ESRF	**Experiment title:** Matrix effect on the local structure of Fe in myoglobin, cytochrome c and photosynthetic reaction center	**Experiment number**: SC-1976
Beamline: BM08	**Date of experiment**: from: 05/07/2006 to: 10/07/2006	**Date of report**: 24/08/2006
Shifts: 18	**Local contact(s)**: Dr. Chiara Maurizio	*Received at ESRF:*

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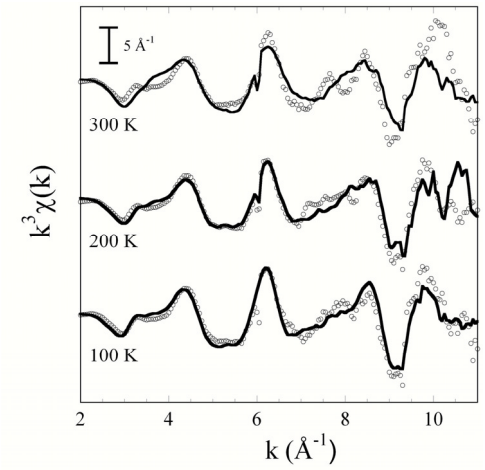
Report:

Trehalose, a disaccharide of glucose, has a peculiar efficacy in preserving the integrity of biostructures under extreme environmental conditions such as dehydration and high temperatures (>70°C) (Crowe et al., 1996). Mössbauer and optical absorption spectroscopy, neutron scattering and molecular dynamics simulations have shown that in soluble heme proteins, as well as membrane protein complexes, embedded in dry trehalose matrices, the conformational protein dynamics is dramatically hindered (for a review see Cordone et al., 2005). Using XAFS spectroscopy (experiment SC1696) we have recently compared the structure and dynamics of the Fe ligand cluster of cytochrome c in solution, in a polyvinyl alcohol (PVA) film and in a dried trehalose matrix. While the weakly interacting PVA matrix negligibly affects structural and disorder parameters as compared to solution, an extensively dehydrated trehalose matrix induces sizeable structural distortions and a drastic decrease of the Debye-Waller factors, indicating that the trehalose matrix dramatically alters the protein energy landscape, hindering the protein internal dynamics and promoting only some conformational substates at the level of local structure (Giachini et al., 2006, submitted to Biophys. J., under revision).

In the present experiment we have performed Fe K-edge X-ray absorption spectroscopy measurements at BM08 in carboxy myoglobin (MbCO) and in a membrane pigment-protein complex (the bacterial photosynthetic reaction center, RC) embedded in trehalose glasses and in a weakly interacting matrix (solution and PVA, respectively). A more extensive investigation has been performed in MbCO samples, for which data were collected at three temperatures (100, 200, 300 K). The aim was to investigate matrix induced structural and dynamical alterations and to compare these effects to those of lowering the temperature. Data of very good quality have been obtained for all samples.

Fig.1



In the case of MbCO we observed significant differences between samples in trehalose (Fig.1, open circles) and in solution (Fig.1, continuous lines). These differences are much greater at 300 K but they are still present at 100 K, indicating that the incorporation in a dried trehalose matrix produces, at room temperature, effects similar (but not equal) to those caused by lowering the temperature in solution. In particular the damping of the EXAFS functions obtained for the samples inserted in trehalose matrices is comparable at all temperatures and it is less pronounced than that observed at 100 K in solution.

This supports the notion that incorporation into a trehalose glass, affecting the protein energy landscape, strongly hinders the dynamics. Moreover, alterations in the EXAFS functions, probably due to subtle structural modifications, are observable in the trehalose matrix around 8 Å^{-1}.

In the case of the bacterial reaction center even more significant differences are observed between the PVA (continuous lines) and the trehalose samples (dotted lines), both in the XANES (Fig.2) and in the EXAFS region of the spectrum (Fig.3). These differences involve mainly the region at low k values, suggesting a reorganization of the first ligands. In the dehydrated trehalose matrix, a preliminary first-shell analysis indicates 6 N/O atoms as ligands, in agreement with previous measurements performed in weakly interacting matrices in the eighties (Eisenberger et al., 1982; Bunker et al., 1982). At variance, for the sample in PVA, the coordination number decreases, suggesting the loss of 2 ligands. These observations seem to indicate that at the relatively high photon flux currently used at BM08 the structure of the Fe site is damaged in a weakly interacting matrix (PVA) and that the trehalose matrix can protects against X-ray damage even under high irradiation.

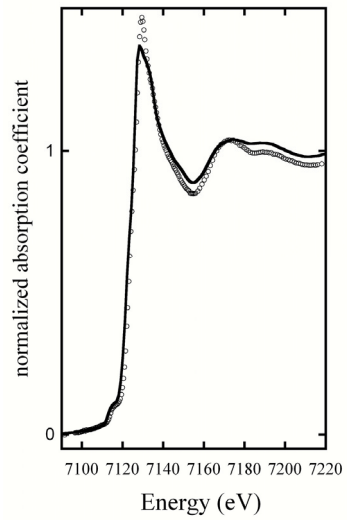


Fig.2

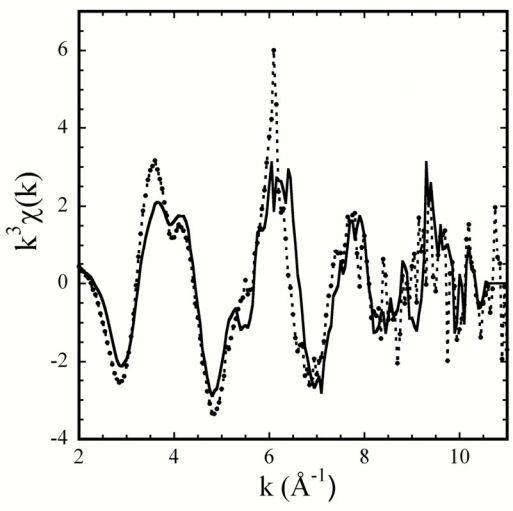


Fig.3

A detailed data analysis is in progress.

References

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