ESRF	**Experiment title:** Electronic structure of intermediates in photosynthetic water oxidation studied by time-resolved high-energy-resolution manganese K-alpha-emission experiments	**Experiment number**: SC2610
Beamline: ID26	**Date of experiment**: from: 18.02.2009 to: 27.02.2009	**Date of report**: 13.03.2009
Shifts: 24	**Local contact(s)**: Dr. Tsu-Chien Weng, Dr. Kristina Kvashnina	*Received at ESRF:*

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Report: Understanding the mechanism of oxygenic photosynthesis (photosystem II) is crucial for development of light-driven hydrogen production for biotechnology. Using time-resolved X-ray spectroscopy, we have characterized novel intermediates in the reaction cycle of water oxidation at the Mn complex of photosystem II (PSII) [1-4]. X-ray experiments at high oxygen pressure excluded that a thermodynamic product backpressure effect limits the efficiency of the reaction [3]. Now in-depth characterization of the electronic state of intermediates is on the agenda. For this, we employed high energy resolution X-ray emission and absorption spectroscopy, in combination with time resolved approaches and laser flash excitation of protein samples in the X-ray beam, using the new spectrometer for XES at beamline ID26.
The present experiments were focussed on XES measurements at the manganese K-alpha and K-beta emission lines. A series of Mn model compounds with relevance for the PSII Mn complex, in particular for the S2-S3 transition, and PSII in its four S-states of the Mn complex as populated by laser flashes were investigated. Significant shifts of the emission lines were observed as function of the oxidation state of the PSII Mn complex, for the first time for K-alpha, which now are interpreted in comparison to the model spectra and using theoretical approaches. K-alpha RIXS at the moment can only be done on PSII with low signal-to-noise ratio, due to fast photoreduction of Mn. For the next measurements, the RIXS spectrometer will be improved by integration of five new Ge crystals (Ge111) for Mn K-alpha to increase the count rate, which will be purchased by our group using a recent grant (to MH) from the German research council.

Experimental: Partially dehydrated PSII membrane fragment samples were prepared as previously; a total of ~10,000 samples was used. XES measurements on PSII were performed at the Mn K-alpha and K-beta lines, at room temperature. Due to fast photoreduction, emission line measurements on PSII were done by recording a timescan of the X-ray fluorescence at 20 emission energies, each on a fresh sample; only the first 10-20 ms of the timescan data were used to construct the emission spectra of the S-states. S-state population was achieved by the application of 0, 1, 2, 3 laser flashes immediately (within ~100 ms) prior to the measurements. Mn model compounds were provided by the groups of Drs. M. Anderlund and A. Magnuson (Uppsala University Sweden) and Prof. K. Wieghardt (MPI Bioanorganic Chemistry, Mülheim Germany). On the models, Mn EXAFS and XANES spectra, K-alpha, K-beta, and K-beta satellite lines, and complete K-alpha RIXS planes in the Mn pre-edge region were measured.

Results: (1) EXAFS, XANES, Mn K-alpha and K-beta lines using non-resonant excitation (6800 eV), and complete RIXS planes in the pre-edge (resonant excitation) were recorded on 10 model compounds (Fig. 1). The high-quality data allow to adress changes in the emission line shapes and energies and in the RIXS planes, in releation to the structures of the compounds from EXAFS and crystallography. In addition, studies for site-selective EXAFS were performed on mixtures of Mn compounds using the K-alpha emission. These data are of relevance for the planned site-selective studies on Fe and Mn-Fe proteins. The data currently is analyzed, e.g., employing theoretical approaches.

