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

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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).

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Keywords: metalloproteins, radiation damage, redox states

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

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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.

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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*

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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-