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Report:

Our experiment at the ESRF facility concerns the XAS characterization of the active site of the met forms of Hc from both molluscs and arthropods and of their derivatives coordinating azide as exogenous ligand(s) in different conditions of pH. It is our aim to obtain precise information from the EXAFS analysis on shell averaged distances (particularly the Cu-Cu distance) and coordination number, and to derive significant information on the copper site symmetry from the features of XANES spectra.

In this experimental session (11-13 April 1998) we have measured six samples of hemocyanin from mollusc (oxy-, met- and metazide-Hc) at pH 7.5 and 5.5. The samples of proteins were in phosphate buffer solution, in the presence of sucrose. The spectra were run at 77K. One measurement of the Met-Hc form at 273K has also been done. Spectra of $CuSO_4$, as a standard model for the Cu(II) ion, were run at 300K and 77K.

The XAS spectra were collected in the range 8700-9700 eV in vacuum in the fluorescence mode. To calibrate the energy scale we have used a Cu metal foil.

To achieve a good signal to noise ratio and to avoid any radiation damage several scans with a short-time of acquisition were collected and averaged on the same sample in the two different vertical positions allowed by geometry of the cell. Hence, each sample was measured for about seven hours.

During the analysis of the data we have noticed shifts of the whole spectra on the same sample in different consecutive acquisitions.
The interpretation of this effect was not immediate.
Successively, these effects have been attributed to concomitant factors:
i) instability on the monochromator calibration due to electronic instability;
ii) reduction of the protein due to high intensity of the beam.
This second effect was observed by another user group in a precedent run on a another biological sample.