Damage by X-rays: A Case Study for Metallo-Protein Crystallography

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All complex life forms on earth rely on oxygen. Only because of constant regeneration of oxygen through photosynthetic water oxidation by green plants and cyanobacteria is this all important substance abundant in the atmosphere. Yet, it is still not known how exactly this reaction is carried out. The structure of the catalytic Mn_4Ca complex that is part of a multi-protein membrane system known as photosystem II (PS II), that performs the reaction of splitting and oxidation of water has been the subject of intense study ever since Mn was identified as an essential element. The detailed structural features of the Mn_4Ca complex during the four intermediate steps of water oxidation have been studied extensively with X-ray absorption, EPR, and FTIR spectroscopies. X-ray absorption spectroscopy studies have established that the four Mn atoms and the Ca atom in the catalytic complex are linked by di- and mono-μ-oxo bridging atoms. The salient structural features determined by these studies are that the Mn_4Ca complex contains three Mn-Mn distances at 2.7-2.8 Å that are characteristic of di-μ-oxo bridges, and one or two Mn-Mn distances at 3.3 Å and two Mn-Ca distances at 3.4 Å that are characteristic of mono-μ-oxo bridges. In addition, recent x-ray crystallography studies of PS II have added

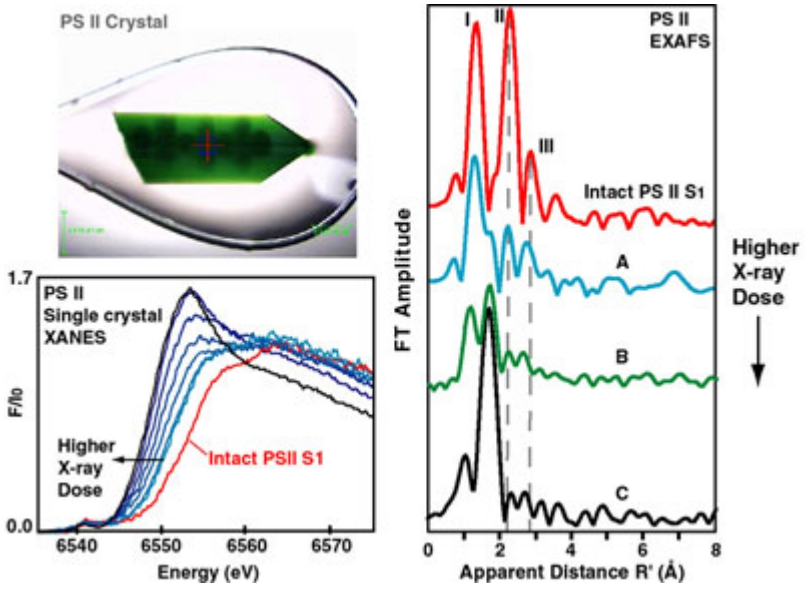

Fig. 1. Mn XANES and EXAFS of single crystals of photosystem II as a function of x-ray dose. As the x-ray dose increases, Mn in PS II normally present as $Mn_4(III_2,IV_2)$ is reduced to Mn(II) as seen by the changes in XANES spectra (left bottom). The changes in the corresponding EXAFS spectra (right) show that the three Fourier peaks characteristic of Mn-bridging-oxo, Mn-terminal, and Mn-Mn/Ca interactions (dashed vertical line) are replaced by one Fourier peak characteristic of a Mn(II) environment. A PS II crystal subsequent to x-ray exposure is also shown (left top). The bright green color is from the chlorophyll molecules in PS II and the dark spots show the areas of the crystal used for x-ray diffraction data collection.

very valuable information to our knowledge about the structure.[1-4] However, at present there are serious discrepancies among the structural models for the Mn_4Ca complex derived from these studies,[5] and inconsistencies with X-ray,[6,7] EPR[8,9] and FTIR[10-12] spectroscopic data. This disagreement is predominantly a consequence of x-ray-induced damage to the catalytic metal site as shown in this study, and also differences in the interpretation of the electron density at the presently available resolution (3.2 to 3.8 Å).

