ESRF	**Experiment title:** The calcium binding site at the manganese complex of oxygenic photosynthesis: Investigations by calcium XAS on native and biochemically treated photosystem II	**Experiment number**: SC1228
Beamline: ID26	**Date of experiment**: from: 30.04.2003 to: 12.05.2003	**Date of report**: 20.08.2003
Shifts: 33[&]	**Local contact(s)**: Dr. Thomas Neisius	*Received at ESRF:*

[&]Block allocation for SC1228 and SC1229 (see separate report for SC1229)

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Report: Photosynthetic water oxidation by photosystem II (PSII) is the source of atmospheric dioxygen and thus of fundamental biological importance. Water oxidation takes place at the tetra-manganese complex of PSII. This complex additionally contains one calcium ion which is essential for the functioning of water oxidation. The location of the Ca ion relative to the four Mn atoms and its coordination environment were here investigated by XAS at the Ca K-edge, a particularly challenging BioXAS experiment.

Partially dehydrated multilayer samples for XAS (~200) of highly active PSII membranes were prepared on Ca-free Mylar tape. Depletion of bulk Ca was achieved by a Chelex-100 treatment. Chelex-treated samples contained 2 ±1.5 Ca atoms per 4 Mn atoms as determined by AAS. XAS at the Ca K-edge was performed at 50 K using a newly constructed evacuated cryostat where 4 samples were mounted simultaneously on a helium-cooled turnable coldfinger. XAS spectra were collected at 45° in fluorescence mode using a large-area photodiode placed within the cryostat as a detector. A Ca-free, 150 nm thick Si-nitrite window facilitated I_0 detection; no other foils were passed by the incident X-ray beam (spot size ~1 mm^2) or the X-ray fluorescence prior to detection. XAS spectra were measured in the rapid-scan mode of ID26 within 30 s (scan range 3900-4500 eV). The energy axis was calibrated by use of reference substances.

The following results were obtained:

(1) Figure 1 compares XANES spectra of a PSII sample which contained bulk calcium with a sample where the Ca content was reduced to ~2 Ca / 4 Mn (Chelex-treated). Reproducible differences in the XANES spectra of ~2 Ca / 4 Mn containing samples (higher pre-edge peak, by 0.3 eV reduced edge energy, lower principal maximum) are likely indicative of a lower coordination number and/or symmetry of Ca bound to PSII compared to its more symmetric coordination by 7-8 water molecules in the bulk.

(2) The difference in the Ca K-edge magnitudes (see arrow in Fig. 1) was employed to address radiation damage. At an excitation energy of 4038 eV the X-ray fluorescence was recorded as function of time with Chelex-treated samples. The increase of the fluorescence intensity within ~400 s (at 50 K, Figure 2) likely indicates the release of specifically bound Ca from its binding site into the bulk due to radiation damage. Within the duration of the XAS scans (30 s), however, the Ca K-edge magnitude remained unchanged (Fig. 2, arrow) meaning that Ca stayed bound to the Mn complex; radiation damage was therefore negligible.

(3) Figure 3 shows the Fourier-transforms (FTs) of EXAFS oscillations (inset) of the ~80 Ca / 4 Mn and the Chelex-treated samples. The spectrum of the high-Ca sample is well simulated using a single Ca-O shell (Table 1, fit I) and with parameters which are anticipated for fully hydrated bulk Ca.

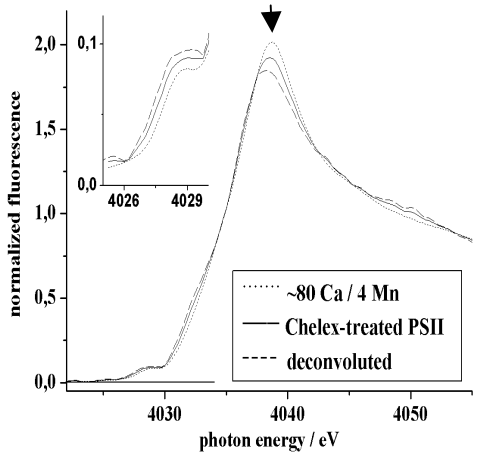

Figure 1: Ca XANES spectra of PSII samples. Dashed line: tentative pure spectrum of the Ca bound to the Mn complex obtained by deconvolution. Inset: pre-edge peak region.

