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A time-resolved vibrational spectroscopy glimpse into the oxygen-evolving complex of photosynthesis

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Oxygenic photosynthesis is arguably one of the most important biochemical processes occurring in the biosphere. Photons produced by solar fusion are harvested by photosynthetic organisms and are used to drive the production of reducing equivalents in the form of NADPH and energy equivalents in the form of ATP, both of which are subsequently used in carbohydrate biosynthesis. Central to this process is the extraction of electrons from water with the concomitant production of molecular oxygen. This extraction is carried out by an exquisite molecular machine called photosystem II. Photosynthetic organisms consequently generate virtually all of Earth's atmospheric oxygen and produce the carbohydrates that lie at the base of nearly all food chains. In this issue of PNAS, Barry *et al.* (1) describe the use of time-resolved vibrational spectroscopy to detect protein-centered intermediates in the oxygen-evolving process. This study paves the way for the identification of the protein domains responsible for these transient signals, which should in turn provide critical details for unraveling the mechanism for oxygen evolution.

Photosystem II is an oligomeric energy-transducing membrane protein complex containing at least 20 protein subunits. Recent x-ray crystal structures (2, 3) at moderate resolution (3.0–3.5 Å) have provided an initial glimpse into the overall architecture of the photosystem and the location of its active site metal cluster. This metal cluster, which is unprecedented in other biochemical systems, contains a mixed valence tetramanganese (III_2–IV_2)–calcium cluster with a closely associated chloride ion. The manganese ions appear to be coordinated by a mixture of di-μ-oxy and mono-μ-oxy bridging ligands, some of which are provided by nearby carboxylates. A redox-active tyrosine termed $Y_Z^{\bullet}$ is in close proximity to the metal cluster and serves as the primary electron acceptor from the oxygen-evolving complex (4). The structural details of the metal cluster are quite controversial. Barber *et al.* (2) favor a distorted cubane structure, whereas Loll *et al.* (5) favor the trimer + 1 architecture that had been proposed based on EPR/ENDOR experiments (6). Additionally, none of the current crystallographic studies allow direct resolution of the Mn–Mn and Mn–Ca distances; these studies all fit distances derived from extended x-ray absorption fine structure (EXAFS) experiments to their crystallographic observations (7). Finally, although the functional metal cluster contains high valence manganese, the manganese in the crystals is rapidly photoreduced to Mn(II) under the conditions used to collect crystallographic data (8); this finding has important negative consequences on the reliability of the proposed architectures of the manganese-calcium cluster modeled in all of the currently available structural studies.

