Two decomposition mechanisms of nitrosyl iron complexes $[Fe_2(\mu\text{-}SR)(NO)_4]$

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Two decomposition mechanisms of nitrosyl iron complexes (NICs) $[Fe_2(\mu\text{-}SR)(NO)_4]$ in aqueous medium are known. One mechanism (for instance, in the case of complex $[Fe_2(\mu\text{-}SC_4H_3N_2)_2(NO)_4]$) involves irreversible and rapid hydrolysis of NIC with the NO release accompanied with the formation of the products of further NO transformations. In the other mechanism (for instance, in the case of complexes $[Fe_2(\mu\text{-}S(CH_2)_2NH_3)_2(NO)_4]SO_4 \cdot 2.5H_2O$ and $[Fe_2(\mu\text{-}SC_5H_{11}NO_2)_2(NO)_4]SO_4 \cdot 5H_2O$), no hydrolysis occurs but NICs reversibly dissociate to release both NO and thiolate ligand into the medium. In the present work, the difference in the mechanisms of the NIC decomposition is explained by the difference in the NIC redox potentials. The experimental evidences of this fact are given.

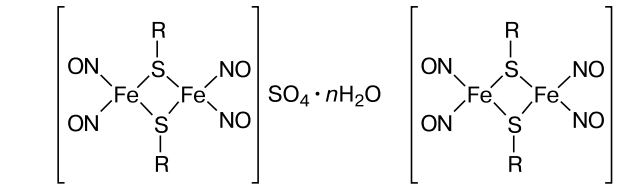
Key words: nitrosyl iron complexes, nitric oxide donor, cytochrome C, redox potential.

Numerous recent studies revealed an essential role of nitric oxide (NO) as the most important physiological regulator.[1,2] This prompted an increase in the interest to the synthesis and study of new compounds capable of NO delivering to target tissues under biological pH values and being the candidates for design of a new generation of NO-releasing drugs.[3] Among these compounds, thiolate nitrosyl iron complexes (NICs) $[Fe_2(SR)_2(NO)_4]$ hydrolyzing in protic media similarly to S-nitrosothiols[4] and diazenium dialates[5,6] with the NO release[7] are outstanding and comprise a new class of universal NO donors for pharmacological applications.[8—10]

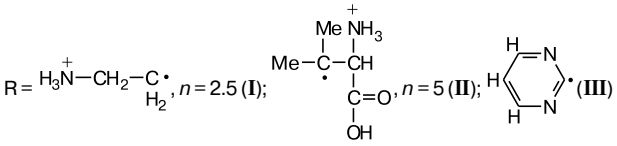
The studies focused on the mechanism of action of NICs and their transformations are of special interest. Earlier,[7,11] we have found that sulfur-nitrosyl iron complexes spontaneously decompose in protic media to release NO and the decomposition rate constants depend on the molecular structure of the complexes. Introduction of deoxyhemoglobin (Hb) into the medium resulted in the HbNO adduct formation. This adduct has a role of a nitric oxide depot and is not only a storage pool of NO (the lifetime of free NO in the living cell is a few seconds[12]) but also determines prolonged action of NICs as NO donors.

Apart from Hb, ferricytochrome C (cyt c^{3+}) can also form relatively stable nitrosyl complexes despite the fact that the heme iron of cyt c^{3+} is completely coordinated to four N atoms of porphyrin core and amino acids (histidine-18 and methionine-80).[13] We have recently found[14] that introduction of NIC $[Fe_2(\mu\text{-}SR)(NO)_4]$ bearing cysteamine thiol ligands (**I**) into the cyt c^{3+} solution gives the NO-cyt c^{3+} complex. The reactions of cyt c^{3+} with nitric oxide significantly affect the mitochondrial respiratory chain.[13] For instance, the formation of the NO-cyt c^{3+} complex can inhibit electron transfer. Moreover, the reaction of NO with cyt c^{3+} can modify the peroxidase activity of cyt c^{3+}, which is apparently applicable for the apoptosis control.[13]

Complexes **I** and **II** show vasodilation[15] and antitumor[16] activities. Complex **I** induces apoptosis of human erythroleukemic cells (K562) and human colon cancer cells (LS174T).[17]



I, II **III**



Earlier, we have described two mechanisms of the decomposition of NICs $[Fe_2(\mu\text{-}SR)(NO)_4]$. One mechanism involves hydrolysis of NIC **III** resulting in its complete decomposition;[18,19] the other mechanism is a reversible dissociation of cationic NICs $[Fe_2(\mu\text{-}SR)(NO)_4]$ (complexes **I** and **II**) to release both NO and thiolate ligand into the medium.[20,21] Due to this, complexes **I** and **II** undergo ligand exchange in the presence of excess gluta-

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thione to give more stable complexes with glutathione ligand.[17] The detected exchange between thiolate and glutathione ligands may result in the regeneration of the glutathionylated essential enzymes and the recovery of their antitumor activity. The discovered ligand exchange reaction occurring in NICs is also of interest for predicting antitumor activity of NICs. The aim of the present work is to find the reasons for the difference in decomposition mechanisms of NIC **III** and NICs **I** and **II**.

Experimental

Commercially available 2-amino-2-hydroxymethylpropane-1,3-diol (Tris) (Serva, Germany), $Na_2HPO_4 \cdot 6H_2O$, $NaH_2PO_4 \cdot H_2O$, safranin T, methylene blue (MP Biomedicals, Germany), methyl viologen, iron(II) sulfate, D-penicillamine, and cytochrome C from equine heart (SIGMA, USA) were used. Water was purified by distillation using a Bi/Duplex apparatus (Germany).

Complexes **I** (CCDC 663194), **II** (CCDC 680286), and **III** (CCDC 681094) were synthesized following the known procedures.[16,18,22]

Elemental analyses of the crystalline samples were carried out in the Analytical Centre of Collective Use of the Institute of Problems of Chemical Physics of the Russian Academy of Sciences.

