



	Experiment title: "The manganese complex of photosynthetic water oxidation studied by calcium EXAFS"	Experiment number: SC924
Beamline: ID26	Date of experiment: from: 10.02.2002 to: 14.02.2002	Date of report: 10.08.2002
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Report. Photosynthetic water oxidation takes place at a tetra-manganese complex bound to the D1 subunit of a metalloenzyme, photosystem II (PSII). The metal site additionally contains, presumably, one calcium atom; its location with respect to the four manganese atoms and its function in the catalytic cycle are under debate. The manganese complex has been extensively studied by XAS at the Mn K-edge, yielding tentative evidence for the binding of calcium at a distance of ~ 3.3 Å from one to two Mn atoms [1,2]. Here, we undertook first attempts to perform the reverse, clearly more difficult experiment, namely XAS measurements at the Ca K-edge, i.e. "looking from the Ca at the Mn atoms".

Partly dried PSII membrane multilayer samples on Mylar tape with Mn in the dark-stable, so-called "S₁" oxidation state were prepared by a new Chelex-treatment protocol to remove access Ca and to finally achieve a content of only about 2-4 Ca atoms per four Mn as verified by atomic absorption spectroscopy. Samples were mounted in an evacuated cryostat (directly connected to the beamline vacuum) on a newly constructed turnable coldfinger which was cooled by liquid helium to 20-80 K. A Si-nitrite window (~ 9 mm², 200 nm thick) served as I₀-detector window in front of the sample. XAS spectra of a Kapton foil (containing Ca) were used for energy calibration. EXAFS spectra of PSII samples at the Ca K-edge (scan range 4000-4650 eV) were measured within 15-30 s, using the rapid-scan mode of ID26. A photodiode mounted inside of the cryostat detected the excited X-ray fluorescence.

The following results have been obtained:

(1) By the construction of an evacuated helium-cooled cold-finger cryostat which contained a photodiode detector in vacuum, by the optimization of the cold-finger to minimize X-ray scattering, and by improving further technical details, the ability for XAS measurements at the Ca K-edge was established at beamline ID26.

(2) XANES spectra at the Ca K-edge were measured in control PSII samples and in PSII which have been treated with hydroxylamine (known to cause the release of Mn and Ca from their binding site). The Ca K-edge spectra of these samples reveal subtle differences (Fig. 1A). They strongly differ from the XANES spectrum of an aqueous CaCl₂ solution (Fig. 1A). These results indicate that in the PSII samples Ca is

coordinated not only by water molecules as may be expected for unspecific Ca contaminations. We conclude that our method of removal of excess Ca from PSII seems to work, yielding samples which presumably contain at least one Ca atom bound to the manganese complex. This notion was corroborated by measurements of a TRIS-washed PSII sample (this treatment is expected to remove all Ca and Mn atoms). In the latter, no indication of Ca was obtained (Fig. 1A).

(3) Fig. 2B shows EXAFS spectra of control PSII samples, and of samples which have been inactivated by excess X-ray irradiation plus total drying due to prolonged vacuum exposure (these factors are expected to cause the release of Ca and Mn from their binding sites). The Fourier-transforms of control PSII reveal two major peaks at reduced distances of about 2 Å and 3 Å. Only the peak at 2 Å was observed in inactive PSII and in a CaCl₂ sample. The latter peak is due to light scatterers (O,N) in the first coordination shell of Ca. The FT peak at about 3 Å may be taken as first evidence for the presence of Mn atoms at a distance of 3.3-3.5 Å from Ca (as indicated by tentative simulations of EXAFS spectra).

(4) The signal-to-noise ratio in the Ca-XAS measurements was low, due to the low metal content of samples and due to glitches and, presumably, monochromator instabilities at higher X-ray energies. These factors limited the useful EXAFS range to an upper value of ~400 eV above E₀ (4050 eV).

