Hemoglobin-stabilized tetranitrosyl binuclear iron complex with pyridin-2-yl in aqueous solutions

N. A. Sanina, L. A. Syrtsova, N. I. Shkondina, E. S. Malkova, A. I. Kotel´nikov, and S. M. Aldoshin*

Institute of Problems of Chemical Physics, Russian Academy of Sciences, 1 prosp. Akad. Semenova, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 (496) 515 5420. E-mail: sanina@icp.ac.ru

The tetranitrosyl iron complex with pyridin-2-yl $[Fe_2(SC_5H_4N)_2(NO)_4]$ (**1**) has higher NO-donating activity in 3% aqueous solutions of DMSO (pH 7.0, 25 °C) than the organic NO donor, *viz.*, adduct of NO with diethylenetriamine (NO-adduct). The NO concentration was determined by the spectrophotometric method based on the formation of an NO complex with hemoglobin (Hb). The apparent first-order rate constants of the studied reactions are $(6.15\pm0.6)\cdot10^{-1}$ s^{-1} and $(0.8\pm0.08)\cdot10^{-1}$ s^{-1} for complex **1** and the NO-adduct, respectively, at an Hb concentration of $2\cdot10^{-1}$ mol L^{-1} and the ratio [NO donor]/[Hb] = 10. The effect of Hb and [NO donor]/[Hb] ratio on the rate of NO generation from a solution of complex **1** was studied. For a fourfold decrease in the concentration of complex **1** the reaction rate constant decreases to $0.5\cdot10^{-4}$ s^{-1}, whereas the fourfold increase in the Hb concentration results in the stabilization of complex **1**.

Key words: NO donors, nitrovasodilators, hemoglobin, sulfur—nitrosyl iron complexes, 2-mercaptopyridine thiol, NO-adduct with diethylenetriamine.

Medical effect of the most known nitrovasodilators, namely, trinitroglycerol (TNG) and nitrosorbide, is caused by the reaction of NO formed due to their biotransformation with guanylate cyclase heme. It is known that during metabolism TNG and other nitro esters also form the nitrite ion as the first product. This reaction is catalyzed by aldehyde dehydrogenase, hemoglobin (Hb), and several other enzymes. The nitrite ion is transformed into NO in the reaction with Hb and other heme- and thiol-containing proteins. Unlike nitro esters and other synthetic (organic and inorganic) NO donors, the nitrosyl [1Fe—S] and [2Fe—2S] complexes generate NO without activation: due to hydrolysis in a protic medium.[1] These compounds have been synthesized[2—5] for the first time at the Institute of Chemical Physics, Russian Academy of Sciences. They are models of active centers of the nitrosyl [1Fe—2S] and [2Fe—2S] proteins, which are unique "depots" of endogenic NO.

The purpose of this work is the comparative study of the NO-donating ability of the $[Fe_2(SC_5H_4N)_2(NO)_4]$ complex (**1**) and the known organic NO donor, *viz.*, NO-adduct with diethylenetriamine. Compound **1** is a diamagnetic complexes of the μ_2-S type[6] and classified as a member of the family of Roussin´s "red salt" esters. Unlike $[Fe_2(SR)_2(NO)_4]$ esters (R = Et,[7] Bu^t,[8,9] $(CH_2)_4Me$,[8] C_6H_5F,[10] Ph [11]), which generate NO upon photoactivation[12] and can be promising for photodynamic therapy,[13—15] complex **1** contains the aromatic ligand (structural analog of pyrimidinic DNA bases), and its NO-donating ability has not been studied earlier.

Experimental

Materials. Bovine hemoglobin (MP Biomedicals, Germany), Sephadex G-25 (Pharmacia, Sweden), sodium dithionite (Merck, Germany), $Na_2HPO_4\cdot6H_2O$ and $NaH_2PO_4\cdot H_2O$ (MP Biomedicals, Germany), Na adduct $C_4H_{13}N_5O_2$ (Sigma, USA), and $NaNO_2$ (Aldrich, USA) were used. Single crystals of **1** were synthesized by a published procedure.[6] DMSO was purified using a standard procedure.[16] Water was distilled in a Bi/Duplex distillator (Germany).

