Summary

Photosynthetic water oxidation at the Mn_4Ca-cluster of Photosystem II (PSII), a process of fundamental importance for the atmosphere and biosphere on the earth, is only insufficiently understood. X-ray absorption spectroscopy (XAS) on the structure of the Mn_4Ca-cluster and, in particular, on the structural changes in the catalytic cycle has resulted in central contributions to the worldwide endeavor to unravel the mechanism of photosynthetic water oxidation. In the presented thesis two specific contributions are described: (i) the methodical developments that eventually facilitated monitoring the protein-bound metal complex in its functional cycle by time-resolved X-ray studies at room temperature and (ii) XAS at the Ca K-edge to get insights in the position of the Ca^{2+} ion relative to the Mn ions.

(i) *Room-temperature and time-resolved XAS on intermediates in the catalytic cycle*

Recently intermediate formation in the catalytic S-state cycle of PSII has been traced in a time-resolved X-ray study; the results were published in Science, a particularly renowned journal [Haumann et al., 2005a]. In an associated commentary in the same issue of Science, J. E. Penner-Hahn and C. F. Yocum [2005] point out the enormous experimental difficulties of this conceptually straightforward experiment. And indeed, the experiment long had been thought to be impossible to carry out so that measuring time at synchrotron radiation sources was denied because of infeasibility. Only on the basis of improved methods for sample preparation and XAS measurements, the time-resolved investigations became possible. Development and optimization of the following methods/techniques had been the essential basis for these time-resolved studies and for other room-temperature XAS investigations:

- Optimal signal-to-noise ratio requires a minimal water content of the samples. I could show that drying of PSII membrane particles previously layered by a specific centrifugation protocol is possible down to water content of only 50 % (weight per weight), which is similar to the water content of PSII crystals.

- Using a electronic dispensing system the automated preparation of 20 ‚dots' of PSII samples on specifically designed ‚samples stripes' has been achieved. For optimized parameters of the sample suspension and the vacuum-drying I could reach a sufficiently low water content, without the time-consuming centrifugation step to layer the PSII membrane particles and without significant activity loss.

- An apparatus for automated sample change during the XAS measurements has been developed that facilitated Laser-flash application and XAS measurements on up to 10,000 PSII samples per beamtime period.

The above methodical developments are described in Chapters 3; they are also described in two articles in the Journal of Synchrotron Radiation [Haumann et al., 2002a, 2005c].

Only by means of the improved methodology, a series of investigation on the Mn_4Ca-cluster in its catalytic cycle became possible. These investigations had been

a joint effort of the research group of Prof. Holger Dau. Specifically Dr. Michael Haumann (development of experimental strategy with Prof. Holger Dau, data analysis and interpretation) and Dr. Peter Liebisch (XAS measurements, development of data analysis tools, XANES simulations) contributed significantly to these studies. The following has been achieved:

- The rate and temperature dependence of the X-ray induced reduction of the Mn_4Ca-cluster was thoroughly characterized. This has facilitated the development of experimental protocols that exclude any significant influence of X-ray photoreduction on the XAS results [Haumann et al., 2002a; Grabolle et al., 2006], Supporting Information in [Haumann et al., 2005b].

- For the first time, structure and oxidation state of the Mn_4Ca-cluster at room temperature (functional and quasi-native conditions) has been characterized for all four semi-stable intermediate states of the S-state cycle and compared to data collected at 20 K [Haumann et al., 2005b]. Of particular importance is that we obtained evidence for Mn oxidation coupled to μ-O bridge formation and transition from five-coordinated Mn^{III} to six-coordinated Mn^{IV} in the heavily disputed $S_2 \rightarrow S_3$ transition.

- Time-resolved XAS measurements at 10 μs resolution facilitated discovery and identification of a novel reaction intermediate [Haumann et al., 2005a]. The results lead to an extension of the fundamental S-state cycle and bear important mechanistic implications.

(ii) *XAS at the Ca K-edge on the position of Ca coordination environment*

At the beginning of the year 2001 little was known about the location of calcium ion in the Mn_4Ca-cluster of PSII. Some XAS experiments on PSII samples depleted of calcium and repleted with Sr^{2+} or other ions had lead to conflicting results (see Chapter 1.4.3 and 6.6); also the then available crystallographic data [Zouni et al., 2001] was inconclusive with respect to the location of calcium. Therefore I approached to investigate the coordination environment of calcium directly by means of XAS at the Ca K-edge. It is important to realize the unusual experimental difficulties associated with this challenging experiment: (i) In typical PSII samples there are several hundred calcium ions per PSII; reduction to a level of less than 2 - 3 Ca per PSII is required for meaningful XAS experiment on the single Ca^{2+} ion associated with the Mn_4Ca-cluster. (ii) All glasses, most plastic container, skin, hair and the human breath are significantly contaminated by calcium. These contaminations lead to extreme problems in preparation of low-Ca samples and, especially, in the assessment of the Ca^{2+} content in elementary analysis by atomic absorption spectroscopy (AAS). (iii) XAS measurements at the energy of the Ca K-edge absorption are experimentally challenging due to the strong absorption of these low-energy X-rays by gases, water and window materials; due to the low penetration depth in the PSII samples and due to the high rate of X-ray radiation damage. In the framework of the present thesis the following has been achieved:

- By optimization of a procedure for removal of excessive calcium, I could reduce the Ca^{2+} content down to a level of 2 - 3 Ca per PSII, without significant activity loss. However, I found the variability in the Ca^{2+} content determined by AAS to be surprisingly high. The variability in the properties of the PSII plant material

as well as uncontrollable calcium contaminations in the course of the dilutions required for AAS are the likely source for this problematic variability [Müller et al., 2004]. (In future experiments, the use of an alternative technique for elementary analysis on undiluted PSII samples could likely solve the experimental problems in the determination of the Ca^{2+} content, but the corresponding instrument will be available in the research group of Prof. Dau not before February 2006.)

- In spite of the experimental difficulties, it became possible to collect and analyze XAS data for PSII samples in the S_1-state [Müller et al., 2005]. First attempts to characterize the S_2-state were made, however due problems to verify that the S_2-state has been reached in the majority of PSII, I consider these results to be inconclusive.

- The calcium is at a distance of ∼3.3 Å to more than one manganese ion. This result is in good agreement with recent crystallographic data [Loll et al., 2005b] and with the XAS results of the research group of K. Sauer and V. K. Yachandra in Berkeley (see Chapter 6.6, [Cinco et al., 2002]). The XANES and EXAFS spectra suggest that the calcium of the Mn_4Ca-cluster may be coordinated by only 5 or 6 light atoms (likely oxygens), which is a lower number than in solution (7 - 8 ligands); the coordination environment seems to be less symmetrically than in solution. There are no indications that chloride is a Ca ligand in PSII.