How Plants Make Oxygen

A biochemical mechanism called the water-oxidizing clock enables plants and some bacteria to exploit solar energy to split water molecules into oxygen gas, protons and electrons

by Govindjee and William J. Coleman

Because molecular oxygen is a necessity of life for human beings, it is easy to forget that simple organisms lived without it for hundreds of millions of years. For those early anaerobic organisms, oxygen was a toxic substance that could steal essential electrons from the molecules in their cells. It may seem surprising, then, that many of these anaerobic cells engaged in a form of photosynthesis, because photosynthesis produces all the oxygen in the atmosphere. The exact process by which oxygen is generated in photosynthesis has been something of a mystery, but now the mechanism can be described in some detail. It is a "water-oxidizing clock" that generates a molecule of oxygen with every four ticks.

The fundamental task of photosynthesis is to make it possible for cells to convert carbon dioxide into carbohydrates with energy absorbed from the sun. The production of oxygen is not crucial, which is why anaerobic cells could photosynthesize without making molecular oxygen long ago and why they have continued to do so to this day.

GOVINDJEE and WILLIAM J. COLEMAN have collaborated on studies of the mechanism of oxygen evolution in green plants. Govindjee is professor of biophysics and plant biology at the University of Illinois at Urbana-Champaign. He was born in Allahabad, India, and received his Ph.D. in biophysics from Urbana in 1960. He has written many books, papers and reviews on the subject of photosynthesis, including two previous articles for *Scientific American*. Coleman is a National Science Foundation postdoctoral fellow studying bacterial photosynthesis in the department of chemistry at the Massachusetts Institute of Technology. He received his bachelor's degree in biology and literature from the University of Pennsylvania in 1979 and his Ph.D. from Urbana in 1987 for his work with Govindjee.

If oxygen is toxic, why and how did green plants and their ancestors ever begin producing it through photosynthesis? The answer to the first question involves energy metabolism. Sunlight provides the energy that drives life on the earth, but cells cannot store or employ that light energy directly; it must be converted into a more usable, chemical form. Electrons are part of the common "currency" of biological energy conversion: many energetic reactions in cells can be generally understood as the transfer of electrons between molecules.

To live, therefore, cells need a source of electrons. Anoxygenic photosynthetic bacteria typically oxidize, or draw electrons from, organic acids and simple inorganic compounds. These substances are relatively rare, however; consequently, anoxygenic bacteria survive today only in sulfur springs, lake bottoms and similar environments where such molecules are sufficiently plentiful.

Approximately three billion years ago, however, some photosynthetic cells learned how to spread into virtually any environment by tapping the electrons in a nearly ubiquitous substance: water. They evolved the ability to split pairs of water (H_2O) molecules into electrons, protons (H^+'s, or hydrogen nuclei) and molecular oxygen (O_2). The electrons and protons were energetically useful; the O_2 was simply a by-product. In short, the evolution of O_2 was a breakthrough for photosynthetic organisms not because O_2 was important in itself but because it meant that photosynthetic cells could exploit water and invade new, more diverse environments.

How cells make oxygen is a far more complicated question. Developing the ability to draw on water as an electron source was no simple feat, and it demanded several modifications to the established photosynthetic mechanism. Because water molecules give up their electrons only grudgingly, the relatively weak oxidant (electron-accepting molecule) that anoxygenic photosynthetic bacteria were able to generate with sunlight had to be replaced with a much stronger one. Even so, the energy available from a single photon, or quantum unit, of visible light is not sufficient to split a water molecule. This problem appears to have been solved by drawing the energy from four photons to split two water molecules, thereby releasing four electrons and four protons. Such a mechanism creates yet another difficulty, however, because the photochemical apparatus can handle only one electron at a time.

To solve that problem, photosynthetic cells developed the special water-splitting catalyst that we call the water-oxidizing clock: a unique biochemical ratcheting mechanism for stabilizing intermediate stages of the water-splitting reaction so that the electrons could be transferred one by one. In recent years much has been learned about the workings of the water-oxidizing clock and its place in the overall process of photosynthesis.

