The origin and evolution of oxygenic photosynthesis

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The evolutionary developments that led to the ability of photosynthetic organisms to oxidize water to molecular oxygen are discussed. Two major changes from a more primitive non-oxygen-evolving reaction center are required: a charge-accumulating system and a reaction center pigment with a greater oxidizing potential. Intermediate stages are proposed in which hydrogen peroxide was oxidized by the reaction center, and an intermediate pigment, similar to chlorophyll d, was present.

THE ADVENT of oxygen-evolving photosynthesis is one of the central events in the development of life on Earth. Before the evolution of this metabolic capability, the atmosphere of the early Earth was largely anaerobic. The development of advanced eukaryotic life forms did not take place until the free oxygen in the atmosphere rose to a sufficient level. While significant questions remain over when oxygenic photosynthesis began, no other known process, either biogenic or non-biogenic, is capable of producing the large quantities of molecular oxygen that demonstrably changed the course of life on Earth. Understanding the evolutionary origin of this metabolic process is therefore of considerable importance.

All known oxygen-evolving photosynthetic organisms contain two photosystems linked in series. Water oxidation is carried out by photosystem II and ferredoxin reduction is mediated by photosystem I. Several groups of anoxygenic phototrophs exist, and in a variety of eukaryotic organisms cyanobacteria and related prokaryotes, whose chloroplasts were formed by endosymbiosis of a simpler photosynthetic organism. The mechanism of oxygen production in all known oxygenic photosynthetic organisms appears to be very similar, and involves charge separation.

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by a chlorophyll-containing photosynthetic reaction center and charge accumulation by a Mn-protein complex prior to the actual conversion of water into molecular oxygen\(^{10-15}\). Water is a very stable compound, and its oxidation to molecular oxygen requires a powerful oxidizing agent (E\(_{\text{red}}\)):

\[
2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4e^- \quad (1)
\]

The midpoint reduction potential for this process is E\(_{\text{red}}\) = 0.82 V (Fig. 1); thus an oxidant with redox potential greater than 0.82 V is needed to decompose water into molecular oxygen.

Two major evolutionary developments are required before water oxidation can take place: the development of an oxidant with a sufficiently high redox potential and the ability to collect and store oxidizing equivalents, formed by the photochemical events in the reaction center, that transfer at most one electron per photon absorbed. Neither of these properties is found in any of the known anoxygenic photosynthetic reaction centers, nor are any intermediate forms known. The midpoint redox potential of P680, the reaction center photoactive chlorophyll of photosystem II, is greater than 1 V (Refs 12, 13), fully half a volt above the bacteriochlorophyll-containing reaction centers found in anoxygenic bacteria\(^{14}\).

The simultaneous evolutionary development of both the high redox potential species needed to oxidize water and the charge accumulation system needed to carry out the four-electron chemistry of water splitting (Eqn 1) would seem impossible, as each characteristic is almost certainly the result of several independent molecular changes in the reaction center proteins. What is needed is a series of transitional forms, in which one of these capabilities arises first in a simplified, but still functional, form and the other characteristic then arises later. Olson\(^{1}\) proposed a series of nitrogen compounds of increasing redox potentials as possible transitional electron donors to a reaction center that gradually increased its redox potential as the more easily oxidized species were depleted. However, only one of these compounds is able to be oxidized by any known photosynthetic reaction center, and this proposal also does not provide a plausible transition to the present Mn-containing system in which the electron donor is water.

Here, we propose an evolutionary scenario that provides for functional intermediate forms that both benefited the organisms that contained them and linked to the current system. The first proposal is that hydrogen peroxide may have been a transitional electron donor on the early Earth and that the current oxygen-evolving complex may be structurally related to Mn-containing catalase enzymes. The second proposal is that a pigment related to chlorophyll may have been an intermediate pigment between bacteriochlorophyll-containing reaction centers and chlorophyll e-containing reaction centers.

