Photosynthetic Reaction Centers

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THE CONCEPT AND PHYSICAL REALITY OF REACTION CENTERS (RCS)

The capture of solar radiation and the conversion of its free energy into chemical energy involves a sequence of reactions that occur within a physical structure called the photosynthetic RC. Following the initial capture of a photon by antenna pigments, the photon is transferred to the RC pigments, where it gives rise to a separation and stabilization of charge across the photosynthetic membrane. Figure 1 depicts this process and illustrates the time scales typically involved. One feature of the photochemistry is that all photosynthetic RCs undergo charge separation with a quantum yield approaching unity, which makes them marvelous molecular machines.

The first notions of the operation of a photosynthetic RC originated with the photosynthetic unit experiments of Emerson and Arnold (9), which demonstrated that approximately 2,500 chlorophyll molecules were involved in the release of just one molecule of O₂. Thus, a photosynthetic unit contains numerous pigments but the photochemically active chromophores are present in much lower concentration. This pioneering concept led to the distinction of two types of pigments: the light-harvesting, but photochemically inactive, antenna chromophores; and the photochemically active RC pigments. The antenna pigments physiologically increase the absorption cross section of the RC dramatically. Moreover, they ensure that the potentially reactive intermediates containing unpaired electron spins (e.g. semiquinones) generated by single photon photochemistry are efficiently converted by a second photochemical event to products (e.g. hydroquinones) that contain only paired spins. For efficient energy transfer between the antenna and the RC, the RC absorbs at longer wavelengths, effectively forming a trap for excitation energy. Despite these conceptual advances, more than 35 years passed before the first physical isolation of a pigment protein RC complex was reported (17). Since that time, many other RCs have been isolated and characterized biophysically and biochemically.

Structural and Operational Insights

Insight into the molecular organization of the RC has been derived, initially, from spectroscopic studies and, subsequently, from the development and analysis of high-resolution crystal structures of several photosynthetic organisms. The first RC structurally resolved (3 Å) was of the purple bacterial RC from *Rhodopseudomonas viridis* (7), for which the 1988 Nobel Prize was awarded. This was soon followed by the elucidation of several other purple bacterial structures. We are now witnessing the appearance of detailed RC structures from oxygenic systems, most notably the 4Å structure of photosystem I (PSI; 13). Good progress is also being made toward achieving two- and three-dimensional structures of photosystem II (PSII) crystals. It is surprising that the structures of all of the different RCs show a dimeric core with a pseudo-C₂ axis of symmetry. This feature is illustrated in Figure 2 in the example of a purple bacterial RC. The holoprotein is shown on the left. The charge-separating RC pigments contained within the structure (Fig. 2, right) are aligned along the C₂ symmetry axis with the two photochemically active (bacterio) chlorophyll pigments positioned in close proximity. Exciton coupling between these two pigments provides a red shift in the optical spectrum that contributes substantially to forming the low-energy trap discussed above. The conversion of photons to chemical potential involves photoexcitation and initial charge separation to produce an oxidized (bacterio) chlorophyll and reduction of one of the other chlorin pigments in the RC. From this chlorin, the electron migrates to reduce a quinone in less than a nanosecond (Fig. 2). It is interesting that the strength of the dimer exciton coupling has changed substantially during the course of oxygenic RC evolution from photosynthetic bacteria. The bacteria usually have strong couplings, approximately 2,000 cm⁻¹, whereas the plant and algal RCs have a much weaker coupling, typically approximately 300 cm⁻¹ (8). The weaker coupling in the oxygenic RCs increases the thermodynamic efficiency of photon capture so that a significant improvement in useful free energy capture from the photon is realized. Subsequent proton-coupled electron transfer steps (Fig. 1) stabilize the charge separation effectively and ensure the near-unity quantum efficiency of photosynthesis.

A remarkable aspect of the RC structures is the occurrence of two almost identical electron acceptor pathways arranged along the C₂ axis relative to the
primary charge-separating dimer (bacterio) chlorophyll (Fig. 2). This finding posed a key question: Does electron transfer involve both branches? In the purple bacterial RC, only one branch is active although the inactive branch can be forced into operation with modification of amino acid side chains on the active branch (1). The strong asymmetry imposed on primary charge separation photochemistry in the purple bacterial RC results from two homologous polypeptides that function as a heterodimer. A heterodimer is also involved in the core of the RCs of PSI and PSII. However, some RCs, such as heliobacteria (2) and green sulfur bacteria (6, 18), contain two identical homodimeric polypeptides, and electron transfer is potentially bifurcated.

Genetic sequence information has greatly improved the understanding of the origin of the RC proteins. From the sequence analysis, it became clear that the purple bacteria RC is remarkably similar to that of PSII, and PSI was also discovered to have similarity with that of the green sulfur bacteria (6, 10). Further elaboration with 16S-rRNA phylogenetic trees (5) and broader homology comparison (14) revealed a close interrelationship among many RCs. Recent structural comparisons between PSI and PSII, for example, show a distinct structural homology, which suggests that even these two RCs likely share a common ancestor (13).

