins with nitrogen monoxide

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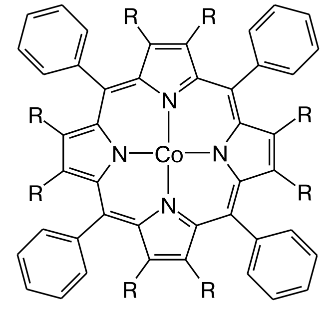
Complex formation of NO and the NO_2^- ion with cobalt porphyrins bearing various substituents in the porphyrin macrocycle, *viz*., tetraphenylporphine (**1**), β-octabromo-*meso*-tetraphenylporphyrin (**2**), protoporphyrin IX (**3**), and 5,10,15,20-tetra(4*N*-carboxymethylene-pyridyl)porphyrin tetrabromide (**4**), was studied. The stability constants of the nitrosyl and nitrite extracomplexes in water and in ethanol were determined. Porphyrin **4** forms the most stable extracomplexes.

Key words: coordination compounds, cobalt(II) porphyrins, nitrogen monoxide, nitrite ion, nitrosyl complexes, nitrite complexes, stability constants.

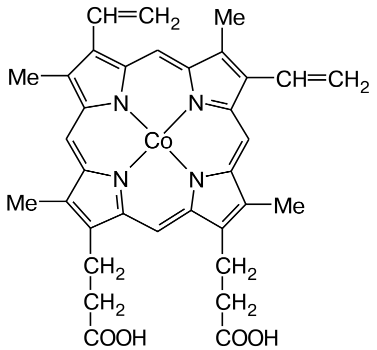
Nitrogen monoxide is produced by enzymatic systems in mammals and acts as a versatile regulator of metabolic processes.[1—3] Nitrogen monoxide is found[4,5] to be an endogenic vasodilator and neurotransmitter and oppresses thrombocyte aggregation; the superoxide radical and NO during oxidative stress act in conjugation.

Nitrogen monoxide is produced in biological systems by NO synthases from L-arginine, which is classified as an enzyme forming the $NO^{\bullet}$ free radical and citrulline as a by-product.[6] In the organism nitrogen monoxide is rapidly oxidized to stable end products, NO_2^- and NO_3^- ions. The nitrite ion *in vivo* oxidizes oxyhemoglobin and catalyzes reactions involving NO. The mechanism of toxic effect of nitrites is mainly attributed to the oxidation of hemoglobin to methemoglobin accompanied by the violation of oxygen transport to tissues due to which hemic hypoxia is developed. Nitrogen monoxide can coordinate to thiols, sulfur—iron proteins of plasma, secondary amines, and hemoglobin and be consumed in reactions with oxygen, superoxide radicals, and hemoglobin.[5—8] Therefore, non-heme models of iron-containing proteins are being developed and their nitrosyl complexes are under study.[9] However, the single compensatory adjustment form of NO depositing and utilization is complex formation with hemoglobin that occurs *via* axial coordination on the protoheme.[10] Therefore, investigation of binding (axial coordination) processes of nitrogen monoxide and its metabolite (nitrite ion) by metal porphyrins in solutions is key for understanding of the essence of biological processes involving NO.

In the present work, we studied the equilibrium of formation of the axial NO and NO_2^- complexes with Co^{II} porphyrins of different structural types: tetraphenylporphine (**1**), β-octabromo-*meso*-tetraphenylporphyrin (**2**), protoporphyrin IX (**3**), and 5,10,15,20-tetra(4*N*-carboxymethylenepiridyl)porphyrin tetrabro-



1, 2



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R = H (**1**), Br (**2**)

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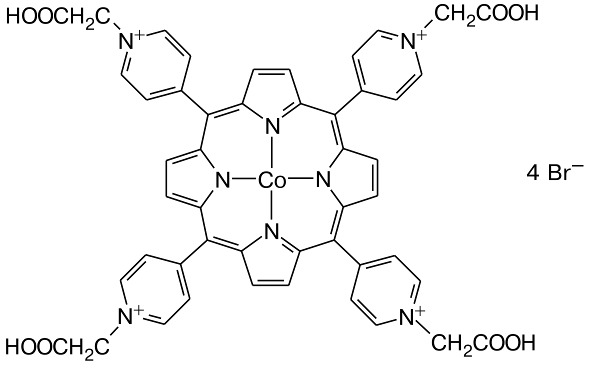
mide (**4**). The complex of protoporphyrin IX (**3**) was chosen as the closest analog of natural protoheme.