Hydrogen peroxide as a transitional electron donor
Hydrogen peroxide is capable of both an oxidant and a reductant. The oxidation of hydrogen peroxide to oxygen can be carried out by a modestly oxidizing species (E\(_{\text{red}}\) = 0.27 V), which is fully within the oxidative capabilities of re- action centers from existing anoxygenic photosynthetic bacteria (Fig. 1).

\[
\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + 2e^- + \text{O}_2 \quad (2)
\]

A bacteriochlorophyll-containing reaction center is thermodynamically capable of oxidizing hydrogen peroxide to molecular oxygen. This oxidation is similar to part of the mechanism of catalase enzymes. The reaction cycle of catalase involves both oxidation and reduction of hydrogen peroxide\(^{16}\). Mn catalase enzymes are known that have a binuclear metal center that is structurally similar to half of the proposed geometry of the tetranuclear Mn center that makes up the photosynthetic oxygen-evolving complex\(^{14}\) (Fig. 2). This structural similarity has been previously noted\(^{16-20}\). Under certain conditions, the photosynthetic Mn complex can act as a catalase\(^{17-19}\). The possibility of hydrogen peroxide serving as a transitional electron donor has also been suggested previously\(^{20-22}\).

An association of an anoxygenic photosynthetic reaction center and a Mn catalase enzyme might produce a primitive system that evolved oxygen, using hydrogen peroxide as an electron donor. Subsequent developments could convert the binuclear Mn site to a tetranuclear site capable of accumulating up to four oxidizing equivalents. The gene sequence for the Mn catalase from Lactobacillus plantarum has recently been published\(^{20}\). There are no obvious sequence similarities to the loop regions of the D1 protein, which is thought to provide the majority of the ligands to the Mn cluster in photosystem II. However, the precise ligands to the Mn in both the oxygen-evolving center and the Mn catalase have not yet been conclusively identified, so a definite conclusion as to whether a distant homology exists is not yet possible. Additional sequences from Mn catalases will be useful in this analysis.

Was hydrogen peroxide present on the early Earth? The early atmosphere is thought to have been mildly reducing or neutral in overall redox balance\(^{1,2}\). Water photolysis by UV light can produce hydrogen peroxide, which then might be concentrated by rainfall in certain protected environments\(^{22}\). Thus, the presence of hydrogen peroxide on the early Earth is possible, although only a small amount might have accumulated due to its intrinsic reactivity. Therefore, it seems unlikely that hydrogen peroxide was ever the principal electron donor to the primitive photosystem because of the limited amounts available. A more likely candidate for an early electron donor is Fe\(^{2+}\), which was present in substantial quantities in the Archean oceans. Purple photosynthetic bacteria that can oxidize ferrous iron to the ferric form have recently been described, although the enzyme complexes that catalyze this oxidation have not yet been identified\(^{20}\).
We propose that the Mn catalase developed to detoxify the hydrogen peroxide that was present in a local environment on the early Earth. This catalase was then recruited to extract electrons from the hydrogen peroxide, producing the first oxygen-evolving complex. Finally, the dinuclear center evolved to become the four-manganese center of the oxygen-evolving center so that it could extract electrons from water.

The development of a highly oxidizing reaction center

While the scenario described above for the origin and evolution of the Mn center provides a plausible way in which the early reaction center might begin to produce oxygen from hydrogen peroxide, it does not address the issue of how the highly oxidizing species that is needed to split water developed. To understand this issue, it is important to appreciate that the essence of the primary electron transfer process in photosynthesis is an oxidation of the excited state of the reaction center primary donor pigment to generate a reduced acceptor molecule and an oxidized donor. The effective redox potential of the excited state is a function of the ground state redox potential of the donor and the excitation energy. The ground state redox potential of the primary donor in purple bacterial reaction centers is approximately +0.5 V. This potential is determined by both the intrinsic redox behavior of the bacteriochlorophylls and the details of their environment in the reaction center protein. Recent work has shown that the redox potential of the primary donor bacteriochlorophyll a in reaction centers from the purple bacterium Rhodobacter sphaeroides can be raised dramatically by engineered mutations that increase the number of hydrogen bonds to the bacterial reaction center. This has been shown for the reduction of the primary donor, bacteriochlorophyll a, to produce oxygen from hydrogen peroxide, producing the first oxygen-evolving complex. Finally, the dinuclear center evolved to become the four-manganese center of the oxygen-evolving center so that it could extract electrons from water.