TYPES OF RCS

The general details of RC structure and function described above persist among photosynthetic organisms, but differences in detail have become apparent. Today, we recognize six different classes of photosynthetic RCs. The principal variations lie in the RC pigments (chlorophyll versus bacteriochlorophyll), the size and nature of the antenna pigment array, the associated longest wavelength maximum and strength of the pigment exciton coupling, and the thermodynamic coupling of the primary donor chlorophyll dimer (P) to its acceptor system (i.e., its midpoint reduction potential). Figure 3 presents a summary of the various RCs, cofactors, and electron transport chains. The six classes of RC divide into two forms: the type I and type II RCs (10, 15). The type I RCs comprise PSI, the gram-positive heliobacteria, and the green sulfur bacteria, all of which share iron-sulfur clusters as electron acceptors. The type II RCs from PSII, purple bacteria and the green filamentous bacteria, share quinone acceptors that serve as two-electron reductants. Two of these RCs, from heliobacteria and the green filamentous bacteria, have only been recognized quite recently and there may be others that await discovery—the field continues to progress rapidly.

Further differentiation in photosynthetic organisms is found in the structure and arrangement of the antenna pigments associated with each RC. The RC from heliobacteria features a very simple organization with a core containing approximately 40 chlorophyll g and no additional auxiliary peripheral antenna proteins (2). Building on this organizational theme are RCs from PSI and green sulfur bacteria, which contain large numbers (~100) of pigments attached directly to the polypeptides that bind the RC components (13), as well as an extensive external antenna array with which the RCs communicate in a controlled way. At the other extreme are the RCs from purple bacteria and PSII, which contain only six...
to eight pigments arranged along the $C_2$ symmetry axis and are fundamental to the charge separation process. These RCs rely on a substantial antenna system as conduits of excitation energy. This antenna system is bound to polypeptides distinct from the RC polypeptides.

**OXYGENIC PHOTOSYNTHESIS**

The incorporation of two RCs in series during the evolution of plant and algal photosynthesis represents a brilliant strategy for using an inexhaustible supply of water in the unlikely role of reductant without sacrificing the ability to use photons in the red ($\lambda > 600$ nm) region of the spectrum. For a single RC oxidizing water and reducing NADP, photons of about 500 nm or shorter would be required to span the entire redox-potential range between the two products ($O_2$ and NADPH) with sufficient irreversibility to ensure a high quantum yield. Using two photoreactions in series, this energetic requirement is relaxed, and photons in the longer wavelength region ($680$ nm [PSII] and $700$ nm [PSI]) become useful. The overall quantum requirement for water oxidation increases from four photons to eight:

PSII: $2H_2O \rightarrow O_2 + 4 H^+ + 4e^- \\
PSI: 2NADP \rightarrow 2NADPH$

but lower-energy photons, up to $700$ nm, are able to drive the process. Coordination of two photosystems, however, requires significantly greater sophistication to balance the incoming excitation energy to the RCs associated with the two photosystems. To meet this requirement, PSI and PSII demonstrate significant differences in pigment composition and placement of the antenna proteins as a function of the light quality. In plants, this situation is highlighted by the lateral heterogeneity between the two RCs, which results in the physical separation of the PSII RC to the grana-appressed region and the PSI RC to the nonappressed region of the thylakoid (3). This division of RCs forms the basis of the biochemical isolation procedure that has been the cornerstone of much of the biochemical and biophysical work with PSII (4).

The charge-stabilizing reactions that occur following primary charge separation in the RC are coming into sharper focus. A key realization has been that these reactions are often proton coupled; that is, the motion of the electron must be coupled in some fashion to the motion of a proton (Fig. 1). This marks the conversion from pure photon and electron chemistry to chemistry that involves nuclei as well. Oka-mura and his coworkers have produced seminal results on electron/proton coupling on the reducing side in the bacterial RC (16). Results that incorporate these and other thoughts concerning proton/electron coupling in PSII are emerging as the underlying mechanism that drives water oxidation to produce $O_2$.

To oxidize water, potentials upwards of $1$ V must be generated in PSII; moreover, the observations by Joliot and Kok (12) that $O_2$ evolution follows a four-
flash oscillatory pattern necessitates that the oxidizing equivalents produced by P680⁺ must be stored. To accomplish this, P680⁺ (midpoint reduction potential at pH 7, approximately 1.2 V) is intimately coupled to a redox-active Tyr (TyrZ) and an inorganic Mn₄Ca₁Clₓ cluster where the water oxidation reaction is catalyzed. Current insights into the cluster structure have been largely driven by x-ray absorption spectroscopy, which predicts that the catalytic manganese complex is organized as pair of manganese-oxo dimers (20). The water oxidation reaction is more difficult to access. Recent H₂¹⁸O/H₂¹⁶O exchange measurements (11) show rapid exchange of substrate water (ms time regime), supporting the notion that substrate water is bound terminally to manganese. A number of other measurements concerning the water oxidation reaction have led to a metalloradical model for PSII water oxidation. In this proposal, TryZ is directly involved in hydrogen atom abstraction from the substrate water terminally bound at the manganese cluster (19). This proposal, as with most hypotheses, has attracted both supporters and detractors!

It is certain that in the next few years the structure of PSII and the oxygen-evolving complex will be resolved at high resolution, as will be the structures of many other photosynthetic RCs. Already the last 25 years have seen tremendous advances in the understanding of photosynthetic RCs. Long-held concepts have been challenged, reinvestigated, and changed as the results of new structural, dynamic, biochemical, and molecular biological insights. These have been stimulating and exciting developments; undoubtedly they will continue.

**LITERATURE CITED**


17. Reed DW, Clayton RK (1968) Biochem Biophys Res Commun 30: 471–475