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Complex **4** is soluble in aqueous solutions in a wider pH range than complex **3**; this allows one to study its interaction with NO at physiological acidity values of the medium. Although protoheme is a water-soluble complex, in the hemoglobin composition its nearest environment is hydrophobic. Therefore, it seems reasonable to study complexes **1** and **2** that are soluble in organic solvents. These compounds have different sets of peripheral substituents with different donor-acceptor properties and different structures of the central macrocyclic ring: planar for complex **1** and distorted for complex **2**.

Experimental

Electronic absorption spectra were recorded on a Specord M-400 spectrophotometer in 50-mL cells (optical path 100 mm) at 293.0±0.1 K. Complex formation was studied by spectrophotometry. A solution of porphyrin with a concentration of 10^{-6}—10^{-5} mol L^{-1} in ethanol or water was deaerated with an Ar flow. The exact volume of an NO-saturated solution was added to the cell with a microsyringe through a rubber septa to prevent contact with air oxygen.[11]

IR spectra were recorded on an Avatar 360 FT—IR ESP spectrometer in the 400—4000 cm^{-1} interval in anhydrous EtOH and in KBr pellets.

Ethanol used as solvent was purified according to a known procedure.[12] The residual water content determined by titration with the Fischer reagent[13] was 0.05%. Sodium nitrite (reagent grade) was twice recrystallized.[14] Saturated solutions of NO in water and EtOH (solvent was deaerated with an Ar flow) were prepared purging the gas containing 0.46% NO and 99.54% N_2 from the cylinder through the solvent. Nitrogen monoxide was obtained according to an earlier described procedure.[14] For this purpose, copper chips were placed into a two-neck flask and HNO_3 (density 1.10—1.15 g cm^{-3}) was slowly added from a dropping funnel. The reaction mixture was cooled to avoid the formation of other nitrogen oxides. To remove higher nitrogen oxides, the gas formed was passed through a flask containing a 5% solution of NaOH and collected above water. The gas was dried by passing through a tube packed with solid KOH. The concentration of nitrogen monoxide in solutions was determined spectrophotometrically using a special procedure.[15] Purity of porphyrins **1—4** was checked by TLC on aluminum plates with the 0.5-mm fixed layer of silica gel F_{254} (Merck) with a $CHCl_3$—EtOH eluate (ratio of solvents was chosen depending on individual properties of porphyrins) and by the electronic absorption spectra, which corresponded to literature data.[16—18]

Results and Discussion

The electronic spectra of Co porphyrins **1—4** in water and in EtOH were studied with purging a gaseous mixture of nitrogen and nitrogen monoxide (NO content in nitrogen is 0.49%) through the solution and showed the immediate formation of the axial (NO)Co**P** complexes (**P** is porphyrin). This is indicated by a considerable bathochromic shift of the Soret band and a slight shift of the visible spectral band. The spectral characteristics are given in Table 1. The synthesized coordination compounds are rather stable, and their spectra are also invariable for at least 1 day.

On contact with air oxygen the electronic absorption spectrum changes in time: a hypsochromic shift is observed (Soret band for porphyrin **4** is shown in Fig. 1). This is related, most likely, to the oxidation of NO to NO_2^- and is confirmed by the appearance of a new absorption band at 352 nm (see Fig. 1), which is characteristic of a solution of the nitrite ion in EtOH.

Upon the addition of a solution of sodium nitrite to solutions of the cobalt porphyrins, the character of the visible part of the electronic absorption spectrum remains unchanged and typical of the cobalt(II) complexes. In this case, the Soret band shifts hypochromically by 4—9 nm, depending on the nature of the porphyrin ligand (see Table 1).

Table 1. Electronic absorption spectra of the cobalt porphyrin complexes (Co**P**)

CoP	λ/nm (logε)		
	Q band I	Q band II	Soret band
		In ethanol	
1	—	540(4.61)	426(5.68)
NO-**1**		545	432
NO_2^--**1**		542	428
2		562(4.20)	445(5.21)
NO-**2**			462
4	608(3.63)	550(3.75)	429(5.33)
NO-**4**		555	446
NO_2^--**4**			437
		In water	
3 (pH 9)	564 (3.98)	534 (4.01)	417 (5.41)
NO_2^--**3**			420
4 (pH 7.4)	595 (sh)	540 (3.78)	432 (5.02)
NO-**4**		545	440
NO_2^--**4**			437

