Short Review

Optical properties, excitation energy and primary charge transfer in photosystem II: Theory meets experiment

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Abstract

In this review we discuss structure–function relationships of the core complex of photosystem II, as uncovered from analysis of optical spectra of the complex and its subunits. Based on descriptions of optical difference spectra including site directed mutagenesis we propose a revision of the multimer model of the symmetrically arranged reaction center pigments, described by an asymmetric exciton Hamiltonian. Evidence is provided for the location of the triplet state, the identity of the primary electron donor, the localization of the cation and the secondary electron transfer pathway in the reaction center. We also discuss the stationary and time-dependent optical properties of the CP43 and CP47 subunits and the excitation energy transfer and trapping-by-charge-transfer kinetics in the core complex.

1. Introduction

Photosystem II (PSII) captures solar energy by a large antenna system consisting of chlorophylls (Chls) and carotenoids and transfers the energy to the reaction center of the complex, where the excitation energy is used to create a charge separated state. The oxidized donor of PSII is a very strong oxidant capable of driving the oxidation of water to O2. PSII of higher plants and algae consists of the PSII core complex and peripheral light-harvesting complexes. Cyanobacteria contain only the PSII core which is composed of four large subunits (D1, D2, CP43, CP47), Cytb559 constituted by subunits PsbE and PsbF, and several low molecular weight...
membrane-intrinsic subunits. At the lumenal side of the complex three membrane extrinsic subunits are located. The structure of PSII from *Thermosynechococcus elongatus* has been determined by X-ray crystallographic analysis to a resolution of 2.9–3.5 Å [1–3]. Very recently, the resolution has been improved from 2.9 Å to 1.9 Å as reported by Shen et al. on the 15th International Congress of Photosynthesis in Beijing. This improvement offers a significant amount of structural details that could not be reliably determined before. The published X-ray structure of dimeric PSII at a resolution of 2.9 Å [3] identifies 36 transmembrane helices and nearly 100 cofactors which include: 35 Chl, 12 β-carotene, 2 pheophytin *a* (Pheo), one b-type (Cytb559) and one c-type heme (Cytc550), 2 plastoquinones (QA and QB), a non-heme-iron, the Mn4Ca-cluster responsible for water oxidation and 25 integral lipids. CP43 and CP47 bind 13 and 16 Chla, respectively (see Fig. 1). The D1 and D2 protein subunits coordinate the cofactors involved in the light-induced electron transfer reactions (see Figs. 1 and 2). The central part of the RC contains four Chl and two Pheo. Two transmembrane branches of cofactors related by pseudo-C2 symmetry connect the dimer PD1–PD2 and the plastoquinones (see Fig. 2). Each branch is composed of the so-called accessory chlorophyll (denoted as ChlD1 and ChlD2 in Ref. [3]), Pheo (PheoD1 and PheoD2) and one plastoquinone (QA and QB, respectively). The dimer of two chlorophyll *a* molecules, P680, located on the lumenal side is often referred to as P680. Originally, P stood for “pigment” and 680 is the wavelength of the maximum bleaching in the red upon oxidation of this pigment at room temperature as a result of the light-induced charge separation in the reaction center [4]. The reasonable agreement between the absorbance difference spectrum for the oxidation of P680 and that for the oxidation of monomeric Chla has been taken as evidence that P680 is a chlorophyll *a* molecule [4].

A word of caution is necessary concerning the term P680. The spectroscopic term P680 that is generally used in the literature no longer indicates the same entity in its cationic and singlet and triplet excited form but different subsets of the six innermost pigments of the RC [5]. In this review, the lumenal dimer of Chl molecules will be termed PD1–PD2, or “special pair” [1] solely based on the structural model in reference to the special pair P–P of purple bacteria (Figs. 3 and 4). The structural identities of the primary donor (spectroscopic term P680), the oxidized donor (spectroscopic term P680) and the reaction center triplet (spectroscopic term P680) of PSII reaction centers will be discussed in detail in this review. The PSII reaction center is of the same type as that of purple bacteria. Figs. 2–4 exhibit the high structural similarity between the arrangements of pigments. There is an almost perfect overlay of ChlD1–PheoD1 with its counterpart BA–HA in purple bacteria (left part of Fig. 4). A remarkable difference exists between the two special-pair structures (right part of Fig. 4). Whereas the ring systems of the two bacteriochlorophylls constituting the dimer PA–PB in the case of purple bacteria overlap with their pyrrole rings I, the “special pair” of the PSII RC is in a staggered rather than an eclipsed conformation. It seems that a slight tilt of the two “special pair” Chls with respect to each other has disrupted the wavefunction overlap in PSII. This small tilt might have had large consequences for the optical and redox properties of the “special pair”, as will

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**Fig. 1.** Chlorophyll containing subunits CP43, D1–D2, and CP47 of PSII core complexes in *T. elongatus* [3]. The transmembrane helices are shown as cylinders and colored in purple (CP43), yellow (D1), orange (D2) and red (CP47). The Chls (and the two Pheo’s) are shown in green, their phytyl chains have been truncated for clarity. A detailed arrangement of cofactors in the D1–D2 subunit containing the RC is shown in Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 2.** Cofactor arrangement in the central D1–D2 heterodimer of PSII core complexes of *T. elongatus* [3]. The Chls are colored in green, Car’s in red, Pheo’s in yellow, plastoquinones in black, Tyr2 in blue. The Mn4Ca cluster of the oxygen evolving complex (OEC) is shown as purple spheres and the non-heme iron (Fe) as orange sphere. The substituents of the macrocycles of the Chl’s and Pheo’s have been truncated for clarity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 3.** Cofactor arrangement in the purple bacteria reaction center of *R. sphaeroides* [6]. The bacteriochlorophylls are colored in green, the carotenoid in red, bacteriopheophytins in yellow, ubiquinones in black. The non-heme iron (Fe) is shown as orange sphere. The substituents of the macrocycles of the bacteriochlorophylls and bacteriopheophytins have been truncated for clarity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 4.** Left part: overlay of accessory (bacterio-) chlorophylls and (bacterio-) pheophytins of the electron transfer active branch of PSII (grey) and the RC of purple bacteria (black). Right part: overlay of the special pair (bacterio-) chlorophylls of PSII (grey) and the bacterial RC (black) [7].
be discussed in detail further (Section 7.1). Whereas the absorbance spectrum of the RC of purple bacteria (bRC) consists of separate bands that reflect the absorbance of the special pair $P_A^-P_B^+$, the two accessory bacteriochlorophylls $B_A$ and $B_B$ and of the two bacteriopheophytins $H_A$ and $H_B$ of the two branches $A$ and $B$ (e.g. [8]), the spectrum of D1D2cyt655 complexes containing the RC of PSII [9,10] shows almost no structure even at 4 K (e.g. [11,12]). All optical bands strongly overlap. In this review we will discuss approaches to decipher the optical properties of PSII reaction centers despite this “spectral congestion” problem.

The following scheme of primary reactions was established for PSII by various spectroscopic techniques. After absorption of light an electronically excited state of the reaction center chlorin is converted into a short-lived charge separated state. The formation of the radical pair $P_{a,b}^-P_{a,b}^+$ occurs within about 50 ps. The kinetics of excitation energy transfer and light-induced primary charge separation in D1D2cyt655 complexes and intact PSII complexes have been studied intensively by time-resolved absorption and fluorescence spectroscopy [13–17]. However, it is still a matter of debate which step is rate limiting: the transfer of the excitation energy to the reaction center or the charge separation [7,13–15,18].