In higher plants, the primary reactions of photosynthesis take place within specialized thylakoid membranes inside the cell structures called chloroplasts. Embedded in the thylakoid membranes are various protein complexes, each of which contributes to the total photosynthetic reaction. The generation of O_2 takes place entirely within the complex of proteins

OXYGEN-RICH BUBBLES on the leaves of a submerged green plant show that photosynthesis is taking place. The oxygen (O_2) is a product of a light-driven reaction in which pairs of water (H_2O) molecules are stripped of four electrons (e^-) and four protons (H^+). Anoxygenic photosynthetic bacteria cannot split water molecules in this way and must get essential electrons from other sources.

and pigments known as photosystem II, which is found in the cells of all oxygenic photosynthesizers: cyanobacteria, algae and other plants containing chlorophyll pigments.

The essential task of photosystem II is to act as a tiny capacitor, storing energy by separating and stabilizing positive and negative charges on either side of the thylakoid membrane. To do this, an array of specialized pigments in photosystem II absorbs a photon and efficiently converts this light energy into a widening separation of charge.

Orchestrating the movements in the complex process of converting light energy into a separation of charge requires the collaboration of many specialized polypeptides and proteins in the photosystem. Polypeptides are linear polymers of amino acids arranged in a defined sequence; they are often many hundreds of amino acids in length. Proteins consist of one or more polypeptides folded into intricate, orderly structures.

The electron-transfer reactions in photosystem II take place within the so-called reaction center. The major structural components of the reaction center are the large polypeptides named D1 and D2 and a smaller protein named cytochrome b_{559}. A polypeptide with a molecular weight of 33 kilodaltons and at least two others of different weights are bound to the inner surface of the thylakoid membrane. These polypeptides serve as a stabilizing matrix for the pigments and other molecules in photosystem II that perform the electron-transfer and oxygen-producing reactions. Other small polypeptides are associated with photosystem II, but their functions are still unknown. Several organic ions and charged atoms—manganese, chloride, calcium, iron and bicarbonate—are involved in catalyzing electron transfer, maintaining the protein structure or regulating the photosystem's activity.

In addition, large numbers of "antenna" chlorophyll molecules collect light energy and funnel it efficiently to the reaction center. Several hundred antenna pigment molecules are associated with each reaction center.

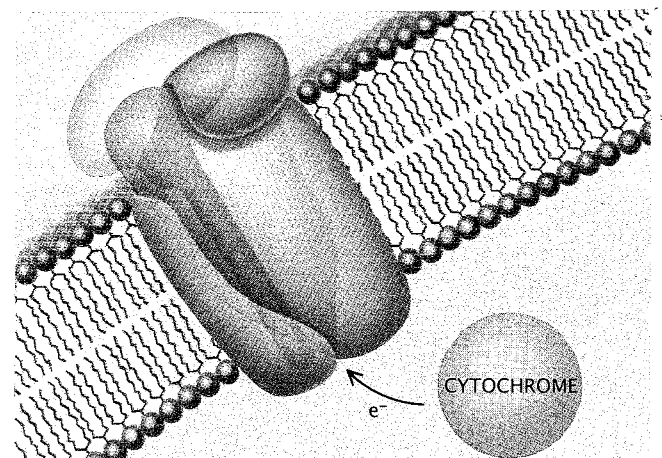
Because the structure of photosystem II is so complicated, many advances in understanding it have come from studies of equivalent complexes in photosynthetic bacteria. The work of Johann Deisenhofer, Robert Huber and Hartmut Michel, who determined the structure of the photosynthetic reaction center in the bacterium *Rhodopseudomonas viridis*, earned them the Nobel prize for chemistry in 1988.

There are many differences between bacterial and green-plant photosynthetic complexes. As previously noted, bacteria do not produce molecular oxygen; moreover, they depend not on chlorophyll but on the pigment bacteriochlorophyll, which absorbs light maximally at a much longer wavelength and is a much weaker oxidant. Yet bacterial photosynthetic complex-

es do catalyze the essential reaction that converts light into an electrochemical potential gradient across a biological membrane [see "Molecular Mechanisms of Photosynthesis," by Douglas C. Youvan and Barry L. Marrs; SCIENTIFIC AMERICAN, June, 1987].