IR spectra were recorded with Spectrum BX-II IR-Fourier-transform spectrophotometer in the KBr pellets (1 mg of the sample for 300 mg of KBr).

All procedures were performed under the inert gas atmosphere as earlier described.[7]

Complex I, $[Fe_2(\mu_2\text{-}SC_2H_7N)_2(NO)_4]SO_4 \cdot 2.5H_2O$. Found (%): C, 8.53; H, 2.77; N, 15.70; S, 17.71. $C_4H_{19}Fe_2N_6O_{10.5}S_3$. Calculated (%): C, 9.10; H, 3.60; Fe, 21.25; N, 15.93; O, 31.87; S, 18.27. IR, ν/cm^{-1}: 3454 (m), 3003 (w), 2927 (w), 1769 (s), 1727 (s), 1461 (w), 1385 (m), 1340 (m), 1266 (m), 1120 (s), 770 (w), 620 (s); 1769, 1727 (νNO).

Complex II, $[Fe_2(\mu_2\text{-}SC_5H_{11}NO_2)_2(NO)_4]SO_4 \cdot 5H_2O$. Found (%): Fe, 15.60; C, 16.72; H, 4.50; N, 11.75; O, 38.02; S, 13.40. $C_{10}H_{32}Fe_2N_6O_{17}S_3$. Calculated (%): Fe, 15.64; C, 16.76; H, 4.47; N, 11.73; O, 37.99; S, 13.41. IR, ν/cm^{-1}: 1771 (s), 1723 (s), 1626 (m), 1375 (m), 1337 (m), 1269 (m), 1189 (m), 1114 (m), 1089 (m), 746 (m); 1771, 1723 (νNO).

Complex III, $[Fe_2(SC_4H_3N_2)_2(NO)_4]$. Found (%): C, 2.00; H, 1.17; Fe, 24.47; N, 23.83; S, 13.96. $C_8H_6Fe_2N_8O_4S_2$. Calculated (%): C, 2.12; H, 1.34; Fe, 24.59; N, 24.68; S, 14.15. IR, ν/cm^{-1}: 3472 (w), 1797 (b.s), 1746 (v.s), 1551 (m), 1425 (w), 1376 (m), 1191 (w), 1151 (m), 1070 (w), 811 (w), 774 (w), 739 (w), 629 (w), 550 (w); 480 (w); 1797, 1746 (νNO).

Hydrolysis of NIC III. A freshly prepared $6 \cdot 10^{-3}$ *M* solution of NIC **III** in DMSO was used. To a weighted sample of NIC **III** place in a vial filled with argon, anaerobic DMSO was added in the amount to obtain a stock solution with concentration of $6 \cdot 10^{-3}$ mol L^{-1}. The mixture was stirred under an argon flow until complete dissolution of the complex (3—5 min). Then 0.1 mL of the resulting solution was placed into an anaerobic experimental cuvette (V = 4 mL, optical path length of 1 cm) containing 2.9 mL of anaerobic 0.05 *M* phosphate buffer (pH 7.0). A reference cuvette contained 0.05 *M* phosphate buffer (pH 7.0). Working concentration of NIC **III** was $2 \cdot 10^{-4}$ mol L^{-1}, concentration of DMSO was 3.3%. The absorption spectra were recorded at selected time intervals at the 450—650 nm range at 25 °C.

Hydrolysis of NIC III in the presence of $K_3[Fe(CN)_6]$. A freshly prepared $6 \cdot 10^{-3}$ *M* solution of NIC **III** in DMSO was used. An aliquot of 0.1 mL of the complex solution was introduced into an anaerobic experimental cuvette containing 2.6 mL of anaerobic 0.05 *M* phosphate buffer (pH 7.0). A reference cuvette contained 2.7 mL of 0.05 *M* phosphate buffer (pH 7.0). The reaction was initiated by addition of 0.3 mL of an anaerobic $4 \cdot 10^{-2}$ *M* solution of $K_3[Fe(CN)_6]$ in 0.05 *M* phosphate buffer (pH 7.0). Working concentration of NIC **III** was $2 \cdot 10^{-4}$ mol L^{-1}, concentration of DMSO was 3.3%. Absorption spectrum was recorded immediately after addition of $K_3[Fe(CN)_6]$.

Decomposition of NIC II. The experiments were carried out with the same stock solution of NIC **II**. To a weighted sample of NIC **II** placed in a vial filled with nitrogen, 0.05 *M* Tris-HCl (pH 7.0) was added in the amount to obtain $6 \cdot 10^{-4}$ mol L^{-1} stock solution. The mixture was stirred for 15 min until complete dissolution of the complex. The solution was collected by an anaerobic syringe and frozen dropwise into liquid nitrogen. The stock solutions of NIC **II** were thawed under a nitrogen flow for 20 min prior to use. Then an aliquot of the solution of 0.75 mL was added into a cuvette (V = 4 mL, optical path length of 1 cm) containing 2.25 mL of 0.05 *M* anaerobic buffer (pH 7.0) to obtain working concentration of NIC **II** of $1.5 \cdot 10^{-4}$ mol L^{-1}. A reference cuvette contained 3 mL of the same buffer. Absorption spectra were recorded at selected time intervals at the 250—650 nm range at 25 °C.

Decomposition of NIC II in the presence of $K_3[Fe(CN)_6]$ was carried out as described for NIC **III** using 0.05 *M* Tris-buffer (pH 7.0).

