Experimental and Theoretical Investigations of Metalloproteins using X-ray Absorption Spectroscopy

Ziyu Wu

Beijing Synchrotron Radiation Facility, Institute of High Energy Physics, CAS, 100049, Beijing, China

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[1,2].

Early works have been mainly focused on Extended X-ray Absorption Fine Structure (EXAFS)[3-9] analysis while research performed applying X-ray Absorption Near Edge Structure (XANES) are almost qualitative[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 *ab initio* full Multiple Scattering (MS) theory that is capable to perform a quantitative XANES analysis[11-13]. This method has been successfully applied to several (biological) systems[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. [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"[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[27,28] in good agreement with experimental results[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

[1] J. Synchrotron Rad., 2003, **10.**

[2] J. Synchrotron Rad., 2005, **12.**

[3] Thomas E. Wolff, J.M. Berg, C. Warrick, Keith O. Hodgson, R.H. Holm, R.B. Frankel, *The Molybdenum-Iron-Sulfur Cluster Complex [Mo$_2$Fe$_6$S$_9$(SC$_2$H$_5$)$_8$]$^{3-}$, A Synthetic Approach to the Molybdenum Site in Nitrogenase*, J. Am. Chem. Soc., 1978, **100**(14), 4630-4632

[4] E. Nordlander, Sonny C. Lee, Wei Cen, Z.Y. Wu, C.R. Natoli, A. Di Cicco, A. Filipponi, Britt Hedman, Keith O. Hodgson, R.H. Holm, *Heterometal Cuboidal Clusters MFe$_4$S$_6$(PEt$_3$)$_4$Cl (M=V, Mo): Synthesis, Structural Analysis by Crystallography and EXAFS, and Relevance to the Core Structure of the Iron-Molybdenum Cofactor of Nitrogenase*, J. Am. Chem. Soc., 1993, **115**(13), 5549-5558

[5] S.D. Conradson, B.K. Burgess, W.E. Newton, A.D. Cicco, A. Filipponi, Z.Y. Wu, C.R. Natoli, B. Hedman, and K.O. Hodgson, *Selenol binds to iron in nitrogenase iron-molybdenum cofactor: An extended x-ray absorption fine structure study*, Proc. Natl. Acad. Sci., 1994, **91**(4), 1290-1293

[6] V.K. Yachandra, V.J. DeRose, M.J. Latimer, I. Mukerji, K. Saucer, and M.P. Klein, *Where plants make oxygen: A structural model for the photosynthetic oxygen-evolving manganese cluster*, Science, 1993, **260**, 675-679

[7] W. Liang, T.A. Roelofs, R.M. Cinco, A. Rompel, M.J. Latimer, W.O. Yu, K. Sauer, M.P. Klein, V.K. Yachandra, *Structural Change of the Mn Cluster during the S$_2$? S$_3$ State Transition of the Oxygen-Evolving Complex of Photosystem II. Does It Reflect the Onset of Water/Substrate Oxidation? Determination by Mn X-ray Absorption Spectroscopy*, J. Am. Chem. Soc., 2000, **122**(14), 3399-3412

[8] J.H. Robblee, J. Messinger, R.M. Cinco, K.L. McFarlane, C. Fernandez, S.A. Pizarro, K. Sauer, V.K. Yachandra, *The Mn Cluster in the S0 State of the Oxygen-Evolving Complex of Photosystem II Studied by EXAFS Spectroscopy: Are There Three Di-μ-oxo-bridged Mn2 Moieties in the Tetranuclear Mn Complex?* J. Am. Chem. Soc., 2002, **124**(25), 7459-7471

[9] Ann E. McDermott, Vittal K. Yachandra, Ronald D. Guiles, Kenneth Sauer, Melvin P. Klein, Kevin G. Parrett, John H. Golbeck, *EXAFS structural study of FX, the low-potential iron-sulfur center in photosystem I*, Biochemistry, 1989, **28**(20), 8056-8059

[10] S.S. Hasnain and K.O. Hodgson, *Structure of metal centres in proteins at subatomic resolution*, J. Synchrotron Rad., 1999, **6**, 852-864

[11] M. Benfatto, A. Congiu-Castellano, A. Daniele and S. Della Longa, *MXAN: a new software procedure to perform geometrical fitting of experimental XANES spectra*, J. Synchrotron Rad. 2001, **8**, 267-269

[12] M. Benfatto and S. Della Longa, *Geometrical fitting of experimental XANES spectra by a full multiple-scattering procedure*, J. Synchrotron Rad. 2001, **8**, 1087-1094

[13] M. Benfatto, S. Della Longa, Y. Qin, Q. Li, G. Pan, Z. Wu and S. Morant, *The role of Zn in the interplay among Langmuir–Blodgett multilayer and myelin basic protein: a quantitative analysis of XANES spectra*, Biophys. Chem. 2004, **110**, 191-201