From these bacterial studies, it appears that the electron-transporting mechanism in the reaction center of photosystem II has five components: a chlorophyll pigment that acts as the primary donor of an electron; a secondary electron donor named Z that reduces the chlorophyll (that is, it replaces the electron that the chlorophyll has lost); pheophytin, a pigment that accepts an electron from the chlorophyll; a primary plastoquinone electron acceptor, Q_A; and a secondary quinone electron acceptor, Q_B.

The chlorophyll pigment in the reaction center is believed to consist of a special pair of chlorophyll molecules that seem to be chemically identical to many of the antenna pigments but are functionally different. The pigment is named P680 because it most strongly absorbs light that has a wavelength of 680 nanometers.



PHOTOSYSTEM II is the protein-pigment complex in the thylakoid membrane of chloroplasts that produces oxygen and traps light energy. The flow of electrons through the photosystem (*above*) is driven by the absorption of a photon by a special pair of chlorophylls (P680). The process involves many other molecules, including pheophytin (Pheo) and two kinds of quinone (Q_A, Q_B) molecules, and such metal atoms and ions as manganese (Mn), chloride (Cl^-) and calcium (Ca^{2+}). In this simplified model, several pigment molecules have been omitted for clarity. Photosystem II reactions near the inner side of the membrane take four electrons from two water molecules and make molecular oxygen; these reactions constitute the water-oxidizing clock. Protons released from these reactions contribute to the synthesis of adenosine triphosphate. A simpler photosystem from an anoxygenic bacterium (*right*) lacks a water-oxidizing clock. Electrons from compounds other than water are passed to this photosystem by a cytochrome protein.

In 1988 the work of Bridgette A. Barry and Richard J. Debus, who were then at Michigan State University, and Willem F. Vermaas of Arizona State University and their colleagues helped to identify Z as one of the amino acids (known as a tyrosine) within the D1 polypeptide. The quinone Q_A is tightly bound to the photosystem II complex, but Q_B can diffuse freely between protein complexes in the membrane when it has accepted two electrons.

During photosynthesis the antenna pigments absorb a photon and funnel this energy to the P680 in the reaction center. There, the excitation energy is converted into a charge separation when the P680 enters an excited state and quickly passes one electron to a nearby pheophytin molecule [*see illustration on next page*]. The pheophytin now carries an excess negative charge, whereas there is a positively charged "hole" on the P680 because it has lost an electron; the P680 has become a $P680^+$. The separation of the charges gets wider when the pheophytin passes its extra electron to Q_A. The distance increases still more when Z donates an electron to the $P680^+$ and picks up the positive charge and the Q_A donates its extra electron to Q_B.

The transfers of charge take place rapidly, especially the initial transfer of an electron from the excited P680 to pheophytin, which occurs within a few trillionths of a second. This was shown by one of us (Govindjee) in collaboration with Michael R. Wasielewski and Douglas G. Johnson of the Argonne National Laboratory and Michael Seibert of the Solar Energy Research Institute in Golden, Colo.

The stepwise transfer of electrons succeeds in pulling far apart the mutually attractive positive and negative charges. Yet the photosynthetic cycle in photosystem II is not complete until all the components of the reaction mechanism are electrically neutral again and ready to begin the charge-separation process anew. How does the Q_B eliminate its negative charge, and how does the Z regain the electron it has lost?

At the Q_B end of the system, the answer is relatively simple. After the Q_B has acquired two electrons and two protons through two photon-absorption cycles, the doubly reduced Q_B diffuses out of the photosystem II complex and is replaced by an unreduced Q_B. The electrons and protons on the freely moving Q_B are carried to still another complex in the photosynthetic pathway. The protons released on the inner side of the thylakoid membrane are eventually exploited to make adenosine triphosphate, an energy-storing compound essential for cellular metabolism.

At the opposite end of photosystem II, it is much harder for the Z to obtain the electron it needs to return to its original state. The electron must come from some oxidizable substance that is available in the cell's environment. Organic acids (such as acetate, malate and succinate) and simple inorganic compounds (such as sulfide and thiosulfate) can be good electron sources, and they are the ones that anoxygenic photosynthetic bacteria exploit; in these bacteria, which lack Z, a cytochrome protein shuttles an electron to the oxidized special pair of chlorophylls in the reaction center.

A molecule far more abundant than organic acids are—and therefore a potentially richer source of electrons—is water. Yet, although the oxidizing strength of $P680^+$ is great, it is not sufficient by itself to strip water molecules of their electrons.

