

Photosystem II: The Reaction Center of Oxygenic Photosynthesis*

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*This article is dedicated to the memory of
Professor Gernot Renger, pioneer in photosystem
II research.

Keywords

oxygen evolution, photoassembly, photoprotection, proton-coupled electron transport, period-four oscillations, water oxidation kinetics, water oxidation thermodynamics

Abstract

Photosystem II (PSII) uses light energy to split water into chemical products that power the planet. The stripped protons contribute to a membrane electrochemical potential before combining with the stripped electrons to make chemical bonds and releasing O₂ for powering respiratory metabolisms. In this review, we provide an overview of the kinetics and thermodynamics of water oxidation that highlights the conserved performance of PSII across species. We discuss recent advances in our understanding of the site of water oxidation based upon the improved (1.9-Å resolution) atomic structure of the Mn₄CaO₅ water-oxidizing complex (WOC) within cyanobacterial PSII. We combine these insights with recent knowledge gained from studies of the biogenesis and assembly of the WOC (called photoassembly) to arrive at a proposed chemical mechanism for water oxidation.

Contents

INTRODUCTION	578
STRUCTURE	580
Cellular Localization and Composition	580
The Reaction Center	581
The Water-Oxidizing Complex	581
PHOTOASSEMBLY OF THE PSII WATER-OXIDIZING COMPLEX	583
A PROPOSED MECHANISM FOR WATER OXIDATION	587
Flash #1: S ₁ → S ₂	587
Flash #2: S ₂ → S ₃	588
Flash #3: S ₃ → S ₄ → S ₀ + O ₂	588
Flash #4: S ₀ → S ₁	589
Additional Considerations	589
DONOR-SIDE KINETICS	589
ACCEPTOR-SIDE KINETICS	591
SECONDARY ELECTRON TRANSPORT WITHIN PSII	591
Tyrosine D	592
Cyclic Electron Transport Around PSII: Cyt b ₅₅₉ , Chl _Z , and Car	592
Charge Recombination	592
LIGHT TO CHEMICAL CONVERSION EFFICIENCY IN PSII	593
PSII Reaction Center Efficiency	593
PSII–Water-Oxidizing Complex Cycling Efficiency	593

WOC:

water-oxidizing complex

PSII: photosystem II

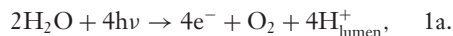
Thylakoid membrane:

lipid bilayer inside cyanobacteria and chloroplasts containing photosynthetic complexes (and respiratory complexes in cyanobacteria)

INTRODUCTION

Natural photosynthesis dominates the biosphere as the most widespread and successful metabolism on Earth (1). Among photosynthetic organisms, the oxygenic phototrophs are the most prolific, comprising all known species of cyanobacteria, algae, and higher plants. The early ancestors of these organisms transformed the surface of Earth beginning circa 3 billion years ago from a drab aluminosilicate composite to a lush green carpet visible from outer space. This transformation was powered by

two highly successful metabolic innovations (2). First, the innovation of reaction center photochemistry and the water-oxidizing complex (WOC) in photosystem II (PSII) produced both redox energy, carried as O₂ and hydrogen in plastoquinol (PQH₂) (Equation 1*a,b*), and the proton motive force (*pmf* ~ Δ*pH*) across the thylakoid membrane. Later, the second innovation, respiration, combined the oxidation of hydrogen carriers with O₂ reduction, *pmf* formation, and ultimately energy storage in phosphate ester bonds:



Remarkably, despite their enormous phylogenetic and ecological diversity, all contemporary oxygenic phototrophs characterized to date use an identical PSII inorganic core and conserved subunits for solar energy conversion. Stated differently, three billion years of evolution under the far-ranging biogeochemical forces since the accretion of Earth have produced only a single blueprint for catalyzing water-splitting chemistry. The evolution of a single enzymatic solution despite access to every ecological niche and the vast time period is exceedingly rare in biology! To learn from this lesson, we must understand the physicochemical principles by which PSII operates (3, 4). This is a main goal of this review. These principles can be applied to designing bioinspired water oxidation catalysts and bioengineering reaction centers (5, 6). The factors determining PSII solar energy conversion efficiency are described below (see PSII Reaction Center Efficiency). We begin here with a general introduction.

The net reaction of PSII (Equation 1) is enabled by chlorophyll (Chl), which both captures light and converts it into chemical energy. Five chemically distinct Chls have been identified in light-harvesting complexes of oxygenic phototrophs: Chls *a*, *b*, *c*, *d*, and *f* (7–9). Most PSII reaction centers utilize Chl *a* [$\lambda_{\text{max}} = 665 \text{ nm}$ in methanol (10)] for primary charge separation. However, the cyanobacterium *Acaryochloris marina* utilizes Chl *d*

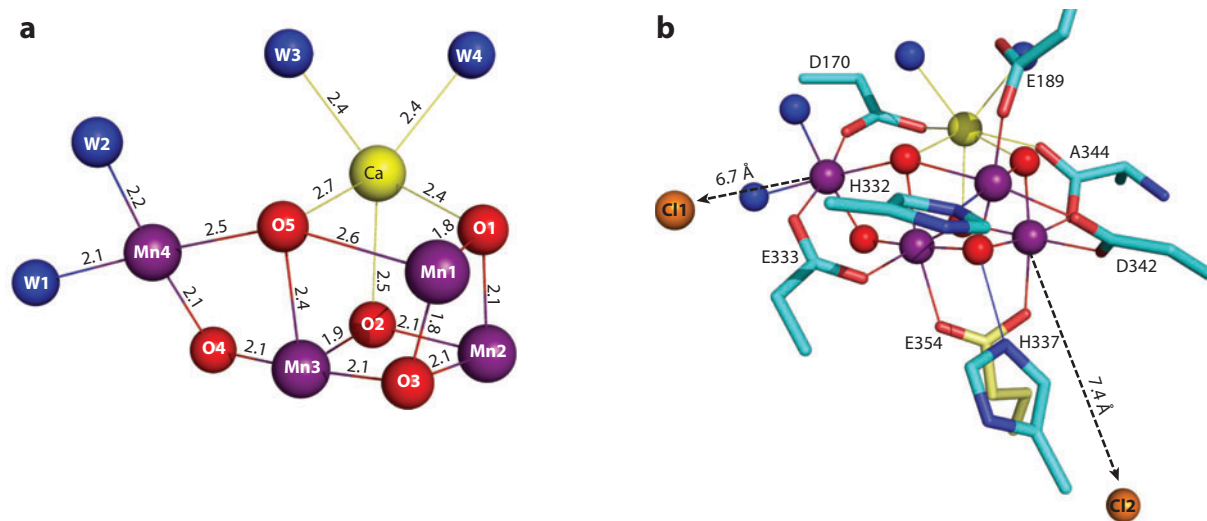


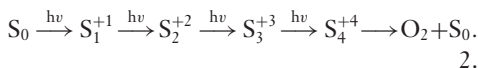
Figure 1

Structure of the photosystem II water-oxidizing complex (WOC) as revealed by the 1.9-Å resolution X-ray diffraction structure by Umena et al. (17). (a) The Mn₄CaO₅(H₂O)₄ core with bond distances in angstroms. (b) First coordination sphere ligand environment of the WOC core. Amino acid residues from the D1 subunit are shown in cyan, and those from the CP43 subunit are in yellow. The relative positions of the two chloride ions are also shown. Images generated in the PyMOL program using Protein Data Bank entry 3ARC (17).

[$\lambda_{\max} = 697$ nm in methanol (11)] to split water. Chl *f* was recently found within a filamentous cyanobacterium derived from stromatolites and has the longest absorbance wavelength of all Chls at $\lambda_{\max} = 707$ nm (in methanol) (11). Whether it too can replace Chl *a* in the PSII reaction center remains to be shown.

Turning to the PSII-WOC, the elemental composition, electronic coupling, and inter-manganese distances within the Mn₄CaO_x core have been established by reconstitution (12) and spectroscopic studies (reviewed in Reference 13). X-ray diffraction (XRD) studies of PSII core protein complexes have revealed the atomic coordinates of the metal-oxo cluster in its dark-stable state, Mn₄CaO₅(H₂O)₄ (**Figure 1**) (14–17). If cells are dark adapted and then subjected to a series of single-turnover flashes (STFs), the greatest yield of oxygen is released on the third flash and every fourth flash thereafter in a damped period-four pattern (**Figure 2b**) (18). All modern proposals concerning the mechanism of water oxidation adopt the time-tested work of Kok and

coworkers (19, 20), who interpreted the data of Joliot et al. (18) by proposing that oxygen is released upon the linear accumulation of four charges through a cycle of four intermediates or S states (Equation 2; **Figure 2a**). Fourier transform (FT) analysis of the oscillations (**Figure 2c**) provides the frequency components that contribute to damping the oscillations. A single frequency is typically obtained and provides a model-independent measure of the turnover probability through the catalytic cycle (the cycle period is also used and has a maximum theoretical value of four flashes):



Several other spectroscopic markers also exhibit period-four oscillations, including the Chl *a* variable fluorescence emission from PSII (F_v , which is discussed in detail in Light to Chemical Conversion Efficiency in PSII, below) (21). When O₂ yield and F_v are monitored simultaneously, overlap of the FTs clearly shows that

Chl: chlorophyll

STF: single-turnover flash

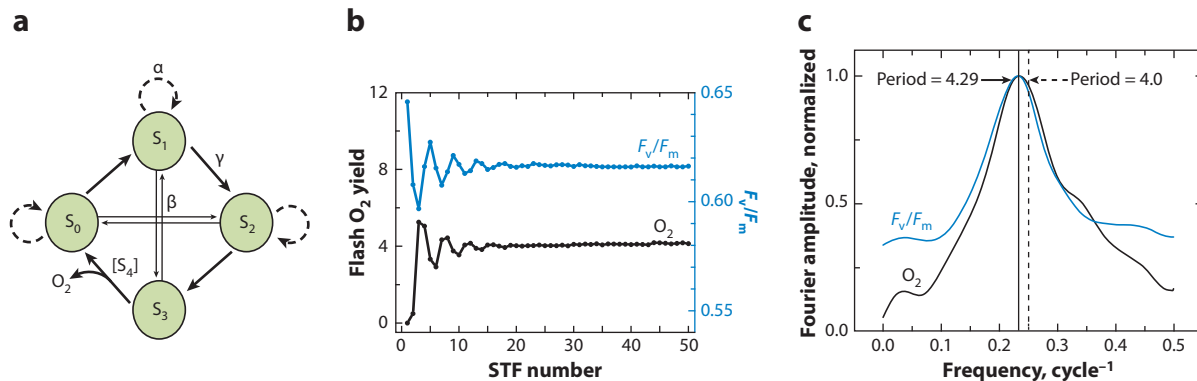


Figure 2

(a) The classic Kok model. Starting from the dark-stable S_1 state, a reaction center progresses to S_2 with probability γ (hit) following a single-turnover flash (STF). O_2 is released following the third flash through the S_4 intermediate, and the cycle resets to S_0 . Damping of oscillations is accounted for by the inefficiency parameters [miss (α) and double hit (β)]. (b) Simultaneously measured oscillations in flash O_2 yield (black) and variable Chl-*a* fluorescence yield (F_v/F_m , blue) in *Arthrospira maxima* whole cells and (c) corresponding Fourier transforms. Based on raw data from Reference 21, in which 50 STFs were applied at 0.5 Hz following 5-min dark adaptation; the signal was averaged 40 times.

both methods are affected by the same physical process (Figure 2). Below (see Light to Chemical Conversion Efficiency in PSII), we discuss how this method has expanded our toolbox to allow measurement of the WOC turnover rate in vivo in many different oxygenic phototrophs.

