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Minireview

Photosynthetic reaction centers

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Abstract **The reaction center is the key component for the primary events in the photochemical conversion of light into chemical energy. After excitation by light, a charge separation that spans the cell membrane is formed in the reaction center in a few hundred picoseconds with a quantum yield of essentially one. A conserved pattern in the cofactors and core proteins of reaction centers from different organisms can be defined based on comparisons of the three dimensional structure of two types of reaction centers. Different functional aspects of the reaction center are discussed, including the properties of the bacteriochlorophyll or chlorophyll dimer that constitutes the primary electron donor, the pathway of electron transfer, and the different functional roles of the electron acceptors. The implication of these results on the evolution of the reaction center is presented.**

Key words: Purple bacterium; Green bacterium; Plant; Photosystem II; Photosystem I; Evolution

1. Introduction

In every photosynthetic organism, the primary events involve an integral membrane pigment-protein complex called the reaction center that creates a charge separation across the cell membrane after light excitation. Coupling to secondary electron donors and acceptors allows the electrons and accompanying protons to be transferred to other components of the photosynthetic apparatus ultimately to be converted into chemically rich compounds such as ATP and NADP. This general pattern is remarkably conserved, although the specific pathway utilized for these processes varies depending upon the organism. In recent years there have been excellent reviews on all aspects of photosynthesis [1–4] including an engaging personal account of bacterial reaction centers [5]. In this review, we compare the structures of different reaction centers and discuss some of the current questions, including those pertaining to the evolution of the reaction center. Many of the presented aspects are based upon extensive mutagenesis studies, in particular on the reaction center from purple bacteria, for which the influence of specific protein-cofactor interactions can be probed due to the availability of the high-resolution X-ray structures. Overall, the focus is on the features common to reaction centers from different photosynthetic organisms with an emphasis on the lessons learned from reaction centers isolated from purple bacteria.

2. A reaction center motif

Photosynthetic reaction centers can be classed into two categories based upon the nature of the electron acceptors (for a review see [6]). Purple bacteria, green filamentous bacteria, and photosystem II belong to the pheophytin-quinone type, while green sulfur bacteria, heliobacteria, and photosystem I belong to the iron-sulfur type (Fig. 1). While anoxygenic bacteria have only one photosystem, cyanobacteria and plants contain both types of photosystems. A structure for each type of reaction center has been determined by X-ray diffraction, and generalizations can be drawn from these structures since sequence comparisons indicate that all the reaction centers within each type are homologous. The reaction center from purple bacteria has four bacteriochlorophylls, two bacteriopheophytins, two quinones, a carotenoid, and one nonheme iron arranged into two symmetrically-related branches (for reviews see [7–9]). Photosystem II has a large number of cofactors and protein subunits, although complexes can be isolated containing only six chlorophylls, two pheophytins, two carotenoids and three protein subunits. Although there is no three-dimensional structure of photosystem II, the cofactors are thought to be arranged in a similar fashion to that of purple bacteria with the additional presence of the oxygen evolving complex (for a review see [10]). The structure of photosystem I reveals a large number of chlorophylls also arranged with a two-fold symmetry axis with three iron-sulfur clusters [11]. While most of the tetrapyrroles in the iron-sulfur type of reaction center function in light harvesting, six central tetrapyrroles and three iron-sulfur complexes appear to constitute the photochemical heart of this reaction center.

A common feature in the structures of the two types of reaction centers is the presence of a two-fold symmetry axis relating both the cofactors and protein subunits. This feature is particularly striking when considering the portion of the reaction centers that contains the components essential for photochemistry. Surrounding the cofactors is a hydrophobic environment provided by amino acid residues from two core subunits, for example L and M in purple bacteria, and PsaA and PsaB in photosystem I. Just as the cofactors are arranged in two symmetrical branches, the two core subunits are symmetrically located about the central two-fold axis. In reaction centers from purple bacteria each core subunit is largely comprised of five long transmembrane helices. The subunits of photosystem I are significantly larger but the pattern of five centrally located and symmetrically related transmembrane helices from each core subunit is still present. The outermost