Kinetics of the reactions of NICs II and III with c^{3+} in the presence of $K_3[Fe(CN)_6]$. The experiments were carried out with the same $2 \cdot 10^{-4}$ *M* stock solution of cytochrome C (commercial cytochrome C contained 10% of ferricytochrome). A weighted sample of cytochrome C was dissolved in 0.05 *M* phosphate buffer (pH 7.0) to obtain a stock solution with concentration of $2 \cdot 10^{-4}$ mol L^{-1}. After stirring for 40 min, the obtained solution was frozen dropwise into liquid nitrogen. The stock solution of cytochrome C was thawed for 20 min in the 5-mL vials under an argon flow immediately prior to use. To 2.6 mL of $2 \cdot 10^{-4}$ *M* cytochrome C solution placed into an anaerobic cuvette, 0.3 mL of $4 \cdot 10^{-2}$ mol L^{-1} solution of $K_3[Fe(CN)_6]$ was added. A reference cuvette contained 2.6 mL of 0.05 *M* phosphate buffer (pH 7.0) and 0.3 mL of $K_3[Fe(CN)_6]$ solution. The absorption spectrum was recorded with the aim to confirm the obtaining of cyt c^{3+}. The reaction was initiated by simultaneous addition of 0.1 mL of $6 \cdot 10^{-4}$ mol L^{-1} solution of NIC **III** in DMSO into both the experimental and reference cuvettes. The volumes of both reaction mixtures were 3 mL. The working concentration of NIC **III** in the experimental cuvette was $2 \cdot 10^{-5}$ mol L^{-1}. The difference absorption spectra were recorded at selected time intervals at the 450—650 nm range at 25 °C.

The reactions of cyt c^{3+} with NIC **II** in the presence of $K_3[Fe(CN)_6]$ were carried out similarly.

Reaction of NIC II with cytochrome C without $K_3[Fe(CN)_6]$. Into an anaerobic cuvette containing 1 mL of 0.05 *M* phosphate buffer (pH 7.0), 1 mL of $1.8 \cdot 10^{-4}$ mol L^{-1} solution of commercial cytochrome C in 0.05 *M* phosphate buffer (pH 7.0) was added. A reference cuvette contained 2 mL of 0.05 *M* phosphate

buffer (pH 7.0). The reaction was initiated by simultaneous addition of 1 mL of $6 \cdot 10^{-4}$ mol L^{-1} solution of NIC **II** in the same buffer into both cuvettes. Working concentration of NIC **II** was $2 \cdot 10^{-4}$ mol L^{-1} in both cuvettes. The difference absorption spectra were recorded at selected time intervals at the 450—650 nm range at 25 °C.

Reactions of NICs I and II with redox indicators. Into an anaerobic cuvette ($V = 4$ mL, optical path length of 1 cm) containing 2 mL of a redox indicator solution with stock concentration of either $3 \cdot 10^{-5}$ or $1.5 \cdot 10^{-5}$ mol L^{-1}, 1 mL of $6 \cdot 10^{-4}$ mol L^{-1} solution of NICs **I** and **II** was added. A reference cuvette contained 2 mL of water and 1 mL of a solution of NIC **I** or **II**. The following working concentrations were obtained: NICs of $2 \cdot 10^{-4}$ mol L^{-1}, redox indicator of either $1 \cdot 10^{-5}$ or $2 \cdot 10^{-5}$ mol L^{-1}. Absorption spectra were recorded at the 450—750 nm range at 25 °C.

Absorption spectra were recorded at 25 °C with a Specord M-40 spectrophotometer equipped with an interface for computer-aided registration of spectra and a thermostatic cuvette holder.

Measurements of amount of cyt c^{3+} and NO-cyt c^{3+}. Amounts of cyt c^{3+} and NO-cyt c^{3+} were estimated using absorption spectra. To find the concentration of NO-cyt c^{3+}, the absorption spectra of the system containing cyt c^{3+} and NO-cyt c^{3+} were resolved into individual components (cyt c^{3+} and NO-cyt c^{3+}) using MathCad software as previously described.[7]

Results and Discussion

Earlier,[18,19] the mechanism of decomposition of NIC **III** with pyrimidine thiolate ligands, (tetranitrosyl)[μ-*S*-bis(pyrimidin-2-ylthio)diiron], has been described. The kinetic dependences of the amount of the NO released by NIC **III** into the media at different pH values and the same other conditions show maxima, which appearance was attributed to the further NO transformations.[18] It was suggested that these transformations proceed in two following steps:

$$\mathrm{LFe(NO)} \xrightarrow{k_1} \mathrm{LFe + NO}, \qquad (1)$$

where L = $[Fe(\mu\text{-}SC_4H_3N_2)_2(NO)_3]$,

$$\mathrm{NO} \xrightarrow{k_2} \mathrm{Products}. \qquad (2)$$

Since nitrous oxide is formed in the solutions of dinitrosyl iron complexes, it was assumed[23] that the final product of the NO transformations is N_2O. Formation of nitrous oxide is indicative of the N—N bond formation. Two molecules of NO cannot be strongly bound to each other. For the N—N bond formation to occur, the reduced form of NO molecule is required. It is known that[24] NO and NO^- react with very high rate to give hyponitrite radical anion $ONNO^-$ exhibiting strong oxidative properties. Further transformations of this species gives nitrous oxide.[25] Two nitroxyls (protonated form of NO^-) dimerize affording unstable molecule HONNOH, which decomposes into N_2O and H_2O. However, protonation of NO^- is a spin-forbidden reaction; therefore, the most probable elementary step producing nitrous oxide is the reaction between NO and NO^-. In both cases, reduction of the NO molecules is the most probable initial stage of the reaction resulting in the nitrous oxide formation: $NO + e^- = NO^-$. To produce NO^- from NO, the reducing agent is required; the standard redox potential of NO is $-0.8(\pm 0.2)$ V.[26] Complex **III** can play a role of the reducing agent after the Fe—NO bond dissociation and coordination of a solvent (water) molecule at an open coordination site. The authors rationalized the enhanced reducing ability of the resulting species in terms of the increased electron density near the Fe atom due to the loss of the partially negative NO ligand and coordination of the donor water molecule. Therefore, two following reactions consuming NO can be suggested. The first reaction (reaction (3)) is the electron transfer oxidation of iron complex

$$\mathrm{LFe + NO} \xrightarrow{k_3} \mathrm{LFe_{ox} + NO^-}. \qquad (3)$$

The second reaction (reaction (4)) is the deep transformation occurring only when the relatively large amounts of the reduced NO^- species are released *via* reaction (3) and accumulated in the medium.

