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Minireview

Some speculations concerning the evolution of photosynthetic function

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Abstract

Following a brief review of the light-driven reactions in photosynthetic membranes, two questions are addressed. (1) Why is the first charge separation reaction in photosynthetic reaction centers so fast; and (2) given what we know about the contemporary structure and function of reaction centers, can we develop a simple model for a much more primitive reaction center? It is proposed that the primary charge separation step in reaction centers is optimized to be ultra-fast principally in order to compete with detrapping into the antenna complex, rather than to compete with radiative and non-radiative losses in the special pair. This leads to a notion of kinetic perfection analogous to that developed for enzymes which operate under diffusion-limited conditions, but elaborated to permit even more 'perfect' function. This hypothesis is testable by changing components in photosynthetic membranes and subjecting them to selective pressures. We speculate that the reaction center is far too complex to have served as an early functional unit, and consider possible roles for the iron-quinone part of the reaction center as a very primitive photosynthetic unit. It is suggested that this working end later became associated with primitive antenna complexes, which then evolved into the elaborate structure we find today.

The role of photosynthesis in the origin of life has been a topic of speculation for many years. It is evident that photosynthetic function is ancient and central. As a person who does not work in the field of evolution, I am not very familiar with much of the speculation that precedes this paper. Proposals and speculation by others are likely based on much firmer ground, and therefore I apologize in advance if some of these ideas have already been suggested by others. In this chapter I take the liberty to speculate on how a structure as complex as the contemporary photosynthetic reaction center (RC) could have evolved from more primitive units, and why it retains some of its remarkable properties and seemingly unnecessary components. Both subjects lead to specific predictions and testable hypotheses.

1. Overview of light-driven reactions in photosynthesis

Contemporary photosynthetic membranes from simple bacteria are composed of several chromophore-protein complexes. The reaction center (RC) is the complex where the initial light-driven charge separation step occurs. Its structure is known to atomic resolution (Deisenhofer and Michel 1989) and is illustrated in Fig. 1. Much is known about the function of RCs, and the relationship between the structure and function has been widely discussed (Feher et al. 1989). The prosthetic groups are intimately associated with two polypeptides, commonly denoted the L and M polypeptides, each containing 5 transmembrane helices. A third polypeptide designated H has one transmembrane helix and a larger globular domain, and does not directly interact with any of the key players in the photochemical reactions. Electron transfer is initiated by photoexcitation of the special pair P consist-