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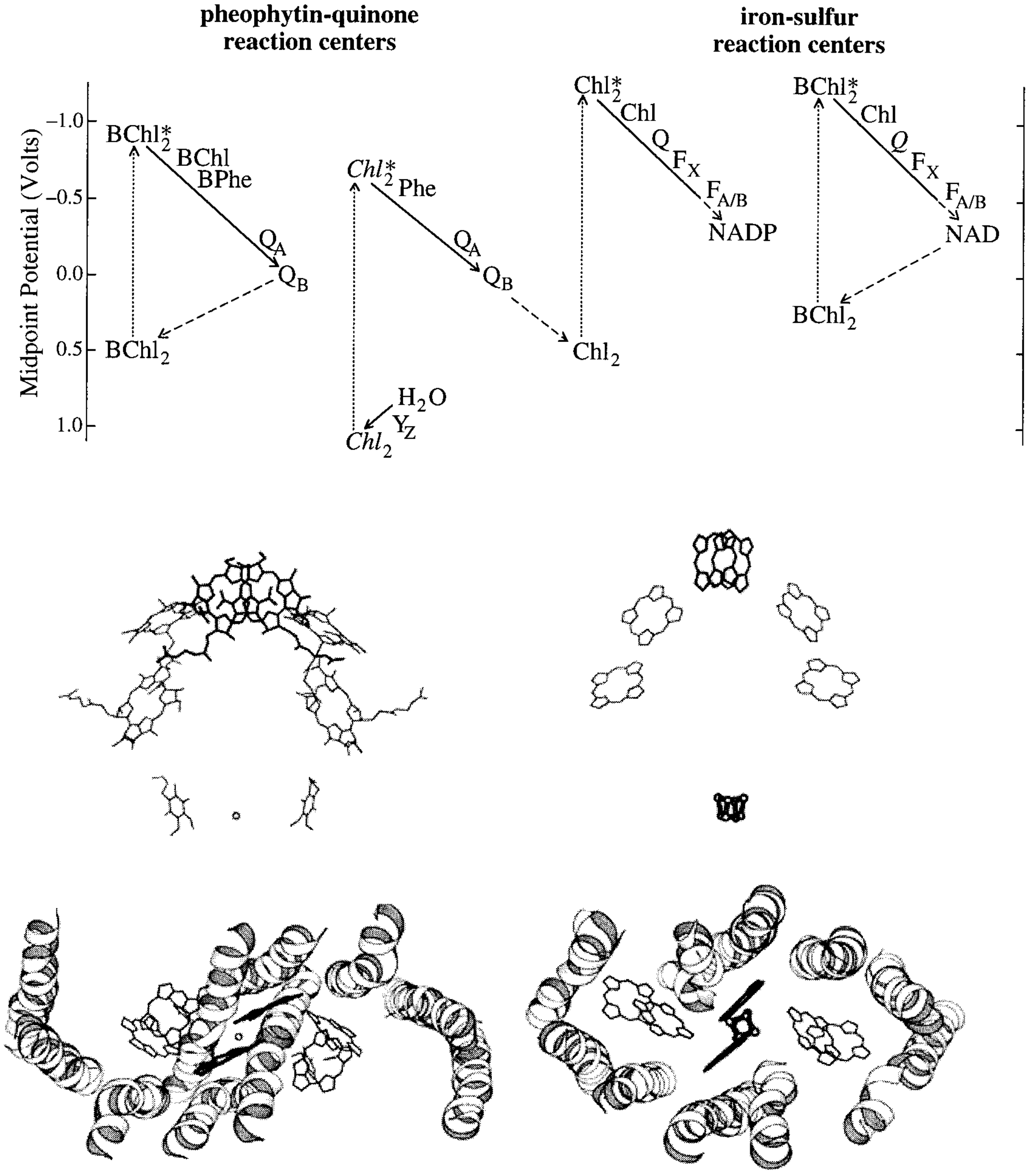

