ESRF	**Experiment title:** The Photosystem II Manganese Complex: temperature dependence of structure and radiation-induced state changes investigated by rapid-scan and time-resolved XAS	**Experiment number**: LS1583
Beamline: ID26	**Date of experiment**: from: 25.04.2001 to: 02.05.2001[§]	**Date of report**: 25.02.2002
Shifts: 10[§]	**Local contact(s)**: Dr. T. Neisius, Dr. L. Jacquamet	*Received at ESRF:*

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[§]block allocation for LS1582 + LS1583, report for LS1583

Report. Exposing samples of layered Photosystem II (PSII) membrane particles to the X-ray beam of modern synchrotron radiation sources results in massive radical formation, X-ray photoreduction of the tetranuclear Mn complex, state changes and, finally, disintegration of the Mn complex. Potentially, such 'radiation damage' affects the structural information obtained by X-ray absorption spectroscopy (XAS, EXAFS) and protein crystallography. (1) Thus, assessment of radiation effects is of clear relevance with respect to structural investigations involving high-brilliance X-ray sources. (2) On the other had, limited X-ray exposure may provide the means to reach specific oxidation states of the metal center. (3) The temperature dependence of the Debye-Waller parameter, σ, of the intact Mn complex provides insights in functionally relevant structural dynamics.

The following results and insights were obtained during our measurements at ID26 in April 2001.

(1) Temperature dependence of the X-ray photoreduction. We investigated the time course of the rate of photoreduction at an X-ray flux of ~10^{12} photons/s for temperatures ranging from 10 K to room temperature for the Mn complex initially in S_1-state (Fig. 1A). The rate of photoreduction increased biphasically (Fig. 1B) with a "breakpoint" around 180 K, characteristic for phenomena related to protein mobility (glas transition of proteins).

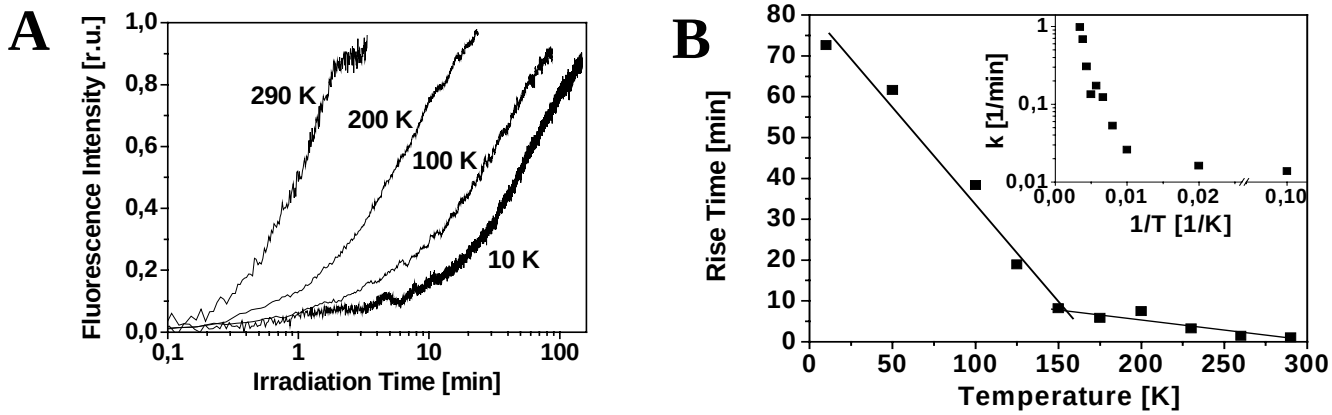

Figure 1: **A**: X-ray photoreduction of the Mn complex at various temperatures, monitored by time-scans of the X-ray fluorescence intensity at a fixed excitation energy of 6549 eV (in the range of the Mn K-edge, see vertical line in Fig. 2). **B**: Temperature dependence of the X-ray photoreduction. The rise-times were obtained from mono-exponential simulations of time-scans as shown in Fig. 1A. (Inset: Arrhenius plot showing the pronouncedly biphasic behavior.)

Fig. 2 shows Mn K-edge spectra exposed to various X-ray illumination periods. The K-edge energy shifted from initially 6551.7 eV (S_1-state of the intact Mn complex) to 6548.7 eV after only 3 min of irradiation.

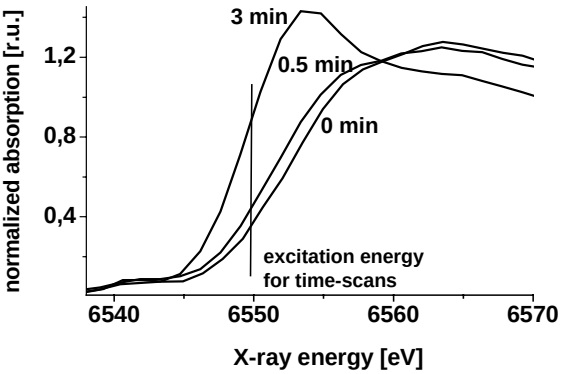

Figure 2: Normalized manganese K-edge spectra for increasing X-ray illumination times at room temperature. The vertical line indicates the excitation energy of 6549.5 eV used for time-scan measurements (see Fig. 1). A downshift of the K-edge energy by ~2 eV is indicative of the photoreduction of the Mn complex from an initial average oxidation state of 3.5 to about 2.3. Thus, three of the four manganese atoms are reduced to Mn^{2+} by 3 minutes of X-ray exposure.

