PERSPECTIVES: PHOTOSYNTHESIS

Splitting Water

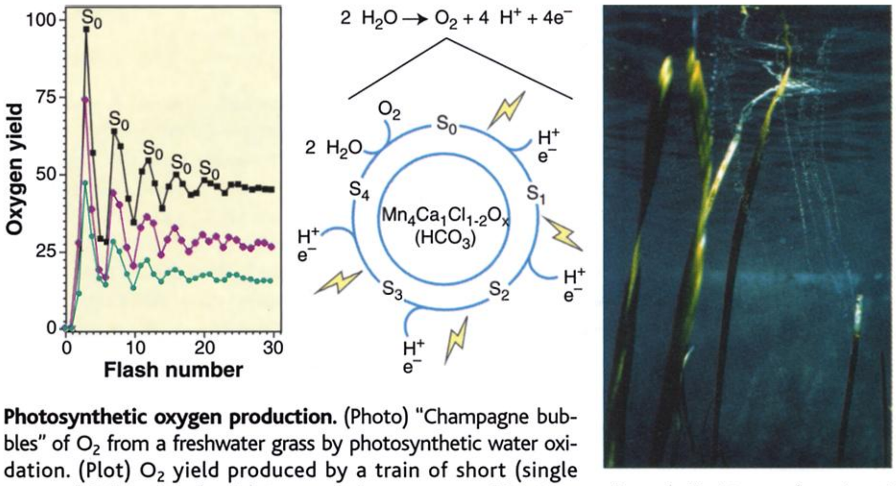
G. C. Dismukes

Photosynthesis is an ancient process (*1*). The emergence of photoreaction center proteins that use light energy to perform sequential one-electron oxidation reactions of reduced compounds through excited chlorophyll pigments is the defining innovation of photosynthesis. The resulting higher energy electrons and liberated protons are used to generate chemical energy and to fix CO_2 for growth. The first organisms capable of photosynthesis could oxidize reduced carbon and sulfur compounds using light as the only energy source. This anoxygenic bacterial photosynthesis evolved through an obscure ancestry (*2*, *3*) possibly linked to bicarbonate as a transitional electron donor (*4*) to become cyanobacteria, organisms capable of splitting water into molecular oxygen, protons, and electrons.

From the perspective of early anaerobic life on Earth, the appearance of O_2 in the atmosphere about 3 billion years ago was the start of the most extensive ecological disaster on Earth (*5*). "Pollution" of the atmosphere with O_2 generated by photosynthesis led to the elimination of much of the anaerobic microbial world. The innovation of water oxidation forever changed the face of Earth, opening an enormous range of new environments for photosynthesis to occur. For the first time, life was limited only by nutrients and light availability, rather than by the availability of electron donors. The photosynthetic systems of the pioneer cyanobacteria were appropriated by all eukaryotic photoautotrophs found in the contemporary world (*6*). Oxygenation of Earth's atmosphere permitted the evolution of more energy-efficient respiratory metabolisms that fostered the evolution of all complex organisms, including humans.

The recent publication of the first x-ray diffraction model of an O_2-evolving photosystem II complex from a cyanobacterium by Zouni *et al.* in *Nature* (*7*) represents an enormous leap in our understanding of O_2 evolution. The authors present a model obtained at a resolution of 3.8 to 4.2 Å that reveals the location of 17 polypeptide subunits, their transmembrane α helices, and their metal and pigment cofactors. Some of these components were revealed partially in prior electron diffraction maps limited to 8 Å resolution (*8*). The current model assigns about 10% of the approximately 45,000 atoms in the structure. Particularly gratifying is the consistency with other photosystem II structural models anticipated by previous spectroscopic and sequence studies (*9*, *10*).

The structure helps to answer many questions, including the origin of the high oxidation potential of the photoactive chlorophyll a pigment, P680. Zouni *et al.* show that unlike the overlapping dimeric bacteriochlorophyll rings in bacterial reaction centers, which are electronically coupled and shifted to lower oxidation potentials, P680 exists as a nonoverlapping array of four monomeric chlorophyll a molecules destabilized by interaction with a neighboring cation (tyrosine-D) (*11*).



