



	Experiment title: <u>Local Structure of charge transfer inhibitors in photosynthetic reaction centers: the case of Zn</u>	Experiment number: 08-01-628 08-01-656
Beamline: BM 08	Date of experiment: From 17/4/03 to 20/4/2003 and from 29/10/03 to 1/11/03	Date of report: 20/2/2005
Shifts: 9+9	Local contact(s): F. D'Acapito and G. Ciatto	<i>Received at ESRF:</i>
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Report:

Photosynthetic reaction centers (RC) are membrane pigment-protein complexes which initiate the photosynthetic process. The binding of heavy metals (like Zn and Cd) has been shown to slow the light-induced charge separation within the bacterial RC, which is the primary event of photosynthesis.

X-ray diffraction (XRD) data at 2.5 Å resolution indicate that Cd and Zn bind at the same location to a cluster formed by AspH124, HisH126 and HisH128. In addition to these three amino acid residues in the case of Zn a water molecule was proposed to interact with the metal (Axelrod et al., Proc. Natl. Acad. Sci. **97**, 1542, 2000). Interestingly, recent data suggest that Cd and Ni compete with protons for the same binding site (presumably HisH126 and/or HisH128) (Paddock et al., Biochemistry **42**, 9626, 2003).

A detailed determination of the local structure is fundamental to shed light into the mechanism which originates the perturbation of charge transfer. For this reason we have performed Zn K-edge X-ray absorption measurements on the GILDA beamline. We have measured a number of RC samples with exogenous Zn at both room and LN temperature and selected reference compounds (Zn-Phthalocyanine, Zn-Porphine and Zn-Heptanedionate). Samples characterized by different Zn/RC ratios were obtained by incubating RCs purified from *Rhodobacter sphaeroides* in a buffer containing ZnSO₄ at different stoichiometries. Excess free Zn was removed by gel filtration chromatography and Zn/RC ratios were

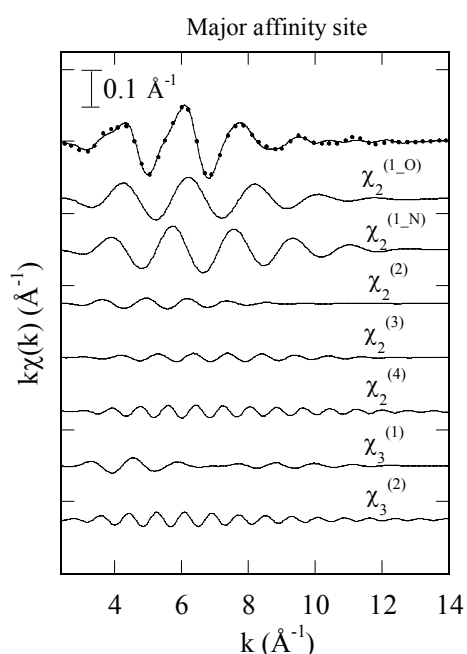
determined by atomic absorption spectroscopy of the metal. Measurements were performed on Zn-doped RCs embedded into polyvinyl alcohol films.

Data analysis of a sample characterized by $[Zn]/[RC] \cong 1$ has been performed using *ab-initio* simulations with FEFF and multiparameter fitting (FEFFIT); structural contributions up to the fourth coordination shell and multiple scattering paths (involving three atoms) of significant amplitude have been included; an example of a typical fit is shown in the figure.

Results for complexes characterized by a Zn to RC stoichiometry close to 1 indicate that Zn^{2+} binds two O and two N atoms in the first coordination shell. Higher shell contributions are consistent with a binding cluster formed by two His, one Asp residue and a water molecule. Analysis of complexes characterized by approximately 2 Zn ions per RC reveals a second structurally distinct binding site, involving one O and three N atoms, not belonging to a His residue.

The local structure obtained for the higher affinity site nicely fits the coordination geometry proposed on the basis of XRD data, but a significant contraction of the first shell is detected. Two possible locations of the second new binding site at the cytoplasmic surface of the RC have been proposed.

Based on these results a paper has been submitted and accepted for publication in Biophysical Journal (Biophysical journal Volume 88 March 2005).



Fit of the EXAFS spectrum of sample characterized by a Zn to RC stoichiometry close to 1. The top curve shows the inverse Fourier transform (continuous line) and its fit (dots); also shown are the seven contributions labeled as described in the text.