Effect of the solvent on the hydrolysis of the iron nitrosyl complex $\{Fe_2[S(CH_2)_2NH_3]_2(NO)_4\}SO_4 \cdot 2.5H_2O$: spectroscopic and kinetic investigations of its monomer and dimer forms

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The hydrolysis of the iron nitrosyl complex $\{Fe_2[S(CH_2)_2NH_3]_2(NO)_4\}SO_4 \cdot 2.5H_2O$ (CysAm) in water is not accompanied by the formation of its monomer form and is a reversible process. According to the ESR, ^{1}H NMR, and spectrophotometric data, the dissolution of CysAm in DMSO affords the monomer form of CysAm. The kinetic parameters of the hydrolysis of CysAm in the dimer and monomer forms were determined by kinetic modeling.

Key words: iron nitrosyl complexes, nitrogen monoxide donors, ESR spectroscopy, ^{1}H NMR spectroscopy, kinetic measurements.

Studies of the structures and properties of nitrosyl intermediates in the decomposition of compounds generating nitrogen monoxide (NO) are of interest due to the role of NO as a polyfunctional physiological regulator and are necessary for the practical application of the knowledge for the targeted delivery of NO to cellular targets.

In the present study, we investigated the water-soluble cationic nitrosyl complex with the cysteamine ligand, *viz.*, tetranitrosylbis(cysteaminylthio)diiron $\{Fe_2[S(CH_2)_2NH_3]_2(NO)_4\}SO_4 \cdot 2.5H_2O$ (CysAm), which has been shown[1] to induce apoptosis of human erythroblastic leukemia (K562) and human rectal carcinoma (LS174T) cell lines. The structure and physicochemical properties of CysAm in the solid state have been described earlier.[2] The compound under consideration is a μ_2-S-type dinuclear cationic iron nitrosyl complex (Fig. 1). It was also found that CysAm liberates NO as a result of the spontaneous hydrolysis without additional activation in polar solvents.[3] For the practical application of CysAm as a potential antitumor agent, it is important to know whether the nature of the solvent has an effect on its NO-donor activity, as well as to know the structures of nitrosyl intermediates that are formed in solution.

It was found that the hydrolysis of μ_2-S-type dinuclear iron nitrosyl complexes can proceed through dissociation into monomers, the hydrolysis of the latter occurring at a higher rate.[4]

The aim of the present study was to investigate nitrosyl intermediates of CysAm, which are formed in water and dimethyl sulfoxide, by spectrophotometry, ESR, and NMR spectroscopy and to perform the kinetic modeling of the hydrolysis.

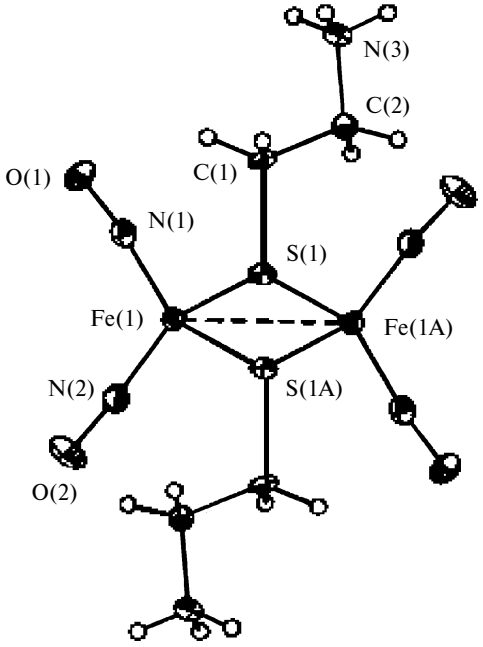

Fig. 1. X-ray diffraction structure of CysAm.[2]

Experimental

The reactants $Na_2HPO_4 \cdot 6H_2O$ and $NaH_2PO_4 \cdot H_2O$ (MP Biomedicals, Germany), $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ and $NaNO_2$

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(Aldrich, USA) were used as is; DMSO (reagent grade, Khimmed, Russia) was additionally purified by distillation according to a procedure described earlier.[5] Water was purified by distillation in a Bi/Duplex distillation apparatus (Germany).