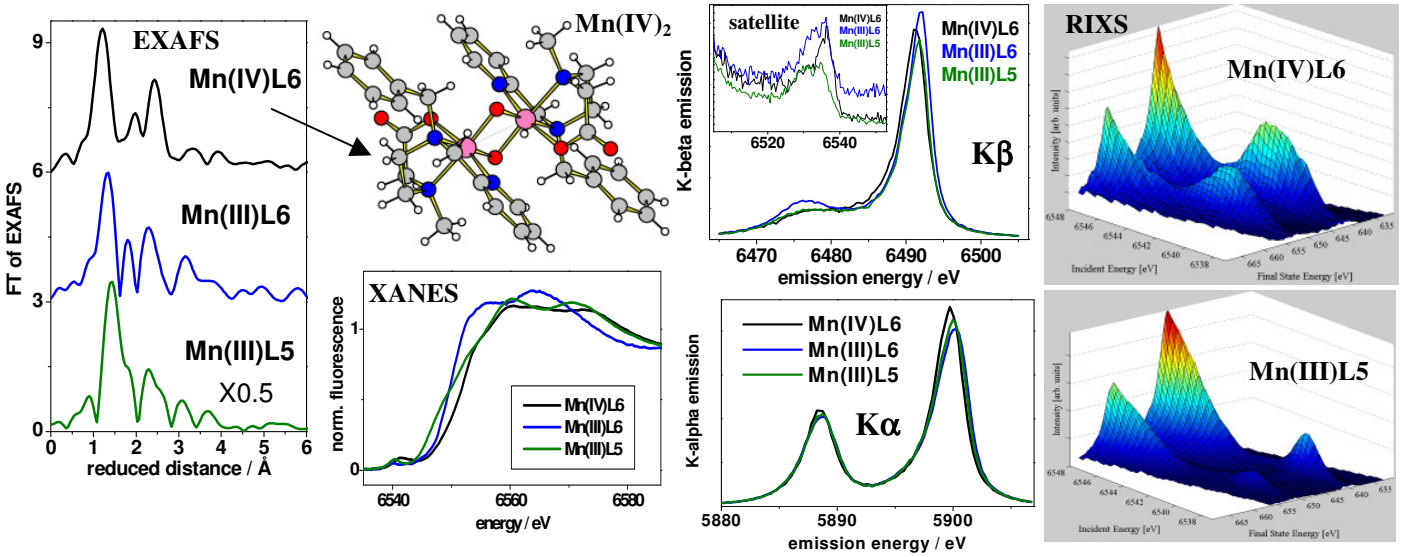

Fig. 1: X-ray absorption and emission data on 3 selected (out of 10 measured) synthetic Mn compounds.

(2) Mn K-alpa and K-beta line measurements on the Mn complex of PSII in four S-states were performed after laser flash excitation of the protein (Fig. 2). For the first time, K-alpha lines were measured for PSII in 4 S-states using a single Ge333 analyzer crystal. The K-beta results reproduced the outcome of our previous experiments (SC2371), but due to the use of 4 analyzer crystals (Ge440) show improved signal-to-noise ratios. Emission lines were recorded in a time interval of only ~20 ms. Significant shifts and shape changes of the emission lines were observed as function of the Mn oxidation state. We are currently aiming at an interpretation of the underlying electronic and structural changes, e.g., in comparison to the model spectra.

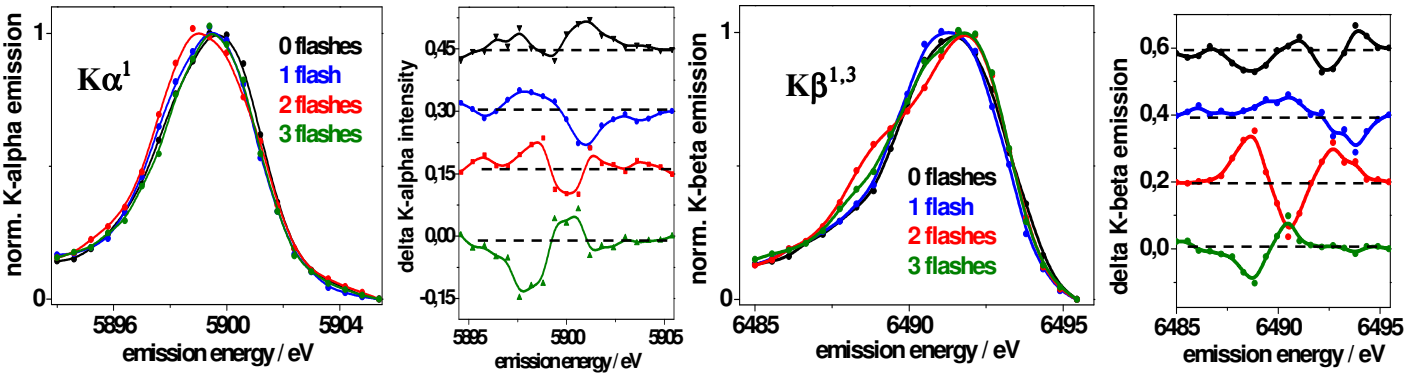

Fig. 2: (left) The first K-alpha line spectra of PSII in 4 S-states and the respective difference spectra. (right) PSII K-beta line spectra in four S-states. Note the similar overall spectral changes occuring at both emission lines upon the laser flashes (but with smaller amplitude at K-alpha), as visible in the difference spectra.

(3) Feasibility studies for RIXS measurements in the Mn pre-edge were performed on PSII. Due to fast photoreduction (reduction of the high-valent Mn ions to Mn(II) within ~2 s), short data acquisition times (10-20 ms) had to be used (if full beam was employed), bringing the countrate down to ~3 counts per 10 ms at the maxima of the pre-edge and K-alpha line (at ~6541 eV and ~5890 eV). In the next experiments we will use 5 new analyzer crystals for RIXS whereby meaningful measurements on PSII will become feasible.

Conclusions: We consider the run SC2610 as highly successful. 10 Mn model compounds were extensively characterized by XAS and XES. XES on PSII at K-alpha and K-beta lines using laser flash excitation at room temperature was established; these techniques are essential for future time-resolved XES measurements. The first K-alpha line spectra of PSII in four S-states were obtained. K-beta line spectra of high quality were measured. The similar spectral changes at both emission lines point to the same underlying processes in PSII, which are now analyzed in detail. A respective publication is in preparation. Valuable information for future RIXS experiments on the 1s-3d transitions in PSII was gained. We will improve the RIXS spectrometer at ID26 for XES studies on proteins by purchasing several sets of analyzer crystals.

References:

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