(2) X-ray induced structural changes. Mn EXAFS spectra obtained at room temperature after various X-ray exposure times (data not shown) were simulated using three coordination shells. The decrease of the coordination number (N) of a Mn-O vector of 1.85 Å length and the simultaneous increase of N of a Mn-O vector of 2.2 Å length is attributed to the formation of Mn^{2+} in the sample (Fig. 3I,II). Mn-Mn vectors of 2.7 Å length indicate the presence of di-μ-oxo bridged Mn pairs in the S_1-state. Their coordination number decreases in parallel to the formation of Mn^{2+} (not shown).

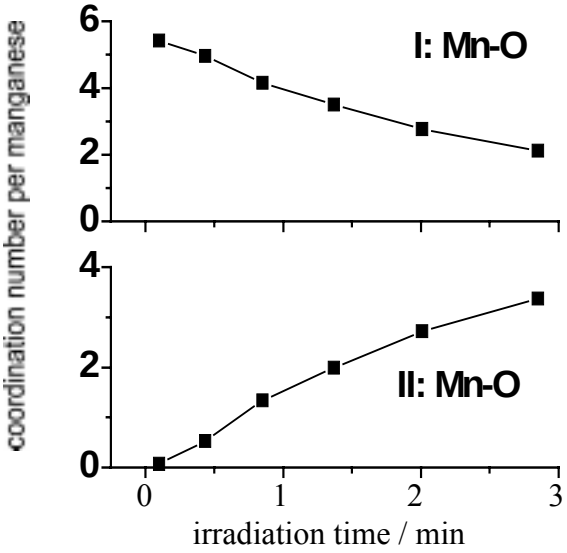

Figure 3: Changes in the coordination of Mn as function of X-ray exposure time at room temperature.

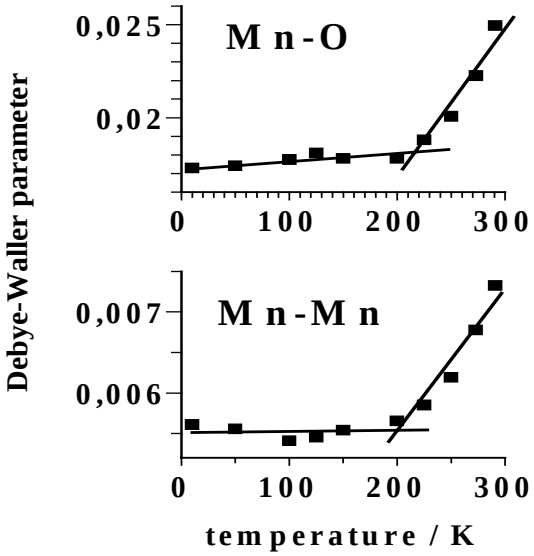

Figure 4: Temperature dependence of the Debye-Waller parameter ($2\sigma^2$) of two coordination shells of manganese (Mn-O at 1.85 Å, Mn-Mn 2.7 Å) in the S_1-state.

(3) Temperature dependence of the Debye-Waller parameter. Fig. 4 shows the Debye-Waller parameter of two coordination shells of the intact Mn complex in the S_1-state as function of the temperature. A similar biphasic temperature dependence as found for the rate of photoreduction was observed (see Fig. 1B).

Conclusions: The temperature dependence of X-ray photoreduction and structural parameters of the Mn complex of PSII were investigated. At room temperature, EXAFS scans of Mn have to be performed within 10-20 s to avoid photoreduction. These experiments can only be performed using the rapid-scan capabilities of beamline ID26.
For the first time, it is shown that structural parameters of the Mn complex (i.e. the Debye-Waller parameter) significantly depend on the temperature. Changes in the structural dynamics (magnitude of σ) of the Mn complex, above 200 K, are detected which may be of functional relevance (coupling of the biological electron transfer to vibrational modes of the protein).

We consider the LS1583 run at ID26 as particulary successful. It resulted in important findings and the following publications:

M. Haumann, M. Grabolle, T. Neisius, H. Dau (2002) The first room-temperature X-ray absorption spectra of higher oxidation states of the tetra-manganese complex of photosystem II. FEBS lett., in press.

M. Haumann, P. Pospisil, M. Grabolle, C. Müller, P. Liebisch, A. Sole, T. Neisius, J. Dittmer, L. Iuzzolino, H. Dau (2001) First steps towards time-resolved BioXAS at room temperature - The manganese complex of oxygenic photosynthesis. J. Synchr. Radiation, submitted.

M. Grabolle, M. Haumann, T. Neisius, and H. Dau (2002) X-ray photoreduction of the manganese complex of PSII - temperature dependence and structural changes. in preparation.