Photosynthetic oxygen production. (Photo) "Champagne bubbles" of O_2 from a freshwater grass by photosynthetic water oxidation. (Plot) O_2 yield produced by a train of short (single turnover) light pulses. (Cycle) Corresponding sequence of five intermediates (called S-states) produced by photosystem II and its water-oxidizing complex (WOC). The inorganic composition of the WOC is given.

Photosystem II is unique in that it does not directly perform sequential one-electron oxidations of its physiological substrate. Rather, it has an integral inorganic catalyst from which it extracts four electrons and then reacts with two water molecules to form O_2 (see the figure). Independent studies have shown that the composition of the inorganic cofactors is $Mn_4Ca_1Cl_{1\text{-}2}O_x(HCO_3)_y$ (*12*) and that the four Mn atoms are electronically coupled within a single Mn_4 cluster through shared bridging atoms (*13*). A photoactive tyrosine radical (tyr-Z) mediates the transfer of electrons from the cluster to P680 (*14*).

In Zouni *et al.*'s model, the edge of the Mn cluster is located 7.0 Å from the side-chain oxygen atom of tyr-Z. No other ordered electron density was identified between tyr-Z and the Mn cluster. If this distance proves to be correct, it is too long to allow direct hydrogen atom transfer to the tyr-Z radical from a water molecule bound to the Mn cluster (*15*). However, mechanisms involving coupled-electron/proton transfer from the substrate water to tyr-Z through an obligatory base are supported by the model (*16*, *17*). Zouni *et al.*'s model does not yet show the location or identity of this critical base nor any of the peripheral atoms surrounding the Mn cluster. Further refinements may provide additional insights into this mechanism.

The dimensions of the inorganic cluster are estimated to be 6.8 Å by 3.9 Å by 3.3 Å, with three bulges defining an isosceles triangle. Anomalous diffraction data confirmed the presence of Mn. At the present resolution and crystallographic goodness of fit (*R* factor = 0.59), the internal structure of the cluster cannot be resolved and conclusions at atomic dimensions cannot be drawn without resorting to other noncrystallographic knowledge. Several different geometries of four Mn atoms can be envisioned within these dimensions, but the volume is too large to accommodate any currently known $Mn_4O_x(OH)_y$ core without additional unaccounted electron density. This may be space occupied by Ca^{2+} and/or Cl^- ions. There is hope that the diffraction data can be extended to higher resolution. Together with spectroscopic methods capable of revealing the electronic and protonic coordinates, we may expect further progress toward a molecular structure and mechanism of this unique photocatalyst (*18*).

Chemists have been fascinated with the possibility of using photosynthetic water oxidation as a blueprint for designing syn-

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thetic catalysts for the commercial production of O_2 and H_2 (*17*, *19*). However, at present, this remains a dream. Two points emphasize the difficulty of catalyzing this reaction. First, the lack of diversity in the composition and structure of the inorganic core in all photosystem II complexes examined to date attests to a unique functionality that has remained essentially unchanged in 3 billion years of evolution. Second, the lack of manmade catalysts, of any kind, for the commercial scale production of oxygen attests to the synthetic challenge. Chemistry is, however, catching up with nature. Recently, two examples of manganese complexes that produce O_2 have appeared (*19*, *20*). Synthetic complexes containing the cuboidal Mn_4O_4 core, a close structural and electronic relative of the photosynthetic core, have been found to exhibit unique reactivity in water oxidation/O_2 evolution (*21*, *22*).

