Exp. report. (MX1275)

Title: Fe local environment in Serum heme-albumin interactions (30.11.2012 - 04.12.2012)
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Figure 1 XANES data. Panel A: normalized Fe K edge XANES measured on HSA-heme-Fe(III) at 20 K as a function of the X-ray exposure time: (*i*) fresh sample (blue line), (*ii*) sample exposed for 5 hours (green line), and (*iii*) sample exposed for 13 hours (red line). The radiation damage is evident after prolonged exposure (red line) resulting in low energy edge shift (arrow) but it is week after 5 hours exposure. Panel B: comparison of Fe K edge XANES measured on HSA-heme-Fe(III), ibuprofen-HSA-heme-Fe(III), and warfarin-HSA-heme-Fe(III). The effect of drugs is mainly evident at the first XANES peak (arrow) while the edge position is largely unchanged, signaling the same Fe electronic state in HSA and drug added samples. All XAS were obtained at pH 7.0 (1.0×10^{-1} M phosphate buffer) and ~ 20 K. For details, see the text.

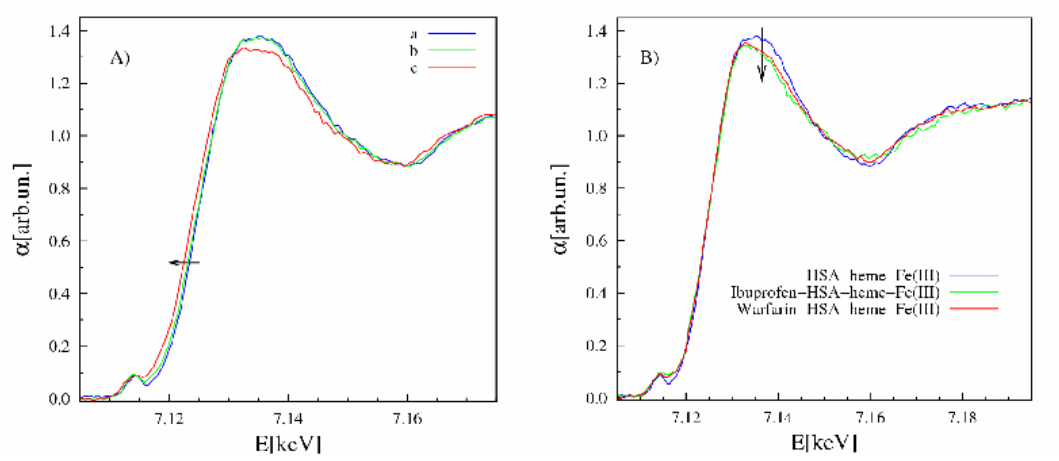
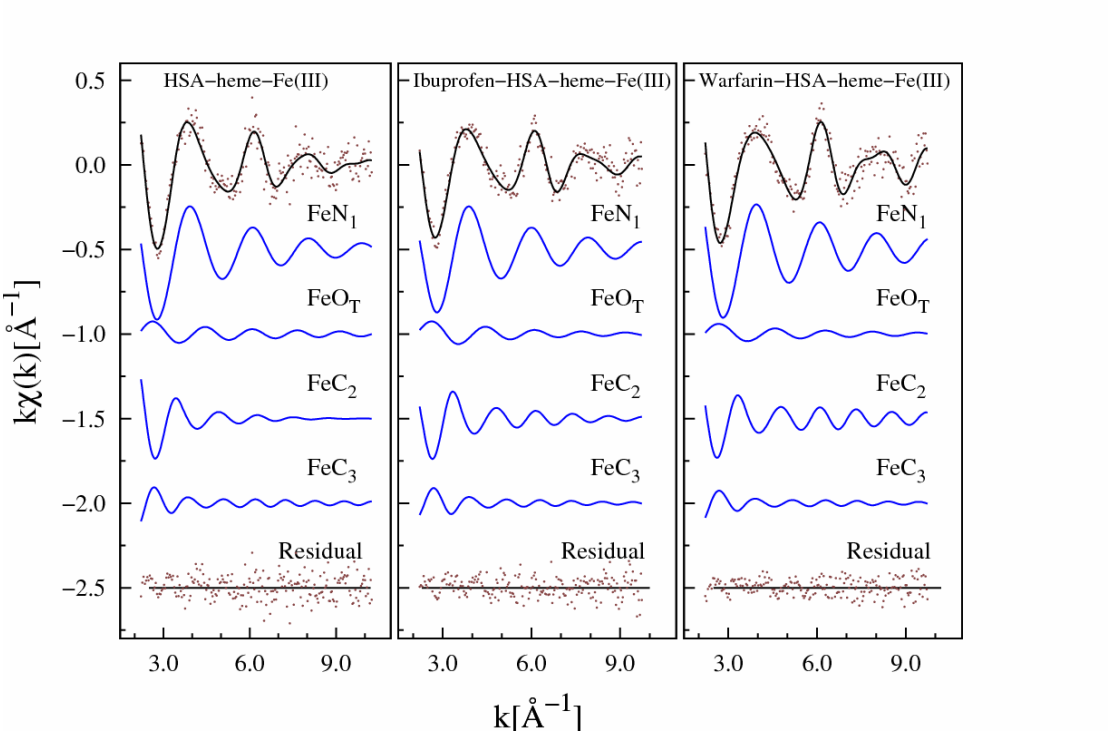


