Investigation of the reaction between phosphoenolpyruvic acid and thiosulfate-nitrosyl iron complex

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Reaction between the nitric oxide donor, tetranitrosyl iron complex with thiosulfate ligand (TNIC), and phosphoenolpyruvic acid (PEP) was studied. Formation of products of the reaction between PEP and TNIC was confirmed by UV-, IR-spectroscopy, and mass spectrometry methods. Also, ions with mass numbers m/z 318 and m/z 256 were identified among the reaction products, belonging to the compounds $[O_3S_2—Fe—PEP]^{2-}$ and $[S—Fe—PEP]^{2-}$, respectively.

Key words: phosphoenolpyruvic acid, sulfur-nitrosyl iron complexes, NO donors, UV spectroscopy, IR spectroscopy, mass spectrometry.

It is well known that the quality of functioning of all structures and components in living organisms depends on the energy state of cells.[1] This explains attention of the scientists to major biological high-energy molecules: adenosine di- and triphosphates (ADP, ATP) and phosphoenolpyruvic acid (PEP).[2—4] The synthesis of ADP takes place during the glycolysis process as a result of the phosphate group transfer from PEP to ADP catalyzed by pyruvate kinase.[5] Phosphoenolpyruvic acid is a strong inhibitor of hexokinase (EC 2.7.1.2), phosphoglucose isomerase (EC 5.3.1.9), phosphofructokinase (EC 2.7.1.11), and aldolase (EC 4.1.2.13) at the initial step of glycolysis.[6] Since the glycolysis rate is higher for cancer cells than for normal ones, the metabolism of PEP and glycolytic enzymes is being under active investigations. This is also important for searches of methods to slow down the glycolysis rate.

Based on nitric oxide-donating nitrosyl iron complexes (NIC), some potential medicines possessing the ability of the targeted delivery of NO to biological targets of the cell may be developed.[7] Nitric oxide is known to inactivate glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12)[8], which blocks glycolytic synthesis of ATP[9] and cell (mitochondrial) respiration.[10] Taking into account our studies of the NCI affects on the energy state of a biological system,[11—13] it was important to verify the possibility of a reaction between PEP and NIC. The thiosulfate nitrosyl iron complex (TNIC) was used as NIC in this work. The discovered therapeutic properties of TNIC were reported earlier.[14,15]

This work is aimed to study the reactivity of TNIC (Fig. 1) as a nitrogen monooxide donor towards PEP by spectral methods.

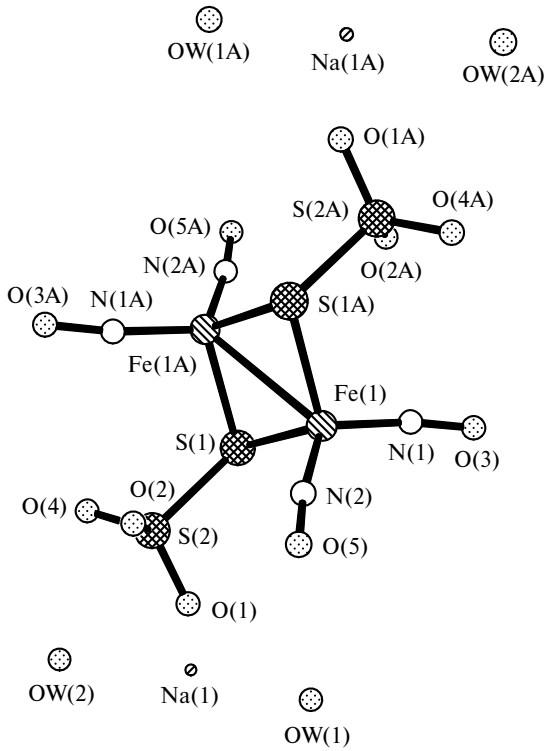

Fig. 1. Molecular structure of the TNIC based on the single crystal X-ray diffraction analisys.[16]

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Experimental

The commercial PEP (purity 99%, Sigma) was used without additional purification.