Operation in a nitrogen atmosphere. All procedures were carried out under nitrogen (high-purity grade), which was additionally purified by passing through a column with a chromium—nickel catalyst. Working solutions (buffer, DMSO) were pre-evacuated and then nitrogen was purged to the magnetically stirred solution for 30 min. Hereinafter these solutions are named anaerobic. All the vessels used and quartz cells were sealed with Rubber Septa seals (Sigma, USA), which allowed one to introduce gases and other necessary components through a needle. A solution was transferred from one vessel to another using syringes with soldered needles or under excess nitrogen pressure using two needles connected with Teflon capillaries. Excess pressure was discharged through an additional needle capped with a Teflon capillary immersed into water.

Cells and vessels with a volume of 4 and 5 mL, respectively, containing weighed samples of the nitrosyl complexes or other reagents were purged with nitrogen through needles for 30 min.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 4, pp. 732—736, April, 2007.

1066-5285/07/5604-0761

Determination of NO. The amount of NO was determined spectrophotometrically from the amount of the HbNO adduct that formed. To determine the HbNO concentration, the absorption spectrum of the reaction system containing Hb and HbNO was decomposed to the components (spectra of Hb and HbNO) by computer processing using the MathCad program. The solution should satisfy the criterion of the minimum of the sum of squared deviations of the experimental spectrum of the mixture from the calculated one :

$$\sigma(\alpha,\ \beta) = \sum_i [A_i - F(A_{\mathrm{Hb}},\ A_{\mathrm{HbNO}},\ \alpha,\ \beta)]^2, \qquad (1)$$

where $\sigma(\alpha, \beta)$ is the root-mean-square deviation; A_i are the experimental data (absorbance) at a certain time moment; F is the desired function of the A_{Hb}, A_{HbNO}, α, and β values; A_{Hb} and A_{HbNO} are the initial absorbances of Hb and HbNO, respectively; α and β are the fractions of HbNO and Hb, respectively. The calculation was performed in a wavelength region of 450—650 nm by 200 experimental points. In the whole series of experiments, the $\sigma(\alpha, \beta)$ value ranges from $2 \cdot 10^{-5}$ to $5 \cdot 10^{-6}$, indicating high quality of simulation of the absorption spectrum of the reaction mixture at each moment and high accuracy of determination of the Hb and HbNO concentrations. The absorption spectra were recorded on a Specord M-40 spectrophotometer with an interface for computer spectra detection and a temperature-controlled cell compartment. The spectra were recorded at 25 °C.

Preparation of an Hb solution. A homogeneous preparation of bovine Hb was obtained from bovine hemoglobin (MP Biomedicals, Germany), being a mixture of methemoglobin (metHb) and oxygenated hemoglobin (HbO_2). A 0.05 *M* phosphate buffer (pH 7.0) was used at all stages of Hb preparation and in all experiments with Hb. To convert a mixture of metHb with HbO_2 to Hb, a column 2×15 cm packed with Sephadex G-25 was prepared and transformed into the anaerobic state. For this purpose, 50 mL (volume of the column) of the anaerobic buffer and then 40 mL of the buffer containing sodium dithionite (5 mL, 100 mg mL^{-1}) were passed through the column. The column was left to stay for 3 min, and then dithionite was washed off by passing the anaerobic buffer (50 mL) until the negative reaction to dithionite (with methyl viologen) was achieved.[17] Commercial hemoglobin (0.5 g) was dissolved with stirring in the buffer (5 mL), nitrogen was purged for 30 min with stirring, and a solution of dithionite (2 mL, 100 mg mL^{-1}) was added. The absorption spectrum of an aliquot of the solution showed that the whole mixture of metHb with HbO_2 transformed into Hb. Then excess dithionite and products of its decomposition were removed on a column with Sephadex G-25. A solution of Hb (5 mL) with a concentration of $6 \cdot 10^{-4}$ mol L^{-1} was eluted. The solution of Hb was stored in the frozen state as balls in liquid nitrogen. Prior to use the Hb solution was thawn out in 5-mL flasks in a nitrogen flow. The indicator of nativity and homogeneity of Hb was the ratio of molar absorption coefficients of all absorption maxima coinciding with published data.[18]