STRUCTURE

Cellular Localization and Composition

PSII exists in vivo in the thylakoid membrane as a dimer, with each monomer containing 19–31 polypeptide subunits (length is species dependent and reviewed in References 22–24). Thylakoid membranes in the chloroplasts of higher plants are arranged in structured stacks (grana) and single sheets (lamella). Assembled PSII in plants is segregated from photosystem I (PSI), as it is preferentially localized in the grana as revealed by biochemistry (25) and electron microscopy studies (26, 27). The chloroplasts of algae typically do not have the elegantly defined grana stacks of higher plants, and less is known about the exact localization of PSII, although isolated appressed thylakoids are enriched in PSII relative to bulk thylakoids (28). The content of PSII in thylakoids relative to PSI and

antenna complexes varies with organism, light intensity, and spectral wavelength in a manner reflecting the need for reductant versus the need for *pmf* and ATP (29).

In cyanobacteria, PSII is found throughout the thylakoid membrane system within the singular cellular compartment. Only in the primitive cyanobacterium *Gloeobacter violaceus* is PSII localized to the cytoplasmic membrane owing to an unusual absence of thylakoid membranes (30).

At least three extrinsic (soluble) subunits are associated with the luminal side of PSII in green algae and higher plants: PsbO (33 kDa, also known as the Mn-stabilizing protein), PsbP (23 kDa), and PspQ (16 kDa). These subunits protect the inorganic core of the WOC and may play a role in proton evolution (for recent reviews, see References 31, 32). Interestingly, the extrinsic (luminal) subunits of cyanobacterial PSII differ from those found in green algae and higher plants. PsbO is conserved, but PsbP and PsbQ are replaced with homologs CyanoP and CyanoQ. In addition, cyanobacterial PSII contains the soluble subunits PsbU (12 kDa) and PsbV (cyt c_{550}), which are not found in green algae and higher plants (31, 32). Although the binding of cyt c_{550} enhances PSII activity by

promoting the binding of PsbO, PsbU, Ca²⁺, and Cl⁻ (33–38), a precise role for the redox-active heme group is unclear (reviewed in Reference 39). The red algae (Rhodophyta) and brown algae (Bacillariophyta or diatoms) also contain PsbU and PsbV in addition to PsbO, PsbP, and a homolog of PsbQ, PsbQ' (31).

The Reaction Center

Whereas the low-molecular-weight and extrinsic subunits of PSII vary among diverse oxygenic phototrophs, the reaction center (RC) core is remarkably conserved. The RC core is defined as the minimal set of subunits required to oxidize water and has been experimentally determined to include D1, D2, CP43, CP47, and cyt b₅₅₉ (α - and β -subunits). The only significant variation observed to date in core proteins is in the D1 subunit of cyanobacteria. At least four classes of D1 isoforms have been identified in cyanobacteria (40). The expression of these protein isoforms is controlled by differential transcriptional regulation of multiple *psbA* gene copies within a single genome (40, 41). Subtle differences in the amino acid sequences of these D1 isoforms control the efficiency of PSII specifically at very high and very low light intensities (42–45) or under low-oxygen conditions (46, 47). By contrast, green algae and plants contain only a single D1 isoform (40) and consequently utilize a different regulation system for dealing with varying light intensities.

The Water-Oxidizing Complex

The structure of the inorganic cluster where water is oxidized has been the subject of significant study for decades, and extensive knowledge has accumulated from spectroscopic and other sources. In 2011, the 1.9-Å XRD structure of PSII from *Thermosynechococcus vulcanus* by Umena et al. (17) provided a significantly higher-resolution map at the atomic level of the PSII-WOC in its dark-stable S₁ state (Figure 1). This structure was built upon earlier XRD reports of a closely related

cyanobacterial PSII-WOC that first established many of the structural insights (14–16).

The WOC core is composed of an irregular cubical cluster or heterocubane, CaMn₃O₄ [as established by Ferreira et al. (15)], bridged by corner Mn³⁺ to Mn⁴⁺ (exocuboidal) via chemically distinct μ -oxos (μ_2 -O4 and μ_4 -O5; see Figure 1a for numbering) differing in their coordination and bonding types. At first glance, the ligand coordination numbers are normal, six for each Mn atom and seven for Ca (Figure 1b). However, Mn1, Mn3, Mn4, and Ca have exceptionally long distances to corner μ_4 -O5 (2.4–2.7 Å), which are longer than typical covalent bond lengths for these atom types and therefore form considerably weaker bonds. The consequences of these weak interactions and this unusual geometry are discussed in the following sections.

Oxygen atoms O1, O2, and O3 are the remaining corner oxos within the Mn₃CaO₄ heterocubane, and each is bonded to three metals. Each of these oxos is more weakly bonded (hydrogen bonded) to a fourth ligand (W923, CP43-R357, and D1-H337, respectively) (17), yielding four-coordinate oxos (μ_4 -oxos) in distorted tetrahedral geometries. The strength and position of the latter hydrogen bonds play an important role in the Mn₃CaO₄ electronic structure and in our proposed mechanism. Ideal tetrahedral geometry imposes sp³ hybridization of the valence oxygen orbitals, essentially eliminating π bonding to Mn and yielding longer and weaker Mn–O bonds. Such bonding is less directional and thus more flexible than, for example, Mn₃(μ_3 -O) in planar geometry, which has stronger sp² + p_z (multiple) bonding and is more inflexible (6). As a result of this bonding dichotomy, two geometrical limits exist for Mn₃O_x core types among inorganic complexes: an incomplete cubane-Mn₃(μ_3 -O)₄ and planar-Mn₃(μ_3 -O)(OH)₃. Prevention of planarization is essential for high water oxidation activity in Mn₄O₄ model complexes. In the WOC, Ca²⁺ helps prevent planarization of Mn₃O₄ by binding to three corner oxos, completing and stabilizing the heterocubane topology (see Photoassembly of PSII Water-Oxidizing

Complex). However, owing to its filled valence electronic configuration, Ca^{2+} has no directional bonding preference at all (ionic bonding). Hence, the oxos coordinated to Ca^{2+} are much less positionally constrained, contrary to the case of a Mn_4O_4 cubane (6). This added flexibility of the Mn_3CaO_4 heterocubane is believed to be a major reason for the WOC's high catalytic activity, as it facilitates the proposed rate-limiting O-O bond formation step between oxos (48). It may also help rationalize the unusual WOC structure observable in the Umena et al. (17) XRD structure, which some characterize as distorted or damaged by X-rays.

Oxygen atom O4 covalently bridges the heterocubane subcluster to the exocuboidal Mn4. This Mn has historically been denoted the dangler (49) or gateway (50) Mn, in reference to its magnetic or redox properties, respectively. Mn4 plays a crucial role in the photoassembly of the WOC (see Photoassembly of PSII Water-Oxidizing Complex, below). O4 is bound to two Mn atoms (Mn3 and Mn4) and strongly hydrogen bonded to a water molecule (W539, 2.55 Å) positioned in the same plane at a trigonal planar site (μ_3 -oxo). This geometry and bond length show that O4 is sp^2 hybridized and hence participates in strong covalent π bonding between Mn3 and Mn4. O4 is surrounded by a constellation of five hydrogen bonds within 3.3 Å (three Asp and CP43-R357), suggestive of a proton transfer network. This bonding arrangement is conducive to tautomerization between O4 and the water molecule W2 located *trans* to O4 (see Donor-Side Kinetics, below), as found in *trans* oxo/hydroxo Mn complexes (51, 52). This tautomerization equilibrates these two forms, $\text{O-Mn4-W2} \rightleftharpoons \text{HO-Mn4-OH(W2)}$ and implies resonance stabilization of W2 resulting from stronger (π) bonding. This interpretation is supported by its short Mn4-O bond length (2.2 Å) and exceptionally low positional disorder (threefold-smaller volume than all other oxos!) (17).

As previously stated, the bonding environment around O5 is most unusual. O5 has 4 + 0 ligands (no ordered H-bonds) and is denoted

μ_4 -oxo. Its geometry is an incomplete octahedron missing two coordination sites *trans* to vectors Mn3-O5 and Ca-O5. This bonding environment indicates that the O5 orbitals are unhybridized and comprise valence $s + 3p$ atomic orbitals directed at the metals and at two open (uncompensated) sites on the octahedron. This geometry is compatible with the possibility that O5 may be a hydroxide ion (protons are not detected in XRD) but is not likely a water molecule. Additionally, the absence of structurally ordered hydrogen-bonding partners to O5 disfavors hydroxide or water at O5. The space *trans* to Mn3-O5 lacks positioned atoms until a methyl group 3.68 Å away (D1-V185) and is presumably filled with disordered water molecules. Similarly, the space *trans* to Ca-O5 lacks positioned atoms until a Cl^- (or HCl) 6.89 Å away. These features all point to an electron-deficient (nearly neutral) O5 atom or hydroxide with atomic $s + p$ orbitals occupied with bonding and antibonding electrons from neighboring metals, resulting in very long bond lengths and an unusual incomplete octahedral geometry. The flexibility of the heterocubane core would facilitate attaining this structure.

The Mn-Mn and Mn-Ca distances revealed by the Umena et al. (17) structure are clearly at odds with previously reported distances from extended X-ray absorption fine structure (EXAFS) measurements by Yano, Yachandra, and coworkers (53, 54). However, the geometry is strikingly similar to a model based on X-ray absorption spectroscopy (XAS) and XRD data by Dau and coworkers (55) and to a separate model based on density functional theory (DFT) calculations by Siegbahn (56, 57). The inherent flexibility of the heterocubane core geometry and intermanganese redox tautomerism have been proposed as a possible reconciliation (58, 59).