[14] S. Della Longa, A. Arcovito, M. Girasole, J.L. Hazemann, and M. Benfatto, *Quantitative Analysis of X-Ray Absorption Near Edge Structure Data by a Full Multiple Scattering Procedure: The Fe-CO Geometry in Photolyzed Carbonmonoxy-Myoglobin Single Crystal*, Phys. Rev. Lett. 2001, **87**, 155501(1-4)

[15] S. Della Longa, A. Arcovito, M. Benfatto, A. Congiu-Castellano, M. Girasole, J.L. Hazemann, and A. Lo Bosco, *Redox-Induced Structural Dynamics of Fe-Heme Ligand in Myoglobin by X-Ray Absorption Spectroscopy*, Biophysical Journal, 2003, **85**, 549-558

[16] Francesc Sepulcre, M. Grazia Proietti, Maurizio Benfatto, Stefano Della Longa, Joaquin García, and Esteve Padrós, *A Quantitative XANES Analysis of the Calcium High-Affinity Binding Site of the Purple Membrane*, Biophysical Journal, 2004, **87**, 513-520

[17] K. Hayakawa, K. Hatada, P. D'Angelo, S. Della Longa, C. R. Natoli, M. Benfatto, *Full Quantitative Multiple-Scattering Analysis of X-ray Absorption Spectra: Application to Potassium Hexacyanoferrat(II) and -(III) Complexes*, J. Am. Chem. Soc., 2004, **126**(47), 15618-15623

[18] P. Frank, M. Benfatto, R.K. Szilagyi, P. D'Angelo, S.D. Longa, K.O. Hodgson, *The Solution Structure of $[Cu(aq)]^{2+}$ and Its Implications for Rack-Induced Bonding in Blue Copper Protein Active Sites*, Inorg. Chem., 2005, **44**(6); 1922-1933

[19] A. Arcovito, D.C. Lamb, G.U. Nienhaus, J.L. Hazemann, M. Benfatto, and S. Della Longa, *Light-Induced Relaxation of Photolyzed Carbonmonoxy Myoglobin: A Temperature-Dependent X-Ray Absorption Near-Edge Structure (XANES) Study*, Biophysical Journal, 2005, **88**, 2954-2964

[20] Shujun Li, Zhaocai Zhou, Wangsheng Chu, Weimin Gong, Maurizio Benfatto, Tiandou Hu, Yaning Xie, Dingchang Xian and Ziyu Wu, *Investigation of zinc-containing peptide deformylasefrom Leptospira interrogansby X-ray absorption near-edge spectroscopy*, J. Synchrotron Rad., 2005, 12, 111-114.

[21] Li Shujun, Zhao Wei, Ma Sixuan, Chu Wangsheng, Niu Liwen, Ten Maikun and Wu Ziyu, *XAS studies on hemorrhagic zinc-metalloproteinase from Agkistrodon acutus venom*, in preparation

[22] Jianping Sun, Jiahai Zhang, Fangming Wu, Chao Xu, Shujun Li, Wei Zhao, Ziyu Wu, Jihui Wu, Cong-Zhao Zhou and Yunyu Shi, *Solution Structure of Kti11p from Saccharomyces cerevisiae Reveals a Novel Zinc-Binding Module*, Biochemistry 2005, **44**, 8801-8809

[23] Peter Pulay, Geza Fogarasi, Frank Pang and James E. Boggs, *Systematic ab initio gradient calculation of molecular geometries, force constants, and dipole moment derivatives*, J. Am. Chem. Soc. 1979, **101**(10), 2550-2560

[24] Geza Fogarasi, Xuefeng Zhou, Patterson W. Taylor and Peter Pulay, *The calculation of ab initio molecular geometries: efficient optimization by natural internal coordinates and empirical correction by offset forces*, J. Am. Chem. Soc. 1992, **114**(21), 8191-8201

[25] P. Pulay and G. Fogarasi, *Geometry optimization in redundant internal coordinates*, J. Chem. Phys. 1992, **96**(4), 2856-2860

[26] Jon Baker, Alain Kessi and Bernard Delley, *The generation and use of delocalized internal coordinates in geometry optimization*, J. Chem. Phys. 1996, **105**(1), 192-212

[27] A. D. Becke, *Density-functional thermochemistry. III. The role of exact exchange*, J. Chem. Phys. 1993, **98**, 5648-5652

[28] C. Lee, W. Yang, R.G. Parr, *Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density*, Phys. Rev. B 1988, **37**, 785-789

[29] B. Gao, S.J. Li, W.S. Chu, W. Zhao, Y.Y. Shi, G. Cibin, A. Marcelli and M. Benfatto, Y. Luo, Z.Y. Wu, The Local Structure around Zinc Ion of Kti11p from Saccharomyces cerevisiae: Studies by Density-Functional Theory and Multiple Scattering Theory Calculations, in preparation.