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PERSPECTIVES: ECOLOGY

The Advantages of Togetherness

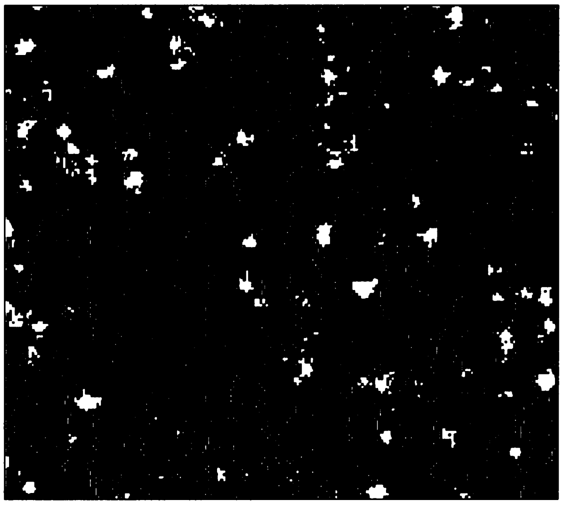
Edward Cox and John Bonner

There is general agreement that unicellular organisms became multicellular early in the course of evolution because the increase in size produced a selective advantage that encouraged their togetherness. Multicellularity arose independently many times, and no doubt the advantages were not always the same each time. On page 504 of this week's issue, Pfeiffer *et al.* (*1*) suggest one advantage that multicellularity conferred on heterotrophs—eukaryotic organisms that take up organic food, such as amino acids and sugars, directly from the environment.

Heterotrophs obtain energy from a food source either in the absence (fermentation) or presence (aerobic respiration) of oxygen. Both pathways yield the energy-rich molecule adenosine triphosphate (ATP), but each pathway makes a different thermodynamic trade-off between the yield and rate of ATP production. Although ATP can be rapidly synthesized by fermentation, the yield is very low. Respiration, on the other hand, yields 10 times as much ATP, but at a much slower rate. The interplay between yield of ATP and its rate of production is an essential feature of evolution, and this is the reasoning that Pfeiffer *et al.* follow.

Through mathematical modeling, the authors play out the consequences of this argument in a model environment in which two types of heterotroph—one a respirator (high ATP yield, low production rate) and the other a fermenter (low ATP yield, high production rate)—compete for a food source (such as sugar) (see the figure). If they compete for the food in a well-mixed environment, that is, one in which the food and the two types of heterotroph are uniformly distributed, then the fermenter may well win the competition. Although the fermenter is less efficient at producing ATP, it has the advantage because it can quickly use up the food—it trades efficiency for rate. Such a fermenting beast will have fewer offspring than the respirator, but will quickly exhaust the food on which the aerobic competitor depends (although in the model the two can come to equilibrium under the right set of conditions).

Now look what happens when the fermenter, respirator and food are allowed to diffuse within the environment in two dimensions. As the mixed population of heterotrophs competes for the food, islands of fermenters and respirators spring up and coexist for a while. In the simplest case—a respirator and a fermenter making their living from a single food source through two different metabolic pathways—the rate at which the food is used up heavily depends on the rate at which both heterotrophs and the food diffuse through the environment. The authors found that when the food has a low influx rate, that is, when it reaches the organisms slowly, respirators are favored, whereas when the food is immediately available and diffuses rapidly, fermenters are favored. When both the food and heterotrophs diffuse slowly, respirators have the advantage because they can use up the food slowly and maximally in their patch, outcompeting fermenters that depend on an inefficient use of the food to produce relatively few offspring. In contrast, when both the food and heterotrophs diffuse rapidly, the fermenters have the advantage because they quickly use up the food, exploiting rate over yield.



The hare and the tortoise. The distribution of heterotrophic organisms—respirators (blue) and fermenters (red)—in an environment containing a food source for which they compete. Yellow denotes areas where both fermenters and respirators are present, black denotes areas where neither organism is present. If the diffusion rate of the food is high, it tends to be more uniformly distributed and the fermenters are favored because they quickly but inefficiently convert the food source into the energy-rich molecule ATP. If the diffusion rate of the food is low and its distribution patchy, the respirators are favored because they can cluster around local concentrations of the food and efficiently but slowly convert it into ATP.

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