Umena and coworkers (17) took great care to minimize the X-ray dose per crystal unit volume. However, some photoreduction of $\text{Mn}^{3+/4+}$ to Mn^{2+} is expected from exposure to 13.3 keV (0.9 Å) radiation. Yano and coworkers (60) showed that a PSII crystal with 61% H_2O content contains, on average, approximately

20% Mn^{2+} during exposure to 0.28×10^{10} photons μm^{-2} (at 100 K, 13.3 keV, the maximal dosage reported by Umena et al.). These values are based on XANES (X-ray absorption near edge structure) calibration standards. The final Mn^{2+} content in the Umena et al. structure is likely less given that their crystals were dehydrated to 57% H_2O (17). Dau and coworkers (61) found similar results suggesting photoreduction in XRD methods. Previous XRD structures were exposed to much higher X-ray doses. For example, the 3.5-Å resolution structure by Ferreira et al. (15, 60) was exposed to 1.75×10^{10} to 3.5×10^{10} photons μm^{-2} (at 100 K, 13.3 keV). At this dosage, approximately 50–70% of $\text{Mn}^{3+/4+}$ was reduced to Mn^{2+} , on average (60). Paradoxically, the overall WOC geometry (CaMn_3O_4 heterocubane plus an exocuboidal Mn4) is quite consistent between the 3.5-Å Ferreira et al. structure and the 1.9-Å Umena et al. structure despite the 6–12-fold difference in X-ray exposure and 2.5–3.5-fold difference in predicted Mn^{2+} content. To us, this suggests that although photoreduction may be occurring, photodamage is not significantly distorting WOC geometry.

By comparing the Mn-Mn distances in the 1.9-Å XRD structure with EXAFS spectra (53, 62, 63), several groups have calculated that the crystal structure reflects a reduced S state lower than S_1 (64–67). This conclusion is based on the assumption that the average oxidation state of Mn is +3.5 in S_1 . However, if a lower oxidation state assignment is used (Mn^{3+} average in S_1), DFT calculations suggest that the Umena et al. WOC geometry can be readily explained without photoreduction arguments (see sidebar, Mn Oxidation States in PSII: An Ongoing Debate) (58). The foregoing structural analysis is developed in A Proposed Mechanism for Water Oxidation, below.

PHOTOASSEMBLY OF THE PSII WATER-OXIDIZING COMPLEX

The Mn_4CaO_5 WOC core is not chemically stable as a free cofactor and must be assembled within the PSII-apo-WOC complex using

energy derived from PSII charge separation. During biogenesis, the native inorganic cofactors Mn^{2+} , Ca^{2+} , and Cl^- bind to the apo-WOC domain of the newly translated and modified PSII protein scaffold and, through a sequence of light and dark steps, assemble the O_2 -evolving complex (68, 69). Bicarbonate serves as a native cofactor in plant PSII and accelerates the kinetics of this slow process (70), exerting an influence on water oxidation kinetics through the arginine residue CP43-R357 in the holoenzyme (71). This maturation process, historically known as photoactivation (12, 68), occurs naturally during de novo biogenesis of PSII and repair of damaged PSII centers. Photoinactivation of PSII is a natural process that occurs across all phylogenetic species of oxygenic phototrophs during O_2 evolution at high light flux. It involves irreversible damage to the D1 subunit, which binds the WOC (40). Photoinactivation would limit photosynthetic productivity without the repair process, in which the PSII complex is recycled by removal of the damaged D1 and replacement with a new copy, followed by photoassembly of a new WOC (reviewed in Reference 12).

The pioneering work of Cheniae and coworkers (69, 72–74) showed that photoassembly is a multiquantum process, and the basic kinetic scheme derived from these original studies on spinach PSII membranes remains intact, although the molecular details have been significantly extended (Figure 3) (12, 75–78). Beginning from a dark-adapted PSII-apo-WOC precursor plus inorganic cofactors (IM_0), photoassembly starts by photooxidation of one Mn^{2+} to form intermediate IM_1 , which then undergoes an essential dark-rearrangement step to form IM_1^* before further productive photooxidation can continue (Figure 3). Ca^{2+} affinity increases during this dark step, and Ca^{2+} addition specifically accelerates this step (79–81). Electron paramagnetic resonance (EPR) spectroscopy has revealed that this interaction involves binding to form a shared bridging (hydr)oxo ligand between Mn^{3+} and Ca^{2+} (82). The formation of IM_1 has a very low quantum yield ($\sim 0.25\%$).

S_i: Kok-cycle intermediate ($0 \leq i \leq 4$)

Electron paramagnetic resonance (EPR): spectroscopic technique used to study changes of the spin states of unpaired electrons

Mn OXIDATION STATES IN PSII: AN ONGOING DEBATE

Based upon the multiline EPR signals of the S_2 and S_0 Kok-cycle intermediates, all four Mn ions of the WOC are electronically coupled to produce a single set of cluster electronic states with spin multiplicity of $S = 1/2$ (160, 161). Additionally, EPR, ^{55}Mn -ENDOR (electron-nuclear double resonance), and XAS spectroscopic analyses agree that S_2 contains a combination of Mn^{3+} and Mn^{4+} (49, 162–165). Therefore, the catalytic cycle of water oxidation begins with S_0 as either $\text{Mn}^{2+}(\text{Mn}^{3+})_3$ [low oxidation state (LOS) assignment] or $(\text{Mn}^{3+})_3\text{Mn}^{4+}$ [or equivalently $\text{Mn}^{2+}\text{Mn}^{3+}(\text{Mn}^{4+})_2$] [high oxidation state (HOS) assignment].

The HOS paradigm is preferred by many in the field based on its interpretation of the PSII Mn K-edge (63, 166–170), Mn $K\beta$ (59, 169, 171), and RIXS (K pre-edge) (54, 172) spectroscopic features when compared with model compounds. For a detailed analysis of these studies, see the recent review by Pace and coworkers (173). ^{55}Mn -ENDOR data suggest that Mn^{2+} is not present in S_0 and therefore support the HOS model (174, 175). This conclusion is based on the relatively narrow S_0 spectral envelope (attributable mainly to ^{55}Mn magnetic hyperfine terms) compared with octahedral Mn^{2+} complexes used as references (invariably large magnetic hyperfine). However, the ligand field symmetry around Mn4 is not octahedral according to the XRD structure of S_1 (**Figure 1**). Rather, it is five-coordinate (the $\text{Mn}_4\text{-O}_5$ distance, 2.5 Å, is too long for appreciable bonding character) and lacks inversion symmetry, an essential requirement to observe large scalar ^{55}Mn hyperfine interactions (176). Therefore, we propose that the Mn^{2+} model complexes used to calibrate this assignment may not accurately reflect the ligand field or the ^{55}Mn hyperfine interactions found at the Mn4. This suggestion needs more evidence going forward.

Two methods have been used to count the number of charge equivalents in the PSII-WOC. Yocum and coworkers (177) titrated intact PSII-WOC samples with excess chemical reductants and observed the loss of O_2 evolution activity and the release of Mn^{2+} . Their data suggest S_1 is present as $(\text{Mn}^{3+})_2(\text{Mn}^{4+})_2$ (HOS assignment). However, the reductants utilized (specifically hydroxylamine) interact with and reduce other species besides $\text{Mn}^{3+/4+}$ (178, 179). In contrast, our group has determined the number of quanta of light (STFs) required to photoassemble apo-PSII-WOC starting from Mn^{2+} and Ca^{2+} . Two studies utilizing different flash intensities and durations have found that O_2 is first released after seven flashes (one-electron transfer steps) (79, 86). These results correspond to the LOS S_1 assignment of either $(\text{Mn}^{3+})_4$ or $\text{Mn}^{2+}(\text{Mn}^{3+})_2\text{Mn}^{4+}$. We emphasize that titration and photoassembly methods do not rely on model complexes for calibration. Hillier & Wydrzynski have also argued for the LOS assignment of S_1 [$(\text{Mn}^{3+})_4$] based on the comparison of water exchange kinetics in Mn^{3+} -containing model complexes and PSII (180). More experiments are needed to fully resolve this critical debate in PSII research.

Additionally, IM_1 decays if the light flux is very low, or is unproductively photooxidized if the dark process does not complete first. EPR spectroscopic data support all of the suggested structures for IM_0 , IM_1 , and IM_1^* in **Figure 3** (83). The next intermediate, IM_2 , forms by photooxidation of a second Mn^{2+} . The structure in

Figure 3 is only postulated as no direct spectroscopic evidence currently exists to characterize IM_2 . All light-induced intermediates after IM_1^* form with much higher quantum yield and have not yet been isolated for characterization.

Experiments by Burnap and coworkers (84) using cyanobacterial cells have used paired

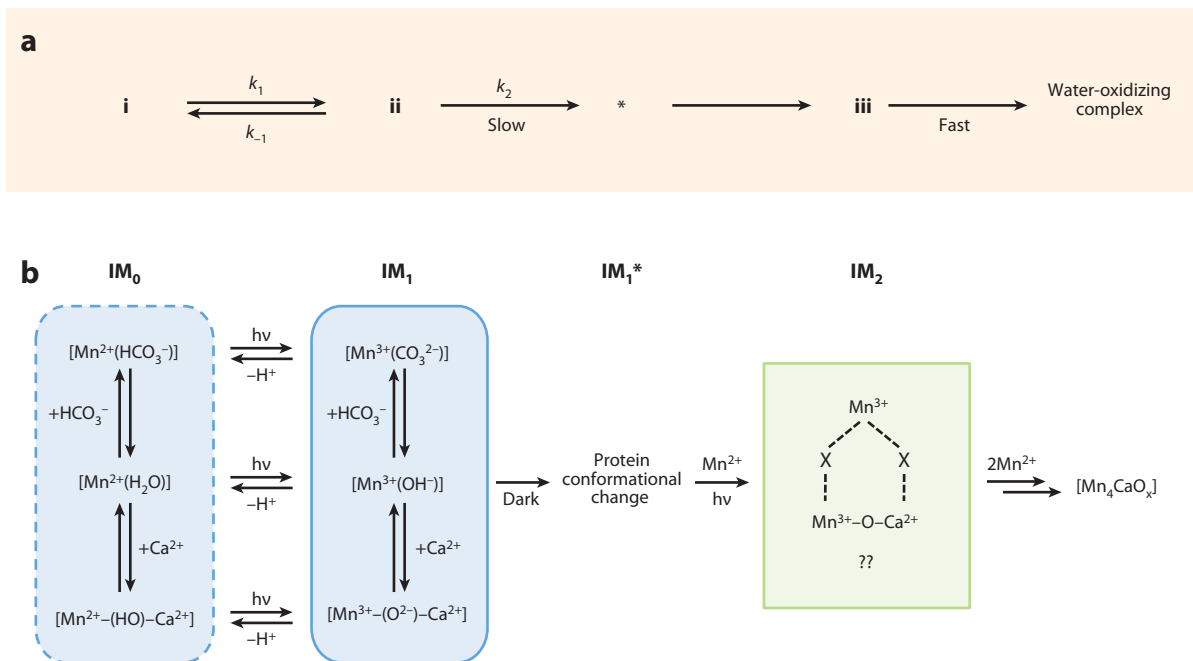


Figure 3

The sequence of kinetic intermediates (panel *a*) and proposed chemical formulation (panel *b*) of the intermediates formed during the major photoassembly pathway in spinach photosystem II membranes. Figure adapted from Reference 12.

flash experiments to reveal a third kinetic phase of photoassembly in a minority of centers, presumably representing an alternate pathway to the second intermediate, IM₂. The rate to this intermediate is 5–10 times faster than the rate-limiting dark-rearrangement step that is normally observed in plant PSII. Assuming that the dark-rearrangement step is a protein conformational change, Burnap and coworkers propose the possibility that a fraction of the centers may already exist in the rearranged state during or shortly after the first light-induced step. These centers are thus able to process the second quantum without the prior delay required by the conformational change. Burnap's observation fits well with photoassembly measurements using spinach PSII membranes, in which Ca²⁺ was replaced by Cd²⁺, an inhibitor of O₂ evolution (85). This change produces a tenfold acceleration of the dark step in Cd²⁺-reconstituted centers, which bind competitively and more strongly to the native Ca²⁺ site.