Absorption spectra were recorded on a Specord M-40 spectrophotometer equipped with an interface enabling its link to a computer and a temperature-controlled cell holder. The absorption spectra in 0.05 *M* phosphate buffer, pH 7.0, were recorded under a nitrogen atmosphere with the use of a freshly prepared solution of CysAm at a concentration of $1 \cdot 10^{-4}$ mol L^{-1}. The absorption spectra of a dimer—monomer mixture were measured under a nitrogen atmosphere with the use of a freshly prepared solution of the mixture in DMSO (see below) diluted by a factor of 10 with the same solvent.

ESR spectra were measured at room temperature on a SE/X 2544 radio-frequency ESR spectrometer (Radiopan, Poland). The modulation amplitude of the magnetic field was 0.125 mT; the microwave power was approximately 5 mW. The concentration of paramagnetic particles was determined by comparing the second integrals of the ESR spectra of the samples under study and of the reference (an aqueous solution of stable nitroxide at a concentration of 1 mmol L^{-1}). The concentration was determined with an accuracy of approximately 20%. The spectra of a powder of CysAm and its solutions in DMSO and water were investigated. Solutions at a concentration of 1 mmol L^{-1} were prepared immediately before the measurements of the ESR spectra. The spectra were recorded at different intervals after the preparation of the solutions, which allowed us to estimate the stability of the complexes in solution with time.

^{1}H NMR spectra of freshly prepared solutions of CysAm in D_2O (99.8 at.% D) or DMSO-d_6 (Aldrich, 99.5% deuterated) were recorded on a Bruker AVANCE III 500 spectrometer operating at 500 MHz at 23 °C.

The technique of operation under a nitrogen atmosphere has been described previously.[6]

Synthesis of CysAm was carried out according to a known procedure.[2] The elemental analysis of the polycrystalline CysAm powder was performed at the Analytical Center for Collective Use of the Institute of Problems of Chemical Physics of the Russian Academy of Sciences. Found (%): C, 8.53; H, 2.77; N, 15.70; S, 17.71. $C_4H_{19}Fe_2N_6O_{10.5}S_3$. Calculated (%): Fe, 21.25; C, 9.10; H, 3.60; N, 15.93; S, 18.27. ^{1}H NMR (D_2O), δ: 3.27 (t, 2 H, CH_2—S, J = 6.7 Hz); 3.37 (dt, 2 H, $C\underline{H}_2$—NH_4^+, J = 6.7 Hz, J = 1.9 Hz).

Preparation of the monomer (the CysAm dimer—monomer mixture). Anaerobic anhydrous DMSO was added to a weighed sample of the CysAm complex in a 5 mL vessel filled with nitrogen in such a way as to prepare a solution of the complex at a concentration of $1 \cdot 10^{-3}$ mol L^{-1}. The mixture was stirred at room temperature under a nitrogen flow for 15 min until the complex was completely dissolved.

Hydrolysis of CysAm and a dimer—monomer mixture at pH 7.0. A 0.05 *M* phosphate buffer, pH 7.0, (2 mL) was intoduced into an anaerobic 4 mL test cell with an optical path length of 1 cm. Anaerobic 0.05 *M* phosphate buffer, pH 7.0, was added to a weighed sample of the CysAm complex in a vessel filled with nitrogen in such a way as to prepare a solution of the complex at a concentration of $6 \cdot 10^{-4}$ mol L^{-1}. The solution was stirred for 15 min until the complex was completely dissolved, and then 1 mL of the resulting solution was intoduced into a test cell containing 0.05 *M* phosphate buffer (2 mL); the final concentration of CysAm was $2 \cdot 10^{-4}$ mol L^{-1}. The reference cell contained 0.05 *M* phosphate buffer (3 mL). After the addition of CysAm, the absorption spectra were recorded in the wavelength range of 450—650 nm at 25 °C for 3 h at 5—15 min intervals. The hydrolysis of CysAm in water was studied in the same way. The hydrolysis of the dimer—monomer mixture was studied in a similar way, but the reaction was initiated by the addition of a solution of the dimer—monomer mixture in DMSO (0.6 mL) (the preparation was described above) to a cell containing anaerobic 0.05 *M* phosphate buffer, pH 7.0, (2.4 mL) under a nitrogen atmosphere. The DMSO content was 20%. In all cases, the current concentration of CysAm was calculated from the absorption spectrum based on the extinction coefficient (ε) at 450 nm ($2.5 \cdot 10^3$ mol L^{-1} cm^{-1}).

