


**Experiment title:**

Intermediates in photosynthetic water oxidation studied by time-resolved high energy-resolution X-ray experiments at room temperature using K-beta fluorescence detection

**Experiment number:**

SC2371

**Beamline:**

ID26

**Date of experiment:**

from: 14.02.2008

to:

23.02.2008

**Date of report:**

17.03.2008

**Shifts:**

24

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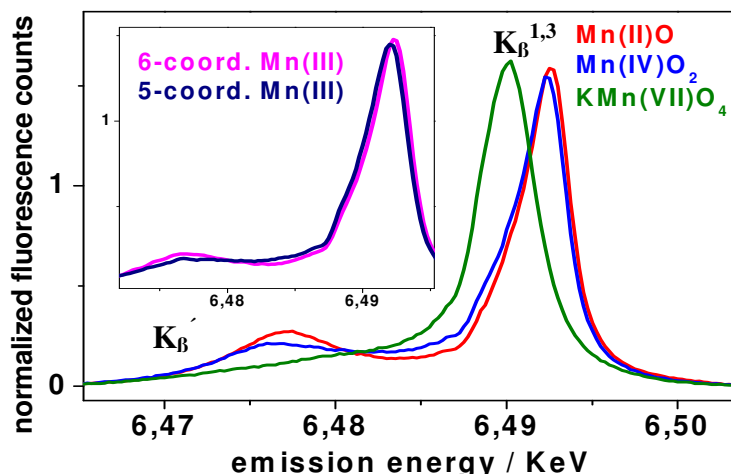
**Report:** Understanding the mechanism of photosynthetic water oxidation is crucial for future biotechnological fuel production. The atmospheric  $O_2$  is produced at a  $Mn_4Ca$  complex of photosystem II (PSII) [1-4]. One important question is whether manganese is oxidized in each of the four redox transitions of the complex during water oxidation [2]. High energy-resolution X-ray emission spectroscopy (XES) at the Mn K-beta ( $K_\beta$ ) and K-alpha ( $K_\alpha$ ) emission lines is expected to yield in depth information on electronic features of the Mn complex (e.g. spin and oxidation state) [5]. Using the spectrometer for resonant inelastic X-ray scattering (RIXS) at beamline ID26, we measured  $K_\beta$  lines of the Mn complex immediately after the application of laser flashes to PSII samples in the X-ray beam, within 500 ms. Shifts of the  $K_\beta$  line energy suggest Mn oxidation in all three transitions between semistable S-states. However,  $K_\beta$  lines of coordination compounds revealed also shifts due to coordination changes at Mn. Accordingly, the  $K_\beta$  line may be affected both by redox and structural changes at Mn. Using Mn coordination compounds, first site-selective XANES spectra using  $K_\beta$ -detection were obtained. In addition, it was shown that future time-resolved XES experiments with laser excitation are feasible at the  $K_\alpha$  line of Mn, using PSII protein preparations.

**Experimental:** XES was performed using the single-crystal RIXS spectrometer at ID26 at room temperature with an emission energy resolution of  $\sim 1$  eV.  $K_\beta$  lines of Mn were recorded on Mn compounds and PSII protein using an excitation energy of 6700 eV. Lines of Mn compounds were measured by continuous scans. On PSII, lines were recorded in a point-by-point procedure where at each emission energy, 0-7 flashes were applied to the PSII samples, and a timescan of the  $K_\beta$  fluorescence of 10 s was performed thereafter. From the first point of these timescans (0.5 s), complete  $K_\beta$  line spectra were constructed ( $\sim 10$  scans were averaged per energy point). Previous determination of the rate of photoreduction [6] under  $K_\beta$  detection conditions and use of appropriate attenuators ensured the absence of this effect in the measurements. Complete RIXS planes at the pre-edge region of Mn XANES spectra were recorded, also in a point-by-point approach (each point of the plane was recorded on a fresh PSII sample). It was estimated that the maximum fluorescence countrate at the  $K_\alpha$  line of PSII samples allows for time resolved investigations at a resolution of 50-100  $\mu$ s per data point.

**Results:**

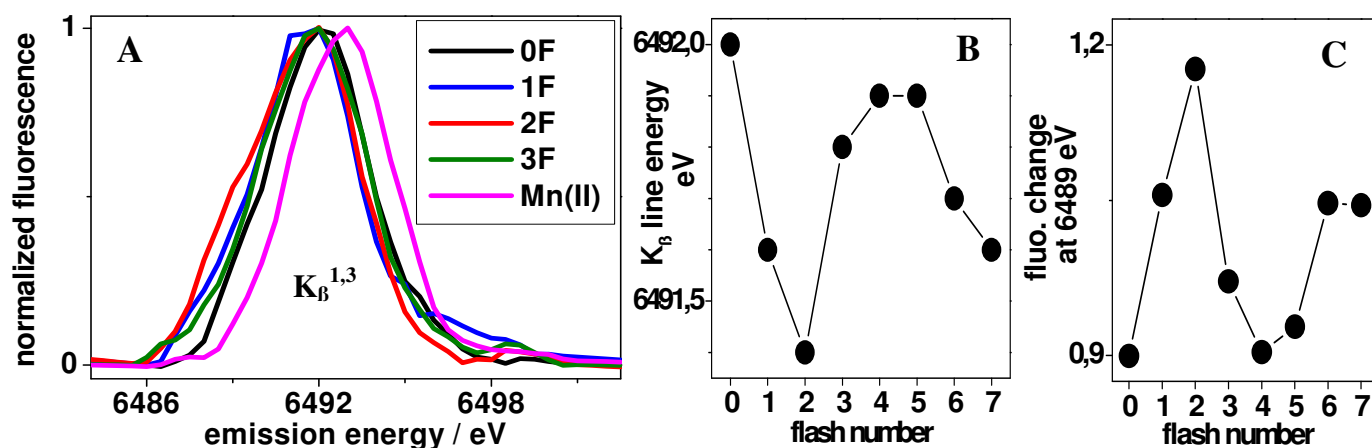
(1)

 Figure 1 shows  $K_\beta$  lines of Mn compounds.

