Two decomposition mechanisms of nitrosyl iron complexes \([\text{Fe}_2(\mu-SR)(\text{NO})_4]\)

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Two decomposition mechanisms of nitrosyl iron complexes (NICs) \([\text{Fe}_2(\mu-SR)(\text{NO})_4]\) in aqueous medium are known. One mechanism (for instance, in the case of complex \([\text{Fe}_2(\mu-\text{SC}_4\text{H}_3\text{N}_2)(\text{NO})_4]\)) 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 \([\text{Fe}_2(\mu-\text{S(CH}_2)_2\text{NH}_3)(\text{NO})_4]\)\(\cdot\)\(2.5\text{H}_2\text{O}\) and \([\text{Fe}_2(\mu-\text{SC}_5\text{H}_11\text{NO}_2)(\text{NO})_4]\)\(\cdot\)\(\text{SO}_4\cdot\)\(\text{H}_2\text{O}\)), 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) \([\text{Fe}_2(\text{SR})_2(\text{NO})_4]\) hydrolyzing in protic media similarly to S-nitrosothiols⁴ and diazenium dialates⁵,⁶ with the NO release⁷ 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 seconds¹²) but also determines prolonged action of NICs as NO donors.

Apart from Hb, ferricytochrome C (cyt c³⁺) can also form relatively stable nitrosyl complexes despite the fact that the heme iron of cyt c³⁺ is completely coordinated to four N atoms of porphyrin core and amino acids (histidine-18 and methionine-80).¹³ We have recently found¹⁴ that introduction of NIC \([\text{Fe}_2(\mu-\text{SR})(\text{NO})_4]\) bearing cysteamine thiol ligands (I) into the cyt c³⁺ solution gives the NO-cyt c³⁺ complex. The reactions of cyt c³⁺ with nitric oxide significantly affect the mitochondrial respiratory chain.¹³ For instance, the formation of the NO-cyt c³⁺ complex can inhibit electron transfer. Moreover, the reaction of NO with cyt c³⁺ can modify the peroxidase activity of cyt c³⁺, which is apparently applicable for the apoptosis control.¹³

Complexes I and II show vasodilation¹⁵ and antitumor¹⁶ activities. Complex I induces apoptosis of human erythroleukemic cells (K562) and human colon cancer cells (LS174T).¹⁷

Earlier, we have described two mechanisms of the decomposition of NICs \([\text{Fe}_2(\mu-SR)(\text{NO})_4]\). One mechanism involves hydrolysis of NIC III resulting in its complete decomposition,¹⁸,¹⁹ the other mechanism is a reversible dissociation of cationic NICs \([\text{Fe}_2(\mu-SR)(\text{NO})_4]\) (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-
thione to give more stable complexes with glutathione ligand.\textsuperscript{15} 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-hydroxy-methylpropane-1,3-diol (Tris) (Serva, Germany), Na$_2$HPO$_4$•6H$_2$O, NaH$_2$PO$_4$•H$_2$O, 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.\textsuperscript{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.\textsuperscript{7}

**Complex I, [Fe$_2$(µ$_2$-SC$_3$H$_7$N$_2$)(NO)$_4$]SO$_4$•2.5$\text{H}_2$O.** Found (%): C, 8.53; H, 2.77; N, 15.70; S, 17.71. C$_{21}$H$_{30}$Fe$_2$N$_4$O$_{11}$S$_5$. Calculated (%): C, 9.10; H, 3.60; Fe, 21.25; N, 15.93; O, 31.87; S, 18.27. IR, v/cm$^{-1}$: 3454 (s), 3307 (vs), 2927 (w), 1769 (s), 1742 (s), 1551 (m), 1425 (w), 1376 (m), 1191 (w), 1151 (m), 1070 (w), 1040 (w), 811 (w), 774 (w), 739 (s), 620 (s). IR spectrum was recorded with the aim to confirm the obtaining of the pure crystalline compound.

**Complex II, [Fe$_2$(µ$_2$-SC$_3$H$_7$N$_2$)$_2$(NO)$_2$]SO$_4$•5$\text{H}_2$O.** Found (%): Fe, 15.60; C, 16.72; H, 4.50; N, 11.75; O, 38.02; S, 13.40. C$_{18}$H$_{25}$Fe$_2$N$_4$O$_{13}$S$_2$. Calculated (%): Fe, 15.64; C, 16.76; H, 4.47; N, 11.73; O, 37.99; S, 13.14. IR, v/cm$^{-1}$: 3171 (s), 1626 (m), 1375 (m), 1269 (m), 1189 (m), 1114 (m), 1089 (m), 746 (m), 1771, 1723 (vN=O). IR spectrum was recorded at selected time intervals at the 450—650 nm range at 25 °C.

**Hydrolysis of NIC III.** A freshly prepared 6·10$^{-3}$ 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 6·10$^{-3}$ 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 2·10$^{-4}$ 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.**

**Kinetics of the reactions of NICs II and III with e$^{3+}$ in the presence of K$_3[Fe(CN)$_6$]$.** The experiments were carried out with the same 2·10$^{-4}$ M stock solution of cytochrome C (commercial cytochrome C contained 10% of ferriyctochrome). A freshly prepared 2·10$^{-4}$ M stock solution of K$_3[Fe(CN)$_6$]$ was added in the amount to obtain 4·10$^{-4}$ M anaerobic cytochrome C solution placed into an anaerobic cuvette, 0.3 mL of 6·10$^{-4}$ M stock solution of K$_3[Fe(CN)$_6$]$ was added. A reference cuvette contained 3 mL of the same buffer. Absorption spectra were recorded at selected time intervals at the 450—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 e$^{3+}$ in the presence of K$_3[Fe(CN)$_6$]$ was added in the amount to obtain 6·10$^{-4}$ M stock solution with concentration of 2·10$^{-4}$ M anaerobic cytochrome C solution placed into an anaerobic cuvette, 0.3 mL of 4·10$^{-2}$ M stock solution of K$_3[Fe(CN)$_6$]$ was added. A reference cuvette contained 2.6 mL of 2·10$^{-4}$ M stock solution of cytochrome C solution placed into an anaerobic cuvette, 0.3 mL of 4·10$^{-2}$ M stock 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 e$^{3+}$. The reaction was initiated by simultaneous addition of 0.1 mL of 6·10$^{-4}$ 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 2·10$^{-5}$ 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 e$^{3+}$ 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 1.8·10$^{-4}$ M anaerobic cytochrome C was added. A reference cuvette contained 2 mL of 0.05 M phosphate buffer (pH 7.0). A freshly prepared 6·10$^{-3}$ M solution of NIC III was added to the cuvette containing cytochrome C. The reaction was initiated by simultaneous addition of 0.1 mL of 6·10$^{-4}$ M stock solution of K$_3[Fe(CN)$_6$]$.