Apparently, the low Ca²⁺ affinity thus controls the rate of the slow dark conformational step, and some other cations that bind more tightly (e.g., Cd²⁺) accelerate conversion to the ready state, IM₁*. Subsequently, photoassembly proceeds, and Cd²⁺ can be exchanged with Ca²⁺ to yield catalytically competent WOC (85).

The three kinetically resolved steps of photoassembly are associated with cofactor uptake by an ordered sequence of steps 1Mn²⁺(1 photon) + Ca²⁺(dark) + 3Mn²⁺(*n* photons), as illustrated in **Figure 4**. The dark step is gated by Ca²⁺ and appears to involve a protein conformational change (D1 carboxyl terminus?) that controls the kinetics (86). This step templates the formation of the binding site for the remaining three Mn ions. Without Ca²⁺, many more than three Mn²⁺ ions bind and photooxidize to form an unproductive intermediate (87).

The Mn²⁺ and Ca²⁺ stoichiometries, their cooperative binding, and their kinetic sequence of uptake as determined by photoassembly

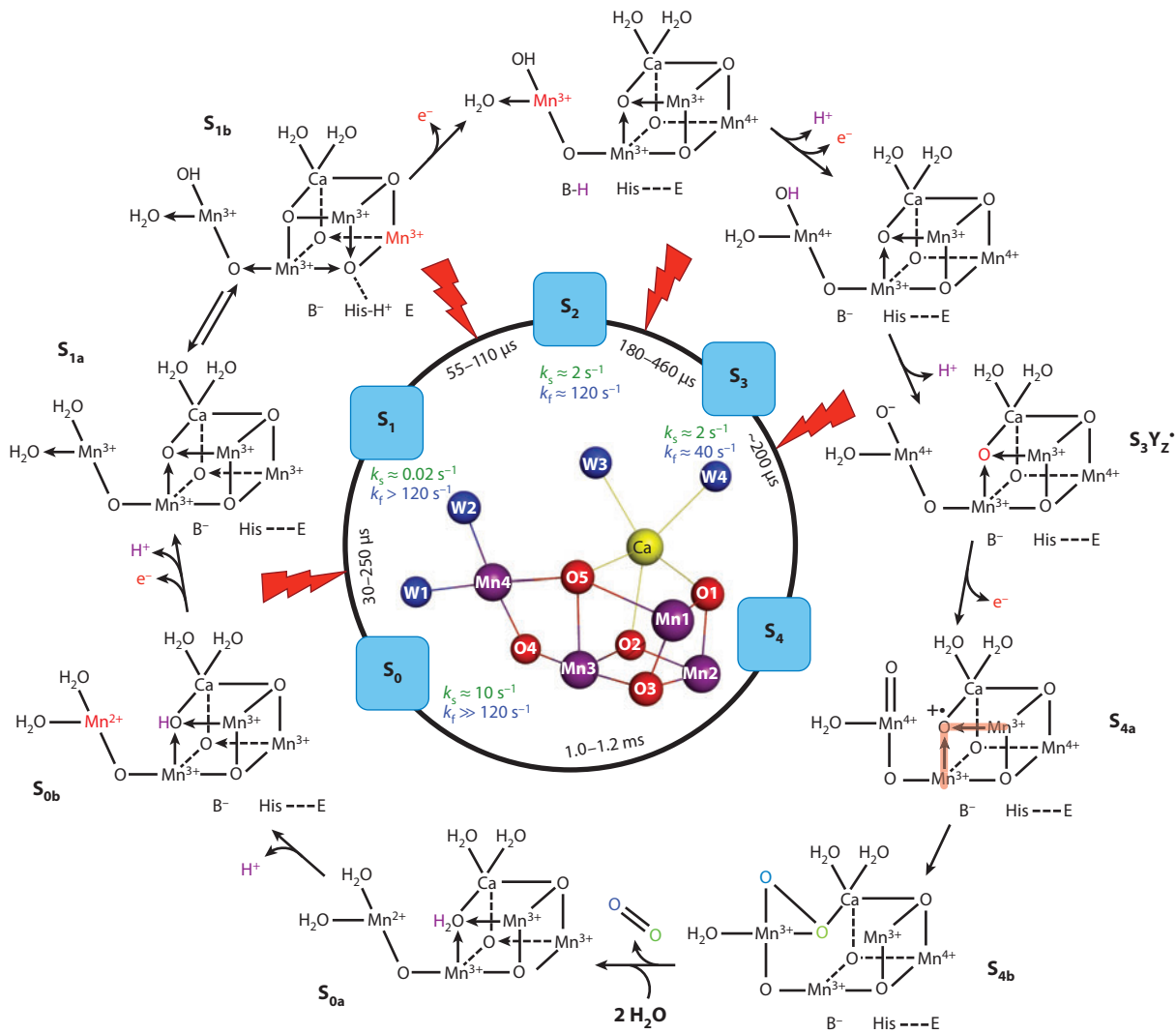


Figure 4

Proposed mechanism of water oxidation by the photosystem II (PSII) water-oxidizing complex (WOC). Arrows within chemical structures represent the alignment of the Jahn-Teller axis on each Mn³⁺, corresponding to the antibonding e_g¹(d_{z²) orbital. Rate constants for S-state transitions (black text) are detailed in Table 1. Fast and slow substrate water exchange rates (k_f and k_s, respectively) were measured at 10°C in spinach PSII membranes (186, 187). The inset structure of the PSII-WOC is analogous to intermediate S_{1b} in our mechanism and was generated in the PyMOL program using coordinates from Protein Data Bank entry 3ARC (17).}

actually forecast the structural organization of these cofactors in the WOC, as seen in recent XRD studies (reviewed in References 12, 75). The initial Mn²⁺ site, corresponding to Mn4, was further identified as the dangler Mn by EPR spectroscopy of the holoenzyme (49). EPR spectroscopy of photoassembled

cyanobacterial PSII-apo-WOC core complexes has given further evidence describing the ligand field of (Mn4)³⁺ in IM₁ (88). A μ-oxo (formally μ-oxido or di-μ-hydroxido) bridge forms between Mn³⁺ and Ca²⁺ during photoassembly, as identified by EPR spectroscopy (82). This appears to be the O5 bridge from Mn4 to

Ca^{2+} (Figure 1). During photoassembly, Ca^{2+} locked in a fixed ligand field at $(\text{Mn4})^{3+}$ independent of the solution pH; in the absence of Ca^{2+} , this $(\text{Mn4})^{3+}$ ligand field varied systematically with pH. Thus, a critical role of Ca^{2+} is to work together with $(\text{Mn4})^{3+}$ to form an ionized oxide bridge (O5) that is chemically and kinetically stable over a wide pH range and provides the template for the cooperative binding and high-quantum-yield photooxidation of the remaining three Mn^{2+} (82). These remaining steps assemble the Mn_3O_4 -incomplete cubane to the precursor $[(\text{Mn4})(\text{O5})\text{Ca}]$, forming the Mn_4CaO_5 catalytic core (see Structure, above).

Chloride is essential for O_2 activity of PSII (89) but not for photoassembly (12). Although no protein chaperones are required for photoassembly (the process can proceed in vitro with purified apo-PSII-WOC), (bi)carbonate is an important cofactor that accelerates the rate and increases the yield of photoassembly. During photoassembly of spinach PSII, it acts through two binding sites: One is a general base site, and the other is specific to (bi)carbonate (70, 80). Electron spin-echo envelope modulation (ESEEM) studies of ^{13}C -bicarbonate binding established that the latter interaction involved direct coordination to $(\text{Mn4})^{2+}$ and contributed to the ligand field around $(\text{Mn4})^{3+}$, possibly by delivery of (hydr)oxide for formation of a μ -oxo bridge to Ca^{2+} (90). Although (bi)carbonate appears not to be essential for O_2 evolution activity in some PSIIIs (91), it significantly influences the kinetics of O_2 evolution in a cyanobacterial strain mediated by CP-R357 (71) and is absolutely essential for the operation of hypercarbonate strains (21), in which it appears to be important for proton evolution (92).

A PROPOSED MECHANISM FOR WATER OXIDATION

Next we propose an atomic mechanism for water oxidation that uses the low oxidation state assignment and builds on a prior mechanism that adopted this assignment (48). We apply the results highlighted in other sections of this review, the atomic positions taken from the

1.9-Å structure (17), and recent DFT calculations. Two remarkable features of the XRD structure that we develop in our proposed mechanism are the unusual ligand field at O5 and the very highly ordered W2 (threefold-lower positional disorder). In our mechanism, we suggest that these two oxygens are derived from substrate waters by applying the principles of chemical bonding and reactivity from inorganic chemistry (93). Notably, we explicitly include the consequences of the Jahn-Teller (JT) effect. This vibronic term causes tetragonal elongation around Mn^{3+} owing to distortion around the asymmetric electronic configuration ($d_{x^2-y^2}$ hole and antibonding electron in d_{z^2}) and results in a large energy stabilization of 0.5–1 eV for Mn^{3+} in oxide ligand fields (93). This effect cannot be overlooked in chemically accurate mechanisms. We also include the mechanistic and energetic consequences of the proton ionization of water molecules bound to $\text{Mn}^{2+/3+/4+}$ cations ($\text{p}K_a$ differences). Notably, a factor of seven to eight difference in $\text{p}K_a$ exists for water molecules bound to axial versus equatorial positions of JT-distorted Mn^{3+} (94). These terms are critical to account for the large differences in substrate water exchange rates with the S state, as determined by ^{18}O -isotope mass spectrometry (95). To our knowledge, the S-state dependence of these rates has not been successfully incorporated within any self-consistent mechanism.

