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Additional iron ions binding sites in hemoglobin: XANES modeling

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The contributions of additional Fe-binding sites and heme iron to the experimental spectrum of urea-influenced hemoglobin were evaluated based on theoretical analysis of X-ray absorption spectra. The theoretical Fe *K*-edge X-ray absorption spectra were calculated for structural models of the supposed additional Fe binding sites and heme. Linear combination fit of theoretical spectra showed that in treated hemoglobin iron ions are more likely to be in the additional Fe-binding sites, which is surrounded by the following amino acids: cysteine-93, histidine-146, and asparagine-94 (Cys 93, His 146, Asp 94).

Keywords: hemoglobin, XANES, additional iron-binding sites, endogenous toxicants.

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One of the most significant protein structures for human life is hemoglobin. When metabolic processes in the body are disrupted, physiological metabolic products in high doses, such as urea, become toxic. Under the influence of such unfavorable factors, a violation of the spatial structure of proteins is observed, and the ability to form complexes with transition metals (Zn, Fe, Cd, Ni) is acquired. As a result, stable protein aggregates with reduced functional activity appear in the cell [1,2].

X-Ray Absorption Near Edge Structure (XANES) is a promising tool for studying the biological systems [3,4]. New opportunities for studying the mechanisms of dysfunction of protein molecules in pathological conditions are opened by model experiments on individual molecules under conditions that reproduce the effects of endogenous (arising due to internal causes) in the body. In [5,6] based on XANES spectroscopy data of protein films, show their ability to bond metal ions under the influence of damaging factors by additional binding sites in the structure of proteins. In this work, the method of theoretical analysis of XANES spectra behind the *K* edge of iron was used to determine the local atomic structure of the environment of Fe-bonding centers in hemoglobin.

Methods

Structural data of hemoglobin were taken from the Protein Data Bank (PDB) [7]. For the structural model of the heme plane (porphyrin core containing iron), the structure 3ODQ was used, for Fe-binding sites — 1BZ1. For these models, a geometric optimization procedure was carried out based on Density Functional Theory (DFT) using the B3LYP exchange-correlation potential and the TZP basis set in the ADF program. Theoretical analysis of XANES spectra for Fe K-edges was carried out using the finite difference method in full potential using the FDMNES [8]. The calculated clusters were 6 Å. The Lorentz convolution procedure was applied.

Results and discussion

In the work [9] to quantify the content of metal ions bound by molecules of intact (initial hemoglobin, without treatment) and urea-treated hemoglobin (hereinafter — treated hemoglobin), the intensity of the fluorescence release of sulfur ions from amino acid residues was used cysteine and methionine. A comparison of the peak intensities of SKα and Fe*K* (R_{Fe}/S) for intact and treated hemoglobin was 0.44 and 0.56, respectively, which exceeded the R_{Fe}/S values for hemoglobin (0.33). This indicated the appearance of additional binding sites for Fe ions in hemoglobin, in addition to heme, after its treatment with urea.

The ability to bond metal ions (Fe and Zn) with amino acids was discussed in works [5,10]. It is known that in most ($\sim$ 90%) proteins, zinc ions are coordinated with cysteine (Cys), histidine (His), asparagine (Asp) and glutamine (Glu) residues [11,12]. Based on these data, for theoretical analysis of XANES spectra, structural models of heme and Fe-binding sites containing combinations of amino acids (Cys93, His146, Asp 94), (Cys93, His146, Glu90, Asp94), (Cys93, His146, Asp94, His97) (Fig. 1, *b*). In model structures (Cys93, His146, Asp 94), (Cys93, His146, Glu90, Asp94), iron is coordinated by sulfur (Cys), two nitrogens (His/Asp), two oxygens (Asp/Glu/Cys). In the model (Cys93, His146, Asp94, His97), iron is coordinated by sulfur (Cys), two nitrogens (His), and oxygen (Asp). For the obtained models, XANES spectra were calculated and compared with experimental data (Fig. 1, *a*).

