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Analysis of the electronic structure of human hemoglobin from soft X-ray emission

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Abstract

We present X-ray emission spectra (XES) of human hemoglobin excited near the iron $L_{2,3}$ threshold and the nitrogen and carbon K-edges. The experiment is compared with our calculations of the corresponding spectra and gives good agreement. We find that the Fe L_3 emission is affected by the nearest nitrogen atoms located in the heme plane around the central iron atom. The distribution of the partial electronic densities of states of hemoglobin is determined.

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1. Introduction

Hemoglobin is a protein supporting the red blood cells in transporting oxygen from lungs to tissue and returning CO_2 from the tissue to the lungs. It is a tetramer made of two alpha and two beta chains with each having a heme. The active metal site in the heme is occupied by an iron ion. Understanding hemoglobin functionality is of fundamental importance hence hemoglobin is sometimes referred to as the hydrogen atom of biochemistry. The relation between structure and functionality for hemoglobin has been studied for decades [1]. Despite most of the studies use X-ray diffraction (see for example [2]), the advances of synchrotron radiation facilities revolutionized another X-ray technique X-ray absorption fine structure or XAFS, which has been used to refine the metalloprotein structure with subatomic resolution [3]. It has been shown that XAFS is sensitive both, to the local atomic structure around the active metal site [4,5] and to the electronic structure [6]. Theoretical analysis made it also possible to distinguish details of XAFS spectra that depend on the structure of the molecule and details that depend on the electron state (configuration) of the iron atom in the active center of the metalloprotein [7]. Soft X-ray spectroscopy provided additional information regarding the spin state of myoglobin and its photolysis products [8]. Recent advances in the analysis of XAFS spectra made it possible to study fine details of the local atomic structure around the active metal center in myoglobin [9,10].

In order to tie structural information to the functionality of the metalloproteins one has to study details of the electronic structure such as the density of electronic states and the character of chemical bonding. X-ray emission spectroscopy (XES) is a powerful experimental tool for this task [11] since it is very sensitive to changes in chemical bonding of the X-ray emitting atoms [12].

2. Experiment and calculations

The Fe $L_{2,3}$ (3d 4s $\rightarrow$ $2p_{1/2,3/2}$ transitions), carbon and nitrogen Kα (2p → 1s transition) emission spectra were

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recorded at the soft X-ray spectroscopy endstation of the undulator beamline 8.0.1 at Advanced Light Source at Lawrence Berkeley National Laboratory. The energy resolution of the iron $L_{2,3}$ XES, carbon and nitrogen Kα XES is about 0.8, 0.3 and 0.4 eV, respectively. All emission spectra are normalized to the number of incoming photons measured by a mesh current.

Calculations of Fe L_3, nitrogen and carbon Kα X-ray emission spectra were performed using ab initio multiple-scattering theory, based on a self-consistent real-space Green's-function formalism with final-state potentials. The approach uses spherical muffin-tin potentials and inelastic losses are included in terms of an energy dependent self-energy. This theory is implemented in the ab initio XANES and electronic structure code FEFF8.2 [13].

The scattering potential is approximated by 15% overlapping spherical muffin-tin potentials. Many-body effects are incorporated approximately in terms of a complex, energy-dependent Hedin-Lundqvist self-energy within the plasmon-pole approximation, which has been successfully applied to X-ray absorption spectroscopy [14].

3. Results and discussion

The Fe L_3-XES spectra were calculated for the experimentally determined structure as well as for the idealized symmetric cluster of the human hemoglobin molecule. The structure of the 'real' molecule was taken from the Protein Data Bank (http://www.rcsb.org) entry 3HHB. The real hemoglobin molecule contains four iron atoms; the number of non equivalent iron positions is equal to 2 and the Fe L_3-XES calculations were performed for these two non equivalent iron positions. The results are presented in Fig. 1 (curves labeled (a) and (b)). The spectra calculated for the two positions of iron do not differ visually. The resulting theoretical Fe L_3-emission spectrum was obtained by averaging of the XES spectra for two iron positions taken with the weights 1:1 (curve (c)). Using the coordinates of atoms obtained from the X-ray diffraction data for large biological molecules like hemoglobin, one has to bare in mind possible insufficient accuracy in the atomic positions.