For kinetic modeling, the proposed reaction scheme describing the hydrolysis of CysAm was considered. The rate constants for the steps of hydrolysis of CysAm were determined by the least-squares method based on the numerical solution of the corresponding differential equation system. The concentrations of the complex, which were determined from the absorption spectra, were taken as the experimental data.

Results and Discussion

It is known that CysAm liberates NO in a buffer at pH 7.0 as a result of the spontaneous hydrolysis without additional activation.[7] To perform a comparative study of the monomer and dimer forms of CysAm, we set ourselves the task of finding the conditions for the preparation of the monomer form of CysAm. For this purpose, we studied an aqueous solution of CysAm by ESR and ^{1}H NMR spectroscopy (Figs 2 and 3). A CysAm powder does not show the ESR spectrum because the CysAm dimer is diamagnetic due to a short distance between the iron atoms. After the incubation of the aqueous solution of CysAm for 3 h, the ESR signal was also not observed. The monomer is formed upon dissolution of CysAm in DMSO. Thus, the solution in DMSO (see Fig. 2, *a*) exhibts the ESR spectrum at the *g* factor of 2.030 characteristic of iron nitrosyl complexes. The spectrum displays a poorly resolved hyperfine structure belonging, apparently, to nitrogen nuclei. The concentration of paramagnetic centers, which is initially equal to 0.35 mmol L^{-1}, decreases with time (see Fig. 2, *b*). Evidently, the diamagnetic dinuclear dimer decomposes into mononuclear moieties, which show a ESR signal; the further decomposition leads to a decrease in the ESR signal (see Fig. 2, *b*). Solutions of the dimer in water do not show the ESR signal due, apparently, to the fact that the hydrolysis of the dimer does not involve the formation of the monomer and the resulting fragments are diamagnetic. According to the estimate of the spin concentration (0.35 mmol L^{-1}) at a CysAm concentration in DMSO equal to $1 \cdot 10^{-3}$ mol L^{-1}, the monomer form accounts for 17.5% of the complex. After heating to 50 °C, the ESR signal disappears, which indicates that the heating leads to an increase in the rate of the decomposition of CysAm in DMSO. Therefore, under the conditions used (the

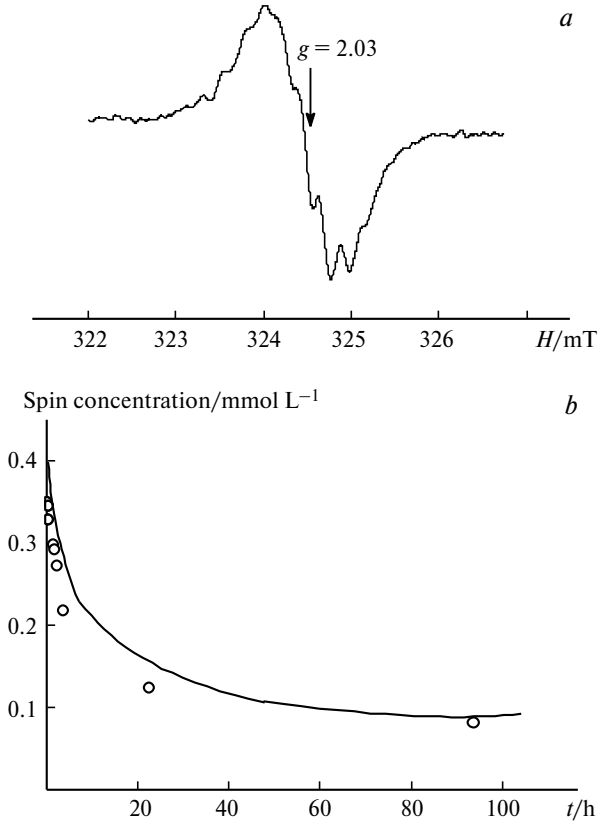

Fig. 2. ESR spectrum of a solution of CysAm in DMSO at a concentration of $1 \cdot 10^{-3}$ mol L^{-1} 15 min after the preparation of the solution (*a*) and the decrease in the concentration of paramagnetic centers in this solution with time (*b*).

storage of a solution of CysAm at a concentration of $1 \cdot 10^{-3}$ mol L^{-1} in DMSO at 20 °C for 15 min), the dimer dissociates into monomers, resulting in the establishment of the equilibrium between the dimer and monomer forms of CysAm, the concentration of the latter in the solution being 0.35 mmol L^{-1}. Hereinafter, we will refer to this solution as a dimer—monomer mixture.