Kinetics of the reaction of Hb complex 1 and NO-adduct. The buffer (2.8 mL) and a solution of Hb ($6 \cdot 10^{-4}$ mol L^{-1}) were introduced into an evacuated 4-mL experimental cell with an optical path length of 1 cm, and the absorption spectrum was recorded. Anaerobic DMSO was added to a weighed sample of nitrosyl complex **1** or NO-adduct in a vessel filled with nitrogen in such an amount that a solution of complex **1** with a concentration of $6 \cdot 10^{-3}$ mol L^{-1} would be obtained. Then the solution was stirred for 3—5 min until the complex was dissolved completely, and 0.1 mL of the obtained solution was introduced into the experimental cell with Hb and into the reference cell already containing 2.9 mL of the anaerobic buffer. The final concentration of complex **1** or NO-adduct was $2 \cdot 10^{-4}$ mol L^{-1}, and that of DMSO was 3.3%. Then the differential absorption spectra were recorded: the first spectrum was recorded 0.5—1 min after the beginning of the reaction, the further spectra were recorded at 3-min intervals for the first 30 min of the reaction, and then the intervals were 15, 30, and 60 min (only the part of these spectra are shown in the figures for clarity). The spectra were recorded until Hb transformed completely into HbNO, when the spectrum stopped changing.

In a special run the final concentration of complex **1** was $5 \cdot 10^{-6}$ mol L^{-1}. For this purpose complex **1** with the initial concentration $1.5 \cdot 10^{-3}$ mol L^{-1} was used. To carry out a run with concentrations of Hb and complex **1** of $8 \cdot 10^{-5}$ and $2 \cdot 10^{-4}$ mol L^{-1}, respectively, a solution of Hb (2 mL, $8.4 \cdot 10^{-5}$ mol L^{-1}) in the buffer with a solution of the complex (0.1 mL, $4 \cdot 10^{-3}$ mol L^{-1}) in DMSO, and the resulting solution was introduced into a 1-mL quartz cell with an optical path length of 0.2 cm.

Results and Discussion

The method based on the absorption spectrum of HbNO was used to detect nitrogen monoxide. Oxygenated hemoglobin is a trap for NO (binding constant is $3 \cdot 10^{10}$ L mol^{-1}, binding rate is close to diffusional[18]) and has a characteristic absorption spectrum that changes during NO addition (Fig. 1). This method is more universal and appropriate for kinetic measurements than other methods of NO analysis. For instance, the chemiluminescence method of NO analysis[19] is not quantitative. The quantitative electrochemical[20] is not suitable for prolong kinetic measurements, because the NO-sensor elec-

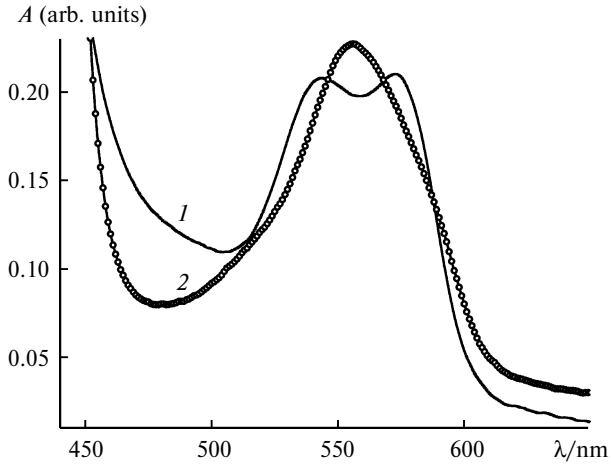

Fig. 1. Absorption spectra of a solution of HbNO (*1*) prepared by the equilibration of a solution of Hb ($4.7 \cdot 10^{-6}$ mol L^{-1}) in 0.05 *M* buffer and this Hb solution (*2*) (pH 7.0, 1 atm NO, 25 °C).

trode used is polarized in time and requires permanent regeneration.