Flash #1: $\text{S}_1 \rightarrow \text{S}_2$

We begin with the dark-stable S_1 intermediate in the low oxidation state $(\text{Mn}^{3+})_4$ (Figure 4). In our model, S_1 can exist as two proton tautomers as recently hypothesized by Pace and coworkers (96). The first S_{1a} species (derived from S_0) features W2 as an aqua ligand to Mn4. Based on energetics (93) and by analogy to those observed from a $(\text{Mn}^{3+})_4\text{O}_4$ model cubane (97), the JT axes of Mn1 and Mn3 are predicted to point toward O5 and that of Mn2 is predicted to point toward O2 in S_{1a} . The other (preferred) tautomer S_{1b} is predicted to form upon net movement of a proton from W2 [bound at the more acidic non-JT axis on

Jahn-Teller (JT) effect: tetragonal elongation of six-coordinate Mn^{3+} ($3d^4$) resulting from asymmetric electronic configuration (hole in $d_{x^2-y^2}$ and antibonding electron in d_{z^2})

(Mn^{3+})₄] to D1-H337. This is likely mediated by the water network connected to W2 and by an internal proton donor, BH. S_{1b} is driven to tautomerization by coordination of the protonated imidazolyl cation on H337 to O3 via a strong hydrogen bond [2.6 Å in the Umena et al. structure (17)]. This tautomerization drives the reorientation of the JT axes of Mn1 and Mn3 toward O3. The S_{1b} vibronically stabilized tautomer can explain the decrease in substrate water exchange rates measured by Hillier & Wydrzynski (Figure 4; reviewed in Reference 95). As shown in Figure 4, the rearrangement of two JT axes in tautomer S_{1b} produces a more tightly bound O5 owing to the loss of two antibonding interactions from Mn1 and Mn3. This stronger bonding is consistent with the observed 500-fold slowing of the substrate exchange rate in S_1 relative to the (OH)5 exchange rate in S_0 (Figure 4), assuming that O5 is the slow-exchanging substrate water site.

Species S_{1b} continues in our proposed catalytic cycle. Following photochemically driven charge separation, Y_Z^\bullet oxidizes Mn2 ($Mn^{3+} \rightarrow Mn^{4+}$), triggering the following steps: (a) internal movement of a proton from the histidyl cation H337- H^+ to a base B^- caused by the loss of an electron and stronger covalent bonding within the Mn2(O1)(O3)Mn1 rhombus [the ($Mn1$)³⁺ $d_{x^2-y^2}$ hole is directed at O1 and O3, and $d_{z^2}^1$ is pointed at O5], and (b) reorientation of the JT axis on Mn3 along O5 to accommodate this stronger covalent bonding. As a result of this realignment of two JT axes with their antibonding interactions directed at O5, the water exchange rate of O5 is predicted to increase substantially from its S_1 value, consistent with the experimental data showing a 100-fold increase at the slow substrate site in S_2 (Figure 4). The faster exchanging site (W2) is not directly affected and slows by a much smaller value (Figure 4).

Flash #2: $S_2 \rightarrow S_3$

After the second flash, a rapid deprotonation event is followed by a metal-centered oxidation (98). This redox-leveling process partially

mitigates the energy barrier of the second oxidation by decreasing the formal charge of the WOC. We propose that the internal base BH associated with H337 (Figure 4) is deprotonated to the bulk in this step. Neutral H337 may be stabilized by hydrogen bonding to the backbone carbonyl of E333 [noted as E in Figure 4 and located 3.37 Å from H337 in the Umena et al. structure (17)]. The hole injected into the Mn₄Ca cluster by Y_Z^\bullet is postulated as localized to Mn4 ($Mn^{3+} \rightarrow Mn^{4+}$). Thus, the fast-exchanging water (W2) should bind more tightly than in the previous intermediate, and the bonding environment of the slow-exchanging site (O5) is not significantly affected in the $S_2 \rightarrow S_3$ transition (Figure 4).

Flash #3: $S_3 \rightarrow S_4 \rightarrow S_0 + O_2$

O₂ is released following the third flash through a complex series of intermediates. First, we propose that the formation of Y_Z^\bullet facilitates the deprotonation of W2 to the bulk via the adjacent water network (species $S_3Y_Z^\bullet$ in Figure 4). Next, the Y_Z^\bullet hole is subsequently transferred to the cluster, generating an oxyl radical cation at O5 (S_{4a}) that is stabilized by delocalization of the hole between the antibonding $d_{z^2}^1$ electrons on Mn1 and Mn3, as highlighted in Figure 4. Ultimately, this configuration evolves to form a bond between O5 and Mn4, as shown in Figure 4.

The O5 oxyl radical is in close proximity to the terminal oxo of Mn4 (W2). Terminal O-Mn⁴⁺ bonds *trans* to oxido (O4) are uniquely weak (and long) owing to a half-filled d_{π^3} shell with three antibonding electrons (99). This configuration favors formation of an intramolecular O-O bond, shown as the peroxo intermediate S_{4b} in Figure 4. The peroxo intermediate that forms at Mn4 in our mechanism is formally similar to the peroxo intermediate that forms during O₂ release from permanganate (MnO_4^-) following photoexcitation in the gas phase (100, 101). Based on DFT calculations and assuming the high oxidation state assignment, Siegbahn (67, 102) found that an oxo-oxyl radical coupling mechanism for O-O

bond formation in S_4 has significantly lower energy than the nucleophilic attack mechanisms preferred by Brudvig and coworkers (103). Importantly, spin states are consistent with bond formation in the Siegbahn oxo-oxyl radical coupling mechanism. The unpaired electron on O5 is predicted to have spin β , which would populate a bonding orbital with a spin α electron from the oxo of Mn4 (102). In a seminal recent paper by Lubitz and coworkers (104), a bridging oxo (O5) was shown to be exchangeable at rates compatible with those previously reported for substrate water exchange (95), which leads directly to possible oxo-oxyl radical coupling mechanisms involving O5, as shown here.

Conversion to O_2 by internal charge transfer is downhill in energy (105, 106). O_2 is released upon uptake of the next two substrate water molecules at sites W2 and O5 (S_{0a} in **Figure 4**). In the resulting S_{0b} state, the water molecule at position O5 spontaneously deprotonates via the adjacent water network to the bulk, forming a μ_3 -hydroxide ligand that assumes sp^3 hybridization with weak bonds to Mn1 and Mn3 and ionic association with Ca^{2+} . There is no appreciable bond strength between Mn4 and O5 in S_0 , in agreement with the long 2.5-Å distance seen in the XRD structure of S_1 (17). This conformation of S_0 results in the fastest exchange rate of the slow substrate water site (O5, 10 s^{-1} ; **Figure 4**). The fast-exchanging water (W2) is bound to $(Mn4)^{2+}$ and also exhibits its most rapid rate in S_0 ($>120\text{ s}^{-1}$; **Figure 4**).

Flash #4: $S_0 \rightarrow S_1$

Following the fourth flash, a metal-based oxidation step precedes deprotonation (98). We propose that the five-coordinate Mn4 is oxidized from Mn^{2+} to Mn^{3+} in this step. The final deprotonation event is centered at O5, and during it the hydroxo is converted to a corner μ_3 -oxo weakly bridging Mn1, Mn3, and Mn4 and ionically associated with Ca^{2+} . As previously discussed, the absence of ordered hydrogen-bonding partners of O5 in the Umena et al. (17) structure suggests that O5 is more likely a bridging oxo rather than a hydroxo in S_1 .

Additional Considerations

The foregoing mechanism emphasizes the most recent structural, electronic, and chemical attributes of the WOC, specifically the oxygen atomic coordinates, the lower oxidation state, and water exchange rates. We stress that our proposed mechanism for WOC turnover, although based on established principles of chemical bonding within transition metal-oxo complexes, may need further refinements from consideration of other experiments or computations.

DONOR-SIDE KINETICS

The redox-active tyrosines Y_Z (D1-Tyr161) and Y_D (D2-Tyr160) are located symmetrically in the D1 and D2 subunits of the homodimeric reaction center core, nearly equidistant to P_{680} ; Y_Z is between P_{680} and the $CaMn_4$ site (**Figure 5**). They have been extensively researched (107). Their environments are highly ordered with different residues and water accessibility that confer different electrochemical potentials, as understood from synthetic models (108). For example, only Y_Z^\bullet has sufficient thermodynamic driving force to advance all S-state transitions of the Kok cycle, whereas Y_D actually acts as an inefficient reductant to the S_2 and S_3 states (109–111). These differences account for their substantially different kinetics of electron donation to the Mn_4CaO_5 complex. The kinetics of the reduction of P_{680}^+ by Y_Z are triphasic and have time constants of 20–50 ns (fast nanosecond kinetics), 300–600 ns (slow nanosecond kinetics), and 30–35 μs (microsecond kinetics) (112–115). The fast nanosecond kinetic reaction is facilitated by a hydrogen-bonding interaction between Y_Z (hydroxo) and D1-His190. The two slower kinetic reactions are related to changes in the protein environment that favor Y_Z oxidation. The 300–500-ns kinetic reaction is controlled by changes in the local dielectric protein environment, whereas the 30–35- μs kinetic reaction reflects proton rearrangement processes. The energetics of these relaxation processes contribute

P_{680} : reaction center
Chl *a* dimer

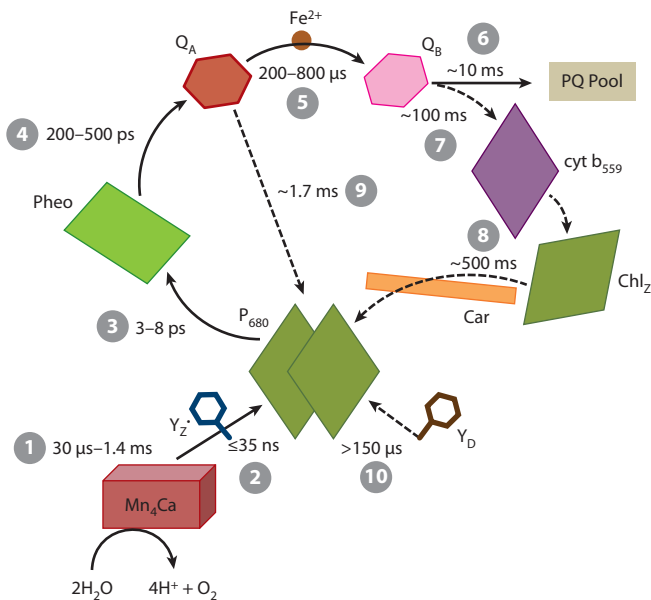


Figure 5

Kinetics of electron/hole carriers in photosystem II (PSII). Dotted lines represent secondary electron transfer pathways as described in Light to Chemical Conversion Efficiency in PSII. ① Y_Z^* oxidation kinetics are S-state dependent, as detailed in Table 1. ② The reduction of P_{680}^+ by Y_Z^* is multiphasic (112–115). ③ The reduction of Pheo (188–190) is approximately 100-fold faster than the ④ subsequent reduction of Q_A (188, 190–192). ⑤ Q_B accepts two electrons from Q_A ($\tau = 200\text{--}400\text{ }\mu\text{s}$ for $Q_A^-Q_B \rightarrow Q_A^-Q_B^-$ and $500\text{--}800\text{ }\mu\text{s}$ for $Q_A^-Q_B^- \rightarrow Q_A^-Q_B^{2-}$) (134–137). ⑥ Q_B preferentially exchanges with the PQ pool (193) or ⑦ can reduce cyt b_{559} (194). ⑧ P_{680}^+ can be reduced via the network of Car, ChlZ, and cyt b_{559} (195). ⑨ Alternatively, P_{680}^+ can be reduced by direct charge recombination (quantum tunneling) with Q_A (150). Indirect (nonradiative) recombinations of $[P_{680}^+Pheo^-]^1$ and $[P_{680}^+Pheo^-]^3$ occur with half-times of $> 1\text{ ns}$ and $\sim 200\text{ ps}$, respectively (151, 196). ⑩ The reduction of P_{680}^+ by Y_D has a half-time of $> 150\text{ }\mu\text{s}$ at pH 6.5 (142). The oxidation of S_0 by Y_D^* is much slower and occurs on the order of 5–40 min (111). Abbreviations: Car, carotenoid; PQ, plastoquinone.

approximately 0.1 eV of stabilization energy for this reaction, with contributions of 10, 25, and 60 meV for each of the three phases, respectively (116). Renger & Renger (13) noted that the contribution of the slower phases increases upon replacement of Ca^{2+} by Sr^{2+} in the Mn_4Ca site, revealing that the relaxation-dependent phases of Y_Z oxidation involve degrees of freedom extending to the inorganic core.