In this study, x-ray absorption spectroscopy was used at the Mn K-edge to systematically investigate x-ray induced radiation damage to the oxygen evolving Mn_4Ca site of PS II single crystals. The study, led by a group from LBNL's Melvin Calvin Laboratory, involves a collaborative effort between groups involved in x-ray spectroscopy (LBNL, Max-Planck-Institut für Bioanorganische Chemie, Mülheim, ESRF and SSRL) and x-ray crystallography of PS II (Technische Universität and Freie Universität, Berlin). The methodology for collecting single crystal XAS data from PS II was developed in collaboration with the Structural Molecular Biology group at SSRL.

The Mn XANES data from PS II single crystals show that following x-ray doses characteristic of the recently published x-ray diffraction measurements, the Mn is largely reduced to Mn(II) from $Mn_4(III_2,IV_2)$ present in the native dark-adapted PS II complex (S_1-state). Moreover, the EXAFS spectrum changes significantly, from one that is characteristic of a high-valent multinuclear oxo-bridged Mn_4Ca complex to one that is typical of mononuclear hexa-coordinated Mn(II) in solution (Fig. 1). These studies reveal that the conditions used for structure determination by x-ray crystallography cause serious damage specifically to the metal-site structure, and provide quantitative details. The results show furthermore that the damage to the active metal site occurs at a much lower x-ray dose compared to the loss of diffractive power of the crystals as established by x-ray crystallography. The damage is significantly higher at wavelengths used for anomalous

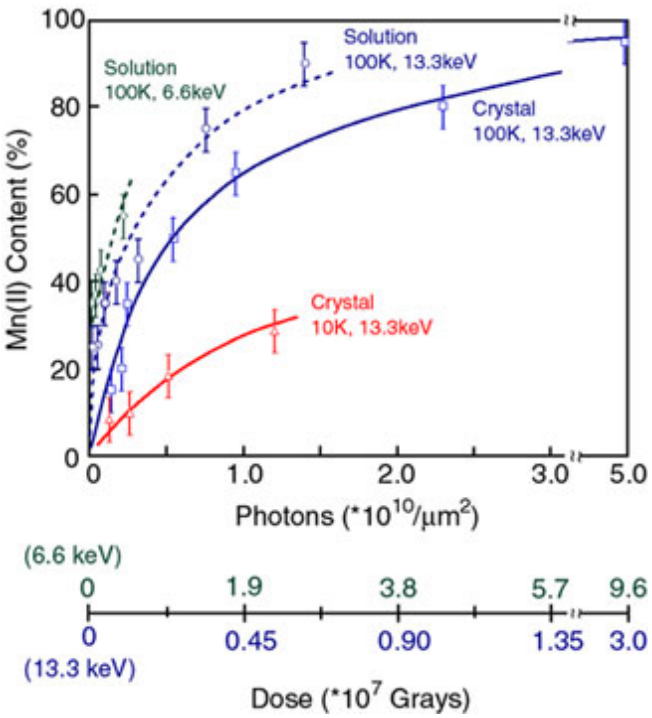

Fig. 2. Increasing Mn(II) content in PS II due to radiation damage. (Solid blue line) Mn(II) content in PS II crystals as a function of x-ray irradiation at 13.3 keV (0.933 Å) at 100 K. The conditions are similar to those during x-ray diffraction data collection. The dose on the abscissa is given in Grays and in photons/unit area, units that are commonly used for crystallography and spectroscopy experiments, respectively. At 66% of the dose ($2.3x10^{10}$ photons/μm^2) compared to the representative average dose of ($3.5x10^{10}$ photons/μm^2) used for crystallography, PS II crystals contain ~80% Mn(II). (Dashed blue line) The damage profile for PS II solution samples is very similar to that seen for crystals. (Dashed green line) The generation of Mn(II) is considerably greater when the x-ray irradiation is at 6.6 keV (1.89 Å) which is the energy at which the anomalous diffraction measurements for PS II were conducted. (Solid blue line) The Mn(II) produced by damage in crystals is considerably decreased when the irradiation is conducted at 10 K. This provides a method that could be used to mitigate the effects of radiation damage during crystallography measurements.

diffraction measurements and is much lower at liquid He temperatures (10 K) compared to 100 K were the crystallography experiments were conducted (Fig. 2).

For future x-ray crystallography work on the Mn_4Ca complex it will therefore be imperative to develop protocols that mitigate the x-ray induced damage.

More generally, these data show that in redox-active metalloproteins careful evaluations of the structural intactness of the active site(s) is required before structure-function correlations can be made on the basis of high resolution x-ray crystal structures.

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