Fig. 1. Comparison of the properties of the pheophytin-quinone (left) and iron-sulfur (right) type of reaction centers. The arrangement of the cofactors in electron transfer for purple bacteria, photosystem II, photosystem I, and green sulfur bacteria is depicted at the top of the figure. The dotted line represents the absorption of light by the primary electron donor ($BChl_2$ or Chl_2). The solid line shows the energy transfer steps in the reaction center, from the tyrosine residue (Y_Z) in photosystem II, through the monomer bacteriochlorophyll (BChl), monomer bacteriopheophytin (BPhe) or pheophytin (Phe) and quinones (Q_A and Q_B) in the pheophytin-quinone type of reaction center, and through the monomer chlorophyll (Chl), quinone (Q), and iron-sulfur centers (F_X, F_A and F_B) in the iron-sulfur reaction centers. The specific nature or role has not been established for two components shown in italics: the primary donor of photosystem II [10,13], and the quinone of iron-sulfur reaction centers in green sulfur bacteria [28]. The dashed line indicates electron transfer events that take place outside of the reaction center. Views of the essential cofactors and central transmembrane helices are depicted in the middle and bottom of the figure. Shown are the bacteriochlorophyll dimer (bold), monomer bacteriochlorophylls, monomer bacteriopheophytins, quinones, and iron atom of the reaction center from the purple bacterium *Rhodobacter sphaeroides*, and the chlorophyll dimer (bold), central chlorophyll monomers, and the F_X iron-sulfur center of the photosystem I reaction center from *Synechococcus elongatus*. The symmetry axis spans the membrane and is in the plane of the paper in the middle view and rotated by 90° so that it is perpendicular to the paper in the bottom view. The phytyl and isoprenoid chains have been truncated. The structure figures were created using the program MOLSCRIPT [36] and coordinates from the Brookhaven Databank files 4RCR and 2PPS.

of the ten central helices are packed in a similar pattern in the two types of reaction centers. The four innermost helices are most closely associated with the cofactors, and have different relative positions and orientations reflecting the differences in the nature of the electron acceptors. Thus on a broad scale the two types of reaction centers look similar, but the details of the binding sites for the cofactors differ. For example, histidine residues from the inner helices coordinate the non-heme iron in pheophytin-quinone reaction centers, but in iron-sulfur reaction centers the central helices are below the central metal cluster F_X that is coordinated by cysteine residues from extramembrane loops of the core subunits.

In general, both types of reaction centers contain a dimer of tetrapyrrole molecules flanked by four monomeric tetrapyrrole molecules, and an iron-binding site on the opposite side. Both the dimer and the iron atoms are situated near the central two-fold axis of symmetry, with the ligands to the Mg atoms of the dimer and for the metal complex provided by amino acid residues from both of the core subunits. From these comparisons emerges a common reaction center motif of two symmetric branches of cofactors that are bounded by a dimeric tetrapyrrole and a metal site and surrounded by five helices each from two symmetric core subunits (Fig. 1).

3. Primary electron donor

All photosynthetic systems have bacteriochlorophyll or chlorophyll containing complexes that serve to collect the light over a large surface area and focus that energy to the reaction center. In principle any of the tetrapyrroles in these systems could be oxidized and thereby serve as the primary electron donor. However, the light energy is always transferred to a uniquely defined pair of bacteriochlorophylls or chlorophylls. In reaction centers from purple bacteria, a wide variety of measurements has established that the two overlapping bacteriochlorophylls evident in the three-dimensional structure serve as the primary electron donor (Fig. 1). These two bacteriochlorophylls are separated by approximately 3 Å and overlap at the ring A position. The presence of the dimer is not required for assembly of the reaction center but is necessary for functional photochemistry to occur [12]. In photosystem I, two chlorophylls located in a comparable position are thought to serve as the primary donor. The relative orientation of the chlorophylls in photosystem I is unknown but the separation is larger at approximately 4 Å. Models of photosystem II vary considerably, but a chlorophyll dimer is generally thought to be at a location similar to that of purple bacteria [13]. Based upon the structures and mutagenesis studies, the tetrapyrroles forming the dimer in both types of reaction centers have histidine ligands, although coordination to other residues is possible and has been observed in the structures of light harvesting complexes.