The kinetics of individual S-state transitions and the O_2 release rate are considerably faster than the catalytic turnover rate, which

is typically limited on the acceptor side by reoxidation of $(PQH_2)_B$. The kinetics of Y_Z^* reduction and Mn oxidation in the WOC center have been analyzed by time-resolved EPR spectroscopy (117, 118) and optical changes in UV absorption (119). These data show that Y_Z^* is the direct oxidant of Mn during S-state transitions. The time courses for the one-step advancements of S_0 , S_1 , and S_2 satisfy single exponential kinetics, in contrast to the multiphasic Y_Z oxidation by P_{680}^+ (see above). The half-lives of these reactions are given in Table 1 and reflect the kinetics for resting populations in dark-adapted PSII. The rates of $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, and $S_2 \rightarrow S_3$ as measured by EPR and XAS progressively slow in the ranges of 30–70 μs , 70–110 μs , and 180–350 μs , respectively (63, 117, 118). Interestingly, the $S_0 \rightarrow S_1$ transition is supposedly slower than $S_1 \rightarrow S_2$ when UV absorption or FT infrared spectroscopy (FTIR) absorption is used to monitor the reaction kinetics (119, 120). The S_3 oxidation by Y_Z^* is slower by a factor of three to eight compared with the next slowest S-state transition and is the most sensitive to environmental factors. This step has been examined by time-resolved Mn XAS and FTIR and found to resolve into two phases with a lag phase of approximately 200 μs . The latter step correlated with the detection of proton release by an entropically favorable process (63, 120).

Kinetic data have been obtained for the detection of the released O_2 product in solution using electrochemical (18, 121, 122), EPR (123), and photoacoustic (124) methods (Table 1). Because diffusion lengths to the detector and internal sinks for O_2 solubility need to be considered in whole cells, data from isolated PSII complexes are considered more reliable for determination of the intrinsic half-life for O_2 release. The ranges of values found are 1.2–2.2 ms for polarography, 1–2 ms for EPR, and approximately 2 ms for photoacoustic methods. These rates are fully consistent with predicted kinetics of the $S_0 \rightarrow S_3$ transition. The release of O_2 is not reversible even at elevated O_2 pressure (125) and exhibits a driving force of at least 220 mV (126).

Table 1 S-state transition half-lives as measured by EPR (Y_Z reduction), UV absorption, X-ray absorption, and FTIR as well as O_2 release as measured by polarography, EPR, and photoacoustics

S-state transition half-life	EPR ^a	UV ^b	XAS ^c	FTIR ^d	Polarography	Photoacoustics
$S_0 \rightarrow S_1$	30–70 μ s	250 μ s	≤ 30 μ s	130 μ s		
$S_1 \rightarrow S_2$	100–110 μ s	55 μ s	70 μ s	65 μ s		
$S_2 \rightarrow S_3$	180–350 μ s	290 μ s	190 μ s	460 μ s		
$S_3 \rightarrow S_0$	1.0–1.4 ms	1.2 ms	1.1 ms	1.4 ms		
O_2 release	1–2 ms ^e				1.2–2.2 ms ^{f,g}	~ 2 ms ^h

Data refer to dark-adapted samples using single-turnover flashes. Abbreviations: EPR, electron paramagnetic resonance; FTIR, Fourier transform infrared spectroscopy; XAS, X-ray absorption spectroscopy.

^a Y_Z^{\bullet} reduction (117, 118).

^bMn^{ox} formation (119).

^cMn^{ox} formation (63).

^dH⁺ and protein dynamics (120).

^e O_2 detection via EPR-active spin probe (123).

^fReference 121.

^gReference 122.

^hPulsed photoacoustic time shift resulting from O_2 release (124).

ACCEPTOR-SIDE KINETICS

Following the formation of the primary charge-separated state, $[P_{680}^+Pheo^-]$, Pheo⁻ reduces the primary acceptor, Q_A , a tightly bound plastoquinone (**Figure 5**). The thermodynamic driving force of this step is defined by the relative midpoint potentials of Pheo/Pheo⁻ and Q_A/Q_A^- and is controlled by the local protein environment. The Pheo hydrogen-bonding partner D1-130 is a Glu in plants and algae but may be either a Glu or Gln in cyanobacteria, depending on the expressed D1 isoform regulated by light intensity (40). Substitution of Gln for Glu at D1-130 changes the $E_m(Pheo/Pheo^-)$ by -33 mV in *Synechocystis* (127) and -17 mV in *Thermosynechococcus* (44, 128). The E_m of Q_A/Q_A^- also varies based on species (129), D1 isoform (130, 131), and herbicide occupancy of the Q_B pocket (132).

Although Q_A accepts only one electron at a time and no protons, the secondary PQ acceptor, Q_B , is reduced and protonated twice to form PQH_2 . Electron transfer from Q_A^- to Q_B is facilitated by a nonheme iron (Fe^{2+}) equidistant between the two PQ molecules. The nonheme Fe^{2+} is ligated by a bidentate bicarbonate ligand [essential for efficient electron transfer (91)] and four His residues: D1-H215,

D1-H272, D2-H214, and D2-H268 (17). Through computational studies, the E_m of Q_B/Q_B^- is predicted to be dependent on the protonation state of D1-H252 (133). D1-S264 forms a hydrogen bond to Q_B that facilitates forward electron transfer from Q_A^- only when D1-H252 is protonated (133). Q_B is reduced with kinetic time constants of 200–400 μ s for the first transfer ($Q_A^- \rightarrow Q_B$) and 500–800 μ s for the second transfer ($Q_A^- \rightarrow Q_B^-$) (134–137). The resulting $(PQH_2)_B$ diffuses from the binding pocket and is replaced by an oxidized Q_B from the membrane-soluble PQ pool.

SECONDARY ELECTRON TRANSPORT WITHIN PSII

The most efficient electron transfer pathway in PSII is the linear flow of electrons from water to $(PQH_2)_B$ via the WOC, Y_Z , P_{680} , Pheo, Q_A , and Q_B . Although Y_Z is the preferred reductant of P_{680}^+ , it is in competition with Y_D , Car (via cyt b_{559} and Chl_Z), and backward reactions from the acceptor side (e.g., Q_A^-). Additionally, although the combined impact of these secondary pathways (**Figure 5**) may be minimal under continuous moderate light flux, they

Q_A : primary PQ acceptor

Q_B : secondary PQ acceptor

Plastoquinone (PQ) pool: the membrane-soluble sum of oxidized PQ and reduced PQH_2

are essential for PSII survival under excess light fluxes and photoassembly (138).

Tyrosine D

Although the cation radical form of Y_D^\bullet has a high reduction potential [$E_m = 700\text{--}800$ mV (111)], it remains oxidized in the dark for long periods and does not play a major role in PSII turnover under continuous illumination (107). However, Y_D is completely conserved in PSII from the genomes of all oxygenic phototrophs, indicating that it is absolutely essential. Owing to its hydrophobic protein environment, the Y_D^\bullet radical is remarkably dark stable and can be observed via EPR for hours after exposure to light (139). However, Y_D can reduce both the S_2 and S_3 Kok-cycle intermediates (generated after one or two STFs, respectively) (109–111) but cannot reduce S_0 or S_1 . Conversely, Y_D^\bullet can oxidize S_0 , although its kinetics are very slow (110, 111). These features help explain why after several minutes of dark adaptation, WOC centers are composed of approximately 25% S_0 and 75% S_1 , but after many hours of dark adaptation, the population of S_1 approaches 100%. When P_{680}^+ formation is faster than WOC cycling or if the Mn_4Ca is disassembled, Y_D can directly reduce P_{680}^+ (140–142). This ability of Y_D to store an oxidizing equivalent supports photoassembly. The Y_D artificial mutant D2-Tyr160Phe was significantly impaired in the rate and yield of photoassembly of the WOC inorganic core (143).

Cyclic Electron Transport Around PSII: Cyt b_{559} , Chl $_Z$, and Car

The PsbE and PsbF subunits comprise the α - and β -subunits of cyt b_{559} and are essential components of the PSII core. As recently reviewed by Shinopoulos & Brudvig (138), following the formation of P_{680}^+ , cyt b_{559} may be oxidized, and the oxidizing equivalent is rapidly equilibrated between cyt b_{559} , Chl $_Z$, and Car (144), and subsequently reduced by Q_B^- . This cyclic pathway around PSII functions when the Mn_4Ca cluster is not present, but it has also

been observed experimentally *in vivo* in a photoprotective role under excess light conditions (145) when the PQ pool is highly reduced (146).

Charge Recombination

The initial [$P_{680}^+Phe^-$] charge-separated state is short-lived and transfers to [$P_{680}^+Q_A^-$] within hundreds of picoseconds following photochemical excitation of P_{680} (147). Typically, Y_Z reduces P_{680}^+ and Q_A^- reduces Q_B . However, [$P_{680}^+Q_A^-$] may also recombine through various pathways with either photoprotective or destructive consequences [see excellent reviews by Vass & Cser (148) and Rutherford et al. (149)]. First, [$P_{680}^+Q_A^-$] may directly recombine via quantum tunneling. This nonradiative reaction is relatively slow (approximately 2 ms) and may serve a protective role in excess light conditions (150, 151). The free energy gap between Q_A/Q_A^- and P_{680}/P_{680}^+ is large (>1.2 eV). Thus, the relationship between the thermodynamics and kinetics of this recombination is hypothesized to follow inverted Marcus behavior owing to the predicted large reorganization energy (148). As previously discussed, the E_m of Q_A/Q_A^- varies considerably among species and cyanobacterial D1 isoforms (see Secondary Electron Transport Within PSII, above). Therefore, we and others have predicted that this tuning of the Q_A/Q_A^- E_m may be the result of evolutionary selection to maximize photoprotection and/or minimize inefficient back reactions under light-limited conditions (44, 45, 148, 149).