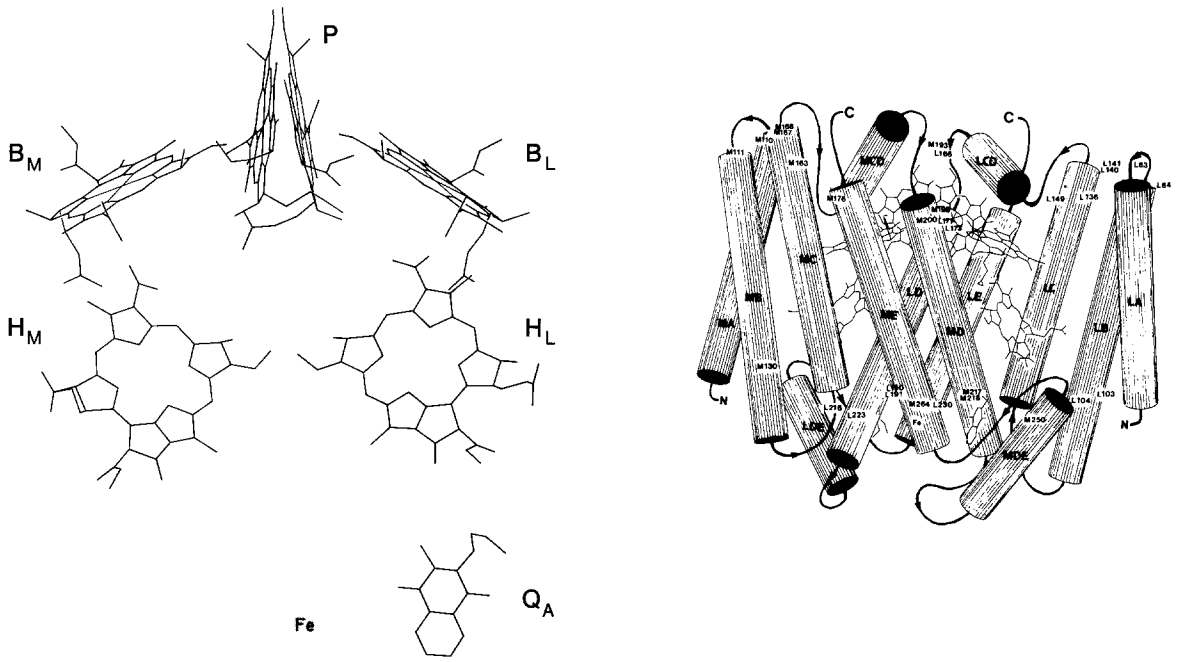

Fig. 1. A schematic diagram of the polypeptides in the bacterial photosynthetic RC of *Rps. viridis* (taken from Michel and Deisenhofer 1988) and an enlarged view of the prosthetic groups which participate in the initial photochemistry. A local C_2 axis of symmetry between the geometric center of the special pair P and the non-heme iron relates all prosthetic groups on the right and left of the diagram. The second quinone, Q_B is not shown, however, it is found at the left of the non-heme iron atom in a position related to Q_A by the same C_2 axis of symmetry.

ing of two closely associated bacteriochlorophylls (BChls). Within a few ps, an electron transfers to a bacteriopheophytin labelled H (Kirmaier and Holten 1987). The mechanism of this electron transfer is the subject of considerable debate, specifically focussing on the role of another monomeric BChl (labelled B). From H the electron moves on to quinone Q_A, then to quinone Q_B. A non-heme iron atom is located approximately mid-way between Q_A and Q_B. The non-heme iron is not redox active, and its precise role is unknown despite many years of study (Feher et al. 1989). It may be crucial for protein folding or stability. The hole left behind on P^+ is refilled by electron transfer from a reduced cytochrome which is not buried in the membrane. With an electron on Q_B and P neutral, the system is ready for another cycle of photoexcitation and electron transfer, delivering a second electron to Q_B, which then picks up two protons and leaves the RC as the hydroquinone (the details of the coupled electron/proton transfers at Q_B are complex (Feher et al. 1989)). The net result is the movement of two electrons across the membrane, and the stored energy is ultimately coupled to ATP synthesis through the cytochrome bc_1 complex and ATPase. These fascinating membrane proteins will not be discussed further.

Both from a functional and evolutionary standpoint, one of the remarkable properties of the RC is the presence of a local two-fold axis of symmetry running approximately between the geometric center of P and the non-heme iron atom. It appears likely that this symmetry, which includes the structure of the transmembrane helices as well as bound prosthetic groups, resulted from an early gene duplication event (Feher et al. 1989, Michel and Deisenhofer 1988). In the contemporary RC, electron transfer proceeds only down the series of chromophores on the right-hand side of the structure as illustrated in Fig. 1 under normal conditions. The molecular basis of this apparent unidirectionality of electron flow is not well understood.

Another interesting feature of the RC from an evolutionary perspective is that the RC apparently functions as a catalyst for the processing of several of its prosthetic groups. This includes the bacteriopheophytins which appear to be formed by loss of Mg from BChl if amino acids which are incapable of coordinating Mg are not present in the binding site (Kirmaier et al. 1991, Colman and Youvan 1990), and the isomerization of all trans-carotenoids to the 15-15′-cis form (Cogdell and Frank 1987).

The other important components of the photosynthetic membrane which interact with light are the antenna complexes. The three dimensional structures of these proteins are not yet known; however, there has been a great deal of speculation on their structure and association with chlorophylls (Zuber and Brunisholz 1991). The polypeptides are quite short, typically a single transmembrane segment, nonetheless they typically bind several BChls, and these transmembrane helices bound to BChls further associate to form the overall antenna structure. The function of the antenna complex is to increase the absorption cross section and deliver excitation energy with minimal loss to the RC. The keys to successful function are that interchromophore interactions are strong enough to allow highly efficient energy transfer without competing non-radiative losses, and that the antenna system is associated closely with the RC. Interestingly, although the typical picture of the antenna involves approximately degenerate energy transfer from pigment to pigment followed by trapping at the RC (Fig. 2A), the lowest singlet electronic transition for the antenna pigments is often about the same or lower in energy than the lowest electronic state of the RC (Fig. 2B). In the latter case, energy transfer into the RC is not expected to be as fast as energy transfer among the antenna pigments, and, most importantly, energy transfer out of the RC back into the antenna is expected to be very fast. In this case, the RC is not a thermodynamic trap for excitation energy, and the large absorption cross section, which is the essence of the antenna function, would appear to be undermined as there is also a large cross section for non-radiative and radiative loss upon detrapping from the RC. This dilemma is solved by the extremely fast initial electron transfer in the RC: so long as electron transfer is fast compared with detrapping, then the RC acts as a kinetic trap for the electronic excitation.