The commercial $FeSO_4 \cdot 7H_2O$ and $Na_2S_2O_3 \cdot 5H_2O$ (Aldrich) were used without additional purification in the synthesis of TNIC The TNIC with general formula $Na_2[Fe_2(S_2O_3)_2(NO)_4] \cdot 4H_2O$ was prepared according to the known procedure.[16] The $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ dianion has binuclear centrosymmetric structure, wherein the iron atoms are bonded with μ-atoms of sulfur of the thiosulfate groups (Fig. 1).

The reaction between PEP and TNIC was carried out in bidistilled water under vigorous stirring and normal conditions. The PEP and TNIC solutions of equal molarity were mixed (concentrations of $1 \cdot 10^{-2}$ mol L^{-1}).

The electronic absorbtion spectra (UV) were recorded on a UV-VIS Lambda EZ 210 spectrophotometer from Perkin Elmer (USA). The absorbtion spectra at different PEP concentrations were recorded with a compensation. The bidistilled water was used as the compensation.

The IR spectra were recorded on a FT-IR ALPHA (4000—500 cm^{-1}) IR-Fourier spectrometer from Bruker (Germany). The measurements were carried out in aqueous solutions from a drop between ZnSe glasses at 20 °C under air atmosphere.

The mass spectra were recorded on a LCMS 20-20 instrument from Shimadzu (Japan) without the separation on a column. The aqueous solution of the analyte was directly injected to the ion source of the mass analyzer. The type of ionisation was electrospray (ESI), the mass analyzer was a quadrupole one. The interval of the measured m/z values was 10—2000, the mass analyzer resolution was 0.6. The mixture of acetonitrile—water (1 : 1) was used as the eluent. The volume of a sample was 0.1—10 μL. Signal assignment in the spectra was based on the abundance of the negatively charged ions. The attribution of the ions with equal mass numbers with TNIC or PEP was determined from the isotope abundance.

Results and Discussion

The solution changed color from yellow-brown to green upon mixing the PEP and TNIC solutions. The solution was gradually becoming transparent. The observed visual changes indicates an ongoing chemical reaction. It may be assumed that the color change takes place due to the formation of a new complex compound.

The recorded UV spectra are shown on Fig. 2. The starting TNIC has maximum absorption at 310 and 360 nm, while the maximum absorption of PEP, the major chromophore in the studied system, is at 212 nm (lgε 10.9823). Figure 2 shows that the absorption spectra of starting compounds and the product of the reaction are significantly different. The reaction product has absorption maximum at 263 nm, *i.e.*, it has a bathochromic shift of the absorption spectral band for 50 nm in a comparison with the starting PEP, accompanied by a hypochromic effect. This indicates a formation of the new compound characterized by π→π* electron transfer and increased level of occupied molecular orbitals due to p,π conjugation between the lone electron pair of an oxygen atom from PEP and the system of p electrons from TNIC. Obviously, there is a classical addition of an electron-donating substituent (OR) having conjugated bonds, but not containing N=N, NO_2, and NO groups. Such substituent R could be $[FeS_2O_3]^{2-}$ fragment of TNIC.

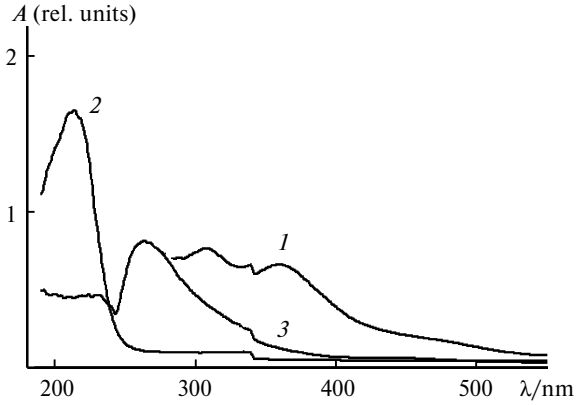


Fig. 2. Electronic absorption spectra of TNIC (*1*), PEP (*2*), and the product of their reaction (*3*). PEP concentration is $1.05 \cdot 10^{-4}$ mol L^{-1}; TNIC concentration is $1.07 \cdot 10^{-2}$ mol L^{-1}.