The heme plane generated according to the 3HHB data set used was not perfectly plane and not symmetric around the iron ion site. Thus, we also used an idealized symmetric cluster of the hemoglobin molecule for the calculations. The model contains the 30 nearest atoms around iron ion including the heme plane and part of the histidine-*F*8 atoms. The Fe L_3 emission for the idealized cluster is shown in Fig. 1 labeled as d. The comparison in Fig. 1 allows to compare the experimental Fe L_3 emission spectrum (curve (e)) with theoretical simulations for both, idealized symmetric and real molecule structures (curves (d) and (c), respectively). Both theoretical spectra agree well with the experimental one and thus one can use the structure of the idealized cluster around Fe ion for theoretical Fe L_3 simulations. In fact the shape of the low energy shoulder labeled A in Fig. 1 of the experimental spectrum is reproduced better by the calculation using the ideal cluster than for the one based on the 3HHB data set.

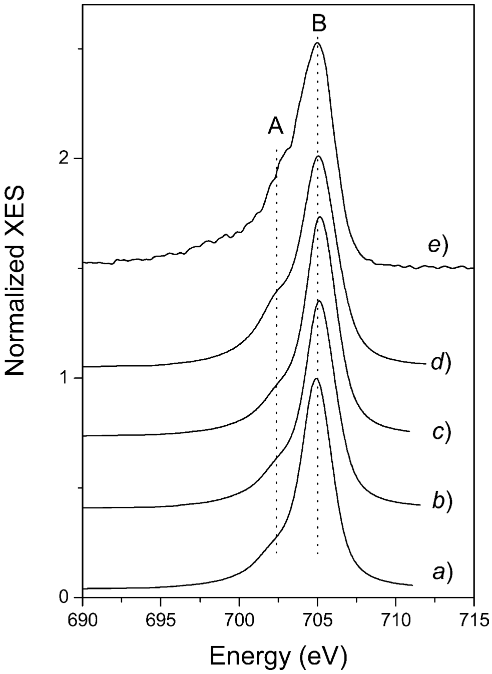

Fig. 1. Comparison of experimental Fe L_3 emission of human hemoglobin (curve (e)) with theoretical simulations. Curves (a) and (b) correspond to the Fe L_3 calculations for alpha and beta chains (PDB entry 3HHB). Curve (c) is obtained by averaging of the spectra (a) and (b). The spectrum for the idealized symmetric part of hemoglobin is also shown (curve (d)).

In order to understand the origin of shoulder (A) of the Fe L_3 emission spectrum, we have calculated the spectrum using the structure of the idealized cluster with the four nearest nitrogen atoms around the central iron atom in heme plane being removed. This spectrum together with the theoretical spectrum for the idealized symmetric cluster containing all atoms is compared with the experimental Fe L_3 emission of human hemoglobin in Fig. 2. We conclude that the low energy shoulder labeled A is suppressed if four nitrogen atoms from the heme, nearest to the iron site, are removed from the cluster. We note that the low energy shoulder of the Fe L_3-XES can be used as a marker for chemical interaction between the central iron ion and its four nearest nitrogen atoms lying in the heme plane. The same molecule potential is used to calculate the partial electronic densities of states (DOS) of iron and nitrogen. The calculations for the idealized symmetric cluster of atoms around the central iron in hemoglobin are presented in Fig. 3. Only the curves for Fe d-states and N p-states are displayed in order to simplify the comparison.

The curve labeled 'N p-states without Fe' corresponds to the N p-DOS calculated for the same cluster, but without Fe ion. The presence of the iron ion results in a significant modification of the density of nitrogen p-states near the active metal center in the heme plane of hemoglobin. The nitrogen p-states show a peak corresponding to the lowest unoccupied Fe d-states of hemoglobin (labeled C) indicating hybridiza-

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