 Clearly, there are shifts and shape changes of the line in response to oxidation state and coordination number changes. Thus, the  $K_\beta$  line seems to be affected by both electronic and structural changes.


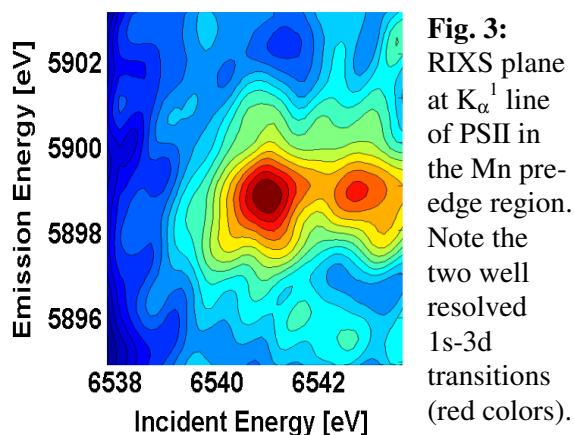
**Fig. 1:** Normalized  $K_\beta$  lines of selected Mn compounds. Shifts of the line energy and shape are discernable in Mn oxides. Similar line energy shifts are detectable between 5- and 6-coordinated Mn(III) compounds (kindly provided by Dr. A. Magnuson, Uni. Uppsala, Sweden). The excitation energy was 6.7 KeV.

(2)  $K_{\beta}^{1,3}$  lines of the Mn complex were recorded using a point-by-point approach and laser excitation of PSII (Fig. 2A). The lines on flashes 1 and 2 reveal shifts of their maxima (from Gaussian simulations) to lower energies by  $<0.5$  eV; on flash 3 the line shifts to higher energies. A quaternary oscillation of the line energy is indicative of the S-state cycling of the Mn complex (Fig. 2B). The line-energy oscillation is similar to that determined from  $K_{\beta}$  fluorescence changes at the low-energy side of the  $K_{\beta}^{1,3}$  line upon the laser flashes.



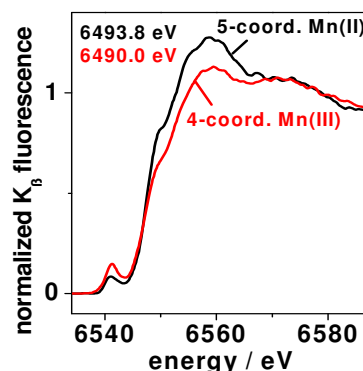
**Fig. 2:** (A) Normalized  $K_{\beta}$  lines of the PSII Mn complex after flashes 0-3 recorded within 500 ms after each flash. The magenta curve was obtained after complete X-ray photoreduction of the Mn complex to Mn(II). (B) Line energies after 0-7 flashes. (C) Fluorescence level changes at  $\sim 6489$  eV in a flash series. Note the similar oscillatory behaviour in (B) and (C) due to the stepping of the Mn complex through the S-state cycle. The line shift after flash 2 inducing the S2-S3 transition seems to be of similar magnitude as that on flash 1, compatible with Mn oxidation on both steps.

(3) Figure 3 shows a RIXS plane of PSII recorded using excitation energies in the pre-edge region of the Mn XANES spectrum. Each point in the plane was recorded on a fresh PSII sample to avoid photoreduction. The experiments revealed that RIXS measurements on PSII using laser flash excitation are feasible at ID26, as well as future time-resolved measurements at the  $K_{\alpha}$  emission lines.



**Fig. 3:** RIXS plane at  $K_{\alpha}^1$  line of PSII in the Mn pre-edge region. Note the two well resolved 1s-3d transitions (red colors).

(4) Site selective spectroscopy is promising for characterization of individual metal ions in multi-nuclear sites. Figure 4 shows  $K_{\beta}$ -detected XANES spectra on a  $Mn_3$  compound containing two 5-coordinated Mn(II) and one 4-coordinated Mn(III) ion (kindly provided by Prof. C. Limberg, Humboldt-Uni. Berlin). Large spectral changes allow for discrimination of the two different Mn species in the same sample.



**Fig. 4:** Site selective XANES of a  $Mn_3$  compound measured at two  $K_{\beta}$  emission energies.

**Summary:** We consider the run SC2371 as highly successful. We showed that  $K_{\beta}$  and  $K_{\alpha}$  spectroscopy is feasible at ID26 on PSII protein using laser flash excitation.  $K_{\beta}$  lines were measured on PSII (after 0-7 laser flashes, within 0.5 s after the flash) and on Mn compounds. X-ray photoreduction was avoided.  $K_{\beta}$  line shifts in PSII are compatible with Mn oxidation on three S-transitions. Shape changes of  $K_{\beta}$  lines currently are analyzed. Experiments at the  $K_{\alpha}$  emission lines revealed that time-resolved  $K_{\alpha}$  experiments aiming at recording of complete RIXS planes are feasible on PSII. Preliminary data show discernable changes of the  $K_{\alpha}$  lines upon laser excitation of PSII, likely related to electronic and structural changes of Mn. These changes will be analyzed in future experiments to characterize novel intermediates in the reaction cycle (recent results of measurements at ID26 are outlined in [7]) and to unravel the mechanism of photosynthetic  $O_2$  formation.

## References:

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