The problem is that the water-oxidizing reaction releases four electrons simultaneously, whereas $P680^+$ can accept only one electron at a time. It therefore became clear to investigators a few decades ago that there must be a catalytic site near Z and the P680 that can, in effect, prolong the oxidation reaction. This water-splitting catalyst must associate with pairs of water molecules and stabilize them during a gradual oxidation process in which electrons are taken away one at a time. The search for this mechanism eventually led to the discovery of the water-oxidizing clock.

An important clue to how this mechanism works was derived from the observation that not all the electrons reach the $P680^+$ chlorophyll molecule at the same rate. Instead the observed transfer time for the electrons varies periodically. This has been demonstrated by experiments in which membranes containing photosystem II reaction centers are placed in darkness and then exposed to brief flashes of light. Each flash is not only very intense but also as brief as possible, so that it sends (on average) only one photon into the photosystem. The observed result is that $P680^+$ recovers an electron in darkness at different rates, depending on the number of light flashes.

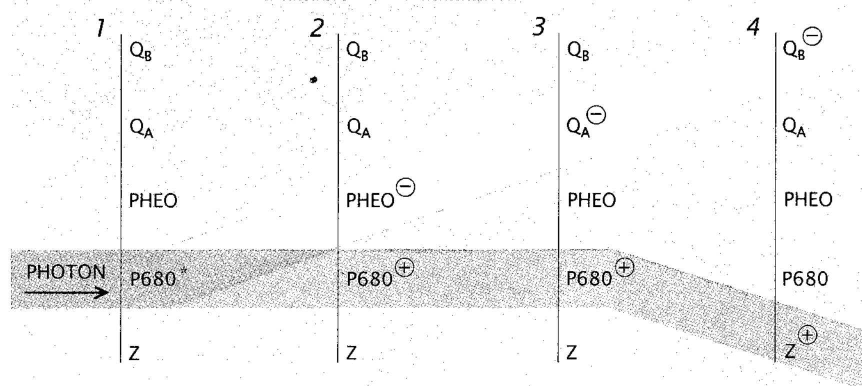
For example, the time for half of the $P680^+$'s to convert back to P680's is approximately 20 billionths of a second after the first and fifth flashes but is much longer after the second, third and fourth flashes. The change in recovery time varies cyclically every four flashes. The four-point periodicity suggests that a cyclic reaction with four steps donates electrons to the reaction center.

These studies of the behavior of P680 were especially important. Pierre Joliot of the Institute of Physicochemical Biology in Paris had previously demonstrated in 1969 that there was a four-point periodicity in the photosynthetic production of oxygen. With a highly sensitive platinum-electrode measuring device that responded to traces of oxygen, Joliot measured the amount of gas that evolved after a series of flashes. There was no O_2 evolution after the first flash and none (or very little) after the second, but there was a maximum release after the third. Thereafter, the amplitude of the O_2 yield oscillated with a period of four until the differences gradually were damped out, or diminished.

Bessel Kok of the Martin Marietta Laboratories in Baltimore proposed a simple hypothesis in 1970 to explain Joliot's results, an idea that came to be known as the water-oxidizing clock or cycle. Kok suggested that the oxygen-producing complex in photosystem II could exist in several different, transient states of oxidation that he called S states. He could not define the chemical nature of the S states precisely, but he hypothesized that each S state made a unique contribution to a four-stage cyclic mechanism.

Kok suggested that, in darkness, the clock settles into one of two S states: S_0 or S_1. The predominant (and more stable) state is S_1, which has one more oxidizing equivalent than S_0; in other words, the complex of molecules corresponding to S_1 has one fewer electron than does the S_0 complex. The chemical basis for the predominance of S_1 is not known.