Subsequent stabilization of charge separation is achieved by electron transfer from $P_{a,b}^-P_{a,b}^+$ to $Q_A$ in 200–500 ps. $P_{a,b}^+$ is reduced primarily in the nanosecond time range by the donor Ty$P$, the redox active tyrosine 161 of the D1-polypeptide. The oxidized Ty$P$ is in turn reduced by the Mn, Ca-cluster. The manganese cluster stores the oxidizing equivalents required for the oxidation of two water molecules to molecular oxygen. In this way, the one-electron photochemistry is connected to the overall four-electron process of water oxidation. At low temperatures ($T < 100$ K), electron transfer from $Q_A$ to $Q_B$ and from Ty$P$ to $P_{a,b}$ is blocked and charge recombination from $P_{a,b}^+Q_A$ occurs with a half-life time between 2 and 5 ms [19,20]. The extent of $P_{a,b}^+Q_A$ formation decreases progressively with successive flashes. This decrease can be explained by an electron transfer from secondary electron donors (Cyt$b$559, Car, Chl$B$) to $P_{a,b}$ that occurs with a low quantum yield in competition with charge recombination of $P_{a,b}^+Q_A$. Such secondary electron transfer leads to a progressive accumulation of long-lived charge separated states (e.g. Car$^+Q_A^-$) with increasing flash number. The electric field caused by the negative charge located on $Q_A^-$ and the positive charge located on the secondary donor leads to shifts of the transition energies of nearby pigments (Stark effect). The formation of these long-lived states has been investigated by absorbance difference spectroscopy and magnetic resonance techniques [for review see [21]]. The quantum efficiency of photo-induced oxidation of secondary electron donors vary widely [22].

A characteristic electrochromic blue shift of the $Q_A$ band of Chl$D_{1}$ is observed around 550 nm (the so-called C550 band shift [23]) and is an indicator of the formation of $Q_A^-$ [24]. In the $Q_A$ region the assignment of the observed electrochromic band shifts is complicated by the strong overlap of the electronic transitions of the pigments in the PSII reaction center and by the stronger exciton coupling in the $Q_A$ region. Only recently, the absorbance difference spectra associated with the light-induced formation of these long-lived states in PSII core complexes could be described quantitatively in the framework of an exciton model that we term asymmetric exciton model. The latter assumes that the mean site energies of the pigments in the two symmetric branches of the RC are different (see below and [7,5]). When the electron transfer to the quinone acceptors is blocked by pre-reduction or by removal of the primary quinone acceptor $Q_A$, then the reaction center triplet state $^3P$ is formed, in high yield at low temperatures by charge recombination of $P_{a,b}^+P_{a,b}^-$ following singlet–triplet mixing in the radical pair state [25,26]. EPR studies with oriented PSII samples have shown that the plane of the CHl which carries the triplet state is oriented like that of the accessory chlorophylls [27], i.e., the triplet state is localized on one of the accessory chlorophylls, most probably on Chl$D_{1}$ [28]. Although the overall reaction scheme is clear, the molecular identities of some of the functional states and the mechanistic and kinetic details are not. This review discusses recent work combining optical spectroscopy, site-directed mutagenesis and theory that enables to elucidate the electronic (excitonic) states of the PSII reaction center chlorines, in particular, the nature of the excited state that drives the primary charge separation and to answer the question where the cation radical of the primary donor and the triplet state formed by charge recombination of the primary radical pair are localized. The next part of this review describes the current knowledge of optical spectra of the antenna subunits CP43 and CP47 and PSII core complexes. The latter will be termed intact PSII complexes because they represent the minimal preparation unit retaining oxygen evolving capacity.

Finally, the structure-based deciphering of the spectral properties offers the opportunity to calculate the excited state dynamics based on a minimal set of common parameters and to relate the results with experimental data obtained by ultra-fast optical spectroscopy. We include a discussion of PSII of the Chl-containing cyanobacterium Acaryochloris marina, since its RC most likely contains both Chl$D_{1}$ and Chl$D_{2}$, which makes the interpretation of optical spectra easier because the spectral overlap of the different bands is less than in the usual PSII that contains only Chl$D_{1}$.

2. Optical properties of the PSII reaction center

2.1. The multimer model

To explain the marked difference in the optical properties of the RCs of purple bacteria and PSII, discussed in the introduction, it was concluded [29,30] that the “special pair” in PSII has a weaker excitonic coupling than in bRC such that all nearest neighbor couplings are of the same magnitude of about 100 cm$^{-1}$, assuming also a similar overall arrangement of the pigments in the two RCs. In these so-called multimer models ([29,31–33]) the excited states where found to be delocalized over several pigments, whereby oscillator strength is redistributed towards the low-energy exciton states. The assumption of high structural similarity between the arrangement of pigments in the two types of RC is supported by an early homology modeling computational study [30] and by the early [34] and recent X-ray crystallographic studies [1–3] (compare Figs. 2 and 3).

Originally [29,30] it was assumed that the smaller excitonic coupling in the special pair of PSII is due to a larger interpigment distance between the two special pair chlorophylls. However, as shown in the right part of Fig. 4 it is rather a tilt than a translation that distinguishes the two special pairs. A quantum chemical analysis of the Coulomb and short-range couplings and site energy shifts revealed that the large excitonic coupling and the low site energy of the special pair in purple bacteria are caused by short-range electron exchange effects [35]. Therefore, most likely the disrupted overlap of $\pi$-electron wavefunctions in particular of one ring of the macrocycles of the pigments, caused by the mutual in-plane rotation of the pigments, is responsible for the smaller excitonic coupling and higher $Q_A$ transition energy of the special pair in PSII. Except for the slight tilt in the special pair, there is a striking similarity between the arrangement of cofactors in the two RCs. For example, there is perfect overlay between the macrocycles of the accessory (bacterio) chlorophylls and the (bacterio) phaeophytins in the two RCs, as shown for the electron transfer active branch in the left part of Fig. 4. The functional implications of this similarity and the difference in the special pair will be discussed later in detail.
The multimer models of PSII assume the same mean local transition energies of the RC pigments. These so-called site energies are defined as the transition energies at which the pigments would absorb light in their local environment defined by the protein binding site for the hypothetical case of absent resonance energy transfer (excitonic) coupling between the different pigments. Roughly speaking, the positions and intensities of optical bands of the coupled pigments are obtained from the eigenenergies and eigenvectors of the exciton matrix that contains in the diagonal the site energies and in the off-diagonal the excitonic couplings. To take into account disorder introduced by slow (compared to the excited state lifetime of the pigments) conformational motion of the protein, one has to diagonalize this matrix many times for different realizations of disorder in site energies.

Usually a Gaussian distribution function of a certain width is assumed for the site energies, correlations in this static disorder at different sites are neglected and the spectra are averaged by a Monte Carlo procedure (e.g. [36]). Protein dynamics which are fast compared to the excited state life times of the pigments lead to homogeneous broadening and to additional shifts of the optical bands. These effects are taken into account by dynamical theories as reviewed elsewhere (e.g. [36,37]). If the same mean site energies are assumed, as in the multimer models, the degree of delocalization and the contribution of the pigments in the different delocalized (exciton) states depends strongly on the particular realization of disorder. In all multimer models a large contribution of the "special pair" in the lowest exciton state was found [29,32,33,38,39]. We note that this result also holds for the pentamer model introduced by Jankowiak et al. [38,39]. In this model, which considered different site energies of the pigments, the pheophytin of the D2-branch of the reaction center is assumed (i) to be decoupled from the other pigments and (ii) to absorb at 668 nm [38,39]. These conclusions were based on optical difference spectra involving sodium dithionite treatment that was believed to reduce the pheophytin of the D2-branch. However, the assumptions of the pentamer model are in conflict with the crystal structure of PSII [3] and the exchange experiments by Germain et al. [12].