To study the kinetics of formation of NO generated by complex **1** and NO-adduct, we detected in time the absorption spectra of the reaction systems containing Hb and studied NO donor (Figs 2—5). All studies were carried out in a nitrogen atmosphere, because NO is known to react rapidly with O_2 forming nitrogen oxides with a rate constant of $2 \cdot 10^6$ (L mol^{-1})^2 s^{-1} (see Ref. 21). Since all sulfur—nitrosyl iron complexes absorb in the visible region, we recorded the differential absorption spectra of the experimental system with Hb and the buffer containing equal amounts of the corresponding complex. The changes in the differential absorption spectra in time for the reaction of complex **1** with Hb are shown in Figs 2—4. It is seen from the data in Figs 2 and 3 that the differential spectra contain three isosbestic points at 551, 570, and 595 nm. This indicates that only Hb and HbNO contribute to the absorption spectrum. The isosbestic points are distorted in the case of the NO-adduct (see Fig. 5) In this case, some amount of the product of the reaction of Hb

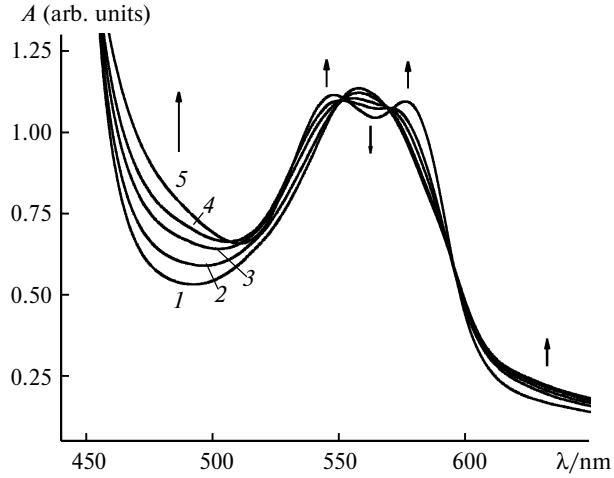

Fig. 2. Change in the differential absorption spectra in time during the reaction of complex **1** ($2 \cdot 10^{-4}$ mol L^{-1}) with Hb ($2 \cdot 10^{-5}$ mol L^{-1}) recording the spectra 0.5 (*1*), 10 (*2*), 28 (*3*), 36 (*4*), and 141 min (*5*) after the beginning of experiment (0.05 *M* phosphate buffer (pH 7.0) containing 3.3% DMSO, 25 °C).

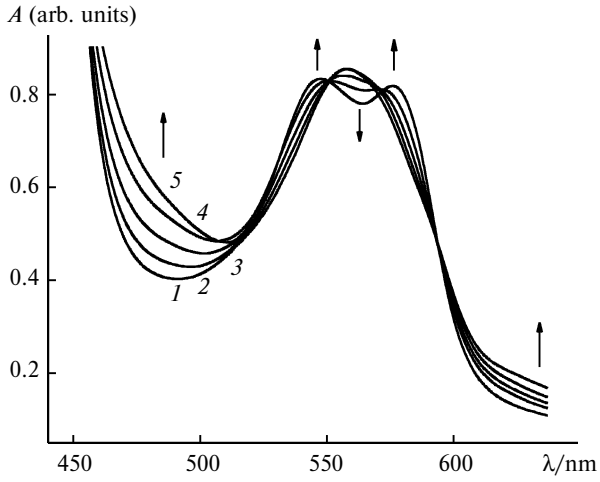

Fig. 3. Change in the differential absorption spectra in time during the reaction of complex **1** ($5 \cdot 10^{-5}$ mol L^{-1}) with Hb ($2 \cdot 10^{-5}$ mol L^{-1}) recording the spectra 0.02 (*1*), 0.5 (*2*), 1.5 (*3*), 3.5 (*4*), and 6.5 h (*5*) after the beginning of experiment (0.05 *M* phosphate buffer (pH 7.0) containing 3.3% DMSO, 25 °C).