$$\mathrm{NO + NO^-} \longrightarrow \mathrm{ONNO^-} \longrightarrow \mathrm{N_2O} \qquad (4)$$

Studying the reactions of NIC **I** with cyt c^{3+},[14] two following important findings were made. First, the number of the NO groups released by NIC **I** can be determined by monitoring the NO-cyt c^{3+} adduct formation. Second, $K_3[Fe(CN)_6]$ oxidizes complex **I** resulting in the release of all NO groups of NIC **I** immediately after addition of ferricyanide. Therefore, we suggested that reaction (3) is a key step causing irreversible decomposition of NICs.

First of all, we studied the kinetics of the decomposition of NIC **III** (Fig. 1, *a*) and its reaction with $K_3[Fe(CN)_6]$ (see Fig. 1, *b*). Immediately after mixing the reagents, intensity of absorption noticeably decreased indicating the decomposition of NIC **III**.

To determine the number of the released NO groups, cyt c^{3+} was used. The absorption spectra shown in Fig. 2 visualize the reaction of NIC **III** with cyt c^{3+}. Due to reversibility of the formation of the NO-cyt c^{3+} adduct *via* reaction cyt c^{3+} + NO $\rightleftarrows$ NO-cyt c^{3+}, the excess of cyt c^{3+} is required.[14] The experiment (see Fig. 2) was conducted under the most optimal conditions. The absorption of the cyt c^{3+} solution with the used starting concentration is nearly 2. To avoid the distortion of the spectra, the cyt c^{3+} concentration cannot be increased. The NIC concentration cannot exceed $2 \cdot 10^{-5}$ mol L^{-1} without loss of accuracy due to small sample volume. The reaction should be performed in the presence of $K_3[Fe(CN)_6]$ since in the absence of potassium ferricyanide cyt c^{3+} is reduced to ferrocytochrome; the latter does not react with NO at pH 7.[27] Figure 2 shows that the reaction results in a rapid

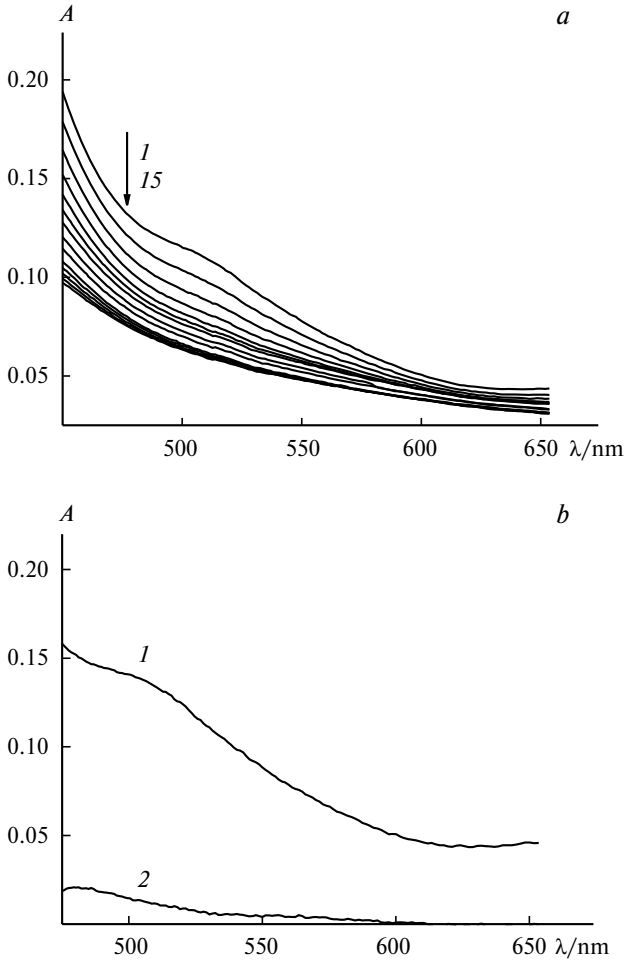

Fig. 1. *a*, Changes in the absorption spectra of NIC **III** ($2 \cdot 10^{-4}$ mol L^{-1}). Spectra were registered after 1 (*1*) and 5 min (*2*) after preparation of the NIC solution. Spectra (*3*)—(*15*) were recorded at 15 min time intervals. *b*, Absorption spectra for the reaction of NIC **III** ($2 \cdot 10^{-4}$ mol L^{-1}) with $K_3[Fe(CN)_6]$ ($4 \cdot 10^{-3}$ mol L^{-1}): *1*, spectrum of the starting NIC **III**; *2*, spectrum recorded immediately after addition of $K_3[Fe(CN)_6]$. Reaction conditions (here and on Figs 2 and 4): 25 °C, 0.05 *M* phosphate buffer (pH 7.0).

formation of $5 \cdot 10^{-5}$ mol L^{-1} of NO-cyt c^{3+}, which indicates the release and binding with cyt c^{3+} of 2.5 NO groups. Under the same conditions, NIC **I** decomposes similarly producing 3.08 NO groups.[14]

In the present work, we also studied the reaction of $K_3[Fe(CN)_6]$ with another cationic complex, namely, NIC **II**. First, we studied the kinetics of the decomposition of NIC **II** (Fig. 3, *a*). Figure 3, *a* shows the time dependence of the absorption of NIC **II**. Similarly to NIC **III**, addition of $K_3[Fe(CN)_6]$ results in a rapid decomposition of the oxidized NIC **II**, which is evidenced by the noticeable decreased in the VIS absorption intensity (see Fig. 3, *b*).

