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Review

Protein ligation of the photosynthetic oxygen-evolving center

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Contents

Abstract

Photosynthetic water oxidation is catalyzed by a unique Mn_4Ca cluster in Photosystem II. The ligation environment of the Mn_4Ca cluster optimizes the cluster's reactivity at each step in the catalytic cycle and minimizes the release of toxic, partly oxidized intermediates. However, our understanding of the cluster's ligation environment remains incomplete. Although the recent X-ray crystallographic structural models have provided great insight and are consistent with most conclusions derived from earlier site-directed mutagenesis studies, the ligation environments of the Mn_4Ca cluster in the two available structural models differ in important respects. Furthermore, while these structural models and the earlier mutagenesis studies agree on the identity of most of the Mn_4Ca cluster's amino acid ligands, they disagree on the identity of others. This review describes mutant characterizations that have been undertaken to probe the ligation environment of the Mn_4Ca cluster, some of which have been inspired by the recent X-ray crystallographic structural models. Many of these characterizations have involved Fourier transform infrared (FTIR) difference spectroscopy because of the extreme sensitivity of this form of spectroscopy to the dynamic structural changes that occur during an enzyme's catalytic cycle.

Keywords: FTIR; Mn cluster; Oxygen evolution; Site-directed mutagenesis; Infrared spectroscopy; S-state cycle

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; EXAFS, extended X-ray absorption fine structure; FTIR, Fourier transform infrared; P_{680}, chlorophyll species that serves as the light-induced electron donor in PSII; PSII, photosystem II; Q_A, primary plastoquinone electron acceptor; XANES, X-ray absorption near edge structure; Y_Z, tyrosine residue that mediates electron transfer between the Mn cluster and $P_{680}^{\bullet+}$; Y_D, second tyrosine residue in PSII that can rapidly reduce $P_{680}^{\bullet+}$.

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1. Introduction

Attempts to identify the ligands of the Mn and Ca ions in photosystem II began shortly after the discovery that the D1/D2 heterodimer contains both P_{680} and Pheo and supports light-induced charge-separation [1,2]. All conserved Asp, Glu, and His residues in the lumenal domains of the D1 and D2 subunits were targeted subsequently by site-directed mutagenesis, mostly in *Synechocystis* sp. PCC 6803, a mesophilic cyanobacterium, but also in *Chlamydomonas reinhardtii*, a green alga. Other conserved residues and regions were also targeted. Mutations are now also being constructed in *Thermosynechococcus elongatus*, the thermophilic cyanobacterium that was used in the recent X-ray crystallographic studies [3,4]. Because of initial difficulties with isolating and purifying PSII particles having functionally intact oxygen evolving complexes from many of the mutants, most mutants were originally characterized by non-invasive methods *in vivo*, primarily by measuring changes in the yield of chlorophyll *a* fluorescence produced by flash or continuous illumination given in the presence or absence of DCMU. The subsequent development of improved methods for purifying PSII particles with conventional chromatography [5] and with metal ion affinity chromatography [6–8] has facilitated the characterization of mutants with a variety of spectroscopic methods such as time-resolved optical absorption spectroscopy, various forms of pulsed EPR spectroscopy, and FTIR difference spectroscopy. Characterizations are now also employing XANES, EXAFS, and time-resolved mass spectrometry. On the basis of the mutant characterizations that had been conducted by late 2003, the residues D1-Asp170, D1-His332, D1-Glu333, D1-His337, D1-Asp342, and the carboxyl-terminus of the D1 polypeptide at D1-Ala344 had been identified as possible ligands of Mn, the residues D1-Asp59, D1-Asp61, and D1-Asp342 had been identified as possible ligands of Ca, D1-Glu189 had been identified as a likely participant in a network of hydrogen bonds that facilitates electron transfer from the Mn_4Ca cluster to $Y_Z^{\bullet}$ during the higher S state transitions, and D1-His190 had been identified as the proton acceptor for Y_Z (for review, see Refs. [9–11]). The recent ~3.5 Å [3] and ~3.0 Å [4] X-ray crystallographic structural models support many of these proposals (*e.g.*, see Fig. 1), but conflict with others, most notably the ligation of Ca and the role of D1-Glu189. The main points of agreement and disagreement between the mutagenesis studies and recent X-ray crystallographic structural models are among the points that are discussed in this review.