We get these conclusions based on the results of ^{1}H NMR investigation of the influence of water and DMSO on CysAm. The ^{1}H NMR spectrum of the iron nitrosyl complex in D_2O (Fig. 3, *a*) shows a triplet at δ_H 3.27 corresponding to the proton of the CH_2—S group and a doublet of triplets at δ 3.37 assigned to the protons of the $C\underline{H}_2$—NH_4^+ group (see Fig. 3, *a*). A more complex form of the multiplet of the CH_2 group at NH_4^+ compared with that of the CH_2 group at the S atom is attributed to the long-range spin-spin coupling between the protons of the CH_2 group and the protons of the ammonium group. The signals for the protons of the ammonium group and water molecules of crystallization are not observed in the spectrum due to the fast exchange with the deuterium atoms of the solvent molecules. Therefore, the ^{1}H NMR spectroscopy confirmed the structure of CysAm proposed earlier,[2] and the fairly high resolution of the spectrum provides evidence for the absence of paramagnetic iron in solution.

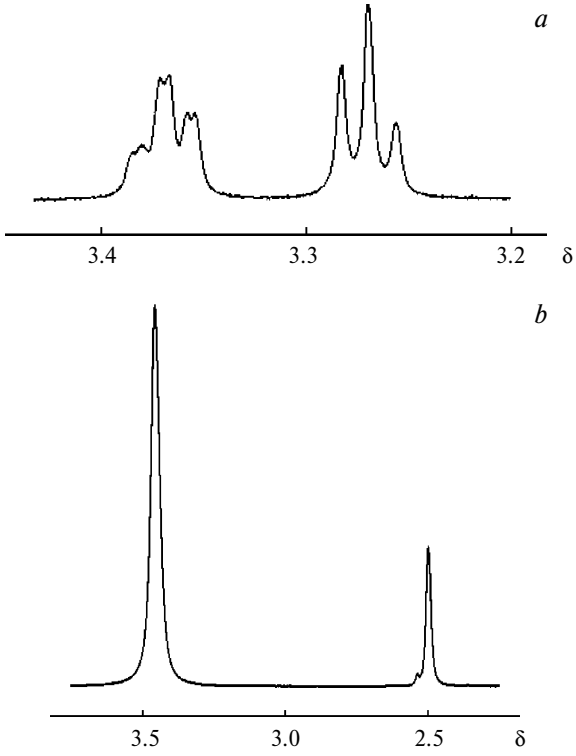

Fig. 3. ^{1}H NMR spectra of CysAm in D_2O (*a*) and DMSO-d_6 (*b*).

The situation is different for a solution of CysAm in DMSO-d_6. In this case, the signals in the ^{1}H NMR spectrum are broadened (Fig. 3, *b*). The half-width at half-height ($\Delta\nu_{1/2}$) of the signal for the protons of water in the spectrum of a solution of CysAm in D_2O is ≈1 Hz; in DMSO-d_6, $\Delta\nu_{1/2}$ of the signal for the protons of admixing H_2O is 17 Hz. Most likely, the broadening of the latter signal indicated the presence of paramagnetic iron in the solution. The appearance of paramagnetic iron is, apparently, associated with the decomposition of the dimer into monomers in a DMSO solution. In addition, a paramagnetic component was not observed in the ^{1}H NMR spectrum of the complex in D_2O even after 30 min, which may be evidence that the hydrolysis of the dimer in water does not afford monomers.