It is clear that complex **II** decomposes immediately after addition of $K_3[Fe(CN)_6]$ as it was found in the case

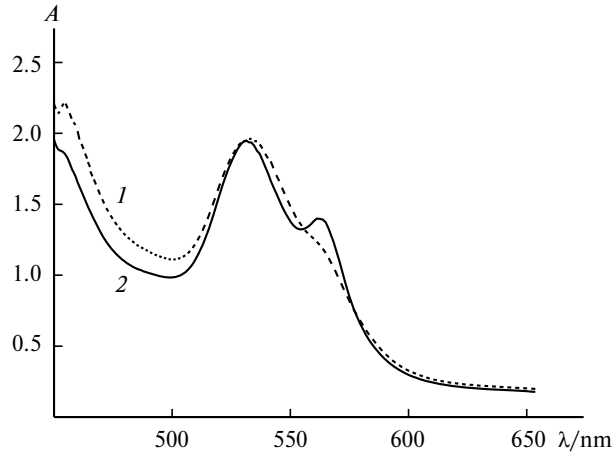

Fig. 2. Changes in the difference absorption spectra for the reaction of NIC **III** ($2 \cdot 10^{-5}$ mol L^{-1}) with $K_3[Fe(CN)_6]$ ($4 \cdot 10^{-3}$ mol L^{-1}) and cyt c^{3+} ($1.5 \cdot 10^{-4}$ mol L^{-1}): *1*, spectrum of a solution containing cyt c^{3+} and $K_3[Fe(CN)_6]$; *2*, spectrum recorded immediately after addition of NIC **III**.

of NIC **III**. To confirm the fast release of the NO groups during this process, we performed the reaction with cyt c^{3+} (Fig. 4). Mixing the reagents causes the rapid formation of adduct NO-cyt c^{3+}. Concentration of this adduct was $6.7 \cdot 10^{-5}$ mol L^{-1}, which corresponds to the release of 3.3 NO groups per 1 molecule of NIC **II**.

At the same time, we found that in other cases (in the absence of oxidizing agents) no hydrolysis of complexes[20,21] $[Fe_2(\mu\text{-}S(CH_2)_2NH_3)_2(NO)_4]SO_4 \cdot 2.5H_2O$ and $[Fe_2(\mu\text{-}SC_5H_{11}NO_2)_2(NO)_4]SO_4 \cdot 5H_2O$ occurs but NICs reversibly dissociate to release both NO and thiolate ligand into the medium. Assuming that ligands (L) are released from the NIC **II** and further from product P_1 formed by the nitric oxide release from NIC **II**, a plausible mechanism of the transformation can be presented as follows:[21]

$$\mathbf{II} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P_1 + NO,$$

$$P_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P_2 + L,$$

$$\mathbf{II} \xrightarrow{k_3} P_3 + L.$$

Earlier,[17] the measurements of the k_1 and k_{-1} rate constants by a sensor electrode gave the following values: $k_1 = (4.6 \pm 0.1) \cdot 10^{-3}$ s^{-1} and $k_{-1} = (9.7 \pm 0.2) \cdot 10^3$ L mol^{-1} s^{-1}. To describe the system completely, the k_2 (the reaction rate constant of the penicillamine ligand releasing from NIC **II**), k_{-2}, and k_3 values should be determined. We suggest that NIC **II** and product P_1 cannot be distinguished spectrophotometrically. Consequently, we can measure experimentally only $x(t) = [\mathbf{II}] + [P_1]$.

The reaction scheme is described by the following kinetic equations:

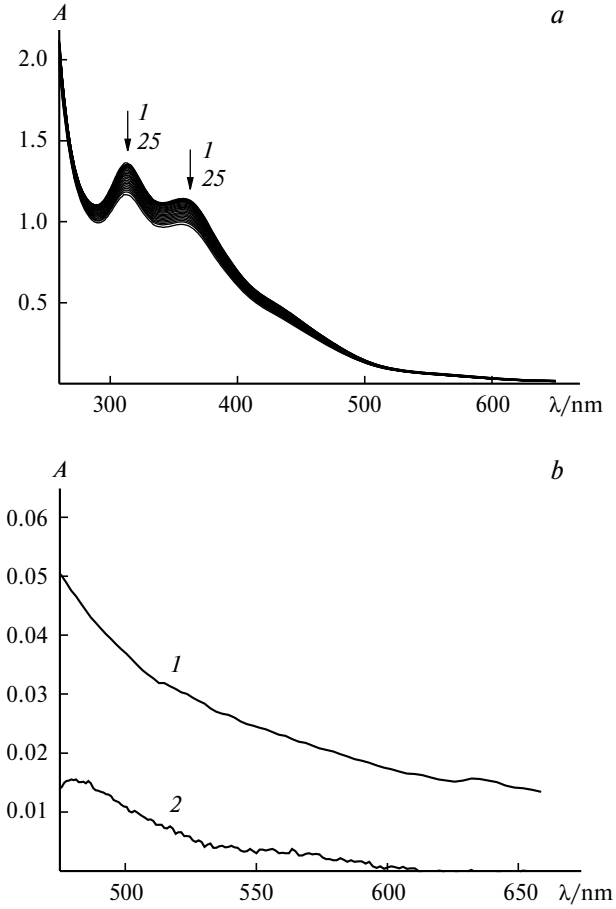

Fig. 3. *a*, Changes in absorption spectra of NIC **II** ($1.5 \cdot 10^{-4}$ mol L^{-1}). Spectra were recorded after 1 (*1*), 5 (*2*), and 10 min (*3*) after preparation of the NIC solution; spectra *4* and *5* were recorded at 10 min intervals, spectra *6*—*8*, at 15 min time intervals, spectra *9*—*25*, at 30 min time interval. *b*, Changes in the absorption spectra for the reaction of NIC **II** ($2 \cdot 10^{-5}$ mol L^{-1}) with $K_3[Fe(CN)_6]$ ($4 \cdot 10^{-3}$ mol L^{-1}): *1*, spectrum of the starting NIC **II**; *2*, spectrum recorded immediately after addition of $K_3[Fe(CN)_6]$. Reaction conditions: 25 °C, tris-HCl (pH 7.0).