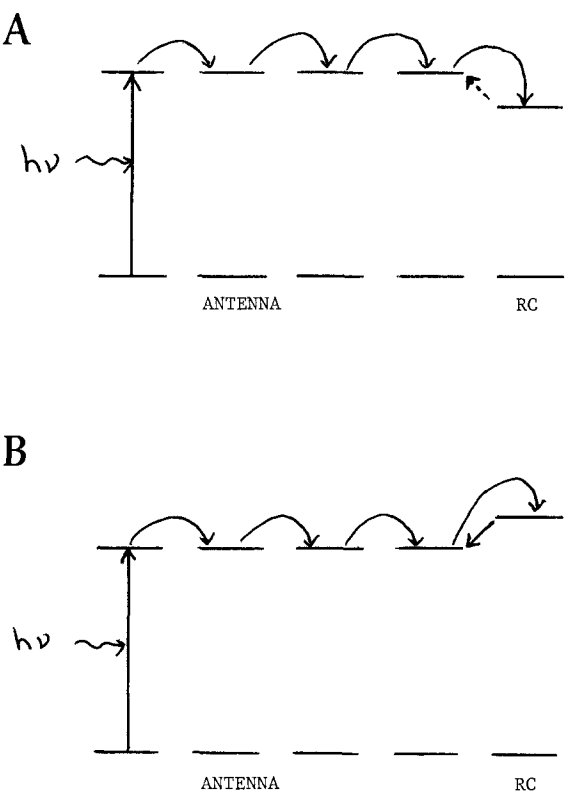

Fig. 2. Highly schematic energy level diagram illustrating the harvesting of energy by an array of antenna chlorophylls followed by trapping at the RC. (A) An arrangement of energy levels in which the lowest allowed electronic excited state of the antenna is considerably above that of the RC. In this case, following rapid energy transfer among the antenna chromophores, irreversible trapping occurs at the RC because the RC levels are much lower and the probability of returning is small. (B) An arrangement of energy levels in which the lowest allowed electronic excited state of the antenna is comparable to or lower than that of the RC. In this case, following rapid energy transfer among the antenna chromophores, energy transfer occurs at the RC, but is rapidly followed by detrapping back into the antenna. In the latter case, in order for the RC to effectively capture the excitation energy, a rapid dissipative process must occur such as electron transfer. Because an antenna provides a significant advantage in terms of harvesting sunlight, this scheme illustrates a strong additional pressure to maintain a very fast charge separation (dissipative) step as well.

The structure of the membrane proteins of higher plant photosynthetic units are less well

characterized. Two photosystems operate in series. The Photosystem II RC bears a strong resemblance in many of its features to the bacterial RC (Michel and Deisenhofer 1988). Photo-oxidation of the primary electron donor in PS II produces a very strong oxidizing agent capable of oxidizing water to liberate O_2. This function is associated with a complex involving several Mn ions which donates electrons to the hole formed by photo-oxidation of the primary electron donor, much as cytochrome donates an electron to reduce P^+ in bacterial RCs. Photosystem I utilizes light to ultimately reduce NADP, and the hole for the primary electron donor in PS I is ultimately refilled by the reducing equivalent produced by PS II. The details of each photosystem, the coupling between them, and alternative branch points are discussed in detail elsewhere (Scheer 1991). Because it is the simpler system, the focus in the following is on bacterial RCs.

2. Kinetic perfection in the reaction center: Evolution of ultra-fast electron transfer

As discussed above, in a functioning photosynthetic membrane, it is likely that detrapping of energy from the RC into the antenna competes with the initial electron transfer event. The intrinsic excited state lifetime of ^1P is not known with certainty; however, recent work on an RC mutant which appears to lack the BPheo electron acceptor indicates that the lifetime is on the order of several hundred ps (Breton et al. 1990). If this decay alone were competing with electron transfer, there would be little evolutionary pressure for the initial electron transfer to be so fast because the quantum efficiency of charge separation would be degraded appreciably only if electron transfer slowed a great deal. We propose that because detrapping can be extremely fast, the RC has become optimized for extremely fast kinetic trapping.