We also studied solutions of CysAm in the buffer and DMSO by spectrophotometry. The intensity of the bands in the absorption spectrum of CysAm in 0.05 *M* phosphate buffer (Fig. 4, *a*) with maxima at 305.1 and 361.6 nm decreased with time. At some time, no changes were observed, and the spectrum measured three days after the preparation of the solution is identical to that measured after 22 h (Fig. 4, *a*, curve *2*). In a solution of the dimer—monomer mixture in DMSO, an absorption maximum at 364 nm appears (see Fig. 4, *b*). In this case, the

absorption spectra of the monomer and the dimer apparently overlap, and it is difficult to separate the absorption maxima of the monomer. The intensity of the absorption bands decreases with time, like the intensity of the ESR lines (see Fig. 2, *b*). After three days, a maximum at 331 nm was observed in the absorption spectrum of the complex in DMSO (see Fig. 4, *b*, curve *4*). The investigation according to the procedure developed in our earlier study[8] (the reaction with cytochrome *c*) showed that the products, which were formed upon storage of CysAm in DMSO for three days, did not contain NO groups. Consequently, it can be concluded that CysAm slowly decomposes in a DMSO solution resulting in the elimination of NO. In a more concentrated solution (see Fig. 2, *b*), the decomposition may occur more slowly. A comparison of Figs 4, *a* and 4, *b* also confirms that the hydrolysis of CysAm in 0.05 *M* phosphate buffer is not accompanied by the formation of the monomer.

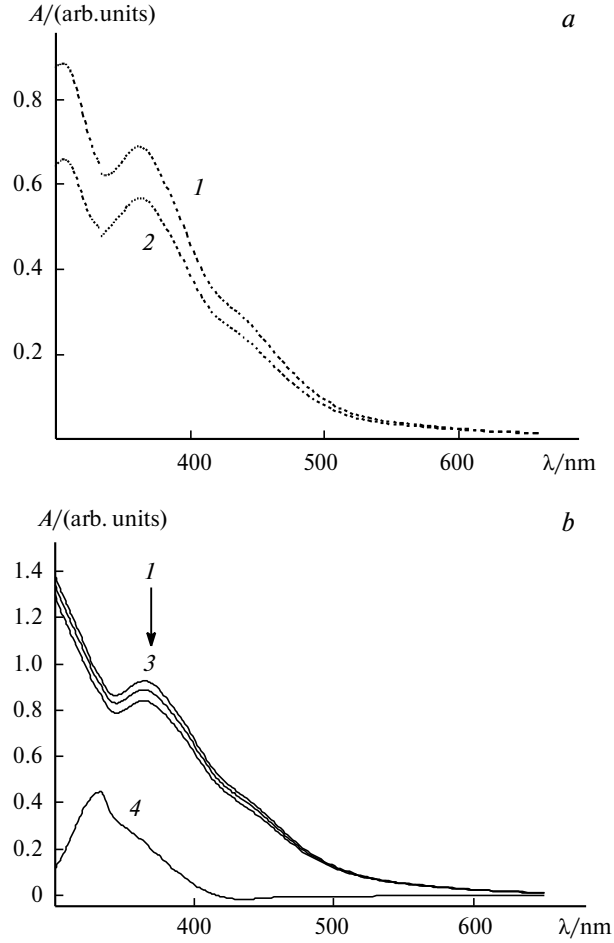

Fig. 4. Absorption spectra of: *a*, a solution of CysAm in 0.05 *M* phosphate buffer, pH 7.0, immediately (*1*) and 22 h (*2*) after the preparation of the solution; *b*, a solution of the dimer—monomer mixture in DMSO immediately (*1*) and 25 (*2*), 60 min (*3*), and 3 days (*4*) after the preparation of the solution ([CysAm] = = 0.1 mmol L^{-1}, 20 °C).

We studied the hydrolysis of CysAm in the dimer and monomer forms. The hydrolysis of CysAm was monitored from the changes in the absorption spectrum of the complex in the wavelength range of 450—650 nm (based on a decrease in the absorbance, see Fig. 4, *a*). It was found[8] that the hydrolysis of a solution of CysAm in 0.05 *M* phosphate buffer, pH 7.0, at a concentration of $2 \cdot 10^{-4}$ mmol L^{-1} for 3 h led to the elimination of 0.43 equiv. of NO, *i.e.*, 10.7% of the CysAm complex undergo hydrolysis. It should be noted that in the presence of hemoproteins, *viz.*, hemoglobin and ferrocytochrome, the nitrosylation at the Fe atom occurs,[7,8] which is accompanied by a 2.7% decrease in the intensity of the absorption band at 450 nm. Therefore, the hydrolysis of the complex resulting in the elimination of NO into solution can be observed based on the changes in the absorption of CysAm.

