ESRF	**Experiment title:** Site-selective XAS/XES on Fe sites of H_2 production in hydrogenases and models	**Experiment number**: SC2733
Beamline: ID26	**Date of experiment**: from: 15.07.2009 to: 21.07.2009	**Date of report**: 05.01.2011
Shifts: 18	**Local contact(s)**: Dr. Tsu-Chien Weng	*Received at ESRF:*

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Report:

Understanding hydrogen production at biological iron sites may pave the road for the development of novel synthetic catalysts. The most active enzyme in hydrogen production is FeFe hydrogenase from bacteria and green algae. This enzyme houses a six-iron site denoted H-cluster, composed of a 4Fe-4S cubane cluster, which is cysteine-linked to a binuclear Fe unit, $2Fe_H$ [1-3]. The Fe ions in $2Fe_H$ carry unusual CN and CO ligands. Atomic-level information on structure and oxidation state of the individual Fe atoms is required to determine the location of H_2-binding and the mechanism of H_2 catalysis.

A rich model chemistry has been developed to mimick relevant features in particular of the $2Fe_H$ active site of the hydrogenases [4-6]. However, often crystallographic information on the models is available only for a "ground-state" structure, which lacks the substrate, i.e. hydrogen, ligands. By XAS/XES, information on structure and electronic configuration in several active states was obtained for compounds in solution.

In this investigation, the XES spectrometer at beamline ID26 together with 2 sets of 5 analyzer crystals purchased by the group of M. Haumann was employed to study three different synthetic models of the 2Fe site and a series of reference Fe compounds (e.g. oxides and sulfides). Radiation damage studies during XES were performed. Iron K-alpha and K-beta emission spectra, K-beta satellite lines (valence-to-core transitions), and high-resolution XANES and EXAFS spectra were recorded. First attempts were made to measure site-selective EXAFS data on binuclear Fe models using narrow-band K-beta emission detection. Preliminary XES data on a FeFe hydrogenase was obtained. These experiments provided a library of reference spectra, revealed further necessary improvements of the set-up, provided high-resolution data on a hydride-coordinated Fe model in solution, and showed the principal feasibility of site-selective XAS methods.

Experimental: XAS/XES spectra were recorded in fluorescence mode using the Rowland-type XES spectrometer at ID26 equipped with 5 analyser crystals (R = 1 m, Fe K-alpha Ge440, Fe K-beta Ge620) and an avalanche photodiode (APD) or a silicon-drift detector for fluorescence detection. Si311 crystals of the monochromator were used. Samples were held in a liquid-helium cryostat of ID26 at 20 K. The emission detection resolution was ~1 eV. We note that due to a public holiday there was a shortage of liquid helium supply of the ESRF for 2 days so that we could not do experiments in this time during the run.

Results: ***(1) Radiation damage:*** X-ray induced modifications of Fe model samples were assessed by measuring XANES spectra with increasing irradiation time. Rapid changes of spectra were observed in particular for compounds with higher oxidation states under XES conditions. Attenuation of the beam was required to avoid photodamage even when using the fast shutter, which was closed during spectrometer movements and open only during data point aquisitions. In future studies, a continuous-scan and/or rapid-scan mode of the XES spectrometer will be implemented for more efficient beamtime usage.

(2) XES/XAS on three models: XES/XAS spectra were obtained for three 2Fe models with different coordination environments of their Fe atoms (1, 2, 3; Fig. 1), and for model 1 in four states in acetonitril solution. Figures 2,3,4 show selected XES and XES spectra of the Fe compounds.

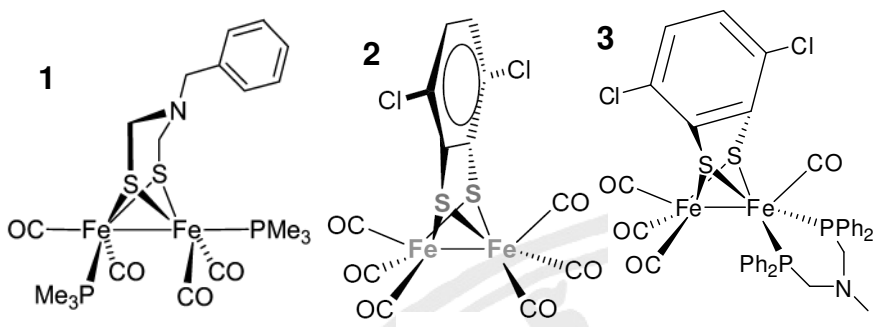

Figure 1: Crystallized biomimetic Fe_2-complexes from our collaborator S. Ott (Uppsala) (*1* [5], *2* [6], *3* unpublished) with variations in the Fe ligation symmetry and bridging ligand.

