fold increase in dose. Comparing structural results from EXAFS to those from crystallography on this and similar proteins, show that x-ray induced photoreduction has impacted the crystallographic data and subsequent structure solutions. These results indicate the importance of using LHe-based cooling for metalloprotein crystallography in order to limit changes at the metalloprotein active sites. The study also illustrates the need for direct measurement of redox states of the metals, through XAS, simultaneously with the crystallographic measurements. The work was performed at SSRL with support from the NIH NCRR BTP program and the US DOE BER. SSRL operations are funded by the US DOE BES.

Keywords: radiation damage, metallo-enzymes, X-ray absorption spectroscopy

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Crystallography with X-ray and optical spectroscopies for metalloproteins structural studies

Samar Hasnain[1], Richard Strange[1], Michael Hough[1], Robert Eady[1], Svetlana Antonyuk[2], Mark Ellis[2]

[1]University of Liverpool, School of Biological Sciences, Life Sciences Building, Liverpool, Merseyside, L69 7ZB, UK, [2]STFC Daresbury Laboratory, Warrington, WA4 4AD, Cheshire, UK, E-mail：s.s.hasnain@dl.ac.uk

Metalloproteins constitute a significant fraction (> 30%) of a genome and use the redox properties of metals to perform essential catalytic processes. The accuracy with which information is required is often not available through X-ray crystallography (1). Furthermore, the effect of intense X-ray beams now available at most synchrotrons on redox centres is very severe and it is not easy to obtain information of the redox state of the metal from a structure. In both of these context, use of XAS will be discussed with some recent examples. In addition, the advantage of combining on-line optical spectroscopy with XAS and crystallography are demonstrated with a specific example of copper nitrite reductase(ref 2).

References

1. Structure of Metal centres in proteins at sub-atomic resolution, S. S. Hasnain & K. O. Hodgson, J. Synchrotron Rad. 6, 852-864 (1999).

2. Crystallography with Online Optical and X-ray Absorption Spectroscopies Demonstrates an Ordered Mechanism in Copper Nitrite Reductase Michael A. Hough, Svetlana V. Antonyuk, Richard W. Strange, Robert R. Eady and S. Samar Hasnain, J. Mol. Biol. (2008) 378, 353－361

Keywords: metalloproteins, radiation damage, redox states

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X-ray absorption spectroscopy for the structure determination of copper transport proteins

Stefano Mangani[1], Lucia Banci[2], Ivano Bertini[2]

[1]University of Siena, Chemistry, Via Aldo Moro 2, Siena, Siena, 53100, Italy, [2]CERM, Univeristy of Florence, Via Luigi Sacconi 6, Sesto Fiorentino, 50019, Italy, E-mail：mangani@unisi.it

All organisms, from prokaryotes to plants and mammals have evolved complex mechanisms to efficiently acquire and properly utilize copper (Rosenzweig, O'Halloran 2000;Wintz, Vulpe 2002). The past ten years have assisted the discovery of many pieces of the sophisticated machinery which is used to efficiently acquire and utilize copper. (Elam et al. 2002;Rosenzweig 2001) At CERM we have focussed our work on the study of copper transport proteins in different organisms by x-ray crystallography and by coupling NMR and x-ray absorption (XAS) spectroscopic techniques that, combined, offer the possibility to achieve the complete structure determination of a metalloprotein in solution and provide unique information on the electronic structure of the metal ion and on how it influences its binding to the protein (Arnesano et al. 2003; Banci et al. 2003; Banci et al. 2004; Banci et al. 2005a,b; Banci et al. 2006). The most recent applications of the NMR-XAS approach to the structure determination of copper proteins involved in the assembly of bacterial and human cytochrome C oxidase will be presented and discussed as well as the comparison with crystallographic results.

References

Arnesano,F., et al. 2003. Proc. Natl. Acad. Sci. U. S. A 100:3814-3819.
Banci,L., et al. 2003. Biochemistry 42:2467-2474.
Banci,L., et al. 2004. J. Biol. Chem. 279:34833-34839.
Banci,L., et al. 2005a. Proc. Natl. Acad. Sci. U. S. A 102:3994-3999.
Banci,L., et al. 2005b. J. Synchrotron. Radiat. 12:94-97.
Banci,L., et al. 2006. Proc. Natl. Acad. Sci. U. S. A 103:8595-8600.
Elam,J.S., et al. 2002. Adv. Protein Chem. 60:151-219.
Rosenzweig,A.C. 2001. Acc. Chem. Res. 34:119-128.
Rosenzweig,A.C. and O'Halloran,T.V. 2000. Curr. Opin. Chem. Biol. 4:140-147.
Wintz,H. and Vulpe,C. 2002. Biochem. Soc. Trans. 30:732-735.