Figure 5, *a* presents the kinetic curves for the hydrolysis of CysAm in 0.05 *M* phosphate buffer, pH 7.0. The experimental data are well approximated by the empirical formula $[\text{CysAm}](t) = a + b \cdot e^{-kt}$, where $a = 0.2136$ mmol L^{-1}, $b = 6.9 \cdot 10^{-3}$ mmol L^{-1}, and $k = 1.87 \cdot 10^{-4}$ s^{-1}. This provides an estimate of the reaction rate of the process. Thus, the maximum rate of the decomposition (at $t = 0$) is $v(0) = k \cdot b = 1.3 \cdot 10^{-6}$ mmol L^{-1} s^{-1}. The relatively large limiting value $a = 0.2136$ mmol L^{-1} indicates that the hydrolysis of CysAm in 0.05 *M* phosphate buffer is not completed. This can be attributed to the reversibility of the decomposition

$$\text{CysAm} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{NO} + \text{products}. \qquad (1)$$

The rate constants $k_1 = (6.6 \pm 0.2) \cdot 10^{-6}$ s^{-1} and $k_{-1} = 11 \pm 1$ mol^{-1} L s^{-1} were determined by the numerical solution of the inverse problem from the experimental data. In Fig. 5, *a*, the experimental data are compared with the theoretical curve calculated from the constants k_1 and k_{-1}. As can be seen from Fig. 5, *a*, the experimental data are adequately described by the reaction (1) with the estimated kinetic parameters.

The experimental kinetic curve for decomposition of CysAm in distilled water is shown in Fig. 5, *b*. Like the hydrolysis of CysAm in the phosphate buffer, the experiment can be described by the empirical formula $[\text{CysAm}](t) = a + b \cdot e^{-kt}$ with the parameters $a = 0.186$ mmol L^{-1}, $b = 4.35 \cdot 10^{-3}$ mmol L^{-1}, and $k = 2.78 \cdot 10^{-4}$ s^{-1}. The rate of the decomposition of the complex in distilled water is similar to the rate of its decomposition in the phosphate buffer $v(0) = k \cdot b = 1.2 \cdot 10^{-6}$ mmol L^{-1} s^{-1}. Taking into account the large limiting value $a = 0.186$ mmol L^{-1}, in this case the reaction (1) can also be considered as the kinetic model of this process. The rate constants $k_1 = (8.2 \pm 0.3) \cdot 10^{-6}$ s^{-1} and $k_{-1} = 17.8 \pm 1.6$ mol^{-1} L s^{-1} evaluated from the experimental data fairly well describe the kinetic curve for decomposition of CysAm (Fig. 5, *b*).

A slight difference in the rate constants describing the hydrolysis of the complex in 0.05 *M* phosphate buffer and distilled water is, apparently, attributed to the influence of the medium on this process.

Figure 5, *c* presents the time dependence of the total concentration of the monomer and dimer forms of CysAm during the hydrolysis in a mixture of 0.05 *M* phosphate buffer and DMSO (see the Experimental section). The empirical description of this dependence by the equation $[\text{CysAm}](t) = a + b \cdot e^{-kt}$ gives the following parameters: $a = 0.0112$ mmol L^{-1}, $b = 0.2216$ mmol L^{-1}, and $k = 2.75 \cdot 10^{-5}$ s^{-1}. The qualitative behavior of the experimental curve and the parameters of its approximation are responsible for the two main differences between the above-considered decomposition of the CysAm dimer—monomer mixture and the hydrolysis of the dimer form of CysAm. First, the rate of the decomposition of the mixture is substantially higher: $v(0) = k \cdot b = 6.1 \cdot 10^{-6}$ mmol L^{-1} s^{-1}, and, second, the limiting experimentally measured value is close to zero. Based on these data with account for the results of ESR and NMR investigations, the following kinetic model of the process under consideration can be suggested:

$$\text{CysAm} + \text{DMSO} \underset{}{\overset{K}{\rightleftharpoons}} 2\,\text{M} + \text{DMSO}, \qquad (2)$$

$$\text{CysAm} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{NO} + \text{P}^1, \qquad (3)$$

$$\text{M} \xrightarrow{k_2} \text{NO} + \text{P}^2, \qquad (4)$$

where P^1 and P^2 are the products.

