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Review

Structural and mechanistic investigations of photosystem II through computational methods☆

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A B S T R A C T

The advent of oxygenic photosynthesis through water oxidation by photosystem II (PSII) transformed the planet, ultimately allowing the evolution of aerobic respiration and an explosion of ecological diversity. The importance of this enzyme to life on Earth has ironically been paralleled by the elusiveness of a detailed understanding of its precise catalytic mechanism. Computational investigations have in recent years provided more and more insights into the structural and mechanistic details that underlie the workings of PSII. This review will present an overview of some of these studies, focusing on those that have aimed at elucidating the mechanism of water oxidation at the $CaMn_4$ cluster in PSII, and those exploring the features of the structure and dynamics of this enzyme that enable it to catalyse this energetically demanding reaction. This article is part of a Special Issue entitled: Photosystem II.

1. Introduction

As the enzyme that first enabled oxygenic photosynthesis to take place around 2.5 million years ago, photosystem II (PSII) has transformed life on Earth, enabling an explosion of ecological diversity that was impossible before the evolution of aerobic respiration. The ability of PSII to harness light energy to accumulate sufficient oxidation potential at the oxygen-evolving complex (OEC) to split water under mild physiological conditions is remarkable to say the least. Yet, despite the enzyme's vital and fundamental importance, structural and mechanistic information about PSII has proven to be most elusive. Attempts to obtain a high-quality X-ray crystal structure of PSII were hampered by difficulties in crystallising this large multi-subunit transmembrane protein complex, as well as the susceptibility of the crucial $CaMn_4$ metal cluster of the OEC to radiation damage upon exposure to X-ray radiation during measurements [1]. Only in recent years have fully refined medium-resolution (2.9–3.5 Å) crystal structures become available, obtained from the thermophilic cyanobacterium *Thermosynchoccocus elongatus*[2–4], and the announcement of a 1.9 Å-resolution structure obtained from *Thermosynechococcus vulcanus* showing the enzyme in atomic detail was greeted with a standing ovation at the 2010 15th International Photosynthesis Congress in Beijing. This structure has very recently been published (see Note added in proof). Nevertheless, despite a lack of crystallographic data, a myriad of biochemical and spectroscopic methods have been used to try to unlock the mysteries surrounding the mechanism by which PSII is able to oxidise water (see reviews in [5,6]).

In addition to these experimental investigations, computational studies have also sought to increase our understanding of the workings of PSII. The earliest structural studies were based on the sequence homologies that were found between the core D1 and D2 subunits of PSII, and the L and M subunits of the bacterial reaction centre from the purple bacteria *Rhodopseudomonas viridis* and *Rhodobacter sphaerodies*, for which crystal structures were available [7–12]. Apart from deducing the secondary and tertiary structures of the D1 and D2 subunits, these studies predicted with remarkable accuracy, given the available information, the positions and orientations of the redox-active amino acid Y_Z, Y_D (the origin of the so-called long-lived tyrosine signal Signal II_{slow}), the binding sites of the various co-factors, and the $CaMn_4$ cluster. This was achieved by a combination of homology structure building against the bacterial reaction centres, both with and without "knowledge-based" searching of other known structures of similar sequences, references to and simulations of a range of literature spectroscopic data, as well as energy minimisation calculations. Although not always correct in every aspect, many of these predictions have been borne out by subsequent experiments, and these studies provided substantial insights into the structure and mechanism of PSII at a time when structure information was still limited.

Abbreviations: Chl, chlorophyll; DFT, density functional theory; EPR, electron paramagnetic resonance; ESEEM, electron spin echo envelope modulation; ESP, electrostatic potential; EXAFS, extended X-ray absorption fine structure; FT-IR, Fourier transform infra-red; HF, Hartree–Fock; OEC, oxygen-evolving complex; MD, molecular dynamics; MEP, minimum energy path; MM, molecular mechanics; P_{680}, the primary donor in PSII; PSII, photosystem II; Q_A and Q_B, the primary and secondary quinone acceptors in PSII, respectively; QM, quantum mechanics; Y_D, tyrosine 161 of the PSII D2 polypeptide; Y_Z, tyrosine 161 of the PSII D1 polypeptide

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Fast forward 20 years, and the availability of crystallographic data as well as the enormous improvements in computing power have greatly expanded the possibilities to study the structure and function of PSII in greater detail with the help of simulations and calculations. This short review will focus mainly on recent computational investigations of two aspects associated with water oxidation in PSII, namely, the detailed molecular mechanism of the S-cycle of the $CaMn_4$ cluster, and the identification and assignment of channels in PSII for the transport of substrates and products to and from the cluster. This will be followed by a brief overview of a few other computational studies related to the structural characteristics and dynamics of PSII.

2. Mechanism of water oxidation at the OEC

Of central importance to the study of PSII is the mechanism of water oxidation at the OEC. While it is known that for each water oxidation event the $CaMn_4$ cluster undergoes transitions through five intermediates (S_0 to S_4) in the S-cycle, the precise mechanistic details such as the sites of oxidation, deprotonation and water binding, changes in the structure of the cluster during the S-cycle, and crucially the mechanism of the O—O bond formation step are still not fully understood. Experimental efforts using techniques such as EPR, FT-IR and EXAFS spectroscopies continue to provide more and more insight into these details, but much remains unknown and under debate (see [13] for a review).