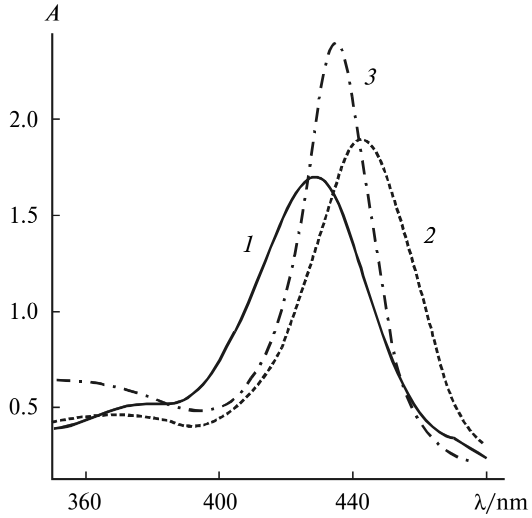

Fig. 1. Electronic absorption spectra of complexes **4** (*1*), NO-**4** (*2*), and NO_2^--**4** (*3*).

Additional bands at 2256, 1925, and 1273 cm^{-1} appear in the IR spectrum of the solution upon the saturation of ethanol with nitrogen monoxide. Based on the theoretical calculations and experimental data,[19—20] these bands should be attributed to stretching vibrations of NO^+, NO, and NO^-, respectively. The study of the IR spectra of complexes **1** and **2** showed that after saturation with nitrogen monoxide all the three types of ligands appeared in their alcoholic solutions: NO^+, NO, and NO^- (bands at 2256, 1925, and 1273 cm^{-1}). In addition, an additional band at 1659 cm^{-1} appears in the spectra. After argon purging, the band at 1925 cm^{-1} disappears and the absorption spectrum returns to the initial spectrum of Co**P**. After air purging, the both bands (1925 and 1659 cm^{-1}) disappear and the electronic absorption spectrum corresponds to that of the Co**P**(NO_2^-) nitrite complex. These data make it possible to assign the absorption bands at 1925 and 1659 cm^{-1} to the free and coordinated nitrogen monoxide, respectively.

The position of the band of stretching vibrations ν_{NO} of the pentacoordinate nitrosyl Co porphyrins changes from 1693 cm^{-1} (KBr) for complex NO-**1** (1683 cm^{-1} in CH_2Cl_2) to 1655 cm^{-1} (Nujol) for the octaethylisobacteriochlorin with NO (see Refs 20—23) and 1660 cm^{-1} (Nujol) for the octaethylporphyrin complex with NO, depending on the nature of substituents in the porphyrin macrocycle. The X-ray diffraction data[20,21] indicate that the cobalt(II) complexes coordinate nitrogen monoxide through the nitrogen atom to form nitrosyl complexes with the nonlinear Co—N—O bonds, the angle between which being ~120°. The axial ligand interacts with the coordination center due to the electron density transfer from the π-orbital of NO to the d_{z^2}-orbital of the cobalt cation. The decrease in the frequency of stretching vibrations of nitrogen monoxide in the axial complex NO-**4** compared to those in the NO-**1** complex is associated with the partial positive charge on the nitrogen atoms of the pyridinium substituents resulting in electron density pulling through the conjugated system of the porphyrin and metal atom from the N—O bond in nitrogen monoxide.

The stability constants (*K*) of the axial complexes of NO and NO_2^- with complexes **1—4** were determined by spectrophotometric titration. The characteristic example for spectral changes during titration of the cobalt porphyrin complexes with a solution of nitrogen monoxide is presented in Fig. 2.

In the general case, the extraligands coordinate with the Co porphyrins according to the equation

$$\mathrm{Co}\mathbf{P} + n\mathrm{L} \rightleftharpoons \mathrm{L}_n\mathrm{Co}\mathbf{P}, \qquad (1)$$

$n = 1$ or 2

where Co**P** is the porphyrin complex with Co^{II}, L_nCo**P** is its axial complex, and L is the axial ligand (in this case, NO or NO_2^-). For the systems studied, *n* is 1. The stability constants of the axial complexes (*K*) were calculated by the equation

$$K = [\mathrm{LCo}\mathbf{P}]/([\mathrm{Co}\mathbf{P}] \cdot [\mathrm{L}]). \qquad (2)$$

The absorbances at the analytical wavelengths were used for the calculation of the equilibrium concentrations of the extra-complex

$$c_{\mathrm{LCo}\mathbf{P}} = c^0{}_{\mathrm{Co}\mathbf{P}}(A - A_0)/(A_\kappa - A). \qquad (3)$$