The type of the reaction products formed in the reaction between PEP and TNIC was investigated by IR spectroscopy (Fig. 3). According to the previously reported data[17], the characteristic absorption in the TNIC spectrum is an intense signal about 1795 cm^{-1} with a shoulder at 1742 cm^{-1}, which belongs to the ν(NO) valence stretch. Such bands are completely absent in the spectrum of the product of the reaction between PEP and TNIC, which indicates the elimination of the NO group during the reaction process. Another characteristic absorption in the TNIC spectrum is the narrow band of medium intensity about 1629 cm^{-1}, which belongs to the ν(Fe—NO) bond vibrations of the iron atom coordinated

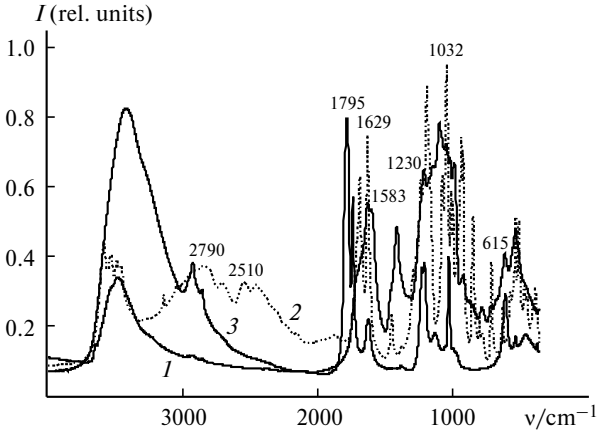


Fig. 3. IR spectra of the TNIC (*1*), PEP (*2*) and the product of their reaction (*3*). Initial concentrations of the PEP and TNIC solutions are $1 \cdot 10^{-2}$ mol L^{-1}.

with NO. This band is broadened in the spectrum of the reaction product, and a shoulder at 1583 cm^{-1} appears for this band. The remarkable feature is that intensity of this band significantly increases, which indicates a possible bonding energy redistribution of the iron atom. Perhaps, the iron atom recoordinated to oxygen atom after NO elimination.

A strong doublet about 1230 cm^{-1} in the IR spectrum of TNIC was assigned to the $\nu_s(SO_2)$ symmetric stretch. The strong band at 1032 cm^{-1} belongs, probably, to the valence vibrations of the sulfur—oxygen bond, ν(S—O).[17—19] Both these bands remain in the spectrum of the product of reaction of PEP with TNIC.

A strong band about 615 cm^{-1} in the IR spectrum of the starting TNIC complex can be assigned to the valence stretch of iron—nitrogen bond, ν(Fe—N). This band is also present in the spectrum of the product. However, there is a possibility that in the spectrum of the product this band belongs to the valence stretching vibrations of Fe—O bond (ν(Fe—O)) or to stretches of COO^- group coordinated with the iron atom, for example, *via* oxygen atom (δ(O—C=O) + ν(Fe—O)), as reported in the literature.[16]

The IR spectrum of PEP contains strong bands in the region 3000—3500 cm^{-1}, which is characteristic for bound water molecules. These bands retain in the obtained product.

A strong band about 2790 cm^{-1} and strong doublets at 2510 and 2490 cm^{-1} belong to ν(C—O) and ν(C—OH) stretches of the carboxyl groups and are characteristic of the IR spectrum of PEP. These bands are completely absent in the IR spectrum of the product. The loss of these bands indicates the formation of a new compound *via* carboxyl groups.