Before undertaking detailed descriptions of mutants and their characterizations, it is worth mentioning that the ligation environment of the Mn_4Ca cluster in the recent ~3.5 Å and ~3.0 Å structural models differ in a number of respects. For example, most of the carboxylate metal ligands are unidentate in the ~3.5 Å structural model [3], whereas most of the carboxylate metal ligands bridge two metal ions in the ~3.0 Å structural model [4] (see Fig. 1). These differences are probably caused by differences in data quality, extent of radiation damage, and approach to interpreting the electron density. Regarding radiation damage, a recent polarized EXAFS study of PSII single crystals (conducted with X-ray doses below the thresholds that cause radiation-induced reduction of the cluster's Mn(III) and Mn(IV) ions) has provided compelling evidence that the structure of the Mn_4Ca cluster in native PSII preparations differs significantly from those depicted in either X-ray crystallographic structural model [12]. This study confirms earlier XANES and EXAFS studies of PSII single crystals [13] and PSII membrane multilayers [14] that provided compelling evidence that the X-ray doses that were used to irradiate the PSII crystals in the crystallographic studies would have rapidly reduced the Mn_4Ca cluster's Mn(III) and Mn(IV) ions to their fully reduced Mn(II) states and significantly perturbed the structure of the Mn_4Ca cluster, disrupting μ-oxo bridges and altering Mn-ligand interactions [13,14]. Consequently, the ligation environment of the native Mn_4Ca cluster may differ in important respects from the ligation environments that are depicted in the ~3.5 Å and ~3.0 Å structural models.

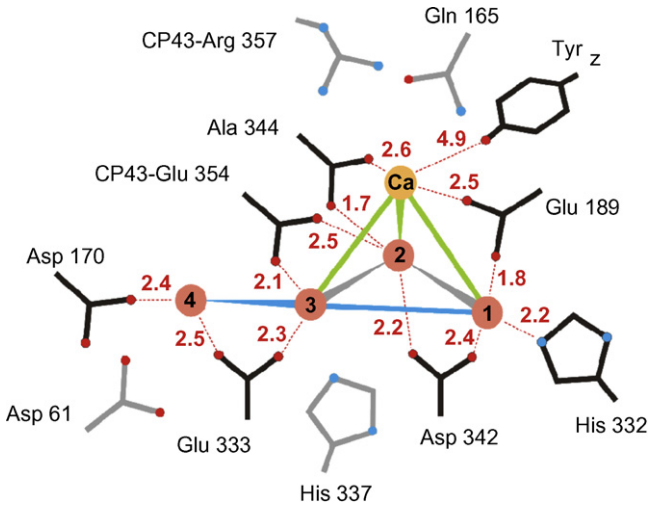

Fig. 1. Schematic view of the Mn_4Ca cluster and its protein environment as depicted in the 3.0 Å X-ray crystallographic structural model. Distances between Mn (red) and Ca (orange) ions in this model are indicated by the connecting lines (grey, 2.7 Å; blue, 3.3 Å; green, 3.4 Å). Amino acid residues in the first coordination sphere are black; those in the second sphere are grey. Distances are given in Ångstroms (reprinted with permission from Ref. [4], copyright 2005 by Macmillan Publishers Ltd., Nature Publishing Group).

Despite these ambiguities, the new structural models mark a new era in which mechanistic hypotheses can be developed and tested in the light of insights derived from structural information. In addition, the new models are serving as valuable guides for experiments designed to identify the specific Mn ion(s) that undergo oxidation during each step in the S state cycle, to identify the amino acid residues that facilitate the deprotonation and oxidation of the Mn_4Ca cluster during the individual steps of the S state cycle, and to identify the amino acid residues that participate in proton transfer pathways leading from the Mn_4Ca cluster to the thylakoid lumen. Many of these experiments involve mutant characterization with Fourier Transform Infrared (FTIR) difference spectroscopy. FTIR difference spectroscopy is an extremely sensitive tool for characterizing dynamic structural changes that occur during an enzyme's catalytic cycle, such as changes in molecular interactions, protonation states, bonding (including changes in