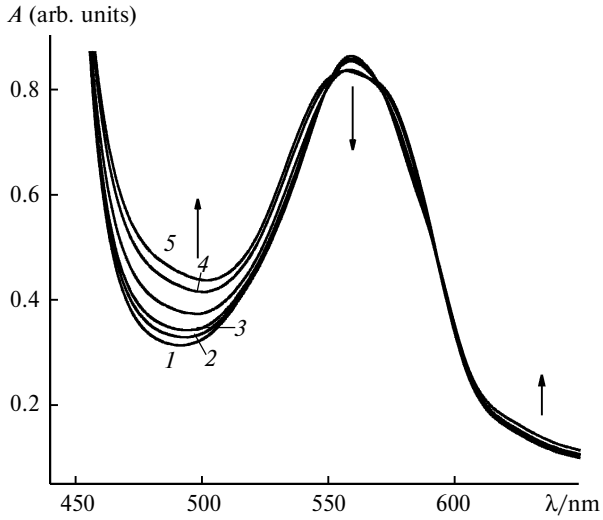

Fig. 4. Change in the differential absorpion spectra in time during the reaction of complex **1** ($2 \cdot 10^{-4}$ mol L^{-1}) with Hb ($8 \cdot 10^{-5}$ mol L^{-1}) recording the spectra 0.15 (*1*), 1.0 (*2*), 2.0 (*3*), 4.3 (*4*), 6.8 (*5*), and 10 h (*6*) after the beginning of experiment (0.05 *M* phosphate buffer (pH 7.0) containing 3.3% DMSO, 25 °C).

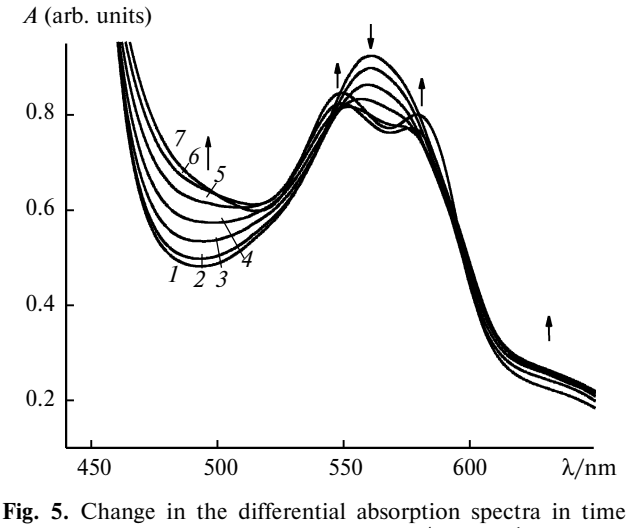

Fig. 5. Change in the differential absorption spectra in time during the reaction of complex **1** ($2 \cdot 10^{-4}$ mol L^{-1}) with Hb ($2 \cdot 10^{-5}$ mol L^{-1}) recording the spectra 0.1 (*1*), 0.3 (*2*), 1.0 (*3*), 2.1 (*4*), 4.1 (*5*), 6.6 (*6*), and 8.6 h (*7*) after the beginning of experiment (0.05 *M* phosphate buffer (pH 7.0) containing 3.3% DMSO, 25 °C).

with the NO-adduct is probably formed and contribute to the absorption spectrum at 450—600 nm.

The data in Figs 2, 3, and 5 show a decrease in the absorbance in the region of the maximum of the absorption spectrum of Hb at 556 nm and an increase in the absorbance at 545 and 575 nm, as well as an increase in the absorbance at 440—540 nm, indicating the formation of HbNO (see Fig. 1).

The data obtained on the kinetics of HbNO formation are presented in Fig. 6. For complex **1** the experimental points were obtained by the data in Fig. 2. It turned out that all kinetic dependences for complex **1** are well described in terms of the formalism of pseudo-first-order reactions. We plotted the theoretical monoexponential curves by the Origin computer program using the function

$$y = a(1 - e^{-kt}), \quad (2)$$

where k is the apparent first-order rate constant, and a is the final concentration of HbNO. The apparent first-order rate constants (k) of the reactions under study are given in Table 1.

Since the rate of the reaction of Hb with NO is close to diffusional (second-order reaction rate constant $k_2 = 1.02 \cdot 10^8$ L mol^{-1} s^{-1}),[18] the obtained rate constants k (see Table 1) indicate that the rate of HbNO formation is described by the step of NO generation to solution from the complex, Thus, the process of NO formation can be described by two consecutive reactions.