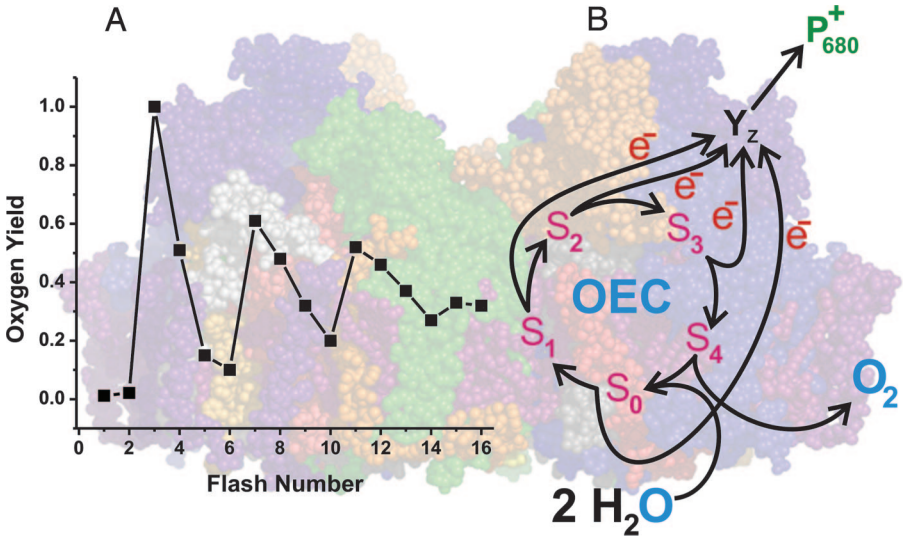

Fig. 1. Oxygen release pattern and Kok S state model for photosynthetic oxygen evolution. (*A*) Typical oxygen release pattern observed for all organisms carrying out oxygenic photosynthesis. The oxygen yield initially peaks on the third flash. Thereafter, the oxygen yield peaks on every fourth flash. This oscillatory pattern dampens with increasing flash number due to small proportions of photosystem II centers that experience misses, double hits, and deactivations during the flash train. (*B*) Kok cycle illustrating the S state transitions. The sequential progression of S states is driven by the primary photoact of photosystem II, the light-driven oxidation of P_{680} to P_{680}^{+}. The oxidizing equivalent is first transferred to $Y_Z^{\bullet}$ and then to the oxygen-evolving complex (OEC), where it is accumulated, with a concomitant S state transition, $S_n \rightarrow S_{n+1}$, taking place. The accumulation of four oxidizing equivalents leads to the release of dioxygen, with the subsequent binding of two water molecules and a resetting of the S state to S_0. For simplicity, the protons released during water oxidation are not illustrated. Shown in the background is the 3.0-Å resolution crystal structure of photosystem II from the cyanobacterium *Thermosynechococcus elongatus* (5).

From a functional standpoint, early studies indicated that dioxygen production followed a biochemically unique pattern. The measurement of oxygen yield after trains of high-intensity light flashes demonstrated that oxygen was initially released on the third flash and subsequently was released with a periodicity of four (9) (Fig. 1*A*). The classical "Kok cycle" has been used to explain this highly unusual pattern of oxygen release (10). In this model, five so-called "S states," numbered S_0 to S_4, represent the number of oxidizing equivalents accumulated on the oxidizing-side of photosystem II (Fig. 1*B*). Each of these oxidations is generated by a light-driven charge separation occurring at the primary electron donor of the photosystem, chlorophyll P_{680}. Upon storage of four oxidizing equivalents in the S_4 state, oxygen is released, two water molecules bind, and the cycle is reset to S_0. Until recently, it was unclear whether the S_4 state was a discrete step or simply a transitional phase in the conversion of $S_3 \rightarrow S_0$. Recent evidence indicates that, indeed, S_4 is a kinetically resolvable state in the Kok cycle (11, 12). In dark-adapted samples, most of the oxygen-evolving complexes are in the S_1 state,

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storing one oxidizing equivalent. Consequently, oxygen is released on the third flash of light in the initial cycle and on every fourth flash of light thereafter, as was observed experimentally.

Since these early seminal observations, a vast number of investigations have focused on elucidation of the properties of the oxygen-evolving complex. These studies have used a wide array of spectroscopic methods spanning nearly the entire available spectrum to probe structure and functional relationships within the photosystem. EXAFS and x-ray absorption near-edge spectroscopy (XANES) (13), UV (11, 14), and vibrational spectroscopy and steady-state and pulsed EPR methods (15), among others, have all been used to probe the function and architecture of the oxygen-evolving site. Time-resolved vibrational spectroscopy is becoming a technique of choice for the investigation of rapid protein changes occurring during the catalytic turnover of enzymes (16). Barry *et al.* (1) have used this technique to observe infrared transients that occur on microsecond to millisecond time scales during each of the four stepwise oxidations of the water molecules bound at the active site of photosystem II. Most importantly, the time signatures of these transients oscillate with the same periodicity as is observed for oxygen release [compare Fig. 1*A* with figure 3 of Barry *et al.* (1)]. This observation directly and unambiguously couples the observed vibrational signals with the S state transitions occurring during the Kok cycle. During the $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions, the observed signals occur with rates similar to those previously observed for manganese oxidation (12). Surprisingly, mathematical fits to the data suggest that, during the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4 \rightarrow S_0$ transitions, the fast transients occur with time constants of 60 and 300 μs, respectively. These values are four to five times faster than the reported rate for manganese oxidation observed by x-ray fluorescence ($t_{1/2}$ values of 190 μs and 1.1 ms, respectively) (12). These results indicate that, during these two S state transitions, protein-centered changes (conformational changes, protonation or deprotonation, etc.) occur before manganese oxidation takes place. It should be noted that Haumann *et al.* (12) also suggested that proton transfer was preceding electron transfer during the $S_3 \rightarrow S_4 \rightarrow S_0$ transition. These results are quite unexpected and will place significant constraints on proposed mechanisms that are responsible for the photosynthetic oxygen evolution process.