A related optimization process has been described and tested for conventional enzymatic catalysis by Albery and Knowles who coined the expression 'kinetic perfection' (Knowles and Albery 1977). There are several interesting aspects of their proposal, but what is relevant for the current discussion is that for conventional enzymatic reactions the maximum reaction rate is determined by the rate at which substrate can diffuse to the active site of the enzyme. This is the diffusion-limited rate, and is approximately $10^9\ l\ mol^{-1}\ s^{-1}$ for a large molecule (enzyme) interacting with a small molecule (substrate). In some cases, it is critical for an organism to have certain reactions whose rates are optimized at this maximum rate, e.g., enzymes involved in rapid turnover of glucose during the fight-or-flight response. Because of the fundamental limitation that the rate can never exceed the diffusion-limited rate, an enzyme which operates at this rate has achieved kinetic perfection and cannot be improved (at least with respect to this rate) by selection. Knowles and coworkers have beautifully illustrated this notion with triosephosphate isomerase (Knowles and Albery 1977, Blacklow et al. 1988). This enzyme operates at or near to the diffusion controlled limit. These investigators have impeded the function of this enzyme by site-directed mutagenesis, placed it under functional selection, and then examined mutations which lead to partial restoration of function. This process can be repeated, essentially subjecting the enzyme to rapid evolution. Indeed, amino acid changes are selected which improve the rate of turnover of substrate, thus the damaged enzyme is again perfected, and there are several pathways for perfection (Blacklow et al. 1988).

I would like to suggest that the phenomenology of rapid energy transfer among antenna chromophores, followed by trapping and detrapping at the RC is similar to the diffusion of a substrate to an enzyme. In this case the 'substrate' is a photon or photoexcitation. This should not be taken to imply that the transport of energy in the antenna system is 'diffusive' in the sense that this word is used by condensed matter physical chemists, rather that the phenomenology is similar to that for a diffusive encounter between an enzyme and substrate. Unlike true diffusion in aqueous solution, the movement of electronic excitation within the antenna complex and to the RC involves elementary steps which can be much faster than the effective bimolecular rate of $10^9\ l\ mol^{-1}\ s^{-1}$. It is not known exactly how fast such steps can be, but estimates by a variety of

techniques suggest that site-to-site hopping times are on the order of ps or less (Sundström and van Grondelle 1991). In contrast to diffusion-limited reactions in fluid solution, the upper limit in this case is set by quantum mechanics and dephasing processes. Thus, the competing charge separation reaction in the RC has a strong incentive to evolve to a very fast rate, much faster than in diffusion-limited enzymatic catalysis, if an antenna system is to be used successfully. Another way to view this is that the capacity of kinetic perfection in a system like the photosynthetic membrane is much greater than in conventional enzymatic catalysis. An important aspect of this enhanced capacity for perfection relative to other biological systems is that the system may also have a greater tolerance against change. The initial electron transfer kinetics in the RC have proven to be remarkably resistant to major change by site-directed mutagenesis (Coleman and Youvan 1991).

If kinetic perfection of the type suggested here does operate in the RC system, then it should be straightforward to test. All of the genes for the antenna and RC proteins have been mobilized on plasmids, and strains in which all these polypeptides are deleted are available (Coleman and Youvan 1991). Furthermore, mutants in which the antenna proteins do not assemble with bacteriochlorophyll have been prepared (Bylina et al. 1988) so that the RC is the only BChl-containing protein in the membrane. If only the RC is present in the membrane and the organism is grown under photosynthetic selection, there is little selective advantage for an RC to have a primary charge separation rate of 3 ps since, as discussed above, the competing non-radiative and radiative processes are several orders of magnitude slower. For example, the quantum efficiency for charge separation for a mutant RC in which the rate is 6 ps (there are several mutants like this (Coleman and Youvan 1990)) would be almost indistinguishable from wild-type, and the pressure to revert to wild-type should be relatively small. On the other hand, in the presence of a functioning antenna, where the detrapping rate is perhaps a few $(ps)^{-1}$ or more, then a small decrease in the rate of the initial charge separation step can have a big impact on the overall quantum efficiency. Because of this competition, more rapid reversion is expected, and it will be very interesting to explore the nature of such reversions. Conversely, it may be possible to engineer the antenna complexes so that the detrapping rate is further enhanced. Under these conditions it may be possible to select for mutations which enhance the initial charge separation rate. Experiments to test the notion of kinetic perfection outlined in this section are currently in progress in our lab.