The [$P_{680}^+Q_A^-$] charge pair is in equilibrium with both the singlet and triplet states of [$P_{680}^+Phe^-$] and can populate with different probabilities and consequences (132, 152, 153). Their formation is controlled by the free energy gap between $Pheo/Pheo^-$ and Q_A/Q_A^- . Similar to that of [$P_{680}^+Q_A^-$], the transient singlet state of [$P_{680}^+Phe^-$] decays harmlessly (and likely protectively) via a nonradiative direct pathway (148). In contrast, when triplet [$P_{680}^+Phe^-$] forms, it decays via triplet $^3P_{680}$, which, owing to its longer lifetime, may react with dissolved molecular oxygen, 3O_2 , to form singlet

¹O₂. This product is a powerful oxidizing agent that causes extensive damage to PSII and surrounding sites (132, 152, 153).

LIGHT TO CHEMICAL CONVERSION EFFICIENCY IN PSII

PSII Reaction Center Efficiency

Here we focus on contributions to the solar energy conversion efficiency of PSII, η , which can be reduced to the product of three terms as in Equation 3 (4, 154),

$$\eta = \text{LHY} \times \text{FEY} \times \text{PQY}, \quad 3.$$

where LHY is light-harvesting yield, FEY is fractional energy yield, and PQY is the quantum yield of product (O₂ + PQH₂) formation.

In PSII, individual quanta of visible light are absorbed directly or transferred from excited states of antenna pigments of comparable or higher energy and form the excited state of the photochemically active Chl, P₆₈₀*. The yield of this reversible process depends on the product states (prior photochemical events) and defines the quantum yield for light harvesting by P₆₈₀ (LHY). Estimates of maximum LHY for oxygenic phototrophs without product inhibition range from 34% to 50%, based on the full spectrum of incident solar radiation at the surface of Earth (3, 4). Subsequently, the excitation on P₆₈₀* is trapped by photochemical conversion steps with a probability that depends on the product state (PQY). These occur as electron and proton transfer steps that form electron/hole pairs on specific neighboring cofactors. As depicted in **Figure 6** (from Reference 4) for PSII, the initial step involves forming a Chl *a* radical cation (P₆₈₀⁺) and a reduced Pheo radical anion (Pheo⁻). Also shown is the FEY relative to P₆₈₀*, defined as the difference in their respective reduction potentials. This step is followed by a series of electron transfer steps to adjacent cofactors that further separate the electron/hole pair at the expense of further loss of internal energy in discrete amounts determined by the cofactor identity and environ-

mental interactions. The FEY decreases overall by 0.49–0.58 V or 27–32% relative to P₆₈₀* upon forward electron transfer on the acceptor side from Pheo⁻ to Q_A⁻ to (PQH₂)_B. Likewise, on the donor side, sequential electron transfers to the high-potential hole of P₆₈₀* are filled by Y_Z, which in turn oxidizes the WOC. There is an estimated overall energy loss of 0.32–0.44 V or 17–24% from P₆₈₀* to the products in Equation 1a (4, 155). This results in a total FEY of 44–56% (ignoring the *pmf* created). This loss is a system design loss of PSII and is not an intrinsic overpotential specific to the WOC. These energy losses following the photochemical step (FEY) are the cost to ensure a high probability of charge transfer between each of the selected carriers and not others (PQY), while also suppressing wasteful charge recombination pathways (see Secondary Electron Transport Within PSII, above) (156). Another trade-off that the cofactor design must avoid is loss of excited state trapping at P₆₈₀* and increased nonphotochemical quenching of P₆₈₀* once it is formed. The magnitude of PQY is related to the turnover efficiency of WOC cycling (see below). Dark-adapted PSII with an oxidized PQ pool and donor-side proton buffers can achieve PQY of up to 95% (1 - α + β) at low light fluxes (21), but the PQY is much lower even at ambient and certainly at full solar intensity as these reservoirs become filled, depending on the species. Returning to Equation 3, we estimate the maximal light to chemical quantum yield of PSII to be approximately 24% (0.5 × 0.5 × 0.95).

PSII–Water-Oxidizing Complex Cycling Efficiency

Most commonly, the efficiency of PSII is reported as a light-saturated rate of O₂ evolution that, when normalized to Chl content, approaches 6,000 $\mu\text{mol O}_2 (\text{mg Chl})^{-1} \text{h}^{-1}$ in vitro (157). As shown in **Table 2**, the sustained turnover rates of the PSII-WOC vary considerably among cyanobacteria, algae, and higher plants and among PSII particle preparation methods. The historical units of $\mu\text{mol O}_2 (\text{mg Chl})^{-1} \text{h}^{-1}$ can be converted

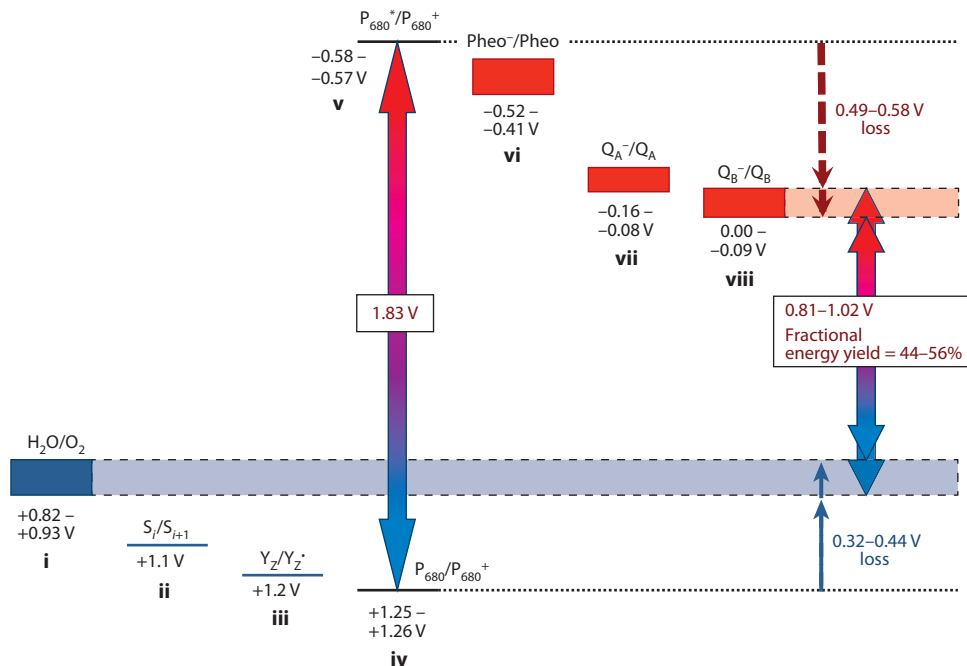


Figure 6

Relative energies of electron/hole carriers in photosystem II (PSII). Adapted from Reference 4.

(i) $E_m(\text{H}_2\text{O}/\text{O}_2)$ values reflect the physiologically relevant pH range of 5–7. (ii) S-state E_m values were indirectly measured in spinach [+1.1 V (197)] or *Synechocystis* 6803 [+1.0 V (198)] with reference to an $E_m(\text{Q}_\text{A}^-/\text{Q}_\text{A})$ value of –80 mV (199, 200). (iii) $E_m(\text{Y}_\text{Z}/\text{Y}_\text{Z}^{\bullet})$ was indirectly measured in spinach (201) with reference to an $E_m(\text{Q}_\text{A}^-/\text{Q}_\text{A})$ value of –80 mV (199, 200). (iv) $E_m(\text{P}_{680}/\text{P}_{680}^+)$ was indirectly measured in *Synechocystis* 6803 [+1.26 V (198)] or spinach [+1.25 V (201)] with reference to an $E_m(\text{Q}_\text{A}^-/\text{Q}_\text{A})$ value of –80 mV (199, 200). (v) The $E_m(\text{P}_{680}^*/\text{P}_{680}^+)$ value is calculated as 1.83 eV (energy of a 680-nm photon) more negative than $E_m(\text{P}_{680}/\text{P}_{680}^+)$. (vi) $E_m(\text{Pheo}^-/\text{Pheo})$ has been indirectly measured in spinach [–410 mV (202), –420 mV (201)] or *Synechocystis* 6803 [–440 mV (198)] with reference to an $E_m(\text{Q}_\text{A}^-/\text{Q}_\text{A})$ value of –80 mV (199, 200), and directly measured by spectroelectrochemistry in *Thermosynechococcus elongatus* containing D1:1-PSII [–522 mV (44)] or D1:2-PSII [–508 mV (128)]. (vii) $E_m(\text{Q}_\text{A}^-/\text{Q}_\text{A})$ was directly measured by redox titrations in spinach [–80 mV (200)] and *Scenedesmus obliquus* [–80 mV (199)], and by spectroelectrochemistry in *T. elongatus* containing D1:1-PSII (–124 mV) or D1:2 (–83 mV) (130), *Chlamydomonas reinhardtii* (–162 mV), spinach (–149 mV), and *Cyanidioschyzon merolae* (–92 mV) (129). (viii) $E_m(\text{Q}_\text{B}^-/\text{Q}_\text{B})$ was indirectly measured in pea chloroplasts [$E_m(\text{Q}_\text{A}^-/\text{Q}_\text{A}) - E_m(\text{Q}_\text{B}^-/\text{Q}_\text{B}) = -70$ mV (135)] or *Synechocystis* 6803 [$E_m(\text{Q}_\text{A}^-/\text{Q}_\text{A}) - E_m(\text{Q}_\text{B}^-/\text{Q}_\text{B}) = -83$ mV (203)].

to the intrinsic turnover rate per PSII from a knowledge of the Chl concentration per RC. This conversion is also given in **Table 2** and reveals that the light-saturated rate using artificial electron acceptors is remarkably similar for PSII from many different organisms, ranging from 27 to 67 $\text{O}_2 \text{ s}^{-1} \text{ PSII}^{-1}$. To the best of our knowledge, the highest sustained rate measured in vitro is 67 s^{-1} in the thermophilic cyanobacterium *Thermosynechococcus elongatus* (157).

The use of artificial electron acceptors with their inefficient reoxidation rate is commonly believed to limit PSII turnover in vitro. As previously described, the Kok cycle operates at less than 1–2 ms per turnover in dark-adapted (resting) enzyme. Thus, the maximal single turnover rate of PSII should be 500–1,000 s^{-1} . Indeed, instantaneous O_2 release as measured by polarography (121, 122), EPR (123), or photoacoustics (124) occurs in 1.2–2.2 ms

Table 2 Light-saturated PSII turnover frequency in vitro (O₂ evolution) and in vivo (variable Chl *a* fluorescence)

Species	Preparation	Turnover frequency (s ⁻¹)
<i>Spinacia oleracea</i>	Membrane fragments	30 ^a
<i>Chlamydomonas reinhardtii</i>	Membrane fragments	45 ^b
<i>Chlamydomonas reinhardtii</i>	Core complexes	27 ^c
<i>Thermosynechococcus elongatus</i>	Core complexes	67 ^d
<i>Synechocystis</i> sp. PCC 6803	Core complexes	27 ^e
<i>Synechocystis</i> sp. PCC 6803	Core complexes	35 ^f
<i>Synechocystis</i> sp. PCC 6803	Core complexes	25 ^g
<i>Chlorella pyrenoidosa</i>	Whole cells	25 ^h
<i>Arthrospira maxima</i>	Whole cells	88 ^h

Unless otherwise specified, all data refer to a temperature range of 20–25°C. Abbreviations: Chl, chlorophyll; PSII, photosystem II.