Here $c_{\mathrm{LCo}\mathbf{P}}$ is the equilibrium concentration of LCo**P**, $c^0{}_{\mathrm{Co}\mathbf{P}}$ is the initial concentration of Co**P**, and A_0, A, and A_f are the initial, equilibrium, and final absorbances, respectively. Experiments were carried out under the conditions where $c^0{}_L \gg c^0{}_{\mathrm{Co}\mathbf{P}}$; the number of added ligands *n* was determined from the slope of the plots of

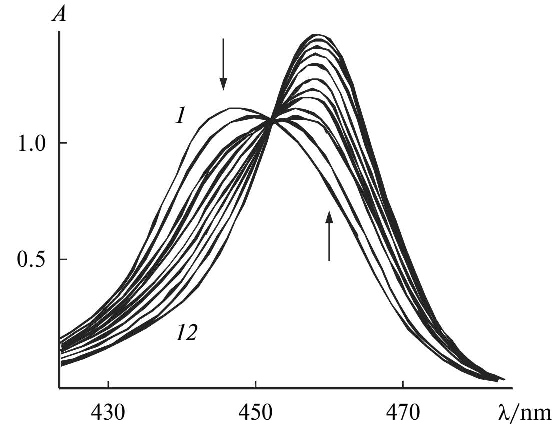

Fig. 2. Electronic absorption spectra of complex **2** ($c^0{}_{\mathrm{Co}\mathbf{P}}$ = $1.2 \cdot 10^{-6}$ mol L^{-1}) with additives of an NO solution (c_L = 10^{-4}—10^{-3} mol L^{-1}) in ethanol at T = 298 K.

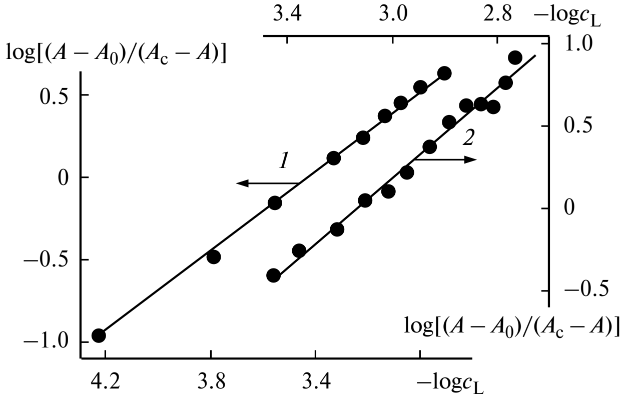

Fig. 3. Plots of $\log[(A-A_0)/(A_c-A)]$ *vs.* $\log c_L$ in ethanol at T = 298 K for **4**—NO (*1*) and **4**—NO_2^- (*2*) systems.

$\log[(A-A_0)/(A_f-A)]$·*vs.* $\log c_L$. Within the determination error, n is unity (Fig. 3). Thus, the Co porphyrins studied form the pentacoordinate nitrosyl and nitrite axial complexes.

As a whole, porphyrin **4** forms more stable axial complexes than porphyrins **1** and **2** (Table 2). The simultaneous introduction of eight bromine atoms into the β-positions of the pyrrole rings and four benzene residues into the *meso*-positions of the macrocycle of porphyrin **2** changes the effective charge on the reaction center due to the electron-withdrawing effect of Br and also considerably distorts the planar structure of the aromatic ligand.[24] This also favors a decrease in the stability of the axial complexes of porphyrin **2** with NO and NO_2^- compared to the complexes of compounds **1**, **3**, and **4** having a planar structure of the macrocycle. The decrease in K of the nitrite complexes compared to the nitrosyl complexes for all systems under study indicates the predominantly electrostatic character of the Co—NO_2 bond.

The experiments with white rats performed at the Ivanovo State Medical Academy confirmed the sorption properties of porphyrin Co**P**(**4**) in the blood and brain of experimental animals. This indicates that regulators of the content of biologically active compounds in the animal organism can be developed and produced.

Table 2. Stability constants of the nitrosyl and nitrite complexes of cobalt porphyrins at 298 K and analytical wavelengths

Porphyrin	Solvent	λ_a/nm	K/L mol^{-1}	
			NO	NO_2^-
1	EtOH	426, 432	10400±500	47±3
2	EtOH	445, 462	2690±200	—
3	EtOH	421		160±20
	H_2O (pH 9)	420		3.9±0.5
4	EtOH	429, 446	24200±500	810±200
	H_2O (pH 7.4)	437		26±4

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