Here, the reaction (2) is the simplest model of the decomposition of the CysAm dimer into monomers (M) in DMSO. It is hypothesized that the condition of the detailed equilibrium $K \cdot [\text{CysAm}] = [\text{M}]^2$ is met for this reaction at any instant of time. The equilibrium constant *K* can be estimated from the ESR data. Thus, after the dissolution of CysAm in DMSO at the initial concentration of the dimer $[\text{CysAm}]_0 = 1 \cdot 10^{-3}$ mol L^{-1}, the concentration of the monomer is $0.35 \cdot 10^{-3}$ mol L^{-1}. Taking into account the reaction mass balance, the equilibrium constant is estimated at $1.485 \cdot 10^{-4}$ mol L^{-1}. The reaction (3) describes the hydrolysis of the dimer in 0.05 *M* phosphate buffer. The rate constants were determined above: $k_1 = 6.6 \cdot 10^{-6}$ s^{-1} and $k_{-1} = 11$ mol^{-1} L s^{-1}. The reaction (4) describes the decomposition of the monomer molecules M. The rate constant k_2 is unknown, and its value should be determined from the experimental data presented in Fig. 5, *c*.

Taking into account the detailed equilibrium, the equation system describing the process under consideration can be written as follows:

$$d[\text{CysAm}]/dt = 2[\text{M}]/(K + 4[\text{M}]) \cdot (-2k_1[\text{CysAm}] + 2k_{-1}[\text{NO}][\text{P}^1] - k_2(K \cdot [\text{CysAm}])^{0.5}), \qquad (5)$$