The first reaction, *viz.*, decomposition of complex **1** with NO generation

Equation

$$\text{Complex } \mathbf{1} \longrightarrow \text{NO}$$

with the reaction rate constant k_a, is the first-order irreversible reaction, and an increase in the NO concentration proceeds *via* the exponential law

$$[NO] = [NO]_\infty(1 - e^{-k_a t}). \quad (3)$$

The second reaction is the formation of HbNO

$$\text{Hb} + \text{NO} \longrightarrow \text{HbNO}$$

with the apparent rate constant of the pseudo-first order $k_b = k_2[Hb]$. For the used Hb concentration equal to $2 \cdot 10^{-5}$ mol L^{-1} (see Figs 2, 3, and 5), k_b is 2040 s^{-1}. This k_b value by 10^7 times exceeds the reaction rate constants of HbNO formation obtained in experiment (see Table 1).

In the case, of consecutive reactions, the accumulation of the final product (HbNO) is described by the equation

$$[HbNO] = [HbNO]_\infty \cdot \cdot[1 - k_b e^{-k_a t}/(k_b - k_a) + k_a e^{-k_b t}/(k_b - k_a)]. \quad (4)$$

For k_b values considerably exceeding k_a, as in the given case, the second part of Eq. (4) can be neglected due to its low value, and the accumulation of the final product proceeds with the rate constant of the first process k_a *via* the equation

$$[HbNO] = [HbNO]_\infty(1 - e^{-k_a t}). \quad (5)$$

Thus, HbNO is formed with the same rate as that of NO generation to solution. The final concentration of HbNO in our experimental is determined by the initial Hb concentration, because the binding constant of Hb with NO is $3 \cdot 10^{10}$ L mol^{-1} (see Ref. 18). The total measurement error of the constants is 10%.

In the case of the NO-adduct (see Fig. 5), the change in the absorption spectra of Hb indicating the formation HbNO occurred more slowly. The apparent first-order rate constant of the reaction of the NO-adduct with Hb is $8 \cdot 10^{-5}$ s^{-1} (compared to $6.15 \cdot 10^{-4}$ s^{-1} for complex **1**, see Table 1) at equal concentrations of the reactants.

The rate constant found for complex **1** shows that complex **1**, unlike the earlier studied complexes of the μ-N—C—S type with azaheterocyclic ligands[1]) generates NO during hydrolysis by an order of magnitude more slowly. Perhaps, this is due to the fact that the NO groups are bonded to the iron atoms in complex **1** more strongly: the Fe—NO bonds in complex **1** are shorted by 0.03 Å than the Fe—NO bonds in complexes of the μ-N—C—S type (1.669—1.677 Å (see Ref. 22), 1.674—1.690 Å (see Ref. 4), and 1.674—1.696 Å (see Ref. 5), ranging from 1.640(2) to 1.660(1) Å (see Ref. 6).

To check whether the rates of removal of different NO groups to solution from complex **1** differ or not, the experimental kinetics of HbNO formation (Fig. 6, curve *1*) was analyzed using the Origin program and function (6) for associative reactions

$$y = y_0 + a_1(1 - e_1^{-k_1 t}) + a_2(1 - e_2^{-k_2 t}) \quad (6)$$

under the assumption that two independent NO groups are removed in the first-order reactions with different rate constants k_1 and k_2. In this case, two equal rate constants were obtained, which were the same as the constant obtained using function (2) for the first-order reaction: $6.15 \cdot 10^{-4}$ s^{-1}. This implies that the NO groups from complex **1** are isolated with equal rates.

Table 1. Rate constant of NO generation (k) from complex **1** and NO-adduct to solution*

NO donor	[NO donor]·10^4	[Hb]·10^5	$k \cdot 10^4$ /s^{-1}
	mol L^{-1}		
$[Fe_2(SC_5H_4N)_2(NO)_4]$	2	0.5	59±6
$[Fe_2(SC_5H_4N)_2(NO)_4]$	2	2	6.15±0.6
$[Fe_2(SC_5H_4N)_2(NO)_4]$	0.5	2	6.15±0.6
NO-adduct	2	2	0.8±0.08

* For experimental conditions, see captions for Figs 2, 3, and 5.

