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ESRF	**Experiment title:** Matrix effect on the local structure of Fe in myoglobin, cytochrome c and photosynthetic reaction center	**Experiment number**: SC1696
Beamline: BM08	**Date of experiment**: from: 29/06/2005 to: 05/07/2005	**Date of report**: 6/08/2007
Shifts: 18	**Local contact(s)**: Chiara Battocchio	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

G. Venturoli and F. Francia, Biology Dept and CNR-INFM, Univ. Bologna, Italy

L. Giachini and F. Boscherini, Physics Dept and CNR-INFM, Univ. Bologna, Italy.

Lorenzo Cordone, Dipartimento di scienze Fisiche ed astronomiche, Univ. Palermo, Italy

Gerardo Palazzo, Istituto per i processi Chimico-Fisici, CNR, Bari, Italy.

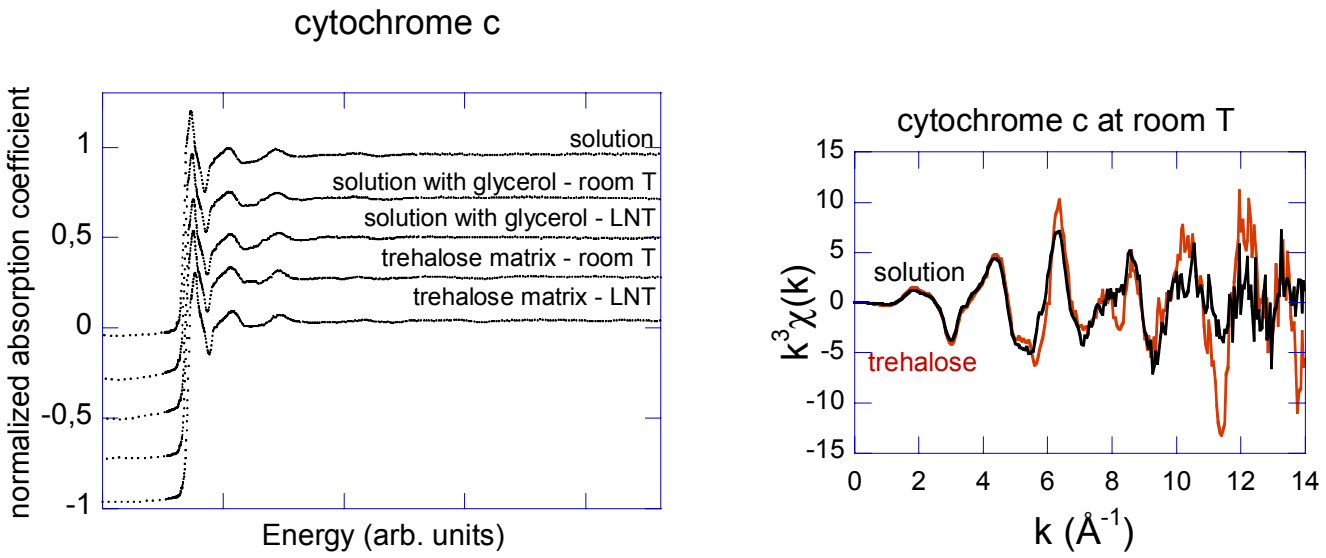
Report:

It is well known that trehalose, a non reducing disaccharide of glucose, found in large amounts in organisms that can survive in a state of suspended metabolism (anydrobiosis), has a peculiar efficacy in the preservation of isolated proteins, membranes and tissues (Crowe et al., 1996). Recent studies based on temperature-dependent optical absorption spectroscopy, Mössbauer spectroscopy, neutron scattering (Cordone et al., 1998; Cordone et al., 1999) and spectral diffusion dynamics (Ponkratov et al.,2002) as well as molecular dynamics simulations (Cottone et al., 2001) show that anharmonic and large scale diffusive motions of Fe in the heme proteins Carboxy Myoglobin (MbCO) and cytochrome c (cyt c) are greatly reduced in a dehydrated trehalose matrix.

In this experiment we have performed Fe K-edge X-ray absorption spectroscopy measurements at BM08 in two extensively studied soluble heme proteins (MbCO and cyt c) and in a membrane pigment-protein complex (bacterial photosynthetic reaction center, RC) in solution as well as in trehalose matrices. The aim was to investigate possible matrix induced structural alterations. In the case of cyt c we have also performed additional measurements at LNT in solution and in trehalose. This was necessary in order to test whether lowering the temperature had a similar effect to that of changing the matrix.

Data of excellent quality have been collected for all samples. As an example we report raw data of cyt c as well as the k^3 weighted EXAFS functions in solution and in trehalose matrix at room T (see figures). Care has been taken to avoid possible radiation damage by checking modifications in the edge region. A number of other control measurements has been performed:

1) cyt c was added in solution with and without glycerol and both solutions have been measured at room temperature to check possible alterations induced by the cryosolvent
2) MbCo was inserted in various trehalose matrices characterized by a different protein/trehalose concentration and by 2 different states of dehydration, dry and extra dry. This was done in order to evaluate the effect of residual water in sugar matrix, the importance of sugar concentration and also in order to measure samples in the same conditions tested in other spectroscopic studies (Cordone et al., 1998; Cordone et al., 1999)
3) RC was measured also in polyvinyl alcohol, a weakly interacting medium, in order to make our data directly comparable with that in literature (Eisenberger et al., 1982).



Left: raw data collected for cytochrome c. From the top to the bottom: cyt. c in solution with no glycerol, cyt.c in solution with glycerol at room T; cyt. c in solution with glycerol at LN T; cyt. c in dry trehalose matrix at room T; cyt. c in trehalose matrix at LN T. **Right:** K^3 weigthed EXAFS functions for cytochrome c in solution and in trehalose matrix at room T.

Changes between solution and trehalose are clearly visible in all proteins, as evident for the case of cyt c reported in the figure. Interestingly, the effect of LN temperature on the EXAFS signal in solution is much smaller than that induced by the trehalose matrix. This indicates that trehalose alters dramatically the protein energy landscape, probably hindering the dynamics and promoting only some conformational substates.

These conclusions have been confirmed by quantitative analysis, the results of which have been published (Giachini et al., 2007).

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