As can be seen from Fig. 1, *a*, the theoretical spectrum calculated for the Fe ion in heme is in good agreement with the experimental spectrum of intact hemoglobin, which

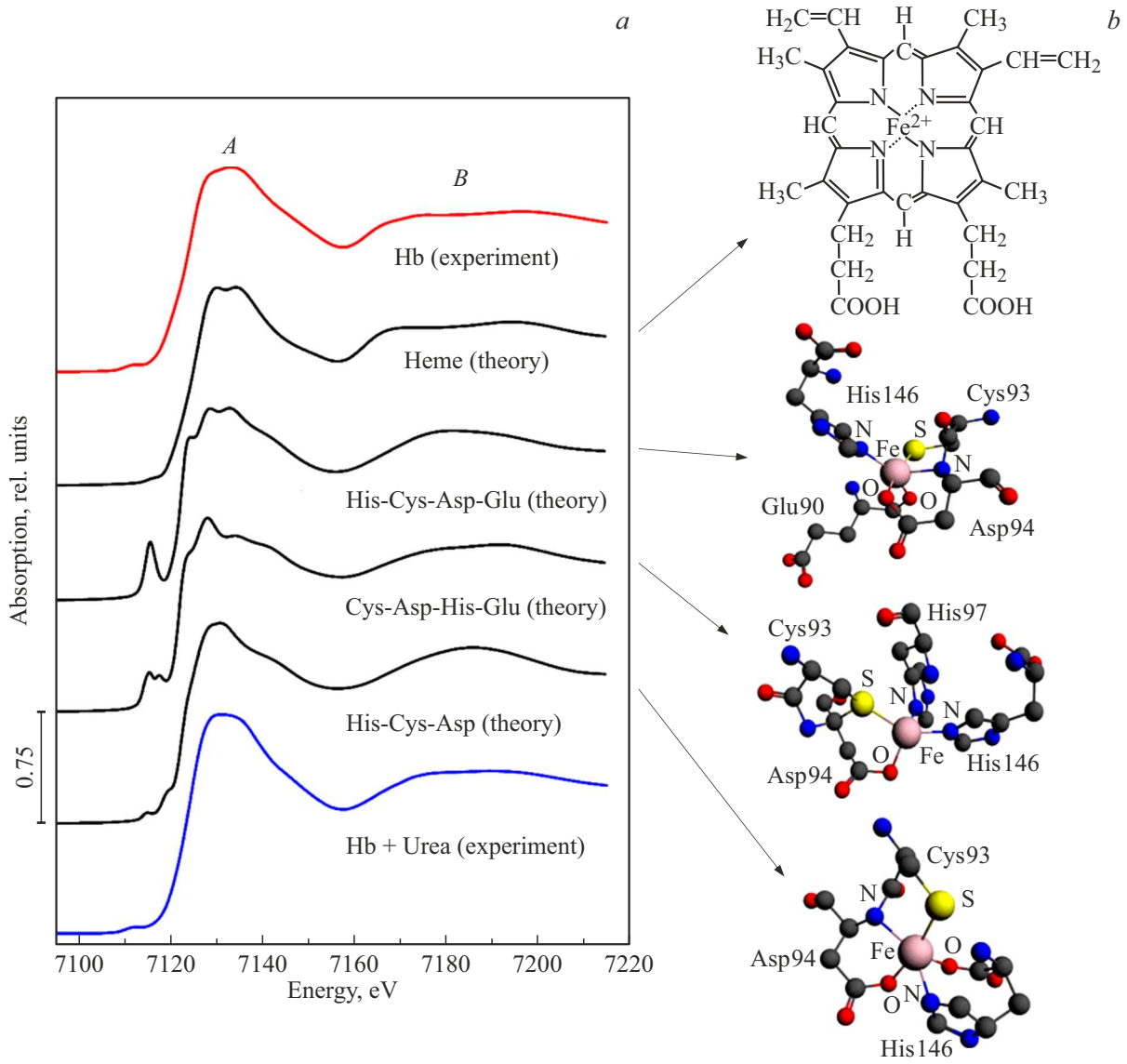

Figure 1. Experimental XANES spectra behind the Fe *K*-edge of intact and treated hemoglobin (from the work [9]) in comparison with theoretical spectra for structural models of heme and Fe-binding sites (*a*); structural models (*b*). Hb — Hemoglobin, Hb+Urea — hemoglobin treated with urea.