Figure 2: EXAFS data fitting. Experimental data (points) and best fit (full lines) for all the analyzed samples are shown. The partial contributions (shells) used for the refinement of each spectrum are shown (vertically shifted for clarity); the structural parameters are reported in Table 1. The residual (experimental data minus best fit) are shown in the bottom for each sample.



Samples: Fatty acid-free HSA, hemin (Fe(III)-protoporphyrin IX) chloride, ibuprofen, and warfarin were purchased from Sigma-Aldrich (St. Louis, MO, USA). HSA-heme-Fe(III) was prepared by adding a 0.8-molar defect of heme-Fe(III) to the HSA solution (1.0×10^{-1} M sodium phosphate buffer, pH 7.0) at 25.0 °C. The final HSA-heme-Fe(III) concentration was 1.0×10^{-4} M. The ibuprofen stock solution (1.0×10^{-1} M) was prepared by dissolving the drug in 1.0×10^{-1} M phosphate buffer, pH 7.0, at 25.0 °C, the final ibuprofen concentration was 1.0×10^{-2} M. The warfarin stock solution (1.0×10^{-1} M) was prepared by stirring the drug in 1.0×10^{-1} M phosphate buffer at pH 12.0 until it dissolved, then adjusting the solution to pH 7.0 with HCl (at 25.0 °C), the final warfarin concentration was 1.0×10^{-2} M. In the presence of 1.0×10^{-2} M ibuprofen or warfarin, HSA-heme-Fe(III) was fully saturated by both drugs; indeed, values of the dissociation equilibrium constant for ibuprofen and warfarin binding to HSA-heme-Fe(III) are $\leq1\times10^{-3}$ M and 2.1×10^{-5} M (at pH 7.0 and 25.0 °C), respectively.

XAFS experiment and data analysis: Fe-K edge (EFe = 7.112 keV) X-ray absorption spectra of HSA-heme-Fe(III) pure and in the presence of ibuprofen or warfarin were collected at the BM23 XAFS in fluorescence geometry. The beam energy was calibrated and monitored during the measurements determining the absorption spectra of a Fe reference metal foil placed after the sample. Solutions of HSA-heme-Fe(III) were enclosed in a plexiglass cell with Kapton windows: 7 mm (vertical) × 12 mm (horizontal). The solutions were cooled to ~ 20 K to preserve the samples from Fe(III) photo-reduction (figure1), and to reduce the thermal contribution to the structural disorder.

In order to check the integrity of sample and monitor the stability of the Fe valence state upon X-ray irradiation before the experiment XANES spectra were collected on a pure HSA-heme-Fe(III) sample as a function of time. This allowed to put in evidence sizable photo-reduction becoming appreciable only after five hours of sample exposure (Fig. 1, panel A).

In order to avoid photoabsorption several spectra were measured shifting vertically the samples (0.8 mm) after each spectrumkeeping fixed the collection time to 1.5 hours for each spectrum, this ensure to collect data from unexposed portions of the sample. Up to 14 spectra were collected for each HSA-heme-Fe(III) sample (pure and drug added) and averaged up. The average statistical noise of the averaged XAS spectra is in the 1×10^{-3} scale.

Data analysis ha been performed in K space including next neighbour shells till around 3.5 A.

The XAFS data analysis demonstrate the increase of Fe coordination state form 5 (pure HSA-heme-Fe(III)) to 6 (drug added HSA-heme-Fe(III)). Theoretical models (Steered molecular dynamic simulations, SMDS) individuate the sixth ligand due to His146 residue. Major details are in the manuscript submitted to Journal of Inorganic Biochemistry, being now under review.