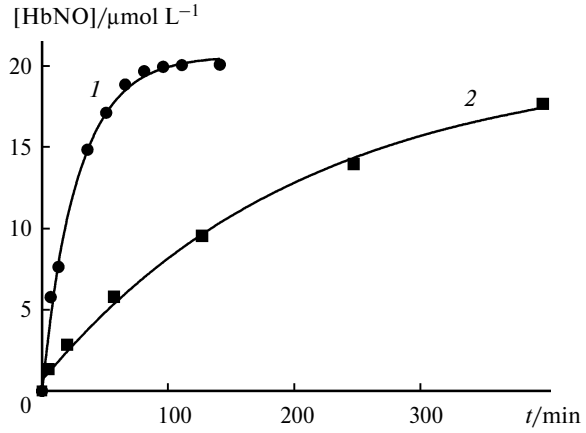

Fig. 6. Kinetics of formation of HbNO by the reactions of complex **1** ($2 \cdot 10^{-4}$ mol L^{-1}) with Hb ($2 \cdot 10^{-5}$ mol L^{-1}) according to the experimental data presented in Fig. 2 (*1*) and NO-adduct ($2 \cdot 10^{-4}$ mol L^{-1}) with Hb ($2 \cdot 10^{-5}$ mol L^{-1}) according to the experimental data presented in Fig. 5 (*2*) (solid lines show the calculated monoexponential curves corresponding to the indicated experimental points and plotted using the function $y = y_0 + a(1 - e^{-kt})$ at $k = (6.15 \pm 0.6) \cdot 10^{-4}$ (*1*) and $(0.8 \pm 0.08) \cdot 10^{-4}$ s^{-1} (*2*)).

We also studied the effect of Hb and the [NO donor]/[Hb] ratio on the rate constant of NO removal to solution from complex **1**. If the reaction of NO generation by complex **1** involved Hb and was bimolecular, then the reaction rate would increase with an increase in the Hb concentration. We found that this does not occur.

The reaction rate constant decreases with a fourfold decrease in the concentration of complex **1** (see Fig. 6, curve *2*). Analysis of the reaction kinetics shows that a good correlation of the theoretical curve with experimental points is obtained using function (6). As a result, it turned out that two processes of NO generation to solution occur according to the first-order kinetic equation. One of the processes coincides with that described above for the experiment presented in Fig. 2 ($k_1 = 6.15 \cdot 10^{-4}$ s^{-1}). The second process proceeds by an order of magnitude more slowly ($k_2 = 0.5 \cdot 10^{-4}$ s^{-1}, see Table 1).

It is known that the rate of NO removal from complex **1** decreases sharply in aprotic media. It is most likely that the observed stabilization of the complex can be explained by the partial absorption of complex **1** by a hemoglobin macromolecule, which decreases its contacts with the aqueous phase.

For a fourfold increase in the Hb concentration complex **1** is stabilized (see Fig. 4). It is seen from the data in Fig. 4 that absorbance increases at 440—540 nm, which corresponds to the formation of HbNO (see Fig. 1). However, the spectra cannot be decomposed correctly and the rate constant of NO generation cannot be determined because of an insufficient change in the absorbance at the absorption maxima of HbNO at 545 and 575 nm due to overlapping with the absorption spectrum of Hb with a maximum at 556 nm. When the Hb concentration decreases considerably ($5 \cdot 10^{-6}$ mol L^{-1}), k becomes equal to $5.9 \cdot 10^{-3}$ s^{-1} (see Table 1).

Thus, the tetranitrosyl iron complex with pyridin-2-yl (**1**) has a higher NO-donating activity in protic media under the same conditions than the NO-adduct: the apparent first-order rate constant of NO generation to solution for complex **1** by hemoglobin is very important for metabolism of the sulfur—nitrosyl iron complexes. Reasons for this effect will be studied further. If the reaction of NO generation from complex **1** involved Hb and was bimolecular, then the reaction rate would increase with an increase in the Hb concentration. We found that this did not occur.

This work was financially supported by the Russian Foundation for Basic Research (Program No. 06-03-32381a) and the Program of the President of the Russian Federation (State Program on Support for Leading Scientific Schools of Russia, Grant NSH-2150.2006.3).