Raszewski et al. [40] revised the multimer model by assuming different mean site energies of the pigments and determining these energies from a fit of the linear absorbance, linear dichroism, fluorescence and circular dichroism spectra of D1D2cyt559 complexes. Afterward the site energies were tested in the simulation of various optical difference spectra of D1D2cyt559 complexes involving chemically modified, oxidized and reduced pigments and pigments in the triplet state [40]. Blue-shifted site energies were obtained for the "special pair" and a red-shifted site energy for the accessory chlorophyll of the D1-branch (ChlD1). In Fig. 5 the resulting density of exciton states (probability that the transition into the exciton state M occurs at the indicated wavelength)

$$d_m(\omega) = \langle \delta(\omega - \omega_m) \rangle_{\text{dis}}$$

(1)

where $\Delta \omega_M$ is the energy transition between the ground state and exciton state M and $\langle \ldots \rangle_{\text{dis}}$ denotes an average over disorder in site energies, is compared with the exciton state pigment distribution function

$$d_m(\omega) = \left( \sum_M |c_{mM}^M|^2 \delta(\omega - \omega_M) \right)_{\text{dis}}$$

(2)

that contains the probabilities $|c_{mM}^M|^2$ to find pigment m excited in exciton state M. As seen in Fig. 5, the lowest exciton state (M = 1) has a large contribution from ChlD1, the second lowest exciton state (M = 2) is dominated by the pheophytin of the D2-branch (PheoD2)

![Fig. 5.](image)

and only the third lowest exciton state (M = 3) contains the excited states of the "special pair" Pd1–Pd2 which are delocalized over the two "special pair" pigments. Because of the large difference between the site energy of ChlD1 and its symmetry partner ChlD2 of the other branch, we term the present model asymmetric exciton model. The difference in site energies leads to partial localization of the exciton states that is seen in Fig. 5 by the dominant contribution of the different pigments m to only one exciton state M, except for the special pair. Nevertheless, shoulders of $d_m(\omega)$ appearing at additional exciton states M show that the excited states are not completely localized, i.e., exciton effects are present.

The rather harsh biochemical treatment of PSII that is necessary to isolate D1D2cyt559 complexes from the PSII core complex has raised the question about the influence of the isolation procedure on the optical and redox properties of the RC pigments (e.g. [41,42]). A difficulty in the interpretation of experiments on PSII core complexes is the overlap of optical bands of the RC pigments with those of the antenna subunits. One way to overcome this problem is to measure optical difference spectra of core complexes where the primary and secondary electron transfer reactions were used to convert particular RC pigments into a different electronic state. Due to the difference, these spectra display the properties of the pigment that has undergone a change in electronic state and of those pigments which are coupled to it. As the couplings between RC pigments and the pigments bound to CP43 and CP47 are weak, the difference spectra reveal direct information about the RC pigments without interference from the antenna pigments. There is, of course, still the problem of overlapping optical bands of the pigments (exciton transitions) in the RC. The latter can be solved.
by combining low-temperature optical difference spectroscopy with site-directed mutagenesis, in which the amino acid residues in the local environment of certain RC pigments are replaced. If this replacement leads only to changes in the local transition energy of the nearest pigment, valuable information about the optical properties of this pigment can be obtained.

Such experiments were performed by Diner et al. [43] on mutants of D1-His198, the axial ligand of P$_{D1}$, and by Schlodder et al. [28] on mutants of D1-Thr179, an amino acid residue nearest to Chl$_{D1}$. The experiments were carried out on PSII core complexes from *Synechocystis* sp. PCC 6803. Important conclusions could be drawn about the identity of functional states at low temperatures, as discussed in detail below.

The P$^\text{+}$Q$_A$ – P$^\text{−}$QA absorbance difference spectra of the wild-type and the mutants are compared in Fig. 6 with calculated spectra, using the exciton Hamiltonian of Raszewski et al. [40] discussed above. The only adjustment of the D1D2cyt559 Hamiltonian necessary to reach agreement with the experimental data on the wild-type is a 4 nm shift of the site energy of Chl$_{D1}$ to even longer wavelengths, i.e. lower energies. In addition, the static disorder of this pigment had to be reduced from 180 cm$^{-1}$ (core complex) for a quantitative fit of the experimental spectra of core complexes [7].

In the calculations of the mutant spectra the site energy of the pigment affected by the mutation was shifted with respect to its wild-type value as indicated in the legend, and the cation was assumed to be localized at P$_{D1}$.

Another point we want to raise about the optical properties of RC pigments is about the relevance of low-temperature experiments for obtaining physiologically relevant information. There is a remarkable change of several difference spectra with increasing temperature [44]. These spectra, which contain a multiple band structure at low temperature, at room temperature all exhibit just a single bleaching at 680 nm. The overlap of different bands at room temperature, of course, is an obstacle in the interpretation of experiments. So the question arises, whether the primary reactions detected at low temperature are the same as those at high T. As shown in Fig. 7, the temperature dependence of the P$^\text{+}$Pheo – P$^\text{−}$Pheo and the P$^\text{+}$Q$_A$ – P$^\text{−}$Q$_A$ spectra can be modelled by assuming the same identity of functional states at different temperatures and taking into account the temperature dependence of the dielectric constant leading to a stronger screening of the Coulomb interaction at higher temperature and thereby to smaller electrochromic shifts in site energies. The increase of $\epsilon_{\text{eff}}$ reflects the higher flexibility of polar side chains above the glass transition temperature (200 K). Similar room temperature values of $\epsilon_{\text{eff}}$ were inferred from Stark shift measurements on photosystem I [45] and on an $\alpha$-helix [46]. In addition, a slight shift in site energies of P$_{D1}$ and Chl$_{D1}$ had to be assumed for a quantitative fit of the data. This shift, however, does not alter the exciton model in that still the lowest exciton state is localized at Chl$_{D1}$. In the case of the T–S spectrum, more than one triplet state contributes at high temperature, as discussed in detail below. The origin of the site energy shift could be a change in the protonation pattern of the protein, or a temperature-dependent mixing of the excited states with charge transfer states, as in the reaction center of purple bacteria [47].

The set of site energies inferred in the asymmetric exciton model by Raszewski et al. [40,7] was confirmed by calculations of linear optical spectra and Stark spectra and comparison with experimental data by Novoderezhkin et al. [48]. The latter model explicitly included mixing of exciton states with a low energy charge transfer state as will be discussed in more detail in the following.
2.3. The asymmetric exciton model with inclusion of a low-energy charge transfer state

From the 2 nm redshift of the Stark spectrum of the D1D2cytb559 complex with respect to the second derivative of its absorbance spectrum, Frese et al. [49] concluded that the low-energy exciton state of PSII RC is mixed with a charge transfer (CT) state. The calculations [7] (solid lines) are compared with experimental data [44] (solid circles). The temperature-dependent wavelengths corresponding to the site energies of PD1 and ChlD1 (PD1/ChlD1) and the dielectric constant εeff used in the calculation of electrochromic shifts are shown as well at each temperature.

The first quantitative simulation of the Stark spectrum was provided by Novoderezhkin et al. [48], who included a low-energy charge transfer state explicit in the exciton Hamiltonian and calculated the spectra in the framework of modified Redfield theory. Therefore, the approach by Novoderezhkin et al. [48] represents an important first step but an extension of the theory, including dynamic localization effects, is needed to draw definite conclusions about the nature of the CT states in the RC of PSII.

The idea of a low-energy charge transfer state in PSII is in line with the finding of Krausz and coworkers [50,51] who detected a long-wavelength homogeneously broadened excited state of the RC that is capable of charge separation. The nature of this state is, however, different from that of the CT state proposed by Novoderezhkin et al. [48]. Krausz et al. [42] reported the absence of CT spectral features in D1D2cytb559 complexes and suggested that the absorption feature in the red region, interpreted as due to CT intensity, is in fact pigment hot band absorption in a 77 K spectrum utilized for the model. We will discuss this unresolved topic further in Section 7 and concentrate in the following on models of primary electron transfer in photosystem II that were inferred from time-resolved experiments.

3. Electron transfer in the PSII RC

3.1. The primary electron donor and the time constant of primary charge separation

van Grondelle and collaborators [13] and Holzwarth and collaborators [15] inferred independently from femtosecond VIS-pump/IR-probe studies and VIS-pump/VIS-probe experiments, respectively, that the primary electron transfer at room temperature occurs between ChlD1 and PheoD1. However, the reported timescale for Pheo reduction differs by a factor of 4–5. Whereas Groot et al. [13] found a 600–800 fs time constant, that of Holzwarth et al. [15] is 3 ps. Taking into account the 30% equilibrium population of ChlD1 at T = 300 K (Fig. 8), these times correspond to intrinsic time constants for primary electron transfer of 200 fs and 1 ps, respectively. The faster of the two time constants is supported from calculations of excitation energy transfer and trapping, as will be discussed in detail below.

