Single Crystal X-ray Spectroscopy of the Mn_4Ca Cluster of the Photosynthetic Water-Oxidation Enzyme

Junko Yano[1,2], Jan Kern[3], Kenneth Sauer[1,2], Johannes Messinger[4],

Athina Zouni[3], and Vittal Yachandra[1]

[1]Melvin Calvin Laboratory, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]Department of Chemistry, University of California, Berkeley, CA, USA, [3]Max-Volmer-Laboratorium für Biophysicalische Chemie, Technische Universität, Berlin, Germany, and [4]Max-Planck Institut für Bioanorganische Chemie, Mülheim, Germany.

Single crystals of photosystem II (PS II) isolated from thermophilic cyanobacteria have been studied by X-ray diffraction, and analyses at resolutions between 3.2 and 3.8 Å have been published. These analyses have localized electron density associated with the water-oxidizing Mn_4Ca cluster within the large complex of PS II peptides, but the limited resolution is short of what is needed to place individual metal atoms precisely in the cluster. In addition, our X-ray absorption spectroscopy measurements show that the conditions used for structure determination by X-ray crystallography cause serious damage specifically to the metal-site structure, which changes from one that is characteristic of a high-valent (III_2,IV_2) oxo-bridged Mn_4Ca cluster, to that of Mn(II) in aqueous solution. Therefore, the reported models of the Mn_4Ca complex at atomic detail are highly speculative, and cannot be based on the diffraction data alone.

Examination of the orientation dependence of the EXAFS of single crystals of PS II can provide structural information about the Mn_4Ca site at a resolution significantly higher than that is presently available from single-crystal X-ray diffraction. We have successfully collected single crystal XANES and EXAFS data from the native S_1 state with the X-ray **E**-vector parallel to the *a*, *b*, and *c* axes of the crystal, under conditions proven to be non-damaging by carefully monitoring the Mn K-edge. These EXAFS spectra show that the Fourier peaks are clearly dichroic, demonstrating an asymmetric Mn_4Ca cluster. We have used the EXAFS dichroism to evaluate the Mn_4Ca cluster geometry based on the published X-ray diffraction structures and found that these data sets are incompatible. We will present a model for the Mn_4Ca complex that is consistent with the EXAFS dichroism of PSII single-crystals.

This case study shows that a careful evaluation of the structural intactness of the active site(s) is required before structure-function correlations of metallo-proteins can be made on the basis X-ray crystal structures.