Some characteristic features of the primary electron donor result from the dimeric organization. For example, the presence of a distinct optical absorption band in the near infrared region for the donor of purple bacteria arises from the exciton interaction generated by the close overlap of the bacteriochlorophylls. For photosystem II the donor absorption band overlaps with the monomer bands and hence the dimer is usually modeled as having a greater separation with a more limited overlap. The distribution of the electron wavefunctions over both tetrapyrroles creates electronic states that are intrinsically different from a monomer and can be described by simple Hückle models, which can be used to predict the oxidation/reduction midpoint potential and the electron density of the donor [14]. In principle, all of the precise properties of the donor and other pigments, including the electron transfer rates, could be explicitly calculated by molecular orbital theory, but in practice the highly interacting nature of the electron donors and acceptors and the critical contribution of the surrounding protein makes such calculations very difficult for these large complex systems. The delocalization of the electrons throughout the conjugated system and hence the limited electron density on the central Mg atoms is reflected in the limited effect of substitutions to the ligands. Replacement of the histidine serving as a ligand to the central Mg can result in an altered cofactor composition [15,16], but when the coordination of the Mg is retained very little is changed in the properties of the donor [17–19]. In contrast, for heme systems substitution of the ligand to the metal results in significant alteration of the protein properties, such as a change in the redox potential by several hundred mV [20].

Both the differences of the chemical properties of the tetrapyrroles and the interactions with the surrounding protein contribute to the observed range in the properties of the donors in different reaction centers. The wavelength of the optical band of the dimer in purple bacteria ranges from 860 nm to 900 nm, with reaction centers containing bacteriochlorophyll *b* having the longer wavelength transition compared to those containing bacteriochlorophyll *a*. For chlorophylls the energy difference between the excited and ground states is larger than for bacteriochlorophylls (Fig. 1), so the absorption spectrum is shifted to the higher energy at 680 nm. In general, mutations that do not alter the cofactor composition have been found not to significantly alter the optical spectrum, presumably due to the neutrality of the ground and excited states. The large range of types of tetrapyrroles found in different organisms enables a precise tuning of the absorption and hence maximum utility of the light spectrum available in each environment of photosynthetic organisms. In solution, chlorophylls are more difficult to oxidize than bacteriochlorophylls by about 0.2 V, contributing to differences in the values of the midpoint potentials of the primary electron donors (for reviews see [21]). Interactions with the protein also contribute substantially to the observed range of over 0.5 V in midpoint potentials. Hydrogen bonds between amino acid residues and the conjugated carbonyl groups of the dimer are one type of interaction that has been identified as significantly affecting the midpoint potential. In purple bacteria, loss of the existing hydrogen bond results in a ~0.1 V decrease in potential while addition of up to 3 hydrogen bonds results in additive increases in midpoint potential up to 0.25 V [22]. Also contributing to a lesser extent are electrostatic interactions from non-ionized but polar amino acids, such as tyrosines, and van der Waals contacts involving aromatic side chains. Since the highly hydrophobic environment of the dimer is unfavorable for charges, it is unlikely that any of the nearby residues are ionized, so that electrostatic interactions from charged amino acid residues may not play a major role in adjusting the energy levels of the dimer. The electron density distribution over the two halves of the oxidized dimer is primarily dependent on differences in the protein surroundings. For example, the addition of each hydrogen bond to the dimer of the reaction center from purple bacteria stabilizes the bacteriochlorophyll containing the proton accepting carbonyl, and this preferential stabilization of one side of the dimer results in both an increase in the midpoint potential and a change in the asymmetry of the donor [14]. The spin density ratio in these types of mutants ranges from almost completely asymmetric to nearly symmetric, depending on the arrangement of hydrogen bonds. The relative contribution of protein interactions to the properties of donors continues to be actively investigated in different photosystems.

4. Electron transfer

A critical aspect of the photochemistry of reaction centers is their ability to perform electron transfer with a quantum yield of almost unity. This high quantum yield is achieved by the utilization of a number of intermediate electron acceptors (Fig. 1). Within 30 ps after excitation a stable charge separated state is formed in all photosystems. Although the nature of the spectral changes with time is complex, the role of many factors driving the initial electron transfer process has been established in purple bacteria (reviewed in [23]). For other types of reaction centers, the larger number of tetrapyrroles and the highly overlapping nature of the optical bands have hindered interpretation of the optical changes, and research continues to delineate the electron transfer processes. In purple bacteria, the rate is sensitive to the free energy difference between the excited state and the charge-separated state but not to the relative distribution of electrons over the two macrocycles of the donor. After extensive studies, the rate is now established to be critically coupled to the properties of the bacteriochlorophyll monomer that lies between the donor and bacteriopheophytin acceptor (Fig. 1). The involvement of the bacteriochlorophyll monomer may give rise to multiple pathways for electron transfer [24] and has been shown to partially determine the asymmetry of the electron transfer along one branch [25]. A set of elegant experiments has demonstrated the coupling of specific vibrational modes with the donor [26]. However, the involvement of protein vibrational modes or other additional factors that give rise to the observed functional asymmetry remains an open question.