$$d[\mathbf{II}]/dt = -k_1[\mathbf{II}] + k_{-1}[P_1][NO] - k_3[\mathbf{II}],$$

$$d[NO]/dt = k_1[\mathbf{II}] - k_{-1}[P_1][NO],$$

$$d[P_1]/dt = k_1[\mathbf{II}] - k_{-1}[P_1][NO] - k_2[P_1] + k_{-2}[P_2][L], \quad (5)$$

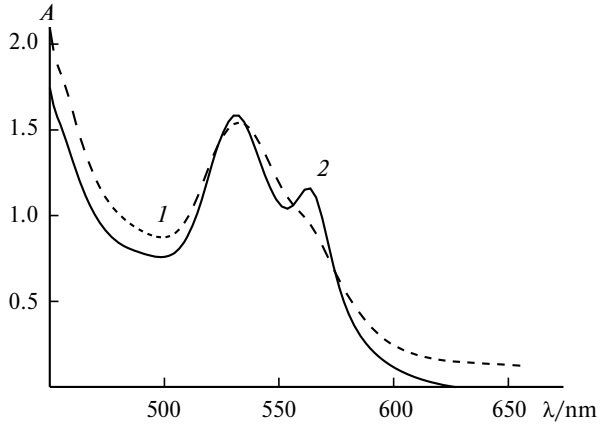

Fig. 4. Changes in the difference absorption spectra for the reaction of NIC **II** ($2 \cdot 10^{-5}$ mol L^{-1}) with cyt c^{3+} ($1.5 \cdot 10^{-4}$ mol L^{-1}) in the presence of $K_3[Fe(CN)_6]$ ($4 \cdot 10^{-3}$ mol L^{-1}): *1*, spectrum of cyt c^{3+} in the presence of $K_3[Fe(CN)_6]$; *2*, spectrum recorded immediately after addition of NIC **II**.

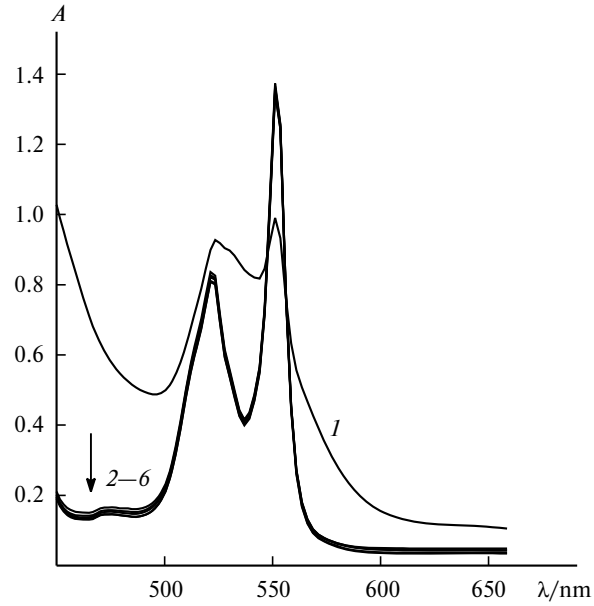

Fig. 5. Changes in the difference absorption spectra for the reaction of NIC **II** ($2 \cdot 10^{-4}$ mol L^{-1}) with Cyt ($6 \cdot 10^{-5}$ mol L^{-1}) in the absence of $K_3[Fe(CN)_6]$. Spectra were recorded after 1 (*2*), 9 (*3*), 24 (*4*), 54 (*5*), and 69 min (*6*) after the reaction onset. *1*, Spectrum of the commercial cytochrome C (a mixture of ferrocytochrome and ferricytochrome). Reaction conditions: 25 °C, 0.05 *M* phosphate buffer (pH 7.0).

Table 1. Redox potentials of the reagents

Redox indicator	Redox potential/V (at pH 7.0)	Color	
		reduced form	oxidized form
Methyl viologen	–0.4	Blue	Colorless
Safranin T	–0.29	Colorless	Red-violet
Methylene blue	+0.01	Colorless	Blue
Cytochrome C	+0.24	—	—
$K_3[Fe(CN)_6]$	+0.35	—	—

$$d[P_2]/dt = k_2[P_1] - k_{-2}[P_2][L],$$

$$d[L]/dt = k_2[P_1] - k_{-2}[P_2][L] + k_3[\mathbf{II}].$$

Solving the differential equations by kinetic modeling technique gives $k_2 = (1.8 \pm 0.2) \cdot 10^{-3}$ s^{-1}, $k_{-2} \approx 0.1$—0.2 L mol^{-1} s^{-1}, and $k_3 \approx 1.0 \cdot 10^{-3}$ s^{-1}.

Thus, if NIC is able to reduce NO ($E_0 < -0.8$ V), it will inevitably decompose on going into the oxidized state; this fact is confirmed by Figs 1, *b* and 3, *b* as well as by earlier published data[18] (for NIC **III**). At the same time, we assume that NICs with $E_0 > -0.8$ V and therefore unable to reduce NO do not transform into the oxidized state and, consequently, undergo reversible dissociation.

To confirm this conclusion, we estimate the E_0 values for NICs **I** and **II** from their reactions with dyes with the known E_0 values (Table 1).

All experiments were carried out under argon since oxygen accelerates the decomposition of given NICs.[18] It has been found[14] that NICs **I** and **II** reduce cyt c^{3+}, whose redox potential E_0 is +0.254 V[28] (Fig. 5).