It is expected that these studies will stimulate a number of future investigations that will examine the basis for these S state-associated signals. Which amino acids are involved? If conformational changes occur, are these a result of catalysis or are they the basis for catalysis? If protonation/deprotonation events give rise to the observed signals, do these occur in a sequential or concerted manner with respect to electron transport? The answers to these questions and many others may help unravel the mechanistic details of this amazing oxygen-producing molecular machine, photosystem II.

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1. Barry, B. A., Cooper, I. B., De Riso, A., Brewer, S. H., Vu, D. M. & Dyer, R. B. (2006) *Proc. Natl. Acad. Sci. USA* **103,** 7288–7291.
2. Barber, J., Ferreira, K., Maghlaoui, K. & Iwata, S. (2004) *Phys. Chem. Chem. Phys.* **6,** 4737–4742.
3. Biesiadka, J., Loll, B., Kern, J., Irrgang, K.-D. & Zouni, A. (2004) *Phys. Chem. Chem. Phys.* **6,** 4733–4736.
4. Debus, R. J., Barry, B. A., Sithole, I., Babcock, G. T. & McIntosh, L. (1988) *Biochemistry* **27,** 9071–9074.
5. Loll, B., Kern, N., Saenger, W., Zouni, A. & Biesiadka, J. (2006) *Nature* **438,** 1040–1044.
6. Peloquin, J. M. & Britt, R. D. (2001) *Biochim. Biophys. Acta* **1503,** 96–111.
7. Yachandra, V. K. (2002) *Philos. Trans. R. Soc. London B* **357,** 1347–1388.
8. Yano, J., Kern, J., Irrgang, K. D., Latimer, M. J., Bergmann, U., Glatzel, P., Pushkar, Y., Biesiadka, J., Loll, B., Sauer, K., *et al.* (2005) *Proc. Natl. Acad. Sci. USA* **102,** 12047–12052.
9. Joliot, P. & Joliot, A. (1968) *Biochim. Biophys. Acta* **153,** 625–634.
10. Kok, B., Forbush, B. & McGloin, M. (1970) *Photochem. Photobiol.* **11,** 457–475.
11. Clausen, J. & Junge, W. (2004) *Nature* **430,** 480–483.
12. Haumann, M., Liebisch, P., Muller, C., Barra, M., Grabolle, M. & Dau, H. (2005) *Science* **310,** 1019–1021.
13. Dau, H., Liebisch, P. & Haumann, M. (2002) *Anal. Bioanal. Chem.* **376,** 562–583.
14. Dekker, J. P., Plijter, J. J., Ouwehand, L. & van Gorkom, H. J. (1984) *Biochim. Biophys. Acta.* **767,** 176–179.
15. Britt, R. D., Peloquin, J. M. & Campbell, K. A. (2000) *Annu. Rev. Biophys. Biomol. Struct.* **29,** 463–495.
16. Kotting, C. & Gerwert, K. (2005) *Chem. Phys. Chem.* **6,** 881–888.