Strong doublets (1710, 1629 cm^{-1}) in the IR spectrum of PEP belong to the ν(P—O) stretches of dimeric and monomeric forms of the phosphate residues. During formation of the new compound, the stretches of the dimer are disappeared, while the stretches of monomer are remained.

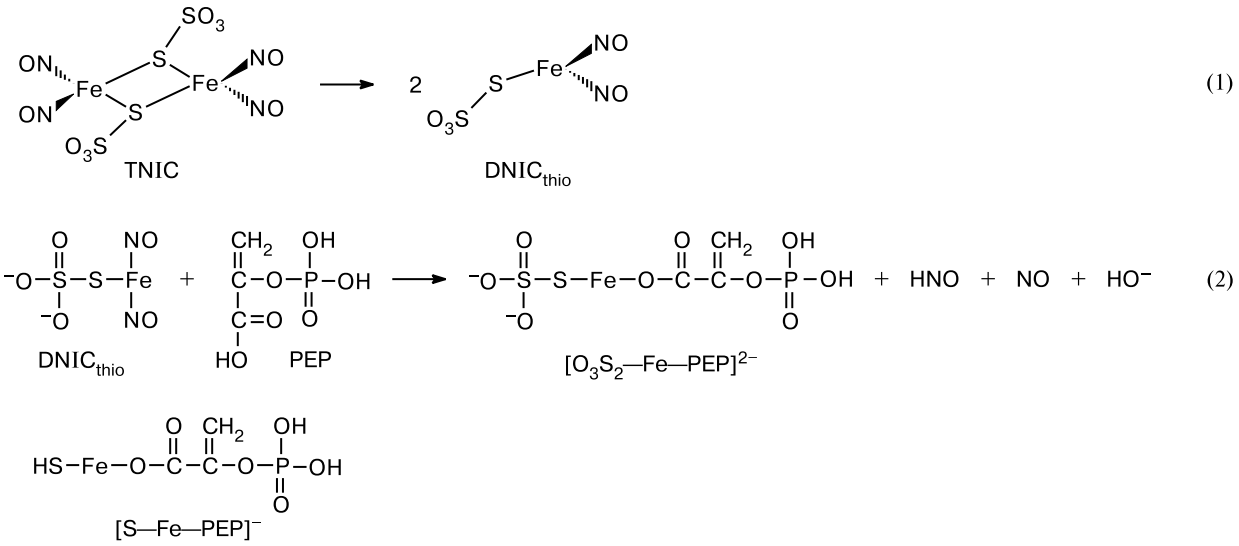
A significant number of bands in the 750—1250 cm^{-1} region in the spectrum of the initial PEP belongs to the valence, deformation, symmetric, and asymmetric stretches of the P—O, P=O, and P—O—C bonds. All these bands retain in the spectrum of the product of the reaction between PEP and TNIC, *i.e.*, the phosphate residues do not participate in the formation of the new compound.

Thus, based on the analysis of IR spectra, it can be concluded that a new compound was produced by forming the bonds between the iron atom from TNIC and the oxygen atom (or atoms) of the carboxyl groups of PEP.

The possible chemical reactions and their products are shown in Scheme 1.

The composition of the TNIC and PEP mixture was analyzed by mass spectrometry before and after the chemical reaction. In fact, some published reports provide suggestions about application of LCMS in the studies of enzymatic processes[20,21] and some particular biological objects,[22] including PEP. According to the known data about the TNIC molecular structure[16] (Fig. 1), this complex contains four molecules of water (OW(1), OW(2), OW(1A), and OW(2A)) in the crystalline state. The binuclear TNIC undergoes a primary decomposition in the protic media with the formation of solvated mononuclear dinitrosyl iron complexes ($DNIC_{thio}$) as it is shown in Scheme 1, Eq. (1). Then the $DNIC_{thio}$ molecules decompose into mononitrosyl complexes and other products of the hydrolysis reaction with the