At the same time, NICs **I** and **II** do not reduce methyl viologen and safranin T (Figs 6 and 7) and only NIC **II** reduces methylene blue (see Fig. 7).

Based on the obtained results, it can be concluded that the E_0 values of NICs **I** and **II** fall in the following ranges:

$$+0.01 \text{ V} < E_0 (\mathbf{I}) < +0.24 \text{ V},$$

$$-0.29 \text{ V} < E_0 (\mathbf{II}) < +0.01 \text{ V}.$$

Consequently, NICs **I** and **II** cannot reduce nitric oxide, therefore these complexes do not undergo oxidation and irreversible hydrolysis in the aqueous medium.

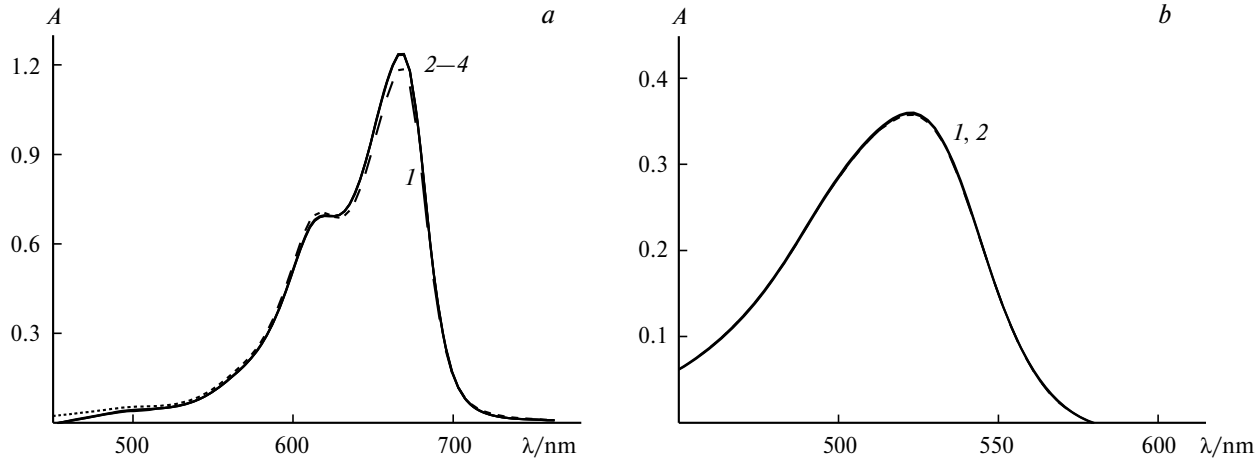

Fig. 6. Changes in absorption spectra for the reaction of NIC **I** ($2 \cdot 10^{-4}$ mol L^{-1}) with methylene blue ($2 \cdot 10^{-5}$ mol L^{-1}) (*a*) and safranin T ($1 \cdot 10^{-5}$ mol L^{-1}) (*b*). Spectra were recorded after 1 (*2*), 5 (*3*), and 10 min (*4*) after the reaction onset. *1*, Spectrum of methylene blue (*a*) or safranin T (*b*). Reaction conditions: 25 °C, water.

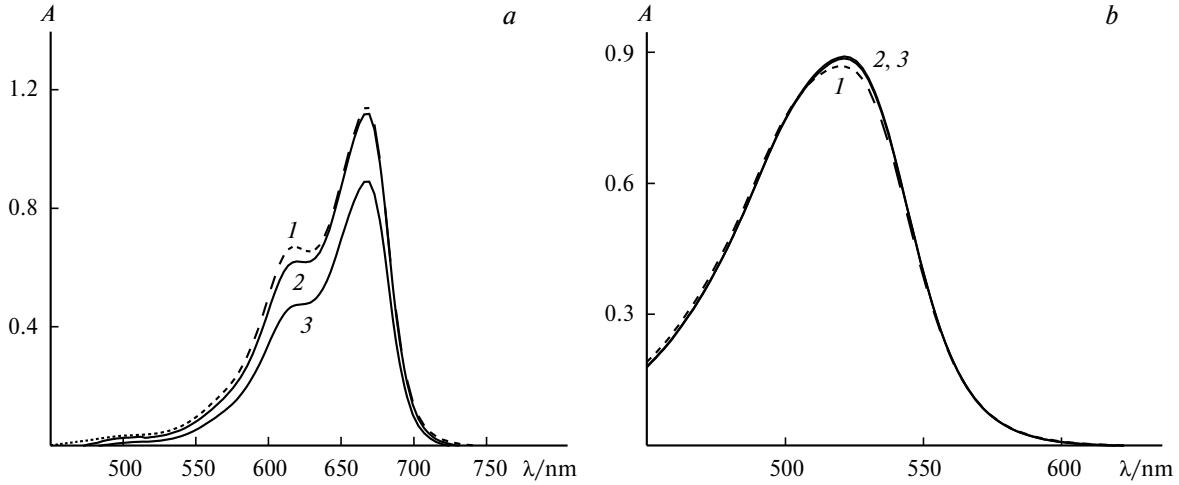

Fig. 7. Changes in absorption spectra for the reaction of NIC **II** ($2 \cdot 10^{-4}$ mol L^{-1}) with methylene blue ($2 \cdot 10^{-5}$ mol L^{-1}) (*a*) or safranin T ($1 \cdot 10^{-5}$ mol L^{-1}) (*b*). Spectra were recorded 1 (*2*) and 5 min (*3*) after the reaction onset. *1*, Spectrum of methylene blue (*a*) or safranin T (*b*). Reaction conditions: 25 °C, water.