After one flash of light, P680 becomes $P680^+$ and must eventually be reduced by an electron. Kok hypothesized that the clock must undergo a change that boosts it into the next-highest oxidation state: a clock that starts at S_1 goes to S_2, and one that starts at S_0 goes to S_1. The transition occurs because one electron is released from the clock to convert $P680^+$ back to P680. A second flash creates another $P680^+$ and boosts the S_2's to S_3's, and a third flash converts the S_3's to S_4's. When the clock reaches the S_4 state, it has released four electrons and is ready to complete the water-



STEPWISE ELECTRON TRANSFER in the photosystem II reaction center stores some light energy in the form of separated positive and negative charges. When the P680 absorbs a photon, it enters an excited state and becomes P680* (*1*). The P680* donates an electron to a pheophytin molecule and is left with an electron deficit, or positive charge (*2*). The pheophytin's negative charge is passed to a primary quinone molecule, Q_A (*3*). Finally, the $P680^+$ takes an electron from Z, an amino acid, and Q_A passes its extra electron to Q_B, another quinone molecule (*4*). The electron-transfer chain returns to its original state when Z accepts an electron from the water-oxidizing clock and the doubly reduced Q_B is replaced by an unreduced quinone.

splitting reaction. The clock then removes four electrons from the two bound water molecules, releases O_2 and drops from S_4 back to S_0, making it possible for the cycle to begin again.

This situation is not unlike that of a base runner in baseball: the player must tag all four bases in sequence to end up where he started. If the player misses a tag, he may be forced to retreat; similarly, there is a possibility that the clocks will not progress smoothly from one state to the next. A small probability exists, for example, that S_1 will not change to S_2 after a flash because the photosystem did not utilize the photon efficiently. There is also a low probability that a photosystem will absorb two photons during one flash (if the flashes are not extremely short) and that the water-oxidizing clock may advance in one step from S_1 to S_3 via S_2.

Kok's mechanism explained Joliot's observations of the clock's oxygen-producing behavior. Because most of the clocks in a dark-adapted sample are in the S_1 state, the maximum release of oxygen takes place after the third flash, when the clocks change from S_3 to S_4 to S_0 and spontaneously release oxygen. The clocks that began in the S_0 state release O_2 after the fourth flash, which is why there is a small oxygen release then.

Those random "errors" that occur when a few clocks fail to advance during a flash or when they advance by two S states can account for the gradual damping-out in the O_2-release oscillations. These processes slowly desynchronize the turnover of the clocks in the sample. After many flashes, there is an equilibrium such that the numbers of S_0, S_1, S_2 and S_3 clocks are roughly equal, and the yield of oxygen after each flash remains steady. The situation is analogous to that of a room filled with grandfather clocks: initially they may all chime loudly and synchronously on the hour, but as the clocks variously gain or lose time, the room begins to reverberate with a continuous soft chime.

Joliot and Kok's discovery of the water-oxidizing clock replaced the black box of oxygen production with a new theoretical mechanism. The theory did not explain, however, the physical makeup of the clock or the interactions of the clock with water molecules. A long search soon began for the chemical nature of the charge accumulator in the clock—the material or materials whose variable oxidation states constitute each of the S states.

From the beginning it was assumed that this elusive chemical entity was a metal atom. Protein-bound atoms of transition metals, such as manganese, iron and copper, are good candidates for catalyzing oxidation-reduction reactions because of their ability to donate and accept electrons alternately.

Manganese (Mn) is believed to make up at least part of the charge accumulator because, as has been long known, O_2 production does not take place unless there are four atoms of manganese in photosystem II for every P680 molecule. Manganese is known to catalyze electron-transfer reactions in other enzymes. It can also assume several relatively stable oxidation states, from +2 to +7; that is, manganese ions can variously share between two and seven electrons with other atoms. When the metal is bound to a large molecule such as a protein, these oxidation states are usually abbreviated as Mn(II), Mn(III) and so on.

Metal-containing proteins have been analyzed extensively by the general technique called spectroscopy because some metal complexes absorb particular forms of electromagnetic radiation. If this absorption is measured carefully, it can serve as a spectroscopic "fingerprint" of the protein-bound metal and provide clues to its nuclear or electronic structure. Spectroscopy is especially well suited to the study of manganese compounds. Many of the biologically relevant manganese complexes are "paramagnetic": the manganese atom contains electrons with unpaired spins, and these electrons, like tiny bar magnets, interact strongly with applied external magnetic fields.

Several highly sensitive measuring techniques have exploited the paramagnetic properties of manganese, most notably electron paramagnetic resonance (EPR) spectroscopy. With EPR, changes in the electronic structure of the manganese complex that follow the absorption of light by photosystem II have been studied. Another informative approach has been nuclear magnetic resonance (NMR) spectroscopy, which can measure the properties of the manganese atoms indirectly by monitoring protons in the water molecules that are in contact with the manganese. While working at the University of Illinois at Urbana-Champaign in the mid-1970's, Thomas J. Wydrzynski pioneered the use of NMR to study dynamic changes in the oxidation state of manganese.