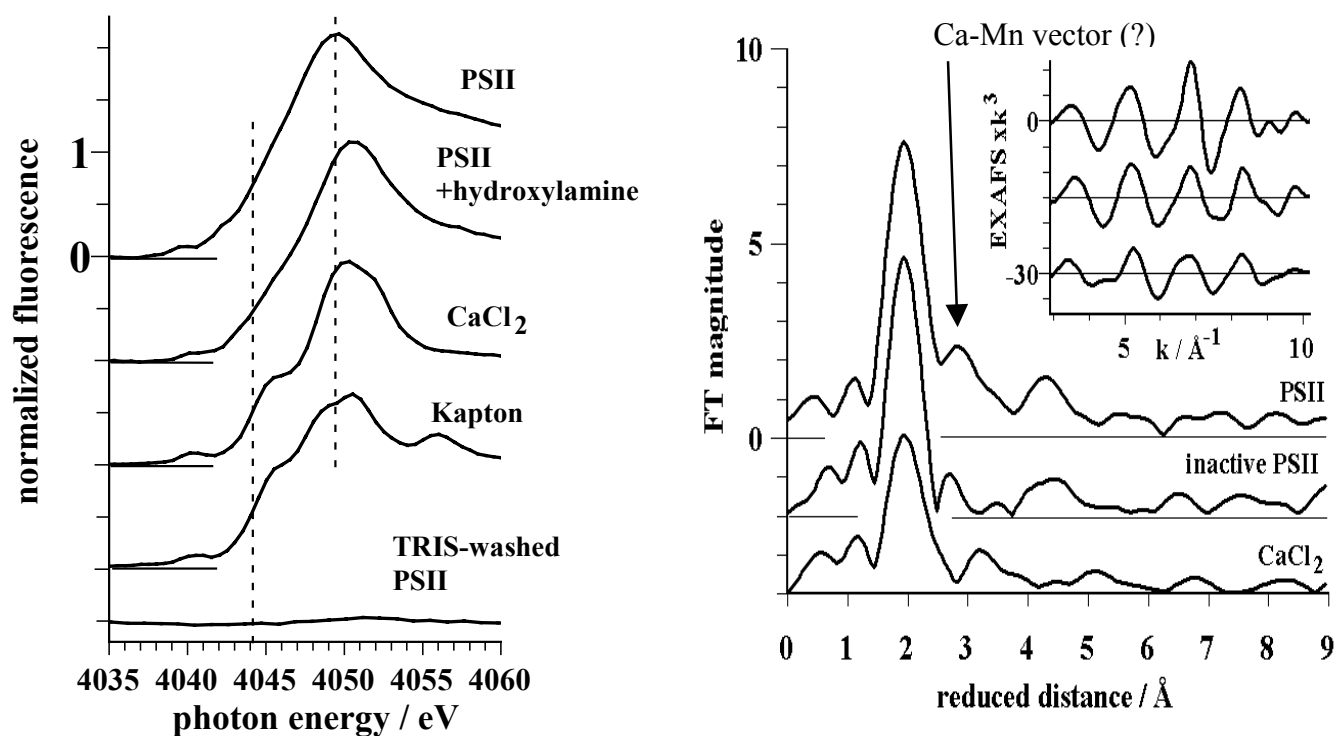


Fig. 1: (A) XANES spectra at the Ca K-edge. (B) Fourier-transforms of EXAFS spectra (calculated from 40-400 eV above E₀ using cosine windows extending over 10 % and 30 % at low and high K-values). Inset: backtransforms into the k -space (window 1-5 Å).

Conclusions:

We consider the particularly difficult experiments conducted during the run in May 2002 as mostly successful. The technical basis for measurements of metalloenzyme samples at the Ca K-edge at beamline ID26 was established. First XAS spectra of photosystem II samples with minimized Ca content have been obtained. The signal-to-noise ratio of EXAFS spectra is still low. Further technical enhancements and optimization of the samples are expected to yield improved data. Ca-EXAFS spectra of PSII provide first evidence for the presence of heavy scatterers, likely Mn atoms, in close vicinity of the bound Ca atoms (Ca-Mn distance ~3.3-3.5 Å).

1. Dau, H., Iuzzolino, L. & Dittmer, J. (2001). *Biochim.Biophys.Acta* 1503, 24-39.
2. Pospisil, P., Haumann, M., Dittmer, J., Sole, A. V., & Dau, H. (2002). *Biophys. J.*, in press.