3. Reaction center complexity: Proposals for a primitive reaction center

The reaction center is a fantastically complex machine, and it is interesting to speculate on how this complexity may have evolved. The first element is the 2-fold symmetry. It is widely believed that the symmetric structure found today in RCs is the result of an early gene duplication event, suggesting that just half of the current RC was sufficient at an earlier time. It is difficult to imagine that this is the case since the binding sites of most prosthetic groups involve contacts with amino acid residues from both polypeptide chains. At least three features are gained by this symmetry: the pair of BChls called P, two quinones, and the possibility (now apparently not used) of redundancy. To date, a special and unique function requiring a dimer has not yet emerged. Possibilities include low-lying charge-transfer states (Boxer et al. 1989), large excited state polarizability (Middendorf et al. 1992), and the related property that the redox potential is especially sensitive to environmental perturbation (Stocker et al. 1992). Likewise there is not an absolute requirement for two quinones in a primitive system, unless there is a system for utilizing a doubly reduced and protonated quinone.

Even if we only had to consider one-half of the current RC, it is still an immensely complex object, and it is interesting to speculate on more primitive forms. The biosynthesis of chlorophylls and bacteriochlorophylls is sequential, both being derived from porphyrins. In a primitive atmosphere, the availability of blue and UV light was much greater than today, so the red-shifted absorption maxima of chlorophylls and bacterio-

chlorophylls compared with porphyrins was a less crucial feature. Porphyrins by themselves are well known to participate in photoinduced redox reactions with a wide range of donors and acceptors, and have been studied extensively as models for solar-energy conversion (Gust and Moore 1989). Most natural porphyrins contain Fe as the central metal; however, the lifetime of the photoexcited states of Fe-containing porphyrins is extremely short, and little productive photochemistry or antenna function can be expected from such complexes (this is true for most transition metal complexes except the Zn-complex whose filled-orbitals lead to properties that are similar to the Mg-complex). One can certainly imagine that complexes between porphyrins and quinones, perhaps associated with simpler materials than proteins, might have served as primitive electron transfer components as in contemporary model systems; however, by itself such a charge separation (which usually leads to rapid charge recombination) does not lead to the storage of solar energy. One solution is to assemble a transmembrane arrangement of the components. It has proved to be quite difficult to spontaneously assemble a chromophore/quinone system which can drive electrons across an interface, even using synthetic methods to covalently link redox components.

Therefore, we would like to propose that the earliest 'photosynthetic' function might have been direct photochemistry in which sunlight drives photoxidation or reduction to produce useful materials. If we look into the contemporary RC for a remnant of a possible early reaction, we are drawn to the Q_A–Fe–Q_B segment. Quinones are considerably simpler than chlorophylls or bacteriochlorophylls, and they absorb in the blue or near ultra-violet. They are photochemically active, however, the photochemistry tends to be complex, especially in the presence of molecular oxygen. In a primitive anaerobic atmosphere with lots of UV light these limitations might be less significant. Reduced iron was ubiquitous on the primitive earth. Therefore, a very primitive photosynthetic system might involve just quinones and ferrous iron. The six-coordinate environment around the Fe(II) in the contemporary RC is comprised of four histidines, a carboxyl-bearing amino acid (Glu) and water to give a distorted octahedral geometry. To date there is no evidence that this iron can be oxidized. However, oxidation of Fe(II) to Fe(III) would represent a significant storage of energy driven by light in a primitive system. One could imagine other metals playing a similar role. It would be interesting to see if a coordination environment like that around iron in the RC can be formed by spontaneous assembly and to explore its photochemical properties.

What remains to be discussed is the question of how such a functional iron-quinone fragment could become associated with chromophores, which in the contemporary system do the primary photochemistry. One can imagine that metalloporphyrins, chlorophylls or bacteriochlorophylls spontaneously associate with simple peptides providing a ligand is available from the amino acid side chain (e.g., a His residue as in most contemporary antenna and RC complexes). By themselves such assemblies are not especially useful, however, were such an assembly to come into close proximity with the proposed iron-quinone primitive functional unit, the result begins to look a bit like the current RC. One can then propose two pathways for further evolution of such a simple chromophore-protein complex: either it becomes integrated with the iron-quinone unit to become an RC or it remains as a simple chromophore-protein complex, which evolves into the contemporary antenna system. Obviously these proposals are highly speculative; however, they do lead to the idea that the arrangement of the chromophores in the antenna and in the RC may have some similarity. To date there are no structures for antenna complexes; however, crystals are available (Zuber and Brunisholz 1991), and we may soon see whether this aspect of the proposal has any validity.

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