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RE-ENGINNEERING PHOTOSYNTHETIC REACTION CENTERS

Steven G. Boxer,* Jon Stocker, Stefan Franzen, and Joshua Salafsky
Department of Chemistry Stanford Unversity Stanford, CA 94305-5080

Abstract: The properties of photosynthetic reaction centers which make them interesting for technological applications are reviewed. Protein engineering has been used to systematically modify the oxidation potential of the initial electron donor in photosynthesis. The driving force and rates of electron transfer reactions in the reaction center have been altered by application of external electric fields. This leads to changes in the population of various intermediates which is reflected in changes in the absorption properties of the sample. The first example of engineering a new site for covalent attachment of new components to the reaction center is described.

1. Introduction to Photosynthetic Reaction Centers

Photosynthetic reaction centers (RCs) are the site of the initial light-driven charge separation steps of photosynthesis. The best characterized RCs are isolated from photosynthetic bacteria. The three dimensional structures of RCs from several species of bacteria have been determined to atomic resolution[1-4]; this structural information provides the basis for our current understanding of the initial steps in photosynthesis. The overall function of the RC is to absorb a photon and transfer electrons over substantial distances with great efficiency.

The precise steps are most easily understood by reference to Figure 1 which is a schematic representation of the redox-active components. The primary electron donor, P, is a pair of strongly interacting bacteriochlorophylls (BChls). It is often called the special pair, and its properties are those of the supermolecular dimer, not a monomer. Following photoexcitation, ^1P transfers an electron within a few ps at room or cryogenic temperatures to H_L, a monomeric bacteriopheophytin molecule (BPheo: BChl where the central

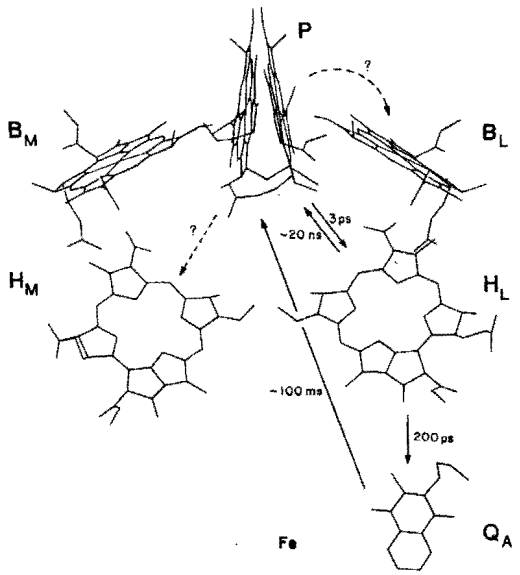

Figure 1: Arrangement of the redox-active components of the reaction center taken from the x-ray coordinates.[1-4] The notation is discussed in the text.

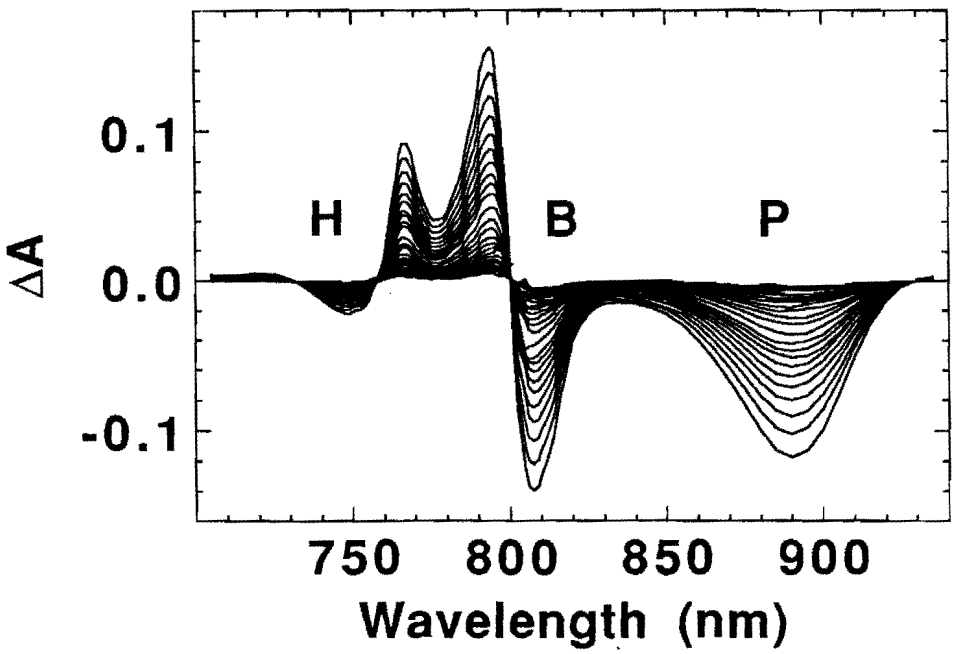

Figure 2: Transient absorption spectrum on the ms timescale illustrating the changes that occur in the near infra-red region following excitation of reaction centers. The bands are labeled corresponding to the chromophores illustrated in Figure 1. A large net change in absorption (a bleach) is observed for the special pair, P. Bandshifts are observed for the B and H bands due to the electrostatic interaction with the $P^+Q_A^-$ dipole.