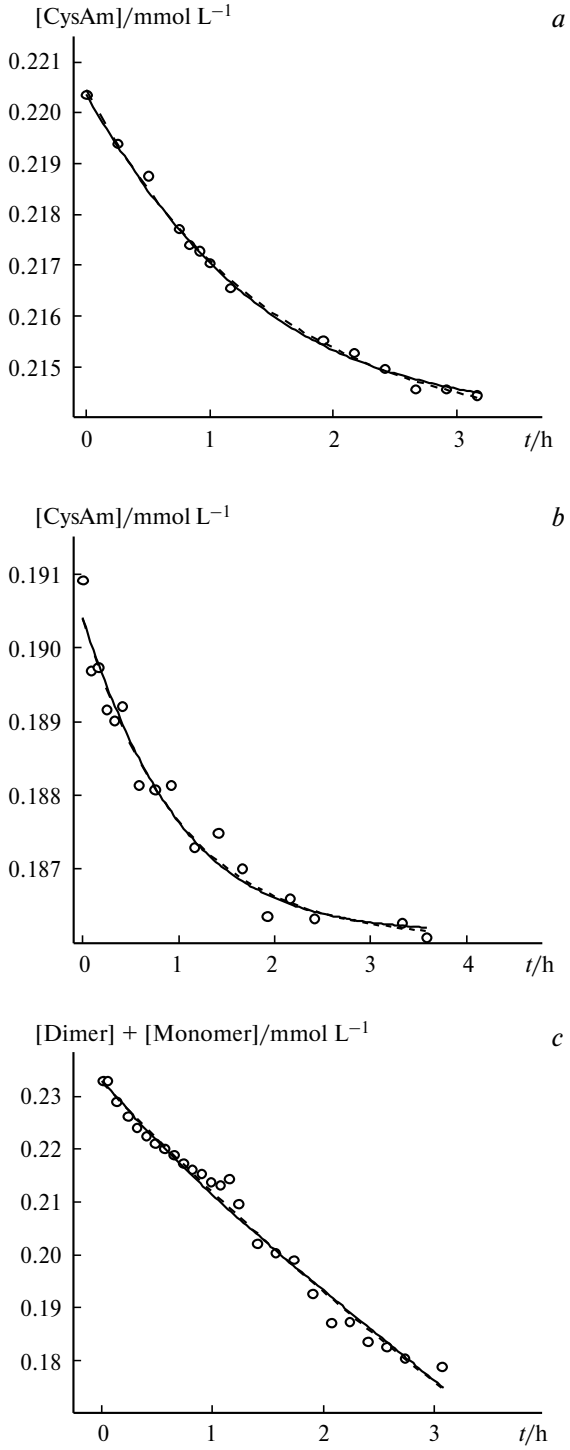

Fig. 5. Kinetics of the hydrolysis of CysAm in 0.05 *M* phosphate buffer, pH 7.0, (*a*) and in water (*b*) at the initial concentration of the complex equal to 0.22 (*a*) and 0.19 mmol L^{-1} (*b*), and the kinetics of the hydrolysis of the CysAm dimer—monomer mixture in 0.05 *M* phosphate buffer and DMSO (*c*). The points correspond to the experimental data, the approximation of the data by the equation $[\text{CysAm}](t) = a + be^{-kt}$ is represented by the dashed line, and the theoretical curves calculated from the constants k_1 and k_{-1} (*a*, *b*) and k_2 (*c*) are shown as solid lines.

$$d[NO]/dt = k_1[CysAm] - k_{-1}[NO][P^1] + k_2(K \cdot [CysAm])^{0.5}, \quad (6)$$

$$d[P^1]/dt = k_1[CysAm] - k_{-1}[NO][P^1], \quad (7)$$

$$d[P^2]/dt = k_2(K \cdot [CysAm])^{0.5}. \quad (8)$$

The experimentally determined function

$$x(t) \equiv [CysAm] + [M] = [CysAm] + (K[CysAm])^{0.5}.$$

The constant k_2 was determined by the minimization of the functional

$$F(k_2) = \sum_i (x_{exp}(t_i) - x_{calc}(t_i))^2,$$

where $x_{exp}(t_i)$ are the values in the experimental dependence at the instants of time t_i, and $x_{calc}(t_i)$ are the corresponding values of $x(t)$ calculated by the numerical solution of the equation system (5)—(7). The value of the constant $k_2 = (7.3 \pm 0.2) \cdot 10^{-5}$ s^{-1} fairly well describes the experimental curve (see Fig. 5, *c*, the solid line), which confirms that the kinetic model (2)—(4) is adequate.

As can be seen, the value of k_2 is approximately an order of magnitude larger than k_1 for the hydrolysis of the CysAm dimer in water and 0.05 *M* phosphate buffer. This means that the rate of the hydrolysis of the monomer form of CysAm is substantially higher than that in the case of other iron nitrosyl complexes.[4]

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References

1. N. A. Sanina, O. S. Zhukova, Z. S. Smirnova, L. M. Borisova, M. P. Kiseleva, S. M. Aldoshin, *Ros. Bioterapevt. Zh.* [*Russ. Biotherapeutic J.*], 2008, **7**, No. 1, 52 (*in Russian*).
2. T. N. Rudneva, N. A. Sanina, K. A. Lyssenko, S. M. Aldoshin, M. Yu. Antipin, N. S. Ovanesyan, *Mendeleev Commun.*, 2009, **19**, 253.
3. T. Rudneva, N. Emel´yanova, N. Sanina, *Abstr. of 3rd European Chemical Congress* (*Nürnberg, Germany, August 29 — September 2, 2010*), Nürnberg, Germany, 2010.
4. 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, *Izv. Akad. Nauk, Ser. Khim.*, 2010, 2148 [*Russ. Chem. Bull.*, *Int. Ed.*, 2010, **59**, 2203].
5. N. A. Sanina, S. M. Aldoshin, T. N. Rudneva, N. I. Golovina, G. V. Shilov, Yu. M. Shul´ga, V. M. Martynenko, N. S. Ovanesyan, *Koord. Khim.*, 2005, **31**, 323 [*Russ. J. Coord. Chem.* (*Engl. Transl.*), 2005, **31**, 301].
6. 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*, 2007, **16**, 181.
7. N. A. Sanina, L. A. Syrtsova, B. L. Psikha, N. I. Shkondina, T. N. Rudneva, A. I. Kotel´nikov, S. M. Aldoshin, *Izv. Akad. Nauk*, *Ser. Khim.*, 2010, 1944 [*Russ. Chem. Bull.*, *Int. Ed.*, 2010, **59**, 1994].
8. N. A. Sanina, L. A. Syrtsova, N. I. Shkondina, T. N. Rudneva, A. I. Kotel´nikov, S. M. Aldoshin, *Izv. Akad. Nauk*, *Ser. Khim.*, 2010, 1528 [*Russ. Chem. Bull., Int. Ed.*, 2010, **59**, 1565].

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