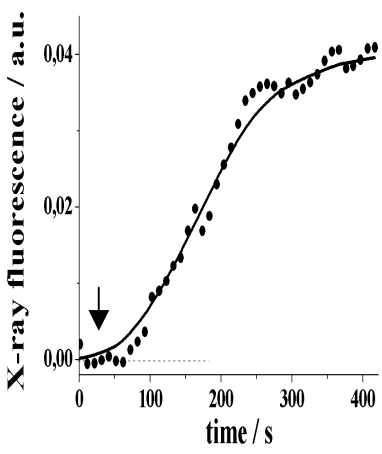

Figure 2: Timescan of X-ray fluorescence at 4038 eV. Arrow: duration of EXAFS scans.

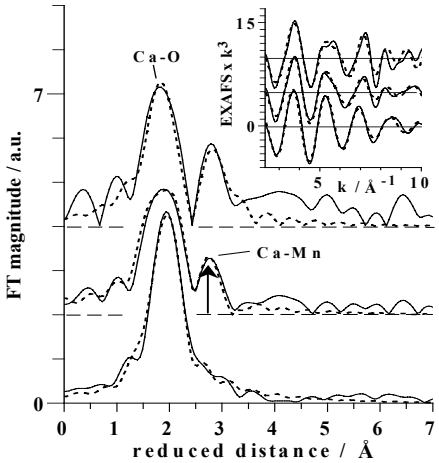

Figure 3: FTs of EXAFS spectra (insets) of ~80 Ca / 4 Mn (bottom) and Chelex-treated PSII samples (middle). Top: tentative pure spectrum of Ca bound to the Mn complex. Dashes, simulations (Table 1).

In the FT of the EXAFS oscillations of the Chelex-treated sample a pronounced second peak at ~2.7 Å of reduced distance emerges (Fig. 3, arrow) which is attributed to Ca-Mn interactions. A good simulation of this spectrum was obtained using two Ca-O vectors (Table 1, fit II) and one Ca-Mn shell. Further quantitative considerations reveal that the Ca bound to the Mn complex is coordinated by 5-6 oxygens and that it is at ~3.3 Å distance from at least two Mn atoms. The new information from Ca XAS in combination with our previous Mn XAS results yields a tentative model for the location of the Ca in the Mn complex (Fig. 4).

(4) Ca XAS spectra were also measured with the Mn complex in one higher oxidation state obtained by illumination of samples in the cryostat at 200 K and with biochemically treated samples. The analysis of the obtained spectra (not shown) is underway and expected to yield, i.a., information on changes in the Ca-Mn distances upon the oxidation of Mn.

Table 1: Parameters of EXAFS simulations (R_F, weighted error sum).

sample	fit	shell	N_i [per Ca]	R_i [Å]	σ^2_i [10^{-3} Å^2]	R_F (1-3Å) [%]
~80 Ca / 4 Mn	I	Ca-O	7.32	2.42	12.3	13.7
~2 Ca / 4 Mn (Chelex treated)	II	Ca-O Ca-O Ca-Mn	2.30 3.37 1.65	2.26 2.43 3.26	1.5 2.5 15.5	12.7

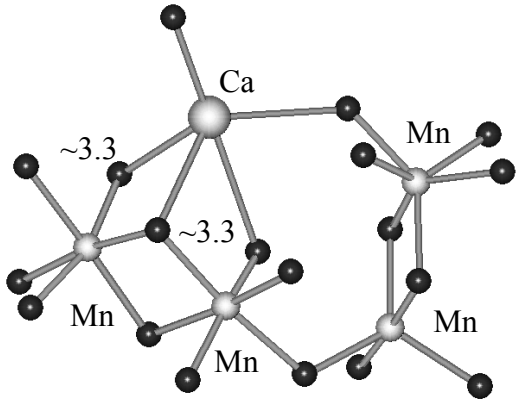

Figure 4: Tentative model of the Mn_4/Ca complex in PSII. Black dots, oxygens; distances are given in Å.

Summary: The measurements on PSII protein preparations revealed that Ca XAS is feasible on biological samples at ID26. Radiation damage can be avoided using the rapid-scan mode of ID126 and well-adapted measuring protocols. For the first time, XANES and EXAFS spectra of Ca bound to the Mn complex of photosynthesis were obtained in two oxidation states of the water oxidation reaction cycle.
The current preliminary status of data analysis already reveals new and valuable information on the coordination of Ca in the Mn complex and of its distance, ~3.3 Å, from the Mn atoms.
In further experiments, the experimental conditions should be modified with respect to the suppression of scattered X-rays to improve the quality of EXAFS spectra and to extent the useful energy range at the Ca K-edge.

The experiments carried out during run SC1228 lead to the following publication:

Claudia Müller, Peter Liebisch, Marcos Barra, Holger Dau, and Michael Haumann (2003) **The location of calcium in the manganese complex of oxygenic photosynthesis studied by X-ray absorption spectroscopy at the Ca K-edge.** *Phys. Scripta*, accepted for publication.