It is interesting to note also that a fast subpicosecond time constant for electron transfer was found by van Brederode and van Grondelle et al. [52,53] for electron transfer between the accessory bacteriochlorophyll B\textsubscript{A} and the accessory phoepxyhin H\textsubscript{A} in the RC of purple bacteria, which is an order of magnitude faster than the 3 ps time constant for electron transfer starting at the special pair. However the strong red shift of the low-energy special pair excited state in the bacterial RC makes it the primary electron donor, since the excited state of B\textsubscript{A} could not accept the excitation energy from the LH1 core antenna which absorbs resonant with the special pair. The fact that the excited states in PSII RC are much closer in energy led van Grondelle and van Brederode [54] to suggest that the fast side pathway in bacterial RCs might be the dominant one in PSII. This idea became more and more alive during the last ten years. But a final proof is still missing. The PSII RC of Chl containing A. marina, discussed below, seems to be a nice model system to solve this problem.

As noted already above, an alternative suggestion of van Grondelle and coworkers [48,16] is the existence of two parallel electron transfer pathways, one starting at ChlD1 and one at PD1, where the relative weight is determined by the particular realization of disorder that determines the equilibrium populations of excited pigment states. Recent time-resolved pump-probe data [16], interpreted within this model, were used to infer the respective...
However, their interpretation includes the assumption that ChlD1 transfer starts at the special pair with a time constant of 0.9 ps.

Shelaev et al. [56] concluded that primary charge pump-probe spectra of D1D2cyt complexes at 77 K and concluded that the primary charge transfer time of 1 ps is attributed to electron transfer starting at the putative primary donor. If, for simplicity, we assume that $k_m^{(\text{intr})}$ does not depend critically on disorder in site energies, the rate constant becomes

$$k_m^{(\text{ET})} = P_m^{\text{eq}} k_m^{(\text{intr})}$$

where the equilibrium population of the excited state of pigment $m$ reads

$$P_m^{\text{eq}} = \left( \sum_M f(M) |c_m^M|^2 \right)_{\text{dis}}.$$  

In Fig. 8 this quantity is plotted for all the six core pigments $m$ in the RC. The manifold of equilibrium populations $P_m^{\text{eq}}$ contains a contribution from the particular m of the primary electron donor of the RC.

We identify the whole family of equilibrium populations $P_m^{\text{eq}}$ of the RC with the spectroscopic state $P^*_{680}$, since it characterizes the excited state of the RC prior to electron transfer. We see that at low temperature, $P^*_{680}$ is almost entirely formed by the excited state of ChlD1, whereas at room temperature the excited states of all six pigments contribute to $P^*_{680}$. Based on the above $P_m^{\text{eq}}$ it seems clear that at low temperature primary electron transfer starts at ChlD1, as it was concluded from mutant difference spectra [43] and from a comparison of the experimental and calculated temperature dependence of primary electron transfer [40]. However, at room temperature electron transfer could in principle also start at the “special pair”, as in the RC of purple bacteria, since there is significant population of the excited states of P680 and P690 (Fig. 8).

Below, we will discuss a PSII RC that even at room temperature exhibits strongly asymmetric equilibrium populations of excited states with a dominant contribution from ChlD1. This system, therefore, would be ideally suited to investigate the kinetic details of the electron transfer reaction starting at ChlD1.

Finally, we would like to mention that the charge separation mechanism and rate may be different in PSII core complexes and isolated D1D2cyt complexes containing only the reaction center pigments. Although the exciton Hamiltonians were found to be very similar [40,7], the electron transfer couplings could be different because they depend exponentially on interpigment distances and therefore are more sensitive to slight conformational differences than the site energies and excitonic couplings. As discussed above, a low lying CT state was only detected for core complexes [42]. Recently, evidence was reported for a low-energy charge separating state in PSII at physiological temperatures [57].

### 3.2. Localization of the cation in the state $P^*_{680}$

After primary electron transfer, by subsequent hole transfer the cation may localize on a different pigment than the primary electron donor. The low-temperature mutant spectra of Diner et al. [43] provided evidence for hole stabilization at the “special pair” chlorophyll P680. In agreement with this conclusion, calculations of the wild-type $P^*Q_A$ – $PQ_A$ spectra by Raszewski et al. [7] give best agreement with the experimental data if the cation is assumed to be localized on P680 (Fig. 9).

Is there any evidence about the identity of $P^*_{680}$ at physiological temperatures? The calculation of the temperature dependence of the $P^*Q_A$ – $PQ_A$ difference spectrum (Fig. 7) suggests that the identity of the state $P^*_{680}$ – $PQ_A$ is the same at all temperatures. EPR studies at room temperature by Zech et al. [58] determined a distance of 27.4 ± 0.3 Å between the negative charge on $Q_A$ and the positive charge on $P^*_{680}$. Based on this distance it is not pos-
sible to distinguish cation localization on \( P_{D1} \) from that on \( P_{D2} \). However, cation localization on \( Chl_{D1} \) or \( Chl_{D2} \) can be excluded as well as a distribution of the cation over all four Chls. ENDOR [59,60] and FTIR [61] studies show a very asymmetric charge distribution between \( P_{D1} \) and \( P_{D2} \) indicating that the cation is stabilized mainly on \( P_{D1} \), the chlorophyll adjacent to TyrZ.

As noted several times above, an interesting model system is given by PSIi of \( A. marina \), which exhibits less spectral overlap of its optical bands. Photosystem II of \( A. marina \) is unique since it is the only PSI known so far that uses Chl \( \alpha \) as the major light harvesting pigment (for recent review see e.g., [62]). This replacement allows this organism to live in the shade of Chl \( \alpha \) absorbing about 35 nm to the red of Chl \( \alpha \) [63]. Interestingly, there is a residual amount of Chl \( \alpha \) bound in \( A. marina \), where the exact amount was found to depend on light intensity [64].

Evidence for the location of one Chl in the binding site of \( P_{D1} \) was reported by Schlodder et al. [65] who compared the \( P’Q_\alpha - P-Q_\alpha \) difference spectra measured on \( A. marina \) with those of Chl containing PSIi in the Soret region around 435 nm and in the NIR region. Whereas the signal in the Soret region reflects the bleaching in absorbance of the pigment that carries the cation, the NIR signal reflects directly the cation absorbance. Both signatures agree very well between \( A. marina \) and Chl containing PSIi, leaving no doubt that the site where the cation localizes, most likely the \( P_{D1} \) site, is occupied by Chl in \( A. marina \).

Renger and Schlodder [66] used the Hamiltonian that Raszewski et al. [40] determined for Chl containing PSIi and modified it to allow for a replacement of Chl \( \alpha \) with Chl \( \alpha \). Different models from the literature concerning the identities of the RC Chls were investigated. A quantitative description of the \( P’Q_\alpha - P-Q_\alpha \) difference spectrum in the Q\(_\beta\) region requires one Chl at the binding site of \( P_{D1} \) and three Chls at the sites of \( P_{D2} \), \( Chl_{D1} \) and \( Chl_{D2} \), besides the two Pheo at the Pheo binding sites [66]. Assuming the binding sites of both “special pair” pigments to be occupied by Chl results in significantly less agreement with the experimental data (Fig. 10). Analyzing the equilibrium population of locally excited states (Eq. (5)) in \( A. marina \), using the site energies determined from the fit of the \( P’Q_\alpha - P-Q_\alpha \) spectrum, allows to identify the primary electron donor as \( Chl_{D1} \), even at room temperature (Fig. 11). Comparison with the respective equilibrium populations in Chl containing PSIii at room temperature (lower part of Fig. 8) demonstrates that the “spectral congestion” problem is much less

Fig. 10. The experimental \( P’Q_\alpha - P-Q_\alpha \) difference spectrum of \( A. marina \) at 77 K [65] is compared with calculations [66] assuming a Chl homodimer or a Chl/Chl hetero dimer in the “special pair”.