Electron transfer in the reaction center culminates at either quinone acceptors or iron-sulfur centers depending upon the type of reaction center. Although a metal atom, usually iron, is coupled to the quinones in the pheophytin-quinone reaction centers, it is not required for electron transfer. The two quinone acceptors in this type of reaction center have different functional properties, with the primary quinone being a transient one electron acceptor and the secondary quinone being a two electron acceptor coupled to the exogenous quinone pool of the cell membrane [27]. Electron transfer in the iron-sulfur type of reaction center proceeds from the chlorophyll acceptor A_0 to the iron-sulfur center F_X (Fig. 1). A quinone is thought to serve as an intermediate acceptor in photosystem I, but the role of quinones in reaction centers from heliobacteria or green sulfur bacteria is not settled [28]. The electron is then transferred to an external protein, ferredoxin, by way of the two iron-sulfur clusters F_A and F_B that are located in the protein subunit PsaC.

Despite the striking symmetry of the cofactors into 2 branches (Fig. 1), electron transfer in the pheophytin-quinone reaction centers proceeds only along one branch with at least a 10:1 ratio. The functional asymmetry is served by the significantly different properties of the two quinones, which arise from protein-quinone interactions. For example, the primary quinone environment is much more hydrophobic than that of the secondary quinone and the hydrogen bonding is more asymmetric. The protonation states of nearby amino acid residues, in particular carboxylate groups, has been clearly established to be crucial to the function of the secondary quinone, which becomes fully protonated and leaves the reaction center. The quinones of photosystem II are thought to bind in homologous sites although the possible involvement of protonatable residues in the electron transfer process requires more detailed studies. Open areas of investigation are delineation of the mechanism of electron transfer from the primary to the secondary quinone and the role of quinone movement during electron transfer [29,30]. Unlike the quinone type of reaction centers, the iron-sulfur reaction centers could ideally be well served by having two functional branches of cofactors. The iron-sulfur clusters are more functionally symmetric than the quinone electron acceptors reflective of their role of single electron carriers to an external protein [31]. Whether electron transfer in the iron-sulfur type of reaction centers is symmetric or asymmetric remains a subject of current investigation.

5. Implications for evolution

The features common to all types of photosystems can be taken as a reflection of a primordial reaction center. The consistency of the overall motif indicates that structural or assembly needs dictate a particular packing arrangement of transmembrane helices that embed the cofactors. The conservation of a tetrapyrrole dimer as the primary electron donor suggests that this is an essential requirement for any functional photosystem. Within this constraint, the individual characteristics of the donor can be modified to adjust to changes in the photosynthetic pathways and to different light conditions. The elasticity of the dimer properties contributes to the ease with which these types of modifications can evolve. The photosystems were able to retain the general donor structure of two coupled macrocycles and adjust the functionality by both changing the protein-donor interactions and varying the chemical nature of the macrocycle from bacteriochlorophylls to chlorophylls. For example, in order to gain the functional capability to oxidize water, it was necessary for the primary donor to both become highly oxidizing and coordinate electron and proton transfer with a metal complex [32].

Although the evolutionary pathways cannot be established uniquely, the process giving rise to the two core subunits that are related by an approximate two-fold symmetry axis can be traced by alterations in the structural genes (reviewed in [6]). Comparison of the biosynthetic pathways of the various tetrapyrroles found in the reaction centers can also be used to track the evolutionary path. Consideration of both the energetic requirements and the pigment composition needed for photosynthetic capability has led to specific scenarios for the stages in the evolution of photosynthesis (reviewed in [33]). Additional clues should be provided by characterization of newly discovered photosynthetic organisms that contain novel cofactors [34,35].

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