References

1. N. A. Sanina, L. A. Syrtsova, N. I. Shkondina, T. N. Rudneva, E. S. Malkova, T. A. Bazanov, A. I. Kotel´nikov, and S. M. Aldoshin, *Nitric Oxide: Biology and Chemistry*, 2007, **16**, 181—188.
2. N. A. Sanina and S. M. Aldoshin, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 2326 [*Russ. Chem. Bull., Int. Ed.*, 2004, 2428].
3. N. A. Sanina, O. A. Rakova, S. M. Aldoshin, G. V. Shilov, Yu. M. Shulga, A. V. Kulikov, and N. S. Ovanesyan, *Mendeleev Commun.*, 2004, **1**, 9.
4. N. A. Sanina, S. M. Aldoshin, T. N. Rudneva, N. I. Golovina, G. V. Shilov, Yu. M. Shul´ga, N. S. Ovanesyan, V. N. Ikorskii, and V. I. Ovcharenko, *J. Mol. Struct.*, 2005, **752**, 110.
5. N. A. Sanina, T. N. Rudneva, S. M. Aldoshin, G. V. Shilov, D. V. Korchagin, Yu. M. Shul´ga, V. M. Martinenko, and N. S. Ovanesyan, *Inorg. Chim. Acta,* 2006, **359**, 570.
6. O. A. Rakova, N. A. Sanina, G. V. Shilov, Yu. M. Shul´ga, V. M. Martynenko, N. S. Ovanesyan, and S. M. Aldoshin, *Koord. Khim.*, 2002, **28**, 364 [*Russ. J. Coord. Chem.*, 2002, **28** (Engl. Transl.)].
7. J. T. Thomas, J. H. Robertson, and E. G. Cox, *Acta Crystallogr.*, 1958, **11**, 599.
8. C. Glidewell, M. E. Harman, M. B. Hursthouse, I. L. Johnson, and M. Motevalli, *J. Chem. Res.*, 1998, **212**, 1676.
9. R. E. Marsh and A. L. Spek, *Acta Crystallogr., Sect. B.*, 2001, **57**, 800.
10. Cai Jinhua, Mao Shaoping, Huang Jinling, and Lu Jiaxi, *Chinese J. Struct. Chem.,* 1983, **2**, 263.
11. T. B. Rauchfuss and T. D. Weatherill, *Inorg. Chem.*, 1982, **21**, 827.
12. C. L. Conrado, J. L. Bourassa, C. Egler, S. Wecksler, and P. C. Ford, *Inorg. Chem.*, 2003, **42**, 2288.

13. R. Ackroyd, C. Kelty, N. Brown, and M. Reed, *Photochem. Photobiol.*, 2001, **74**, 656.
14. R. K Pandey, *J. Porphyrins Phthalocyanines*, 2000, **4**, 368.
15. V. G. Granik and N. B. Grigor´ev, *Oksid azota (NO). Novyi put´ k poisku lekarstv* [*Nitrogen Monoxide (NO). New Routes to Search for Drugs*], Vuzovskaya Kniga, Moscow, 2004, 64 (in Russian).
16. A. Weissberger, E. Proskauer, J. A. Riddick, and E. E. Toops, *Organic Solvents: Physical Properties and Methods of Purification*, Intersci., New York, 1955, 517.
17. S. G. Mayhew, *Eur. J. Biochem.*, 1978, **85**, 535
18. E. Antonini and M. Brunori, *Hemoglobin and Myoglobin in the Reactions with Ligands*, Eds A. Neuberger and E. L. Tatum, North-Holland Research Monographs, *Frontiers of Biology*, North-Holland Publ., Amsterdam—London, 1971, **21**, 276.
19. T. M. Millar, C. R. Stevens, N. Benjamin, R. Eisenthal, R. Harrison, and D. R. Blake, *FEBS Lett.*, 1998, **427**, 225.
20. J. J. Doel, B. L. J. Godber, R. Eisenthal, and R. Harrison, *Biochim. Biophys. Acta*, 2001, **1527**, 81.
21. A. M. Miles, D. A. Wink, J. C. Cook, and M. B. Grisham, *Methods Enzymol.*, 1996, **268**, 105.
22. S. M. Aldoshin, N. A. Sanina, O. A. Rakova, G. V. Shilov, A. V Kulikov, Yu. M. Shul´ga, and N. S. Ovanesyan, *Izv. Akad. Nauk*, *Ser. Khim.*, 2003, 1614 [*Russ. Chem. Bull.*, *Int. Ed.*, 2003, 1702].

Received December 15, 2006;
in revised form March 2, 2007