3.3. Pathways of secondary electron transfer

When electron transfer between TyrZ and \( P_{680} \) is blocked at low temperatures, besides charge recombination reactions, secondary electron transfer occurs in PSIi involving Cars, Chlz and Cytb559, for recent review see, e.g., ref. [21]. From calculations of \( Q_\alpha \), Car” - \( Q_\alpha \) Car optical difference spectra and comparison with experimental data, it can be concluded that only Car\(_{D2} \) and not Car\(_{D1} \) is involved in secondary electron transfer in PSIi [28] (Fig. 12). The linear dichroism of the absorbance band of the Car cation around 1000 nm shows that the oxidized Car is oriented parallel to the membrane plane [67] which supports the assignment that Car\(_{D2} \) is oxidized by \( P_{680} \). The position of Car\(_{D2} \) in the RC (upper part of Fig. 12) suggests that it connects the core pigments with the more peripheral secondary electron donors Chlz\(_{D2} \) and Cyt b559. There has been some discussion about the origin of the low-energy bandshift seen in the \( Q_\gamma \) Car” - \( Q_\gamma \) Car optical difference spectra. Whereas initially an interpretation in terms of a site energy shift of Pheo\(_{D1} \) was reported [68,69], the asymmetric exciton model [40] predicted that the Stark shift of \( Chl_{D1} \) is responsible for this feature. This prediction has been verified experimentally by Schlodder et al. [28] who found that the position of the low-energy bleaching changes if a residue in the vicinity of \( Chl_{D1} \).
Fig. 12. Upper Part: Pigments of the RC of cyanobacteria including the two carotenoids CarD1 and CarD2, the two peripheral ChlD1 and ChlD2, and the Heme of the Cytb559 complex. The two Cars are colored in correspondence to the cation localization assumed in the calculations in the lower part. Lower part: experimental light-minus-dark difference spectra of PSII core complexes of Synechocystis sp. PCC 6803 at 5 K are compared with Qa’ Car’ - Qa Car spectra calculated assuming two different identities for Car’ [28]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 13. T–S spectra at 5 K calculated [7] assuming the triplet state to be localized on the respective RC Chls in comparison with experimental data [44] on T. elongatus. The inset shows the RC pigments colored in accordance with the triplet localization assumed in the calculations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This temperature dependence an energy difference of 10 meV (81 cm⁻¹) was inferred between ³⁷ChlD1 and ³⁷P680 in D1D2cytb559 complexes [40]. Similar values of 8 meV (65 cm⁻¹) and 13 meV (105 cm⁻¹) were reported for this energy difference from FTIR and EPR studies on D1D2cytb559 complexes reported by Noguchi et al. [71] and Kamlowski et al. [72]. The calculations on PSII core complexes gave a very similar value of 11 meV (89 cm⁻¹) [77].

Interestingly, the lifetime of the triplet state in the RC depends strongly on the redox state of Qₐ. In PSII complexes, in which the first quinone acceptor, Qₐ, is removed or doubly reduced (QₐH₂), the lifetime of ³₈₈₀₉₀ is similar to that of the Chl triplet state in solvents (about 1 ms under anaerobic conditions). In the presence of singly reduced Qₐ the P₆₈₀ triplet state was however found to decay two or three orders of magnitude faster [25,73]. The rapid triplet quenching which is observed in closed PSII (singly reduced Qₐ) may represent a protective mechanism to prevent the formation of potentially damaging singlet oxygen. The production of harmful singlet oxygen occurs in D1D2cytb559 complexes or in PSII core complexes after double reduction or removal of Qₐ due to the reaction between the long-lived triplet state and oxygen. In this case, however, the acceptor side is already irreversibly damaged leading to non-functional reaction centers. The quenching mechanism in the presence of singly reduced Qₐ is still an open question. As a possible mechanism, it has been proposed that the rapid triplet decay occurs via a thermally activated triplet–triplet energy transfer from ³ChlD1 to PheoD1, which is followed by a two-step electron transfer process consisting of (1) reduction of ³PheoD1 by Qₐ, and (2) normal forward electron transfer from PheoD1 to Qₐ [25]. The observed efficient triplet quenching by Qₐ led Noguchi to conclude that triplet localization on ChlD1 might be functionally important [74]. The PSII RC had to find a different way for triplet quenching than the one by Cars used in the antenna and in the RC of purple bacteria, because of the high redox potentials of the RC Chls, as discussed before.

4. Triplet localization and quenching mechanism in PSII RCs

Important information about the localization of the triplet state in PSII RCs came from EPR experiments of van Mieghem et al. [27] who concluded that the triplet is localized on a pigment of which the chlorine plane is tilted by 30° with respect to the membrane plane. Taking into account the crystal structure of PSII, this conclusion leaves two possibilities for triplet localization, either on ChlD1 or ChlD2. Calculations of T–S spectra of D1D2cytb559 complexes and comparison with experimental data revealed that the triplet is localized on ChlD1 [40]. Direct evidence was provided by Schlodder et al. [28] from T–S spectra on mutants of PSII core complexes from Synechocystis sp. PCC 6803. In Fig. 13, calculations [7], assuming triplet localization on the different RC Chls in PSII core complexes are compared with experimental data. Agreement between both is only obtained if the triplet state ³Chl is identified as ³ChlD1. A simulation of the temperature dependence of the T–S spectrum revealed that at higher temperatures the thermal population of the state ³P₆₈₀ contributes to the spectrum [40,7]. From this temperature dependence an energy difference of 10 meV (81 cm⁻¹) was inferred between ³⁷ChlD1 and ³⁷P₆₈₀ in D1D2cytb559 complexes [40]. Similar values of 8 meV (65 cm⁻¹) and 13 meV (105 cm⁻¹) were reported for this energy difference from FTIR and EPR studies on D1D2cytb559 complexes reported by Noguchi et al. [71] and Kamlowski et al. [72]. The calculations on PSII core complexes gave a very similar value of 11 meV (89 cm⁻¹) [77].

Interestingly, the lifetime of the triplet state in the RC depends strongly on the redox state of Qₐ. In PSII complexes, in which the first quinone acceptor, Qₐ, is removed or doubly reduced (QₐH₂), the lifetime of ³₈₈₀₉₀ is similar to that of the Chl triplet state in solvents (about 1 ms under anaerobic conditions). In the presence of singly reduced Qₐ the P₆₈₀ triplet state was however found to decay two or three orders of magnitude faster [25,73]. The rapid triplet quenching which is observed in closed PSII (singly reduced Qₐ) may represent a protective mechanism to prevent the formation of potentially damaging singlet oxygen. The production of harmful singlet oxygen occurs in D1D2cytb559 complexes or in PSII core complexes after double reduction or removal of Qₐ due to the reaction between the long-lived triplet state and oxygen. In this case, however, the acceptor side is already irreversibly damaged leading to non-functional reaction centers. The quenching mechanism in the presence of singly reduced Qₐ is still an open question. As a possible mechanism, it has been proposed that the rapid triplet decay occurs via a thermally activated triplet–triplet energy transfer from ³ChlD1 to PheoD1, which is followed by a two-step electron transfer process consisting of (1) reduction of ³PheoD1 by Qₐ, and (2) normal forward electron transfer from PheoD1 to Qₐ [25]. The observed efficient triplet quenching by Qₐ led Noguchi to conclude that triplet localization on ChlD1 might be functionally important [74]. The PSII RC had to find a different way for triplet quenching than the one by Cars used in the antenna and in the RC of purple bacteria, because of the high redox potentials of the RC Chls, as discussed before.