X-ray spectroscopy techniques have made valuable contributions to the study of oxidation states and of the physical environment of the manganese atoms in photosystem II. Other studies of the chemical composition of S states have used optical spectroscopy, because manganese complexes have unique absorption bands in the ultraviolet region of the electromagnetic spectrum.

It is worth pointing out, however, that despite the wide range of applicable spectroscopic techniques, two major difficulties have humbled scientists studying the photosynthetic membrane. First, the membrane is complex, and many of its components have overlapping absorption spectra.

Second, because neither the structure nor the chemical nature of the photosystem II complex is known precisely, the data from spectroscopic analyses of the water-oxidizing clock cannot be interpreted definitively. As a result, we do not yet know conclusively what chemically constitutes the various S states. It has been possible, nonetheless, to develop a tentative picture.

It is clear that the manganese atoms undergo dynamic changes, including changes in their oxidation states, during the S-state transitions. A four-point periodicity has been observed in the manganese oxidation-state changes, as Kok's model had suggested. One surprising discovery is that the manganese atoms do not become consistently more oxidized throughout the cycle. S_2 is more oxidized than S_1, and S_1 is more oxidized than S_0, but there is no discernible change in the manganese oxidation states between S_2 and S_3. It seems, then, that the positive charge the clock acquires during its transition from S_2 to S_3 must be carried on some feature of the clock other than the manganese atoms. One of us (Govindjee), together with Subhash Padhye, Takeshi Kambara and David N. Hendrickson of the University of Illinois at Urbana-Champaign, proposed in 1986 that the amino acid histidine in one of the proteins in the clock could possibly store a positive charge.

Work by Melvin P. Klein, Kenneth Sauer and their co-workers at the University of California at Berkeley and by Robert R. Sharp and his colleagues at the University of Michigan at Ann Arbor has helped define the oxidation states of some of the manganese atoms more precisely. Tentatively, S_0 has been identified with the presence of Mn(II), S_1 with Mn(III) and S_2 with Mn(IV). Both Mn(II) and Mn(III) appear to be stable and long-lived in photosystem II; these observations corroborate Kok's prediction of stable S_0 and S_1 states. In contrast, the Mn(IV) associated with S_2 is a relatively transient intermediate. Recent evidence collected in the laboratory of Horst T. Witt of the Technical University in West Berlin indicates that during the S_0-to-S_1 transition, an Mn(II) ion converts to an Mn(III) ion. The only conversions observed during subsequent transitions are from Mn(III) to Mn(IV).

Low-temperature EPR studies by G. Charles Dismukes and Yona Siderer of Princeton University suggest that the S_2 and S_3 states involve multinuclear complexes with as many as four manganese atoms. For example, the S_2 state may be a mixed-valence group consisting of one Mn(III) atom and one Mn(IV) atom or of three Mn(III) atoms and one Mn(IV) atom.

In summary, dynamic changes in the oxidation states of the manganese atoms bound within photosystem II unquestionably correspond to changes in the S states of Kok's clock. The precise chemical and electronic configurations of these states are still uncertain and are under study.