A major challenge for all such mechanistic studies of water oxidation, both experimental and computational, has been the lack of definitive structures of the OEC during each step of the S-cycle. Until very recently, the PSII X-ray crystal structures available did not have high enough resolution to give detailed assignments of the position of the individual atoms in the cluster [2–4], and as mentioned above, it seems likely that the structures obtained have been affected by radiation damage during measurements [1]. While recent high-quality EXAFS studies (both isotropic [14,15] and polarised EXAFS [16]) have significantly narrowed down the number of possible structural candidates, uncertainty remains. The new crystal structure at 1.9 Å resolution (PDB ID: 3ARC) provides an atomic resolution view of the cluster in the S_1-state. Nevertheless, questions remain for the remaining S states, and there is indication that some degree of Mn-reduction may have taken place even in this structure (though to a very substantially lower extent than for the previous structures). Furthermore, the electronic structure of the cluster during the S-cycle is also under intense discussion between the Berlin [13] and Berkeley [17] EXAFS groups. As yet it is not clear which of the Mn ions are oxidised during S-state transitions, whether Mn-centred oxidation takes place in all steps, or what the nature of the structural rearrangement observed in the $S_2 \rightarrow S_3$ transition is.

A number of computational studies have in recent years tried to solve this mechanistic puzzle. Density functional theory (DFT) has been the quantum mechanical (QM) method of choice for calculating the energetic, geometric and magnetic properties of the $CaMn_4$ cluster and its ligands. DFT is a quantum mechanical method where the ground-state electronic energy and other properties of a system are taken to correspond uniquely to the system's ground-state electron density distribution, i.e. the energy of the system is a functional of $\rho(\mathbf{r})$, the electron density at a given point $\mathbf{r}$ in space. As such, it is not necessary to treat each electron individually. This increases the size of the systems that are amenable to calculations compared to Hartree–Fock (HF) methods. Additionally, DFT takes into account electron correlation effects between electrons which the HF method does not. However, the accuracy of the DFT calculations is sensitive to the form that the functional used for the energy calculations takes, and the formulation of a satisfactory expression for exchange-correlation energy is a very challenging aspect of functional development. (For more detailed overviews of DFT and functionals see [18,19].)

Two different computational approaches have been used to study the water oxidation mechanism. Although they share the common feature of treating the OEC using DFT, different methods have been used to account for the remainder of the PSII complex. More importantly, conceptually different methodologies and criteria have been used to arrive at their respective proposals for the water oxidation mechanism. These are reviewed in turn below. For each approach, an overview of the methodology and protocol used for the computation is first presented (Sections 2.1.1 and 2.2.1), followed by summaries of the results obtained using each approach for elucidating the S-state cycle (Sections 2.1.2 and 2.2.2). Readers more interested in the computational results from these different approaches may wish to proceed directly to Sections 2.1.2 and 2.2.2. An evaluation of the methods used and their implications for the results obtained are presented in Section 2.3.

2.1. Cluster model approach with DFT

2.1.1. Methodology—cluster model DFT

In a long line of studies spanning over a decade [20–32], Siegbahn and co-workers have performed DFT calculations using a cluster model approach. In this method, a cluster consisting of the $CaMn_4$ cluster and a number of atoms from the surrounding ligands are explicitly considered, with the polarisation of the remaining protein medium being approximated by the inclusion of a constant dielectric. Starting from a total of around 30–40 atoms at a time when structural information about the cluster and the surrounding was very limited [20,21], the most recent calculations have expanded to cluster models of around 200 atoms that include the $CaMn_4$ cluster, the full ligand sphere, a second-shell ligand (CP43-R357) some water molecules and a chloride ion (Fig. 1A) [26,30,32]. The positions of the ligand backbone atoms are taken from the London crystal structure (PDB ID: 1S5L, 3.5 Å [2]), and the ligand motifs for the sidechains are akin to those found in the Berlin crystal structure (PDB ID: 2AXT, 3.0 Å [3]).

A key feature of the work by Siegbahn and co-workers is the focus on the energetics of all structural intermediates and mechanistic pathways that have been proposed for the S-cycle. Two central criteria for discriminating between the vast array of possible structures and mechanisms can be discerned from these studies.

The first criterion is that, whatever the intermediate structures in the cycle may be, they must lead to a low barrier O—O formation step with an energy barrier that fits with the millisecond kinetics for O_2 formation (13–14 kcal/mol as estimated from transition state theory). This was the focus of the earlier studies, and extensive studies were made to identify the most favourable mechanism for O—O bond formation, as well as the geometries within the cluster in the S_4 state that would be required to allow this to take place [20,21,24,25]. It was found that nucleophilic attack on an oxygen radical bound to a Mn^{IV} ion in the cluster gave the most energetically favourable mechanism. Initial work had suggested an attack on a Mn^{IV}–$O^{\bullet}$ radical by an external substrate molecule [20]. However, exhaustive studies of thousands of possible structures of the S_4 state and several different mechanisms [24,25] subsequently showed that in fact an attack by a μ-oxo bridge oxygen on such a radical oxygen species led to a much more reasonable energy barrier.

The studies of different spin configurations on the O and Mn atoms involved also revealed an additional spin alignment requirement for a low barrier O—O formation mechanism (Fig. 1B). In order to form the O—O bond, the oxygen atoms involved must have opposite spins. For the lowest barrier mechanism, the Mn atoms must also have opposite spins to their respective bound oxygen atoms. This allows easy Mn^{IV} to Mn^{III} reduction and O—O bond formation without crossing over to another spin surface [26,30,32].

The second principal criterion in determining the structures of the proposed intermediates in the S-cycle is that they should each be the lowest energy structure for the given electronic and protonation state of the S-state in question. As the O—O formation step from the S_4 structure was regarded by Siegbahn and co-workers as the most extensively and reliably studied step, the strategy for reaching the other S-state