^aBBY preparation (25), 450 μmol O₂ (mg Chl)⁻¹ h⁻¹, 270 Chl/Y_D• (181).

^bPSII-enriched thylakoid membrane fragments, 650 μmol O₂ (mg Chl)⁻¹ h⁻¹, 276 Chl/4 Mn (28).

^cHis-tag-labeled D2 subunit, 2400 μmol O₂ (mg Chl)⁻¹ h⁻¹, 46 Chl/ Y_D• (182).

^dHis-tag-labeled CP43 subunit, 6000 μmol O₂ (mg Chl)⁻¹ h⁻¹, 45 Chl/ Y_D• (44, 157) at 45°C.

^e2500 μmol O₂ (mg Chl)⁻¹ h⁻¹, 44 Chl/0.25 O₂ (183).

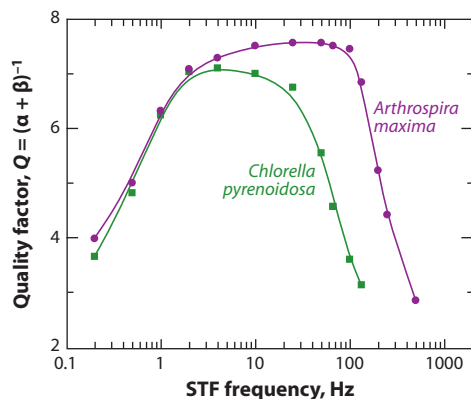
^f2400 μmol O₂ (mg Chl)⁻¹ h⁻¹, 59 Chl/ Y_D• (184).

^gHis-tag-labeled CP47 subunit, 2440 μmol O₂ (mg Chl)⁻¹ h⁻¹, 41 Chl/cyt b₅₅₉ (185).

^hValues correspond to turnover frequency at one-half maximum *Q* value (21).

(see **Table 1**), corresponding to rates of 455–833 s⁻¹.

In vivo, PSII turnover kinetics are readily measured by monitoring variable Chl *a* fluo-

**Figure 7**

Dependence of period-four oscillations in F_v/F_m on the dark time between single-turnover flashes (STFs) for intact cells of the green alga *Chlorella pyrenoidosa* (green) and the cyanobacterium *Arthrospira maxima* (purple). The quality factor values, $Q = (\alpha + \beta)^{-1}$, were obtained by fits to a standard two-parameter Kok model (see **Figure 2a**). The figure is based on raw data from Reference 21.

rescence using fast repetition rate fluorometry (21, 158). The damping rate for the amplitude of period-four oscillations of variable Chl *a* fluorescence (F_v) reflects the intrinsic turnover kinetics of the PSII-WOC (**Figure 2b**). Two analytical approaches are used (21). Fourier analysis of these oscillations gives the period of the oscillations directly and provides a model-independent measure of the dissipation of the catalytic cycle with flashing rate (**Figure 2c**). Alternatively, the amplitude of the oscillations can be mathematically fit to the classic (19, 20) or extended (159) Kok model (**Figure 2a**) to estimate the probability of a miss (α) or double hit (β). The Kok parameters are then used to calculate a familiar quantity in resonator theory called the quality factor of the cycle, $Q = (\alpha + \beta)^{-1}$. When Q drops to half its peak value, the power entering the Kok cycle drops by half. As shown in **Figure 7**, the in vivo upper limit of WOC turnover rate varies considerably by species. The WOC cycling efficiency of the green alga *Chlorella pyrenoidosa* sharply decreases above STF frequencies greater than 25 s⁻¹ and reaches 50% at 100 s⁻¹. Because four flashes are required for one WOC cycle,

the turnover frequency can be estimated as $1/4 \times 100 \text{ s}^{-1} = 25 \text{ s}^{-1}$. The fastest in vivo water splitter is the hypercarbonate cyanobacterium *Arthrospira maxima*. The WOC cycling efficiency decreases above STF frequencies greater than 100 s^{-1} , dropping to 50% at 350 s^{-1} (turnover rate = 88 s^{-1}) (21). This

drop-off in rate was limited by the buffer capacity of the lumen and thus attributed to proton release kinetics (92). In vivo PSII turnover efficiency is strongly affected by the redox state of the PQ pool, which is controlled by downstream electron carriers (including cyt b_6f , PSI, and alternative oxidases) (29).

SUMMARY POINTS

1. The greatly improved resolution of the PSII-WOC atomic structure (O atom chemical specification and coordination revealed) enables a structure-based assignment of the chemical bonding between Mn and O and gives the location of many water molecules.
2. The PSII reaction center core is remarkably conserved across cyanobacteria, algae, and higher plants, but significant differences in low-molecular-weight and extrinsic subunits exist.
3. New information identifying the oxidation states of the Mn atoms in the WOC has come from spectroscopic, computation, and photoassembly studies and indicates discrepancies that are still under debate.
4. Consideration of the improved atomic structure and oxidation states data, together with numerous other studies, has provided new insights that enable formation of likely chemical mechanisms of water oxidation and substrate water exchange.
5. Short-circuiting electron transfer pathways occur within PSII that lower the efficiency of WOC cycling while serving roles in photoprotection.
6. The PSII-WOCs from plants, algae, and cyanobacteria operate at light-saturated rates that are remarkably consistent in vitro and in vivo ($25\text{--}88 \text{ s}^{-1}$).

FUTURE ISSUES

1. Can a high-resolution XRD structure of a green algal or plant PSII be obtained? How would it differ from the thermophilic cyanobacterial structures?
2. Can high-resolution XRD structures of S_0 , S_2 , S_3 , and S_4 be obtained?
3. What spectroscopic, computational, and reconstitution experiments are needed for the field to arrive at a consensus mechanism for WOC turnover?
4. Can the efficiency of PSII turnover in vivo be improved from less than 100 s^{-1} to approach the intrinsic WOC turnover limit of $500\text{--}1,000 \text{ s}^{-1}$?
5. What additional principles can our current understanding of PSII offer to scientists designing more efficient abiotic catalysts using Earth-abundant materials?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Contents

Prefatory

Christian Raetz: Scientist and Friend Extraordinaire <i>William Dowhan, Hiroshi Nikaido, JoAnne Stubbe, John W. Kozarich, William T. Wickner, David W. Russell, Teresa A. Garrett, Kathryn Brozek, and Paul Modrich</i>	1
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Recent Advances in Biochemistry

Mechanisms for Initiating Cellular DNA Replication <i>Alessandro Costa, Iris V. Hood, and James M. Berger</i>	25
The Chromatin Response to DNA Breaks: Leaving a Mark on Genome Integrity <i>Godelieve Smeenk and Haico van Attikum</i>	55
Readout of Epigenetic Modifications <i>Dinshaw J. Patel and Zhanxin Wang</i>	81
Flap Endonuclease 1 <i>Lata Balakrishnan and Robert A. Bambara</i>	119
New Mechanistic and Functional Insights into DNA Topoisomerases <i>Stefanie Hartman Chen, Nei-Li Chan, and Tao-shih Hsieh</i>	139
Arrest Peptides: <i>Cis</i> -Acting Modulators of Translation <i>Koreaki Ito and Shinobu Chiba</i>	171
Structural Basis of the Translational Elongation Cycle <i>Rebecca M. Voorbees and V. Ramakrishnan</i>	203
CRISPR-Mediated Adaptive Immune Systems in Bacteria and Archaea <i>Rotem Sorek, C. Martin Lawrence, and Blake Wiedenbeft</i>	237
Correlating Structure and Energetics in Protein-Ligand Interactions: Paradigms and Paradoxes <i>Stephen F. Martin and John H. Clements</i>	267
Extracellular Chaperones and Proteostasis <i>Amy R. Wyatt, Justin J. Yerbury, Heath Ecroyd, and Mark R. Wilson</i>	295

Molecular Chaperone Functions in Protein Folding and Proteostasis <i>Yujin E. Kim, Mark S. Hipp, Andreas Bracher, Manajit Hayer-Hartl, and F. Ulrich Hartl</i>	323
Sumoylation: A Regulatory Protein Modification in Health and Disease <i>Annette Flotho and Frauke Melchior</i>	357
Ubiquitin Ligases and Cell Cycle Control <i>Leonardo K. Teixeira and Steven I. Reed</i>	387
Molecular Architecture and Assembly of the Eukaryotic Proteasome <i>Robert J. Tomko Jr. and Mark Hochstrasser</i>	415
Design of Protein Catalysts <i>Donald Hilvert</i>	447
Hydrogen Tunneling Links Protein Dynamics to Enzyme Catalysis <i>Judith P. Klinman and Amnon Kohen</i>	471
Methylerythritol Phosphate Pathway of Isoprenoid Biosynthesis <i>Lishan Zhao, Wei-chen Chang, Youli Xiao, Hung-wen Liu, and Pinghua Liu</i>	497
Posttranslational Biosynthesis of the Protein-Derived Cofactor Tryptophan Tryptophylquinone <i>Victor L. Davidson and Carrie M. Wilmot</i>	531
Mitochondrial Complex I <i>Judy Hirst</i>	551
Photosystem II: The Reaction Center of Oxygenic Photosynthesis <i>David J. Vinyard, Gennady M. Ananyev, and G. Charles Dismukes</i>	577
The Voltage-Gated Calcium Channel Functions as the Molecular Switch of Synaptic Transmission <i>Daphne Atlas</i>	607
Sphingosine-1-Phosphate and Its Receptors: Structure, Signaling, and Influence <i>Hugh Rosen, Raymond C. Stevens, Michael Hanson, Edward Roberts, Michael B.A. Oldstone</i>	637
Membrane Fission Reactions of the Mammalian ESCRT Pathway <i>John McCullough, Leremy A. Colf, and Wesley I. Sundquist</i>	663
Signal Recognition Particle: An Essential Protein-Targeting Machine <i>David Akopian, Kuang Shen, Xin Zhang, and Shu-ou Shan</i>	693
Peroxisome Formation and Maintenance Are Dependent on the Endoplasmic Reticulum <i>Henk F. Tabak, Ineke Braakman, and Adabella van der Zand</i>	723

Systemic Amyloidoses	
<i>Luis M. Blancas-Mejía and Marina Ramirez-Alvarado</i>	745
Nanobodies: Natural Single-Domain Antibodies	
<i>Serge Muyldermans</i>	775

Indexes

Cumulative Index of Contributing Authors, Volumes 78–82	799
Cumulative Index of Article Titles, Volumes 78–82	803

Errata

An online log of corrections to *Annual Review of Biochemistry* articles may be found at <http://biochem.annualreviews.org/errata.shtml>