Mg ion is replaced by 2H). The precise mechanism of this reaction, especially the function of the monomeric BChl labelled B_L, is the subject of current debate. H_L^- transfers an electron to Q_A within a few hundred ps. Q_A transfers its electron to Q_B on the hundreds of μs timescale. By this process, the state $P^+Q_B^-$ is formed with essentially unit quantum yield, and it has a lifetime of several seconds. If Q_B is removed, the $P^+Q_A^-$ charge-separated state lives for about 100 ms. If Q_A is removed, the $P^+H_L^-$ charge-separated state lives for about 10 ns. Thus, the rate of formation of each intermediate is always several orders of magnitude faster than its decay ensuring a high quantum yield for each step.

In its natural setting, the RC is embedded in a membrane bilayer. The hole on P^+ created by the processes described above is refilled by electron transfer from a reduced cytochrome which is either bound to the RC or diffuses to it. Because the electron is on Q_B^-, the components on the right hand side as illustrated in Figure 1 are available to repeat the cycle described in the previous paragraph. This delivers a second electron to Q_B, producing $Q_B^=$, which then picks up two protons, and leaves the RC complex as the neutral hydroquinone. This complicated process is essential for the biological function of the RC; however, in the work described in the following, these latter steps are not useful, and only work on Q_B-depleted RCs will be discussed.

Aside from Q_B, two other chromophores are seen on the left hand side of the structure, B_M and H_M, which are BChl and BPheo, respectively. These components, along with the entire RC protein at the level of its secondary structure, are related to the components on the right hand side of the Figure by a local C_2 axis of symmetry running vertically from the center of P to the non-heme Fe atom. For reasons which are not understood, the initial electron transfer steps only occur along the right-hand (L- or sometimes A-) branch of redox-active components. Therefore in the following the subscripts on components will be dropped. This unidirectionality of electron transfer is an excellent example of exquisite molecular-level control of electron movement though an insulating medium. It is therefore a prime example of an elementary component in a molecular electronic

device.

From the perspective of possible techological applications, several other aspects need to be highlighted. First, the reaction forming P^+ from ^1P leads to the complete loss of the strongly allowed, lowest energy electronic transition, known as the Q_y transition, which occurs at approximately 900 nm. Thus, whenever the RC is in the P^+ state, the near infrared absorption spectrum is very different from the neutral state. The changes in the near infrared region are illustrated in Figure 2. In addition, P^+ has a characteristic absorption at about 1250 nm which is absent in the neutral state. The oscillator strength of this absorption is much smaller (10-50 times) than that of the Q_y transition of P. A second key feature is that all of the components in Figure 1 are encased in two polypeptides, known as the L and M polypeptides. These polypeptides bind the redox active components tightly, protect them from exogenous impurities, fix their positions so that diffusion cannot occur, and provide some level of control over the redox properties of the chromophores (see below).' A third polypeptide known as the H-subunit is also associated with the RC complex; however, none of the redox-active components interacts with this protein. The genes which code for the L- and M-polypeptides have been cloned and placed on plasmids which allow for efficient production of RCs in strains where the native RC genes are deleted.[5] Thus, it is possible to change individual amino acids which interact with the redox-active components or larger segments of the protein sequences using standard recombinant DNA methods.

2. Engineering the Redox Potential of the Special Pair

In order to further our understanding of the unidirectional (L-side only) charge separation steps, we have changed a large number of amino acids which are in contact with P and are different on the right and left sides of Figure 1. These are called symmetry mutants because they increase the symmetry of the RC structure. The first of these, named Syml, makes most of the contacts with P into L-side amino acids.[6] Even though a large number of amino acid changes has been introduced (nine), these RCs assemble and can be readily isolated.

Although we had hoped that electron transfer might now occur down the M-side branch of components, we found that it does not. Instead there are relatively small changes in the rates of the charge separation steps. Surprisingly, these alterations in primary sequence substantially affect the mid-point potential for the P/P^+ couple, making it much more difficult to oxidize. This means that the driving force for the initial charge separation step is smaller than in wild-type.

