Metalloproteins are an important class of proteins and perform a variety of fundamental biological processes. Synchrotron radiation (SR) X-ray sources provide the most powerful capabilities for structure-function studies for this class of proteins that constitutes almost 40% of proteins of the entire genome. Metalloproteins exploit the redox and coordination chemistry of biological metals to perform a wide variety of chemical reactions. In order to understand how these metalloproteins utilize the chemistry of metals to perform a particular function, it is mandatory to know the 3D structure of these proteins, in general, and of the metal site, in particular, to a very high resolution. Actually, small changes at the metal centre and of its ligands can be amplified by the protein to perform many complex biological processes.

Recent advances in SR and in the detector technology allowed the determination of a continuously increasing number of structures at atomic resolutions <1.2 Å. However, the number of the resolved structures remains a relatively small fraction of the total, e.g., only 2.5% of the available atomic-resolution structures available in the PDB on March 2005 contain copper, iron and zinc proteins.

X-ray Absorption Spectroscopy (XAS) is a powerful technique capable to determine the local structure around a photoabsorber. It has the ability to define structural differences at a resolution commensurate with the different redox and coordination chemistry of the metal ion regardless of the physical state of sample under studied. Moreover, the XAS methods is synergic with PX and NMR techniques and the combined application of these different approaches recently determined a significant advance in the understanding of this specific class of proteins. Therefore, the application of the XAS technique in this wide research area has been named “BioXAS” and Special issues have been recently published on the subject\cite{1,2}.

Early works have been mainly focused on Extended X-ray Absorption Fine Structure (EXAFS)\cite{3-9} analysis while research performed applying X-ray Absorption Near Edge Structure (XANES) are almost qualitative\cite{10}. Therefore, a quantitative characterization of the local fine structure of metal centers using XANES at the metal edges represents a specific and challenging task. Recently at the LNF Benfatto and co-workers developed a novel software package called MINUIT XANes (MXAN) in the
framework of the \textit{ab initio} full Multiple Scattering (MS) theory that is capable to perform a quantitative XANES analysis\cite{11-13}. This method has been successfully applied to several (biological) systems\cite{14-21}.

Among the many metalloproteins, Kti11p is the only member of the protein family characterized by a CSL zinc-finger domain that has been structurally determined. Its 3D structure has been resolved by NMR, but the zinc ion is silent to NMR and as a consequence, the location of the zinc ion has been proposed but a clear experimental confirmation is missing. In Ref. \cite{22} NMR data combined with EXAFS analysis were applied to check the proposed coordination environment around the Zn ion. A quantitative XANES analysis of the zinc K-edge has not yet available, since the cluster model of Kti11p contains three soft chains, one of which connects the two S atoms around the Zn ion. While, the standard version of MXAN performs structural optimization in polar or Cartesian coordinates, we improved the software introducing an additional coordinate system, similar to the so-called “natural internal coordinates”\cite{23-26} or the Z-matrix coordinate system, well known in quantum chemistry.

Instead to use Cartesian or polar coordinates, the “natural internal coordinates” system defines an atom using three parameters: the inter-atomic distance and bonding angles and the torsional angle. The advantage being a simpler description of the relative atomic position allowing in addition, a simple optimization of the geometrical parameters according to the geometrical constraints. These advantages are particularly useful in systems containing soft chains and rings. By using this new MXAN version, we have adjusted the first shell of Zn ion (4 S atoms), and several features observed in the experimental spectra have been successfully reproduced. This study, based on the new version of MXAN will help also to clarify the origin of the first two peaks in the experimental spectra. Moreover, a cluster model of Kti11p including 111 atoms (57 hydrogen atoms added to the dangling bonds) has been optimized at B3LYP level\cite{27,28} in good agreement with experimental results\cite{29}.

In conclusion, we have performed XAS experiments and compared them with theoretical calculations trying to unravel the local structure around the metallic ions of several metalloproteins. However, these studies demonstrate that only a systematic and efficient approach may clarify the structures and the functions of the metalloproteins. However, the combined theoretical and experimental procedure set up for biological system can be extended with success to many other problems associated to a local structural analysis such as nano-systems.
Reference


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