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. 2005a; 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


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

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Structure in the local environment of Zn2+ 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-Mg2+-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 Mg2+ ion to form the typical octahedral polyhedral1). Further studies showed that not only Mg2+ ion but also several other divalent cations, except Cu2+, Yb3+, Hg2+ cations, are effective, and the structures of HutP-L-histidine-Mn2+ and HutP-L-histidine-Ba2+ revealed to be very similar to that of the HutP-L-histidine-Mg2+ complex2). We recently solved the crystal structure of the HutP-L-histidine-Zn2+ complex, because Zn2+ is the best among divalent cations for mediating RNA-binding and probably antitermination process as well2). Our complex (HutP-L-histidine-Zn2+) revealed that imidazole Nε atoms of not only His residues of HutP but also of the L-histidine ligand undergo four-fold Zn2+ coordination, which differs from the case of octahedral coordination found in our previous complex (HutP-L-histidine-Mg2+). To obtain further insight into the Zn2+-binding site, X-ray absorption both near-