Further examination of the changes in Sym1 suggested that a particular change, Phe(M)195 → His might introduce a hydrogen bond to the M-side BChl comprising P. This was tested by making the single amino acid mutation (M)F195H (see Figure 3, called the His/His mutant because symmetry-related (L)168 and (M)195 are now both His), and, indeed, the special pair in this strain is as difficult to oxidize as in Sym1. Woodbury and co-workers have also produced the reversed symmetry mutant (Phe/Phe) and find that P is easier to oxidize.[7] Extensions of this work to many residues are currently in progress, and changes which affect the redox level of other components in the electron transport chain are in progress in a number of labs. The key point is that engineering of a critical aspect of RC function is possible. This basic result will likely prove to be important in the next generation of applications because it permits us to control the driving force for electron transfer between P^+ and externally appended redox components.

3. Controlling the Rate of Electron Transfer with External Electric Fields

As seen in the previous section, the redox potential for individual components can be shifted in discrete steps by changing amino acids in the environment of the components. An alternative and complementary approach is to change the free-energy level of charge-separated intermediates by application of an electric field. The interaction energy of a dipole with an electric field contributes to the free energy. For a charge-transfer process, the change in dipole moment is: $\Delta\mu = \mu_{final} - \mu_{initial}$, and the interaction energy

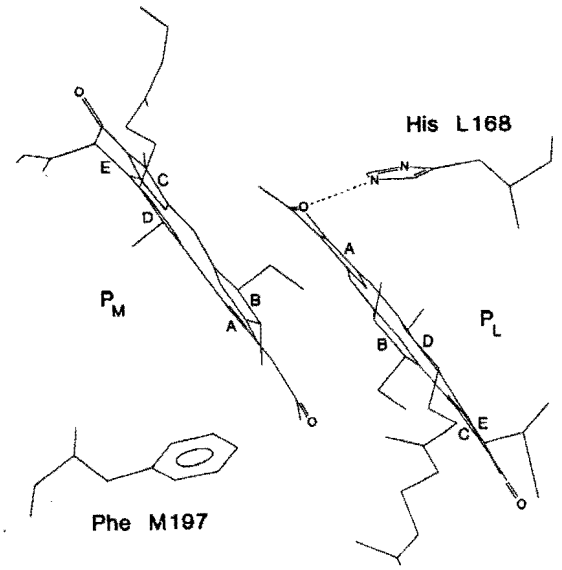

Figure 3: Detailed diagram of the special pair taken from the x-ray coordinates for *Rb. sphaeroides* reaction centers.[3] Residue (M)197 is phenylalanine in wild type and is not capable of hydrogen bonding to the special pair. In the mutant this residue [(M)195 in *Rb. capsulatus*] is converted to histidine which may form a hydrogen bond.

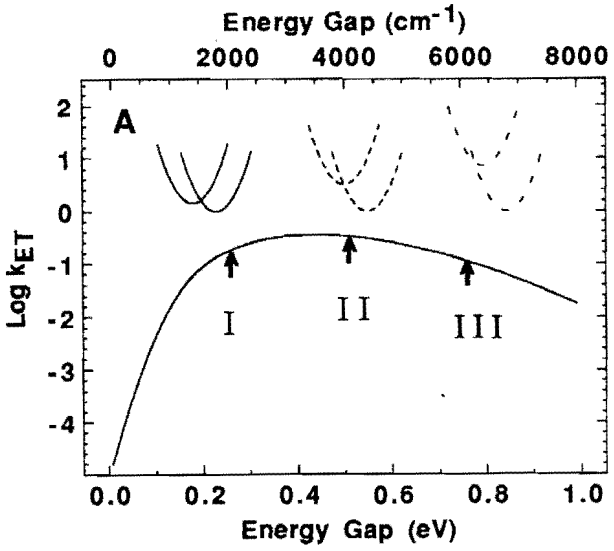

Figure 4: Schematic illustration of the origin of the effect of changing the driving force of an electron transfer reaction on the rate of electron transfer. I, II and III are the normal, activationless (optimally exothermic) and inverted regions, respectively.

with the external field, F_{ext}, is $\Delta U = -\Delta\mu \cdot F_{ext}$. Figure 4 illustrates why changing the energy of a dipolar intermediate will affect the rate of electron transfer. Figure 4 shows a typical calculated dependence of the rate of electron transfer on the free energy change for the reaction. By shifting the free energy upon application of an electric field it is possible to tune the rate of electron transfer. In this sense, the applied field is analogous to the amino acid mutations which affect individual redox potentials discussed in the last section. With the combination of shifts in potential produced by specific amino acid changes and external fields, it is possible to sample a wide range of driving forces.