5. Optical properties of the CP43 and CP47 core antennae

The cross section of the PSII RC to absorb light is enlarged by a number of antenna complexes surrounding the RC. These antenna complexes serve to deliver excitation energy to the RC, where it is used to drive the primary electron transfer reaction. All organisms contain the core antenna complexes CP43 and CP47 (Fig. 1). In cyanobacteria and red algae these core antenna complexes are connected to membrane-extrinsically bound phycobilisomes, whereas in green algae and higher plants membrane-intrinsic pig-
ment–protein complexes serve as distal antennae. Whereas the core antenna complexes contain only Chl a, the distal antennae bind, in addition, Chl b, which absorbs at higher energies, thereby increasing the cross section of the RC also spectrally. Information about the optical properties of CP43 and CP47 core antennae was obtained from high-resolution spectroscopy in the frequency domain \[80,75,78,79,81,82,51\]. Excitation dependent fluorescence line narrowing spectra \[80\] gave first evidence that in CP43 at low temperatures there are two emitting states with different inhomogeneous distributions. From the Stark and triplet-minus-singlet (T–S) spectra evidence about partially delocalized low-energy excited states in CP43 was reported \[80\].

The two quasi-degenerate low energy states in CP43 were further characterized by non-photochemical hole burning (NPHB) and triplet bottleneck hole burning spectra by Jankowiak et al. \[82\] who termed these states A- and B-states. Interestingly, the A-state was found to mainly contribute to the triplet bottleneck spectrum, whereas the B-state was detected, instead, in the NPHB spectrum \[82\]. These findings were explained by assuming a longer life time of the triplet state of A and that most of the excitation energy from higher energy exciton states is trapped by the B-state \[82\]. Some evidence for excitonic coupling of the B-state pigments was reported from high energy satellite holes at 673 nm and 678 nm \[82\]. Hughes et al. \[51\] investigated CP43-complexes with NPHB and non-wavelength selective photoconversion spectroscopy. They proposed a different intrinsic photoconversion (hole-burning) efficiency of the A- and the B-state due to the strong hydrogen bond of the B-state Chls reported earlier by Groot et al. \[80\]. From circular dichroism difference spectra, Hughes et al. \[51\] reported evidence that the B-state is excitonically correlated with higher energy states at 677 nm and 682 nm.

The site energies of the pigments of the CP43 complex were inferred recently by Raszewski and Renger \[77\] from a fit of linear optical spectra (left part of Fig. 14). Three pigments (encircled in the left middle part of Fig. 15) are found to contribute to the lowest exciton state, as seen in the exciton pigment distribution function in the middle left part of Fig. 15. Two of the three pigments, Chls 43 and 45, belong to the same domain, whereas Chl 37 is part of a dimer domain with Chl 34 (see Fig. 15). Hence, there are two degenerate low energy exciton transitions around 682 nm which represent the lowest excited states of the two domains in the luminal (containing Chls 34 and 37) and stromal layer (Chls 41, 43–47). These characteristics fit to the A- and B-states identified in NPHB and triplet bottleneck spectra \[80,82,51\], discussed above. The B-state is identified as the low energy exciton state of the stromal domain with six pigments (shown in grey in the left part of Fig. 15), whereas the A-state is the low energy exciton state of the Chl 34–37 dimer on the luminal side (shown in yellow in the left part of Fig. 15).

Independent evidence for the trap state at Chl 37 was provided by Reppert et al. \[83\] from fitting of absorbance, fluorescence and holeburning data. As a second low-energy pigment state Chl 44 was suggested \[83\], which was not identified in the calculations by Raszewski and Renger \[77\], but is located in the same exciton domain as the low energy Chls 43 and 45 assigned in the latter study.

The low energy states of CP47 were studied in great detail by de Weerd et al. \[75\]. From a Gaussian band fitting of the absorption and linear dichroism spectra, it was concluded that the low-energy state around 690 nm is due to a monomeric Chl with a distinct orientation of its Q_y-transition relative to the membrane plane (with an angle larger than 35°), and that the next higher excited state

![Fig. 14. Optical spectra of CP43 complexes. Left part: Linear absorbance (Abs) at 77 K and 293 K, linear dichroism (LD) and circular dichroism (CD) spectra at 77 K. The calculations \[7\] (solid lines) are compared with experimental data \[75\] (symbols). Right upper part: 77 K pump-probe spectra for excitation with a 60 fs pulse centered at 671 nm and detection by a probe pulse centered at 670 and 682 nm (left part) or by a white probe pulse at two different delay times (right part). The calculations \[7\] (solid lines) are compared with experimental data \[76\] (symbols). Right lower part: Calculated life time density map of excited states of CP43 complexes at 77 K.](image-url)
around 683 nm carries the oscillator strength of approximately three Chls. The finding about the 690 nm emitting Chl supported earlier investigations of van Dorson et al. [81], which provided evidence that the low energy fluorescing state in PSII belongs to CP47. From polarized emission measurements, de Weerd et al. [75] obtained additional evidence for a single emitting state at 690 nm. The depolarized 683 emission was interpreted by assuming that at least two isoenergetic states with non-parallel transition dipole moments contribute.

In agreement with de Weerd et al. [75], the fit of linear optical spectra by Raszewski and Renger [77] (left upper part of Fig. 16) suggest that the lowest excited state of CP47 is localized on a monomeric Chl, namely Chl 29, as seen in the exciton pigment distribution function in the middle right part in Fig. 15. From the 16 Chls in CP47 only Chls 11, 22 and 29 have an angle between their transition dipole moment and the membrane plane larger than 35° and therefore could be responsible for the negative LD signal observed at low energies in CP47.

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Fig. 15. Energy sinks in CP43 (left part) and CP47 (right part) complexes, as determined from a fit of optical spectra [77]. The outer left and right parts contain the arrangements of pigments in the CP43 and CP47 complex, respectively. The pigments are numbered as in Ref. [2]. Chls with the same color belong to the same exciton domain. Arrows indicate a van der Waals contact between the conjugated \( \pi \) system of a carotenoid and a Chl. The three encircled Chls are those with the lowest site energies (trap states). In the case of CP47 angles of Chl transition dipoles with respect to the membrane plane that are larger than 35° are shown in parentheses. The exciton states pigment distribution functions \( d_m(\alpha) \) for the three low-energy pigments are shown in the left (CP43) and right (CP47) middle parts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Fig. 16. Optical spectra of CP47 complexes. Upper left part: Linear absorbance (Abs), linear dichroism (LD) and fluorescence (Flu) at 77 K, and circular dichroism (CD) spectra at 293 K. The calculations [7] (solid lines) are compared with experimental data [75,78,79] (symbols). Right part: 77 K pump-probe spectra for excitation with a 60 fs pulse centered at 671 nm and detection by a probe pulse centered at 670, 677, 683 and 690 nm (right upper part) or by a white probe pulse at two different delay times (right lower part). The calculations [7] (solid lines) are compared with experimental data [76] (symbols). Left lower part: Calculated lifetime density map of excited states of CP47 complexes at 77 K.
In agreement with anisotropy measurements [75], the calculations [77] show that the second lowest optical band (at 683 nm) consists of more than one exciton transition. The two low energy pigments Chls 11 and 24 which are located in different domains contribute to different exciton transitions at this wavelength (Fig. 15).

Jankowiak and coworkers [84] measured linear absorbance, fluorescence and holeburning spectra on improved CP47 preparations that in particular, exhibit a fluorescence maximum at the same spectral position (695 nm) as intact PSII core complexes. In earlier CP47 preparations this maximum was blueshifted by 2–5 nm. Based on an analysis of their spectra, Jankowiak and coworkers [84] concluded that Chl 29 contributes only to the second lowest exciton state, whereas the lowest state is dominated by Chl 26 which is located in an exciton domain well connected to the reaction center. Unfortunately, no linear dichroism and circular dichroism spectra on the new preparations were measured yet. In particular the LD spectrum would be very important as discussed above. We note that the fit of the LD spectrum of Raszewski et al. [7] (Fig. 16) does show some discrepancies around 690 nm, the calculated LD being more negative than the measured one. An explanation of this discrepancy is needed to obtain more direct evidence about which pigment dominates the low-energy exciton state of CP47.