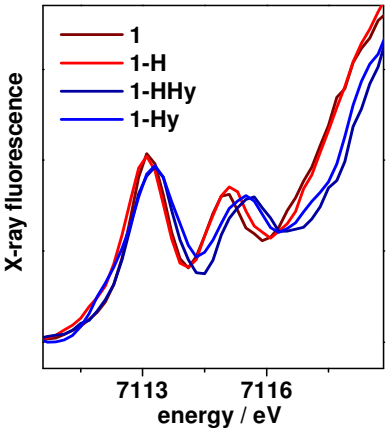

Figure 2: High-resolution XANES spectra of complex 1 [4] in four states in MeCN solution (1, unprotonated; 1-H, protonated at adt-nitrogen; 1-Hhy, FeFe-bridging hydride & adt-N protonated; 1Hy, FeFe-briding hydride). Note the shift of the 1s-3d transitions due to partial Fe oxidation.

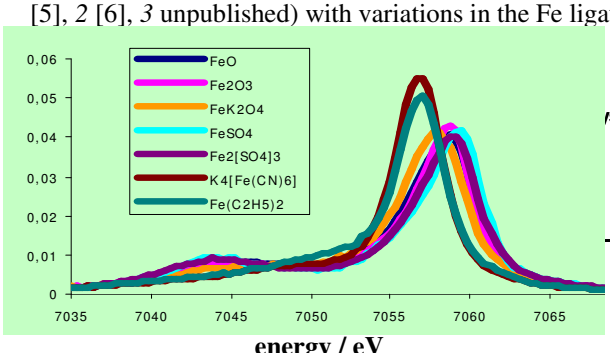

Figure 3: K-beta emission spectra of Fe compounds.

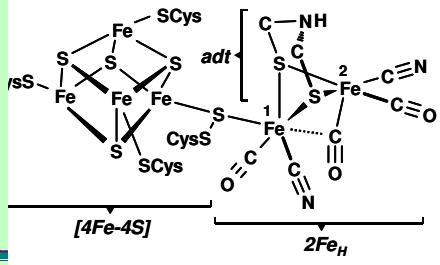

Figure 4: Structure of the active site in FeFe hydrogenase HydA1 from the alga *Chlamydomonas*.

(3) XES on FeFe hydrogenase: First XES data were obtained for the active site iron cluster of FeFe hydrogenase HydA1 (Figs. 4,5). The experiments revealed that further improvements of the set-up are necessary to avoid photodamage of the enzyme under XES conditions in future experiments.

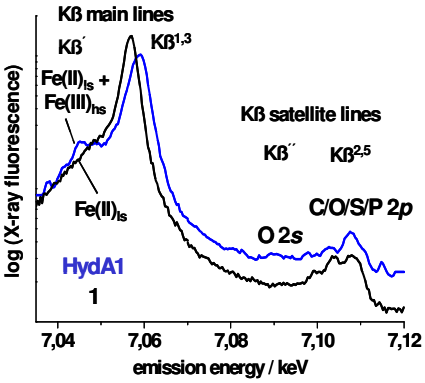

Figure 5: K-beta emission spectra of FeFe hydrogenase HydA1 and complex 1.

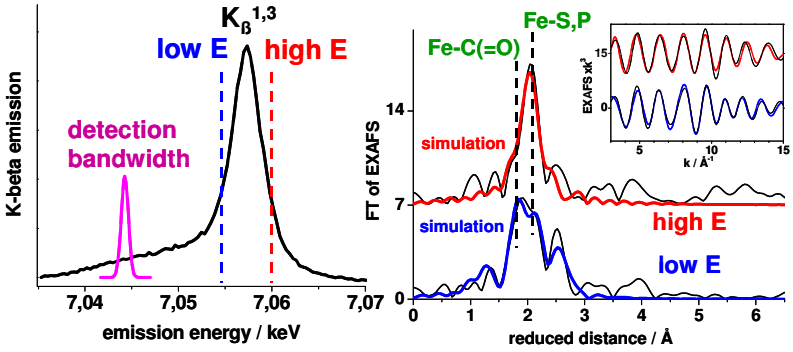

Figure 6: Site-selective XAS spectra on complex 3 using K-beta emission detection at indicated energies. EXAFS simulations (coloured lines) revealed individual Fe-ligand bond lengths and coordination numbers at the two asymmetric Fe ions.

(4) Site-selective XAS:
The asymmetric complex 3 was used for site-selective EXAFS studies using K-beta emission detection (Fig. 6). The data revealed high discriminative power for individual bond lengths and coordination numbers at the two Fe ions and the principle feasibility of the site-selective XAS/XES approach.

Conclusions: We consider the run as very successful. High-resolution XAS and XES spectra using the new instrument at ID26 were obtained for three biomimetic Fe compounds active in hydrogen production and preliminary data for FeFe hydrogenase enzyme. Data analysis unraveled structural and electronic features of the compounds (ms in preparation). The results showed that implementation of a rapid-scan mode for XES detection is desirable for experiments on proteins. The principle feasibility of a site-selective approach for discrimination, e.g., of the 4Fe and 2Fe units in FeFe hydrogenase was demonstrated. This promising approach will be further pursued and combined with time-resolved studies [7] on high-valent metal sites.

References:

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