Decomposition of these complexes in the aqueous medium to release NO and thiolate ligand is reversible.[20,21]

In summary, we developed simple method for prediction of decomposition pathway of NICs. This method is based on the reaction of NIC with methyl viologen. The absence of reduction of the dye with NIC is evidenced in favor of the reversible decomposition mechanism of the complexes, the possibility of the ligand-glutathione exchange, and, in turn, the potential antitumor activity of NIC.

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References

1. J. A. McCleverty, *Chem. Rev.*, 2004, **104**, 403.
2. N. M. Crawford, *J. Exp. Bot.,* 2006, **57**, 471.
3. K. M. Davies, D. A. Wink, J. E. Saavedra, L. Keefer, *J. Am. Chem. Soc.*, 2001, **123**, 5473.
4. R. Butler, I. L. Megson, *Chem. Rev.*, 2002, **102**, 1155.
5. A. S. Dutton, J. M. Fukuto, K. N. Houk, *Inorg. Chem.,* 2004, **43**, 1039.
6. A. S. Dutton, Ch. P. Suhrada, K. M. Miranda, D. A. Wink, J. M. Fukuto, K. N. Houk, *Inorg. Chem.,* 2006, **45**, 2448.
7. N. A. Sanina, L. A. Syrtsova, N. I. Shkondina, T. N. Rudneva, E. S. Malkova, T. A. Bazanov, A. I. Kotel´nikov, S. M. Aldoshin, *Nitric Oxide: Biol. Chem.*, 2007, **16**, 181.
8. O. S. Zhukova, N. A. Sanina, L. V. Fetisova, G. K. Gerasimova, *Ros. Bioterapevt. Zh.* [*Russ. Bioter. J.*], 2006, **5**, 14 (in Russian).
9. A. A. Timoshin, A. F. Vanin, T. R. Orlova, N. A. Sanina, E. K. Ruuge, S. M. Aldoshin, E. I. Chazov, *Nitric Oxide*, 2007, **16**, 286.
10. L. M. Borisova, I. Yu. Kubasova, M. P. Kiseleva, Z. S. Smirnova, N. A. Sanina, S. M. Aldoshin, T. N. Rudneva, *Ros. Bioterapevt. Zh.* [*Russ. Bioter. J.*], 2007, **6**, No. 1, 42 (in Russian).
11. N. A. Sanina, L. A. Syrtsova, N. I. Shkondina, E. S. Malkova, A. I. Kotelnikov, S. M. Aldoshin, *Russ. Chem. Bull.*, 2007, **56**, 761.
12. A. L. Lehninger, D. L. Nelson, M. M. Cox, in *Principles of Biochemistry*, Ed. V. Neal, Worth Publishers, New York, 1993, p. 769.
13. A. N. Osipov, G. G. Borisenko, Yu. A. Vladimirov, *Biochemistry* (*Moscow*), *Special Issue, Biol. Chem. Rev.*, 2007, **72**, 1491
14. N. A. Sanina, L. A. Syrtsova, N. I. Shkondina, T. N. Rudneva, A. I. Kotel´nikov, S. M. Aldoshin, *Russ. Chem. Bull.*, 2010, **59**, 1565.
15. Pat. RF 2460531 C1; *Byul. Izobret.* [*Invention Bull.*], 2012, No. 25 (in Russian).
16. Pat. US 8067628 B2; 2011.
17. N. A. Sanina, O. S. Zhukova, Z. S. Smirnova, L. M. Borisova, M. P. Kiseleva, S. M. Aldoshin, *Ros. Bioterapevt. Zh.* [*Russ. Bioter. J.*], 2008, **7**, No. 1, 52 (in Russian).
18. N. A. Sanina, G. V. Shilov, S. M. Aldoshin, A. F. Shestakov, L. A. Syrtsova, N. S. Ovanesyan, E. S. Chudinova, N. I. Shkondina, N. S. Emel´yanova, A. I. Kotel´nikov, *Russ. Chem. Bull.*, 2009, **58**, 572.
19. L. A. Syrtsova, N. A. Sanina, A. F. Shestakov, N. I. Shkondina, T. N. Rudneva, N. S. Emel´yanova, A. I. Kotel´nikov, S. M. Aldoshin, *Russ. Chem. Bull.*, 2010, **59**, 2203.
20. L. A. Syrtsova, N. A. Sanina, E. N. Kabachkov, N. I. Shkondina, A. I. Kotel´nikov, S. M. Aldoshin, *RSC Adv.*, 2014, **4**, 24560.
21. L. A. Syrtsova, N. A. Sanina, K. A. Lyssenko, E. N. Kabachkov, B. L. Psikha, N. I. Shkondina, O. V. Pokidova, A. I. Kotel´nikov, S. M. Aldoshin, *Bioinorg. Chem. Appl.*, 2014, 9; http://dx.doi.org/10.1155/2014/641407.
22. Pat. RF 2441873 C2, *Byul. Izobret.* [*Invention Bull.*], 2012, No. 4 (in Russian).
23. A. F. Vanin, *Biokhimiya*, 1998, **63**, 924 [*Biochemistry* (*Moscow*) (*Engl. Transl.*), 1998, **63**].
24. S. V. Lymar, V. Shafirovich, G. A. Poskrebyshev, *Inorg. Chem.*, 2005, **44**, 5212.
25. G. A. Poskrebyshev, V. Shafirovich, S. V. Lymar, *J. Am. Chem. Soc.*, 2004, **126**, 891.
26. M. D. Bartberger,W. Liu, E. Ford, K. M. Miranda, C. Switzer, J. M. Fukuto, P. J. Farmer, D. A. Wink, K. N. Houk, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 10958.
27. M. Hoshino, K. Ozawa, H. Seki, P. C. Ford, *J. Am. Chem. Soc.*, 1993, **115**, 9568.
28. A. L. Lehninger, D. L. Nelson, M. M. Cox, in *Principles of Biochemistry*, Ed. V. Neal, Worth Publishers, New York, 1993, p. 388.

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