The new assignment of Jankowiak et al. seems to be in line with holeburning studies by Krausz and coworkers [85,86], who suggested that the high holeburning efficiency of PSII core complexes observed at the energies of the trap states of CP43 and CP47 is due to slow energy transfer to the reaction center and a conformational transition related to primary charge transfer. Due to this transition the excitation energies of the initially excited chromophores (i.e. the trap states of CP47 and CP43) are changed giving rise to the hole in the spectra. Of course, such a mechanism requires the trap states to be situated close to the reaction center.

The excitation energy transfer within CP43 and CP47 was investigated by time-resolved absorption and fluorescence spectroscopy [76] at 77 K. In both complexes, time constants of 0.2–0.4 ps and 2–3 ps were found that were attributed to exciton transfer between Chls in the same, stromal or luminal, layer and that between pigments in different layers, respectively. In CP47, in addition, a slower 17–28 ps component was detected [76] for transfer to the 690 nm trap state. The calculations by Raszewski and Renger [77] provided a structure-based analysis of these pump-probe spectra and also served to check the exciton relaxation times predicted by the theory. The 77 K pump-probe spectra calculated for the CP43 complex are compared in the right upper part of Fig. 14 with the experimental data [76] and that of the CP47 complex in the right part of Fig. 16. Both agree well with the experimental data. As seen also in the lifetime density map of the exciton states in the right lower part of Fig. 14, exciton relaxation in CP43 at 77 K occurs with a main time constant of about 2 ps. Recent pump–probe data of Holzwarth and coworkers [87] show that at room temperature exciton relaxation is somewhat faster with a main component of about 600 fs. The factor of 3–4 difference between the time constants of exciton relaxation calculated at 77 K [77] and measured at room temperature [87] led Holzwarth and coworkers to question the validity of the calculations. Fig. 17 contains the previously unpublished room temperature lifetime density map of the exciton states in CP43. As seen there, exciton relaxation is predicted to proceed with a main time constant of 500 fs, in good agreement with the recent experimental data [87].

Finally, we note that the slow 17–28 ps components measured [76] at low temperatures for relaxation into the lowest exciton state of CP47 complex are due to the large distance between the three trap states in this complex, as explained by the calculations [77]. At low temperatures, the excitation energy transfer to the lowest state is calculated to become dispersive with a broad distribution of transfer times in the 20–60 ps time range (left lower part of Fig. 16), whereas at room temperature it is an order of magnitude faster [77]. The main exciton relaxation time constants in CP47 are calculated to be rather temperature insensitive in the 400–600 fs time range [77].
6. Excitation energy transfer and trapping in PSII core complexes

The research on excitation energy transfer and trapping in PSII has a long history [88]. An interesting finding in studies of the time dependence of fluorescence decay of PSII is that the main decay time roughly scales with the number N of antenna pigments connected to the RC (e.g. Ref. [89]). This number can be systematically changed by biochemical preparation of complexes with different size. If energy (exciton) transfer between the Chls would be fast compared to primary electron transfer in the RC, the latter would start from an excitonically equilibrated state of the whole complex. In such a state all pigments would be excited roughly with a probability of 1/N, neglecting spectral differences between them. Thus the primary donor is excited with this probability and the inverse rate, that is the time constant, of charge separation is expected to scale with N, as observed in the experiments.

The above argument was particularly powerful before crystal structure information became available and led to the excited state radical pair equilibrium (ERPE) model, which assumes exciton equilibration between all antenna states to be fast compared to primary charge transfer [90,91]. In these models the intrinsic rate constant of primary charge transfer is estimated to be about (1–2 ps)$^{-1}$.

For PSII core complexes ($N = 35$) this would result in a fluorescence decay time of 35–70 ps in agreement with experimental data (see, e.g., Fig. 18). In the recent paper of Miloslavina et al. [14] the interpretation was however modified. They assume that exciton equilibration occurs in about 1.5 ps, the apparent rate of primary charge separation is about 6 ps and that the longer time constants result from a dynamical equilibrium between the excited and the first (ChlD1 * PheoD1) and second radical pair (P680 * PheoB1). It has been suggested that charge recombination during the lifetime of the radical pair states can repopulate the excited singlet state of the primary donor, which equilibrates with the antenna pigments, and gives rise to longer-lived recombination fluorescence.

To summarize these models, the original ERPE model is simple because it explains the observed scaling law of the fluorescence decay time with N. The modified ERPE model becomes complex as there is a need to incorporate charge recombination processes so as to account for the major component of the fluorescence decay rate.

Probably the weakest point of the ERPE model is that it does not take into account the crystal structure information. The structure shows that the distance between pigments within the core antenna subunits CP43 and CP47 are much smaller than the inter-subunit pigment–pigment distances between CP43/47 and the six core pigments in the RC. Rapid exciton equilibration, required by the ERPE model, is not consistent with the relatively large distances between pigments.

First calculations of excitation energy transfer, performed by Vasiliev et al. [18] reported much slower exciton equilibration times and located the bottleneck for the decay of excited states as the transfer between CP43/47 and the RC. Despite the many shortcomings of this study (orientation of transition dipole moments of pigments were not known, no optical spectra were calculated, local transition energies of the pigments were not known, localized excited states were assumed) this study reported the first structure-based calculations that questioned the assumptions of the ERPE model.

A detailed theoretical analysis using the site energies determined as described above was performed by Raszewski and Renger to model the excited state decay in whole PSII core complexes [77]. Generalized Förster theory was applied to model excitation energy transfer between different domains of strongly coupled pigments and Redfield theory for excitation relaxation within the domains. No adjustable parameter was used for the excitation energy transfer rate constants. The experimental fluorescence decay (Fig. 18) could only be described by two assumptions: (i) recombination fluorescence does not contribute significantly and (ii) primary charge transfer in the RC is ultrafast, occurring with a time constant of 300 fs, that corresponds to an intrinsic rate constant of (100 fs)$^{-1}$, assuming about 30% equilibrium population of ChlD1 (see Fig. 8 bottom). In order to find the bottleneck for the decay of excited states the ‘exact’ calculations were compared with simpler models, where fast exciton relaxation was assumed to occur in certain domains [77]. The simplest model that could still reproduce the ‘exact’ result is a 3-compartment model containing the six core pigments of the RC in one compartment and CP43-ChlD1 and CP47-ChlD1 in the other two. In this model, excitation energy transfer between the CP43 and the CP47 antenna and the RC occurs with 41 and 50 ps at 300 K, respectively, i.e., light-harvesting is found to be limited by the transfer to the trap (Fig. 19).

Whereas the ERPE model provides a simple explanation of the scaling law of fluorescence decay, a structure-based calculation provides a simple explanation of the slow fluorescence decay time.

Fig. 18. Comparison between experimental fluorescence decay of PSII core complexes at 300 K, measured [14] after excitation with a 100 fs optical pulse that was centered at 663 nm (open circles), in comparison to the ‘exact’ calculations (black solid line) and calculations that assume fast exciton equilibration in compartments consisting of CP43-ChlD1, CP47-ChlD2 and the six core pigments of the RC (green dashed line) or fast exciton equilibration in the whole PSII core complex (blue dotted line) [77]. An intrinsic rate constant for primary electron transfer of (100 fs)$^{-1}$ was obtained from the fit of the ‘exact’ calculation to the experiment. All other parameters were determined independently. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 19. Minimal compartment model [77] that describes the experimental fluorescence decay in Fig. 18. The red numbers show disorder averaged inverse rate constants for excitation energy transfer between the three compartments. The rate constants between the compartments were obtained from the individual microscopic rate constants by assuming fast exciton equilibration between the excited states within the compartments, i.e. without free parameters. The 0.3 ps time constant in the RC characterizes the primary charge transfer between the equilibrated excited state of the RC and the first radical pair state, assuming primary electron transfer starting at ChlD1 with an intrinsic rate constant of (100 fs)$^{-1}$. Pigments colored in the same way belong to the same domain of strongly coupled pigments in which delocalization of excited states occurs.
constants measured. So, the question arises: Can a structure-based calculation also explain the linear scaling of the fluorescence decay time constant with the size of the antenna? Unfortunately, at present there exist no crystal structure data on larger complexes than PSII core complexes that would allow to perform excitation energy transfer calculations as described above. However, there is the following indirect evidence for fast excitation energy transfer between the distal antenna and the core complex.