corresponds to $R_{Fe/S} = 0.44$ for intact hemoglobin. A linear combination fit of theoretical XANES spectra was performed to quantify the contributions to the XANES spectrum of processed hemoglobin from Fe in heme and supposed additional Fe-binding sites (Fig. 2). It follows from Fig. 2 that the differences in the experimental spectra of intact and treated hemoglobin are expressed in an increase in intensity and a change in the shape of the white line *A* and peak *B*. The XANES spectrum — is the average spectrum from all Fe ions present in the sample; therefore, it represents a superposition of signals from Fe ions located in the heme plane and additional binding sites. The observed differences are associated with changes in the ratio of heme Fe/Fe-binding sites. Thus, the spectrum of intact hemoglobin should largely reflect the local environment of Fe ions in heme ($R_{Fe/S} = 0.44$), and the spectrum of processed hemoglobin should contain a signal from additional Fe-binding sites, which is confirmed by the results of a linear combination.

Figure 2 shows that the trend in the experimental spectra is better reflected by the combination of the theoretical spectra of heme and the structural model (Cys93, His146, Asp94), which confirms the highest probability of the presence of these amino acids surrounded by an additional Fe-binding sites. The inset shows the trend of changes in the spectra at different weight ratios. The theoretical spectrum of heme is characterized by a division of the maximum *A*; with increasing weight of the components of the structural model (Cys93, His146, Asp 94), there is a tendency for the disappearance of peak doubling and the coincidence

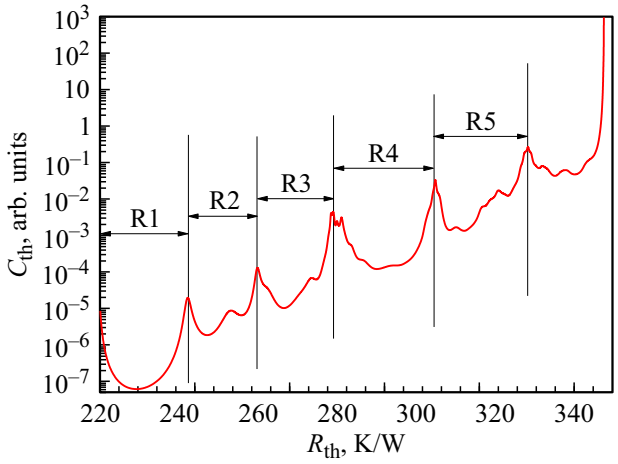

Figure 2. Experimental Fe K-edge XANES spectra in intact and treated hemoglobin [9] in comparison with linear combinations of theoretical spectra for structural models. Inset: trend of theoretical spectra at different heme weights and Fe-binding site model (Cys93, His146, Asp94). Hb — Hemoglobin, Hb+Urea — hemoglobin treated with urea.

of the peak shape with the experimental one. Also, for the *B* peak, with an increase in the contribution of the Fe-binding model, there is an increase in intensity, as for the experimental spectra. For the structural models (Cys93, His146, Glu90, Asp94) and (Cys93, His146, Asp94, His97), there is no this trend for peaks *A* and *B*. Moreover, there is an intensive increase in the spectral feature in the pre-edge region.

Conclusion

There was an assessment of the contributions of additional Fe-binding centers in hemoglobin and heme iron to the experimental spectrum of hemoglobin exposed to urea. The trend of changes in the resulting theoretical spectra of heme and supposed Fe-binding sites (Cys93, His146, Asp 94), (Cys93, His146, Glu90, Asp94), (Cys93, His146, Asp94, His97) obtained as a result of a linear combination at different weights is shown. It has been specified that in processed hemoglobin, Fe ions, in addition to heme, are more likely to be in an additional Fe-binding sites surrounded by the following amino acids: cysteine-93, histidine-146 and asparagine-94 (Cys93, His146, Asp 94).

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Conflict of interest

The authors declare that they have no conflict of interest.

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