Keywords: copper proteins, X-ray absorption, NMR spectroscopy

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Structure in the local environment of Zn^{2+} ion in the anti-termination protein of *Bacillus subtilis*

Paul Fons[1], Dhakshnamoorthy Balasundaresan[2], Hiroshi Mizuno[2], Penmetcha K.R. Kumar[2]

[1]National Institute for Advanced Industrial Science & Technology, Center for Applied Near-Field Optics Research, Higashi 1-1-1, Tsukuba Central 4, Tsukuba-shi, Ibaraki-ken, 305-8562, Japan, [2]Institute for Biological Resources and Functions, AIST, 1-1-1 Higashi, Tsukuba 305-8566, Japan, E-mail：paul-fons@aist.go.jp

HutP is an RNA-binding protein that regulates the expression of the histidine utilization (hut) operon in Bacillus subtilis, by binding to cis-acting regulatory sequences on hut mRNA. Our crystal structure of the quaternary complex (HutP- L-histidine-Mg^{2+}-21-mer RNA) showed that three Nε atoms of imidazole rings of His residues, the backbone nitrogen and carboxyl oxygen atoms of L-histidine, and a water molecule coordinate the Mg^{2+} ion to form the typical octahedral polyhedra1). Further studies showed that not only Mg^{2+} ion but also several other divalent cations, except Cu^{2+}, Yb^{2+}, Hg^{2+} cations, are effective, and the structures of HutP- L-histidine-Mn^{2+} and HutP-L-histidine-Ba^{2+} revealed to be very similar to that of the HutP-L-histidine-Mg^{2+} complex2). We recently solved the crystal structure of the HutP- L-histidine-Zn^{2+} complex, because Zn^{2+} is the best among divalent cations for mediating RNA-binding and probably antitermination process as well2). Our complex (HutP-L-histidine-Zn^{2+}) revealed that imidazole Nε atoms of not only His residues of HutP but also of the L-histidine ligand undergo four-fold Zn^{2+} coordination, which differs from the case of octahedral coordination found in our previous complex (HutP-L-histidine-Mg^{2+}). To obtain further insight into the Zn^{2+}-binding site, X-ray absorption both near-

edge (XANES) and extended (XAFS) at the Zn K-edge have been explored. We will present further insights into the divalent metal ions regulation of the hut operon in Bacillus subtilis, by combining both crystallography and X-ray absorption spectroscopic studies.
1)Kumarevel et al., Nature 434, 183-191 (2005), 2)Kumarevel et al., Nucleic Acids Res. 33, 5494-5502 (2005)

Keywords: X-ray absorption fine structure, protein crystallography with synchrotron radiation, protein refinement

MS.63.5

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The structure of the Amyloid β-peptide high affinity copper II binding site in Alzheimer's disease

Victor A. Streltsov, Stephen J. Titmuss, Vidana Epa, Jose N. Varghese
CSIRO, Molecular and Health Technologies, 343 Royal Parade, Parkville, Victoria, 3052, Australia, E-mail：victor.streltsov@csiro.au