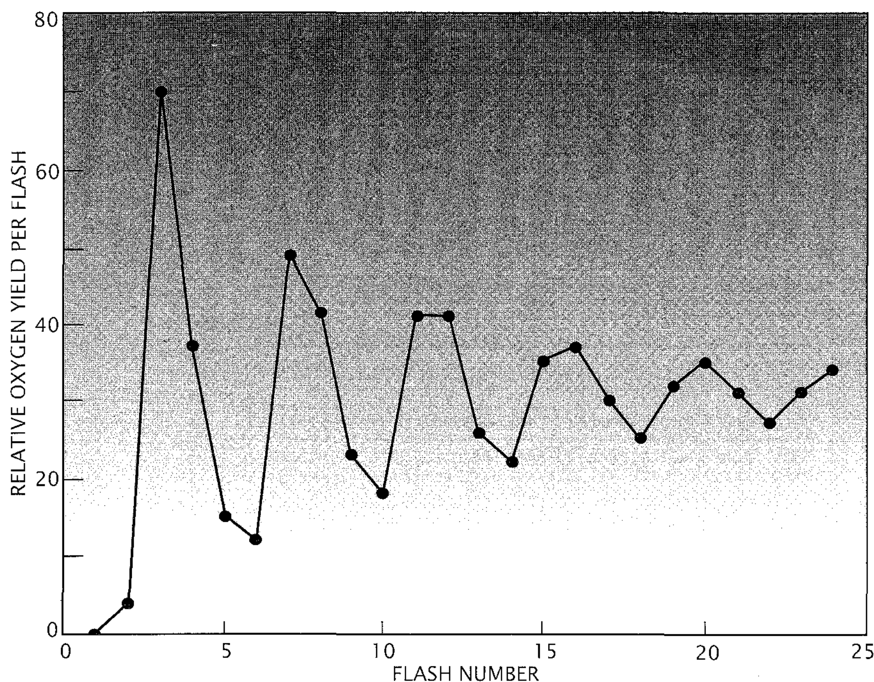
Various experiments have indicated that manganese is probably not bound directly to any of the small polypeptides in the photosystem II complex. This leaves the large D1 and D2 polypeptides as the most likely sites for manganese binding. We have recently proposed that four manganese-binding sites may exist on the D1 and D2 polypeptides on the inner side of the thylakoid membrane, but other investigators have suggested that manganese is bound across the interface between the D1, D2 and 33-kilodalton polypeptides.

X-ray spectroscopy by Klein, Sauer and their collaborators at Berkeley and by Graham N. George and Roger C. Prince of Exxon Research in Annandale, N.J., has revealed some details of the arrangement of the manganese atoms. In the S_1 state, two of the atoms appear to be part of a binuclear complex and are separated by only 2.7 angstroms. (One angstrom is one ten-millionth of a millimeter.) The other pair of manganese atoms is separated by a larger distance. The atoms can be imagined as being at the four corners of a trapezoid.

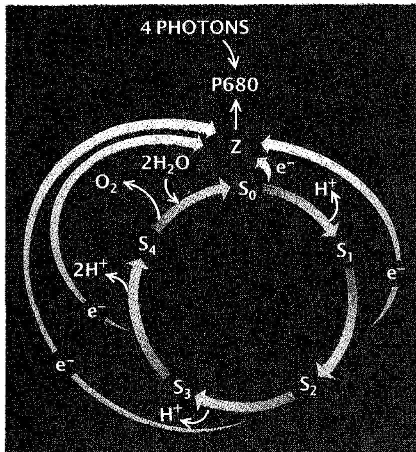
Because of all these studies, much more is now known about how manganese atoms might catalyze the removal of electrons from water to reduce $P680^+$. Electrons, however, are not the whole story: the water-splitting reaction also produces four protons. Are all four protons released at once, simultaneously with the release of O_2, or are they liberated sequentially along with the electrons?

This question has been answered by careful measurements of proton release in response to a series of flashes. Because the release of protons increases the acidity of the surrounding fluid, the timing of proton release can be studied with electrodes and dyes that are highly sensitive to acidity. C. Frederick Fowler of Martin Marietta and, soon thereafter, Satham Saphon and Anthony R. Crofts, then at the University of Bristol in England, discovered that the four protons are released sequentially. One is released during the S_0-to-S_1 transition, none during S_1-to-S_2, one during S_2-to-S_3 and two during the S_3-to-S_4-to-S_0 transition.

These findings have important im-



YIELD OF OXYGEN from photosynthetic membranes exposed to a series of brief flashes oscillates with a four-point periodicity. It is highest after the third flash and peaks again four flashes later, but the variation in the amplitude gradually decreases as the number of flashes increases. The occurrence of the peaks and the damping of the oscillation are explained by the four-step cycle of the water-oxidizing clock.