During the past few years we have investigated the effects of applied fields on the initial charge separation steps and on the long-distance, much slower $P^+Q_A^-$ charge recombination process. This is described in detail in the original papers[8,9]. From a technological viewpoint, these changes are not very interesting because the intrinsic rates (measured at low temperature) turn out to be relatively insensitive to applied field. Therefore, huge fields would be needed to change the populations of states in the reaction scheme to any appreciable extent. This is illustrated in Figure 5 for the $P^+Q_A^-$ recombination reaction, where it is seen that even at a very high applied field, the rate of recombination for a non-oriented sample is only weakly affected at low temperature. However, rather interesting results are obtained at room temperature because of the participation of competing, activated steps which can be greatly enhanced by the applied field. This is also shown in Figure 5, where it is seen that at room temperature the applied field causes a very large change in the $P^+Q_A^-$ decay kinetics. These plots are obtained by subtracting the decay measured in the absence of field from that obtained when the field is on during the decay (the field is off during the formation of $P^+Q_A^-$). Where the difference decay is positive, the rate of decay slows (e.g. at all times in the low temperature data); where the difference decay is negative the decay rate is accelerated by the field (e.g. at early times for the room temperature data).

In order to understand the acceleration in the $P^+Q_A^-$ decay rate

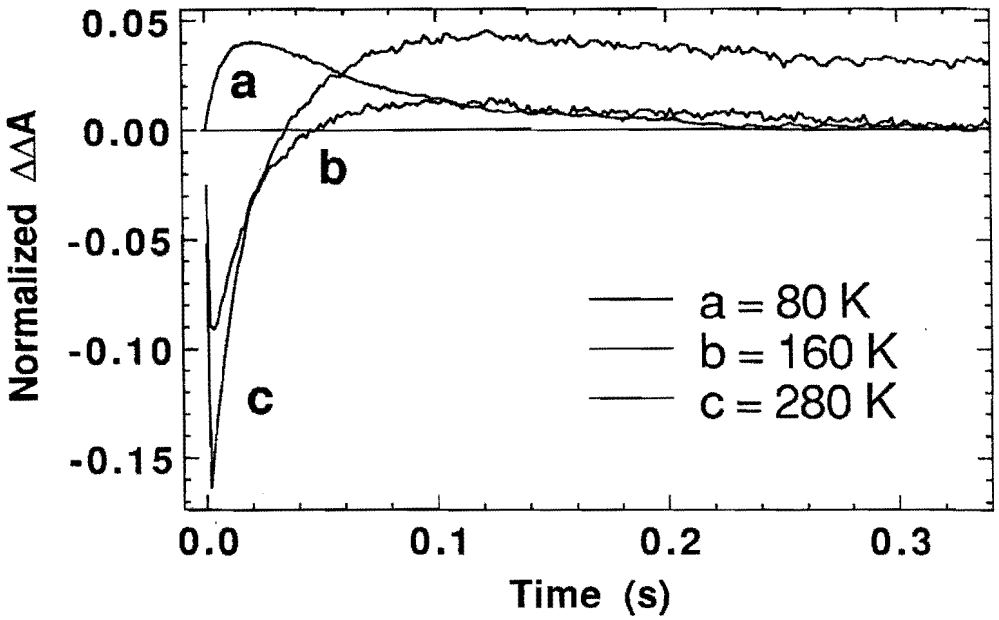

Figure 5: The effect of an applied electric field on the decay kinetics of the $P^+Q_A^-$ state as a function of temperature. Where the normalized difference decay is positive, the rate is slower than in the absence of the field.

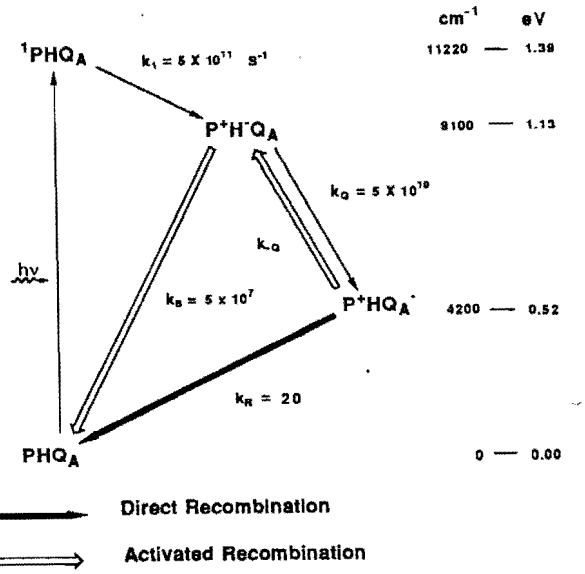

Figure 6: Schematic reaction scheme and approximate energy levels for characterized intermediates in the reaction center. The direct and activated routes for $P^+Q_A^-$ decay discussed in the text are highlighted.