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How might bacteriochlorophyll a be converted into chlorophyll a? In the modern biosynthetic pathways of these pigments, chlorophyll is an intermediate on the way to bacteriochlorophyll a. The biosynthetic pathway of both these pigments includes a reduction of ring D with the formation of a single bond instead of a double bond. A similar reaction would occur in the chlorophyll a biosynthesis, which is the oxidation state of ring A. In the bacteriochlorophyll a biosynthesis, an additional reduction of ring B takes place. This reaction is catalyzed by a set of three enzymes (coded by the bchZ, bchY, and bchX genes) that are clearly homologous to the enzymes (coded by the chlL, chlM, and chlA genes) that reduce rings B and D in photosystem II. Burke and coworkers proposed that the ancestral chlorophyll a was produced from bacteriochlorophyll a by a similar reaction. This pigment is nearly identical to chlorophyll a, which has recently been reported as the potential of the primary donor, that in itself is not enough to create a working reaction center with both a highly oxidizing potential of the donor and a sufficiently reducing potential of the excited state. The only way to do both these things is to increase the photon energy by shifting the absorption maximum from the near infrared into the visible region of the electromagnetic spectrum. This assumes that the redox properties of the electron acceptors are unchanged. The overall reaction center structure and especially the acceptor systems are very similar in photosystem II and the purple bacterial reaction centers.

The chemical structures of bacteriochlorophyll a and bacteriochlorophyll b are shown in Fig. 3. There are two differences, which are shown in red. The first is the oxidation state of ring B, which is oxidized in chlorophyll a and reduced, converting the double bond into a single bond, in bacteriochlorophyll a. The second difference is the presence of the acetyl substituent at the 3 position in ring A in bacteriochlorophyll a instead of the vinyl found in chlorophyll a. The spectral differences between these two pigments are primarily due to the first of these two structural differences.
principal pigment in a newly discovered oxygencaryotic photosynthetic organism. Chlorophyll d has an in vivo absorption maximum at only a slightly longer wavelength than chlorophyll a (716 nm versus 680 nm). This modest change in the biosynthetic enzyme specificity would produce a donor pigment that is structurally similar to bacteriochlorophyll but with a redox potential that is capable of oxidizing water. The acetyl group would fit the preexisting hydrogen-bonding interactions for the bacteriochlorophyll a. Finally, the ability to make the acetyl group at the 3 position was lost and the reaction center pigment became chlorophyll. Information on the pathway and enzymes involved in the biosynthesis of chlorophyll d would be important in evaluating this proposal.

Figure 4 shows the stages of development of the reaction center protein from purple bacterial complex to photosystem II. The intermediate stages involve the association of the Mn catalase and the intermediate pigment similar to chlorophyll d.

Conclusions

The proposed scenario is that the ability to oxidize hydrogen peroxide by an ancestral bacteriochlorophyll-containing reaction center similar to the purple bacterial complex was the first step in the progression that led to the oxygen-evolving reaction of photosystem II. The second step was the conversion from bacteriochlorophyll to chlorophyll, which raised the redox potential of the reaction center pigment sufficiently to oxidize the very weak electron donor water. Additional evolutionary steps that led to two linked photosystems were also needed to produce the present system found in all oxygenic photosynthetic organisms. These steps either could have followed the steps described above, or possibly could have preceded them.

Acknowledgements

The authors thank Drs John Olson, James Allen and Wayne Frasch for helpful discussions. Supported by a grant from the Exobiology program of NASA. This is publication 342 from the Arizona State University Center for the Study of Early Events in Photosynthesis.

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