WATER-OXIDIZING CLOCK is a cyclic mechanism that supplies electrons to the P680 chlorophylls in the photosystem II reaction center. As each photon is absorbed by the P680, the clock advances by one S state, or oxidation state, and thereby releases one electron (e^-). When the clock reaches S_4, it spontaneously releases an oxygen (O_2) molecule and reverts to the S_0 state to close the cycle.

plications for the mechanism of the water-oxidizing clock, although their interpretation depends on whether the released protons come from the water molecules directly or from some other source, such as the polypeptides that bind the manganese atoms. If the protons come from the water, then the water molecules must be undergoing some chemical changes prior to the S_4 state. Conversely, if the sequentially released protons come directly from the polypeptides (and are later replaced by protons from the water molecules), then no water oxidation occurs until the final S_4-to-S_0 transition. The protons' immediate origin has not yet been determined.

Regardless of the source of the protons, it now seems likely that the higher S states (particularly S_2) accumulate some net positive charge. It is possible that a negatively charged ion may be needed to stabilize this positive charge, which could explain the observation that ions such as chloride are essential to keeping the water-oxidizing clock running. Seikichi Izawa of Wayne State University in Detroit was one of the first to demonstrate that chloride ions can turn on the water-oxidizing clock.

In collaboration with Herbert S. Gutowsky and his colleagues at the University of Illinois at Urbana-Champaign in 1982, we began to apply NMR techniques to monitor the binding of chloride ions to photosynthetic membranes. In early studies there, Ion C. Baianu, Christa Critchley and one of us (Govindjee) showed that chloride ions associate with and dissociate from isolated chloroplast membranes freely and rapidly. These findings led us to speculate in 1983 that the binding of a negatively charged chloride ion might be linked with the arrival of a positive charge on the water-oxidizing clock from $P680^+$ and that the release of the chloride ion might coincide with the release of protons.

NMR experiments by Christopher Preston and R. J. Pace of the Australian National University in Canberra suggested that chloride ions bind more tightly in the S_2 and S_3 states than in the S_0 and S_1 states. This finding is consistent with the more positively charged character of the higher S states. X-ray spectroscopy data collected by Klein and his colleagues indicate that chloride does not bind directly to the manganese atoms in the lower S states.

Peter H. Homann of Florida State University and his associates have suggested that chloride probably binds to positively charged amino acids on the proteins of the clock. Working with Gutowsky, we have made observations of chloride binding in photosystem II complexes from spinach. Our measurements indicate that several chloride ions bind to the clock and that they seem to be divided between two major binding sites: one near the manganese, perhaps on the D1 and D2 polypeptides, and the other on the 33-kilodalton polypeptide.

All these experiments suggest that the function of chloride ions in the water-oxidizing clock may be to expedite the release of protons from water. In doing this, the chloride ions may increase the efficiency of the water-oxidation reactions, or they may stabilize the charged manganese ions in the higher S states, or they may do both. The role of chloride is still controversial; it may turn out that chloride organizes the photosystem II proteins into a stable structure.

Another ion, calcium (Ca^{2+}), is essential for both the oxidation of water and the operation of the photosystem II reaction center, and it also appears to be intimately involved with the function of chloride. Experiments in several laboratories suggest that calcium ions can functionally replace two of the polypeptides at the bottom of photosystem II that are involved in the production of molecular oxygen. It has also been observed that the removal of calcium ions seems to block both the turnover of the water-oxidizing clock (by interrupting the S_3-to-S_4-to-S_0 transition) and the fast reduction of $P680^+$ to P680.

It seems likely, therefore, that calcium has a structural or regulatory role in photosystem II. Calcium has been shown to play an important part in controlling a wide variety of proteins in other biological systems: it switches the activity of the proteins on and off and maintains their three-dimensional structure. The calcium ions in photosystem II may put the polypeptides of the water-oxidizing clock into the correct functional conformation.

The elaborate mechanism that makes oxygen during photosynthesis is only one small part of the full photosynthetic pathway in oxygen-producing organisms. Although the general details are similar among all photosynthetic species, significant differences have arisen in the course of evolution.

Most analyses indicate that the differences between the photosystem II of cyanobacteria and that of plants are relatively minor, which suggests that cyanobacteria are ancestors of, or otherwise closely related to, plants. The differences between the reaction centers of cyanobacteria and those of many other photosynthetic bacteria are much more pronounced, revealing a clear division in the evolutionary pathway. More detailed studies of the photosystems by molecular genetics, X-ray crystallography and spectroscopy will undoubtedly refine understanding of the evolution of life.

FURTHER READING

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