at a qualitative level, it is necessary to examine the energies of various intermediate states in the RC as illustrated in Figure 6. Focusing on the formation and decay of P^+Q^-, it is seen that the P^+H^- state is not far in energy above the P^+Q^- level in the absence of a field. The decay of P^+Q^- can occur by the direct route, labelled k_{direct}, or by activated reformation of P^+H^- and decay to the ground state (illustrated with the bold arrows). Upon application of the field, the energies of states move relative to each other depending on the difference dipole moment between the $P^+Q_A^-$ and P^+H^- states. This difference dipole is smaller than for $P^+Q_A^-$, but is sufficiently large that the rate of $P^+Q_A^-$ decay via an activated route (shown with darker arrows) is enhanced by the field.

The effect of the applied field on the activated route can only accelerate the recombination rate because the intrinsic decay via the P^+H^- is many orders of magnitude faster than via the $P^+Q_A^-$ state. On the other hand the effect of the applied field on the direct rate constant can either accelerate or decelerate the rate. Of course, for an isotropic sample, although some orientational subpopulations satisfy the conditions described qualitatively above for rapid activated recombination, others are shifted such that the levels are further separated, and the activated process is inaccessible. To some extent, these orientations can be trapped in the $P^+Q_A^-$ state. However, by reversing the direction of the applied field, the energies of dipoles which shifted down can be shifted up, and this population then decays very rapidly, like that orientational subpopulation which was shifted up in energy originally. Because these changes in population are seen as large changes in absorption in the Q_y band of P, this scenario represents a primitive electrical-to-optical switching device. At the present time the rate-limiting process is slewing the electric field (limited by the RC time-constant of the sample). This can likely be improved considerably by working with much smaller samples. Many possible variations on this theme can be imagined to produce very fast, short-term optical memories, spacial light modulators, and other electro-optical devices.

4. Interfacing the RC to the Outside World

There have been many attempts to duplicate the exquisite function of the RC with artifical components. Although some of these are very elaborate and mimic some aspects of the RC function, these synthetic model compounds are extremely difficult to synthesize, and only very small quantities are available. By contrast, the photosynthetic bacterium synthesizes and assembles all the RC components, and intact RCs can therefore be obtained in very large quantities at low cost. As described briefly in Section 2, it is straightforward to alter individual or groups of amino acids using recombinant DNA methods. Therefore, we wondered if it might be possible to consider the entire RC complex as an intact model system and exploit its function by interfacing it to external components.

As a first step in this direction, we have introduced a Cys residue on the surface of the RC in reasonably close proximity to P. Cys contains a sulfhydryl (-SH) group which can be easily covalently modified. There are no other sulfhydryl groups which appear to be accessible from the aqueous surface. For the first set of experiments, we chose residue (M)189 for modification. Examination of the x-ray structure by computer graphics suggests that this residue is surface-accessible and also close to P. The surface region of the RC near P has a depression or crater which likely serves as the concave surface for binding to the convex surface of cytochrome, which is a small globular (roughly spherical) protein. Residue (M)189 is on the edge of this crater. This may prove to be important for attachment of the RC to relatively flat surfaces (see below). This mutation was introduced and RCs were isolated. Their properties are very similar to wild type.

In order to demonstrate that the sulfhydryl group introduced at position (M)189 is indeed on the surface and amenable to covalent modification, we have attached dye molecules which are functionalized so as to react uniquely with sulfhydryl groups. Mutant RCs were incubated with this dye, and the unreacted dye was separated from the RCs by gel filtration chromatography. As expected, a new absorption band corresponding to the dye is observed in the absorption spectrum

of this complex, and the added absorption corresponds to nearly stoichiometric (1:1) covalent attachment of the dye to the RC. A much smaller amount of non-specific binding was observed for the control wild type. These and related modified RCs are currently being studied by transient absorption to measure energy and electron transfer as well as pH shifts on a fast timescale. This is the first example of a synthetically modified RC, and demonstrates the feasibility of building higher order structures with the RC as the central component. Experiments are in progress where these mutant RCs are being attached to modified glass and gold surfaces, both to orient RCs, and, in the latter case, to attempt investigate electron transfer from the electrode to P^+ following photoinduced charge separation within the RC.

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