Scheme 1



NO release into the solution. The structure of these intermediates was confirmed by mass spectrometry.[7,23]

The results obtained for the identification of molecular ions by the mass spectrometry are given in Table 1. The mass spectrum of PEP contains a molecular ion with *m/z* 168. In the mass spectrum of the reaction mixture, the presence of this molecular ion was being observed during the entire observation time, 36 min. This evidence allows us to conclude that the reaction between PEP and $DNIC_{thio}$ is slow. Based on the chemical structure of the PEP molecule, it was assumed that the PEP molecules in a solution can exist in the form of dimers, but there was no observed ions with *m/z* 336. An anion with *m/z* 318 is probably referred to the first reaction product, compound $[O_3S_2—Fe—PEP]^{2-}$ ($[FeC_3S_2PO_8H_3]^{2-}$, see Scheme 1). The characteristic for the reaction product anion with *m/z* 318 appeared in the spectrum after 2 min from mixing of the TNIC and PEP solutions, while an ion with *m/z* 228 ($DNIC_{thio}$) with two NO groups was also present.

An anion with *m/z* 256 is probably referred to the second reaction product, compound $[S—Fe—PEP]^-$ ($[FeC_3SPO_6H_5]^-$, Scheme 1 and Table 1). These ions appeared in the spectrum 2 min after from the reaction onset. The peak intensity of this ion was increasing during the reaction process until the 36th min of observation. Probably, the $[S—Fe—PEP]^-$ molecular ion with *m/z* 256 was formed from compound $[O_3S_2—Fe—PEP]^{2-}$. After 36 min of observation, the amount of compound $[S—Fe—PEP]^-$ was almost 2.5 times greater than the amount of the $[O_3S_2—Fe—PEP]^{2-}$ compound. Compound $[S—Fe—PEP]^-$ can be also formed in a different way, *via* a parallel reaction of PEP with the $[S—Fe]^-$ ion that is a residue of TNIC.

It is interesting that after 2 min from mixing the solutions of the starting materials an ion with *m/z* 420 appeared in the mass spectrum. Obviously, this is the ion from the third reaction product, in which the TNIC residue is coordinated with two molecules of PEP, [S—Fe—2PEP] (see Table 1).

Apparently, the following processes take place in the system:

Table 1. Mass spectrometry analisys data for products of the reaction of PEP with TNIC after 2 (I) and 36 min (II) from the reaction onset

Reaction products	*m/z*	Relative intensity of ion flow (%)	
		I	II
$[S—Fe—PEP]^-$	256	11.90	57.69
$[O_3S_2—Fe—PEP]^{2-}$	318	33.90	16.03
$[S—Fe—2PEP]^-$	420	19.25	28.21

(1) dissociation of TNIC according to the previously reported scheme;[23]

(2) the reaction between $DNIC_{thio}$ and the PEP molecule, *i.e.*, elimination of the NO group leads to the [PEP-$DNIC_{thio}$] complex, from which an $[O_3S_2—Fe—PEP]^{2-}$ intermediate product with *m/z* 318 is formed. Then, the $[SO_3]^-$ ion is eliminated to give the [S—Fe—PEP] reaction product;

(3) the $[O_3S—S—Fe]^-$ ion, which is a fragment of $DNIC_{thio}$ and is formed as a product of the TNIC dissociation, reacts with PEP to form the same $[O_3S_2—Fe—PEP]^{2-}$ intermediate reaction product, stabilized as $[S—Fe—PEP]^-$.

In summary, the new compound was produced in the reaction between PEP and TNIC *via* formation of the iron—oxygen bond. The mass numbers of the reaction products were determined by the mass spectrometry method. The scheme of the reaction process and the structures of formed products were proposed. The additional kinetic studies are needed to refine the proposed scheme, to determine the quantitative parameters in the steps of the process, and the structure of intermediate products.

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