A major source of neurodegeneration observed in Alzheimer's disease (AD) is believed to be related to the toxicity from reactive oxygen species (ROS) produced in the brain by the Amyloid-β (Aβ) protein bound primarily to copper ions. The evidence for an oxidative stress role of Aβ-Cu redox chemistry is still incomplete. Details of the copper binding site in Aβ may be critical to the etiology of AD. Here we present the structure determined by combining X-ray absorption fine structure (XAFS) and Density Functional Theory analysis of truncated Aβ(1-16) peptide complexed with Cu(II) in solution under a range of buffer conditions. PBS buffer salt (NaCl) concentration does not affect the copper binding mode. The XAFS spectra for truncated Aβ(1-16)-Cu(II) and full length Aβ(1-40/42)-Cu(II) peptides are similar. The novel six-coordinated (3N3O) geometry around copper in the Aβ-Cu(II) complex includes three histidines, glutamic or/and aspartic acid and axial water. The structure of high affinity Cu2+ binding site is consistent with the hypothesis that the redox activity of the metal ion bound to Aβ can lead to the formation of di-tyrosine linked dimers found in AD. X-ray absorption near-edge spectroscopy (XANES) has been used to probe the substrate mediated reduction of Cu(II) to Cu(I) in Aβ-Cu(II) complexes by ascorbate and the neurotoxin 6-hydroxydopamine (6-OHDA), however dopamine and, in particular, cholesterol are incapable of reducing soluble monomeric Aβ-Cu(II) complexes. The results are in agreement with assignment of the redox potentials for Aβ-Cu(II), ascorbic acid and dopamine.

Keywords: beta-amyloids, Alzheimer's proteins, X-ray absorption

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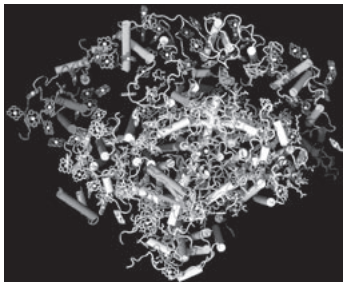
Structural basis of a plant photosystem I sunlight conversion

Alexey Amunts, Omri Drory, Nathan Nelson
Tel Aviv University, Biochemistry, Levanon st., Tel Aviv, Tel Aviv, 69978, Israel, E-mail：amunts@tauex.tau.ac.il

A plant Photosystem I (PSI) is a large membrane super-complex that drives photosynthesis. PSI captures sunlight through sophisticated pigment network and uses the energy to perform transmembrane electron transfer. It consists of the reaction center complex (RC), where the charge separation reaction takes place and the light harvesting complex (LHCI), which serves as an additional antenna system. PSI performs a photochemical activity with the unprecedented quantum yield of close to 1.0, being the most efficient light capturing and energy conversion device. We determined the X-ray structure of intact PSI at 3.4 Å resolution [1]. The crystal structure provides a picture at near atomic detail of 17 protein subunits; 3038 amino acids were assigned, as well as 168 chlorophylls, 2 phyloquinones, 3 Fe4S4 clusters and 5 carotenoids. The remarkable feature of PSI is the unprecedented high content of non-protein components, approximately one third of the total mass of about 650 KDa consists of different co-factors. The structure reveals intriguing insights regarding unique interactions between the RC and the LHCI complexes.
[1] Amunts, A., Drory, O. & Nelson, N. (2007) Nature, 447, 58-63.



Keywords: photosynthesis, membrane protein, electron transfer

MS.64.2

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Inhibitor complexed structures of the Cyt bc1 from the photosynthetic bacterium *R. sphaeroides*

Di Xia[1], Lothar Esser[1], Chang-An Yu[2]
[1]Center for Cancer Research, National Cancer Institute, NIH, Laboratory of Cell Biology, 37 Convent Dr., Building 37, Room 2122C, Bethesda, Maryland, 20892, USA, [2]Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK, 74078, USA, E-mail：dixia@helix.nih.gov

The cytochrome bc1 complex (bc1) is a major contributor to the proton motive force across the membrane by coupling electron transfer to proton translocation. The crystal structures of wild type and mutant bc1 complexes from the photosynthetic purple bacterium Rhodobacter sphaeroides (Rsbc1), stabilized with the quinol oxidation (QP) site inhibitor stigmatellin alone or in combination of with the quinone reduction (QN) site inhibitor antimycin, were determined. The high quality electron density permitted assignments of a new metal-binding site to the cytochrome c1 subunit and a number of lipid and detergent molecules. Structural differences between Rsbc1 and its mitochondrial counterparts are mostly extra membranous and provide a basis for understanding the function of the predominantly longer sequences in the bacterial subunits. Functional implications for the bc1 complex are derived from analyses of 10 independent molecules in various crystal forms and from comparisons with mitochondrial complexes.

Keywords: membrane protein crystallization, cytochrome bc1 complex, mechanism of proton pumping

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Structure and mechanism of the DsbB-DsbA protein disulfide generation system in *E. coli*

Kenji Inaba