In experiments on PSII membrane fragments of spinach by van Amerongen and coworkers [92], the fluorescence decay for two different excitation conditions was compared, one in the Chl b spectral region, that, therefore, excites predominantly the distal antenna and one in the Chl a spectral region with no spatial selection of excitation. The minor difference of about 4 ps between the decay times suggests fast excitation energy transfer between the peripheral and the core antenna complexes. Since this excitation energy transfer is fast compared to the transfer to the trap, i.e., between CP43/47 and the RC, a quasi-equilibrium of excited states is established in the antenna, before the excitation energy is transferred to the RC. This equilibrium gives rise to a linear scaling of the decay constant with antenna size, since the populations of CP43 and CP47 states that transfer excitation energy to the RC scale accordingly. Of course, the question arises: Why is the transfer between more peripheral complexes so much faster than between the core antennae and the RC. The most straightforward explanation lies in the high redox potential of the RC pigments, needed for the water splitting reaction. Nature had to isolate these pigments from the rest in order to avoid an oxidation of antenna pigments.

It is, however, interesting to note that, despite the lower oxidation potential, also purple bacteria exhibit a transfer-to-the-trap limited light-harvesting kinetics [93].

7. Challenging questions and hypotheses

7.1. How fast is the primary charge transfer and what is the mechanism?

The values suggested for the time constants of primary charge separation range from 300 fs from modeling of fluorescence decay data of PSII core complexes [77] to 3 ps inferred from time-resolved pump-probe experiments on D1D2cytD559 complexes [15,16] and PSII core complexes [15].

Raszewski and Renger [77] noted, that using instead of the 300 fs a 600 fs time constant (corresponding to an intrinsic time constant of 200 fs), inferred experimentally from femtosecond IR studies [13], in the calculation of the fluorescence decay (Fig. 18), still results in a reasonably agreement with the experimental data, however a 3 ps time constant cannot describe the data (see supporting online material of Ref. [77]). Of course, such short time constants, described above, raise the question about the underlying mechanism and whether electron transfer between two pigments that do not show considerable wavefunction overlap as needed for the water splitting reaction [96,7].

The multiple ET pathway hypothesis of van Grondelle and coworkers that is based on an interpretation of time resolved optical pump-probe data [16] and a theoretical analysis of the Stark spectrum [48] seems to be in line with the finding of Krausz and coworkers [50,51] of a low lying excited state of the PSII RC capable of initiating primary electron transfer. However, besides the different broadening mechanisms, discussed above, Novoderezhkin et al. [48] predicted that the absorbance of the CT state in D1D2cytD559 complexes extends to wavelengths longer than 690 nm, in disagreement with experimental data of Krausz et al. and coworkers [42], which show that the long-wavelength absorbance of “native” PSII core complexes is lost when D1D2cytD559 complexes are formed. The nature of the persistent spectral hole-burning in D1D2cytD559 complexes was explained [97] by using a simplified Raszewski et al. [40,7] asymmetric exciton model. According to this explanation, it is the fraction of exciton realizations arising from inhomogeneous broadening in which PheoD1D2 dominates the lowest exciton state, that gives rise to efficient persistent spectral hole burning.

The inhomogeneous broadening assumption for the CT state of Novoderezhkin et al. contradicts the large permanent dipole moment of 30 D assumed for this state in the calculations [48]. However, since modified Redfield theory cannot describe dynamic localization effects [47] of the excitonic wavefunction it cannot be applied for strong exciton-vibrational coupling, i.e., homogeneous broadening. It is not clear whether the large inhomogeneous broadening assumed by Novoderezhkin et al. for the CT state can really compensate for the above short-comings of modified Redfield theory. Therefore, the conclusions concerning the nature of the CT state and its role in the primary charge transfer in photosystem II should be reexamined including dynamic localization effects in the description. The hierarchical equation of motion approach developed by Tanimura and Ishizaki [98] seems to be suitable for this task.

We note that, in agreement with the finding of Krausz and coworkers at low temperature, water splitting activity of functional plant leaves [99] and PSII enriched membranes and PSII core complexes from spinach [57] at physiological temperatures, following far-red light excitation, was reported.

7.2. Structure-based calculation of site energies and short-range effects in the “special pair”

An important parameter class of the exciton Hamiltonian is given by the site energies of the pigments. So far these energies were inferred indirectly from a fit of optical spectra of the subunits.
However, to draw conclusions about structure–function relationships, giving rise to these values, a direct structure-based calculation is needed. First successful calculations of site energies of pigment–protein complexes were reported for the Fenna–Matthews–Olson protein, a light-harvesting complex of green sulfur bacteria [100,101], photosystem I core complexes [102] and the light-harvesting complex LHC2 of photosystem II [103]. Applying this methodology, it will be interesting to find out, for example, which protein–cofactor-interactions are responsible for the asymmetry of site energy values in the RC of PSII. Another important question concerns the location of the so-called antenna trap states in CP47 and CP43, i.e., the sites with low-excitation energies [81,80,104,82,75,105,77,83]. Of course, a structure based calculation of site energies should ultimately also take into account the conformational transitions of the protein that are responsible for static and dynamic disorder. For example, it is an open question, why the site energy of ChlD0 differs in D1D2cyt6559 complexes and PSII core complexes and why its inhomogeneous width is smaller in PSII core complexes [40,7], whereas the site energies and inhomogeneous distribution functions of the remaining pigments are the same in the two preparations.

Once a higher resolution crystal structure will be available, a structure based calculation of short-range effects on the excitonic coupling of the “special pair” and of the coupling of excited states to intradimer charge transfer states will become possible. A methodology exists to extract these effects from monomer and dimer quantum chemical calculations [35]. The finding of Krausz and coworkers [50,51], that charge separation can be initiated at low temperature with long-wavelength excitation of a strongly homogeneously broadened excited state raises the question about a low-lying charge transfer state in the RC of PSII. For an accurate calculation of its energy, most likely solvent and protein environmental effects have to be included in the calculations.

8. Functional implications

A major challenge for PSII is to cope with the high redox potential of its four central RC Chls that is needed for the water splitting reaction. The structure based analysis of optical spectra discussed above allows to identify some building principles that make the photosystem relatively stable.

One is the large spatial separation between RC and antenna pigments in order to avoid an oxidation of the antenna pigments. This large separation gives rise to a transfer-to-the trap limited light-harvesting kinetics, where the primary electron transfer is two orders of magnitude faster than the excitation energy transfer between the core antenna complexes CP43 and CP47 and the RC. The ratio of time constants for forward and back excitation energy transfer between the core antenna complexes CP43 and CP47 and the RC. The ratio of time constants for forward and back excitation energy transfer between the core antenna complexes CP43 and CP47 and the RC. The two orders of magnitude faster primary charge transfer time allows an efficient trapping of excitation energy despite an unfavorable ratio of forward and back excitation energy transfer rate constants between the core antennae and the RC. Moreover, by slowing down the primary charge transfer time, the RC could switch the PSII core complex into a photoprotective mode where a large part of the excitation energy can escape the RC back into the antennae [77].

Another building principle is the limitation of unavoidable photophysical damage to the D1-protein. This limitation is obtained by using one branch for primary and the second branch for secondary electron transfer. In this way only the D1-protein needs to be replaced in a controlled way within the repair cycle of PSII. One of the deciding factors that guide primary electron transfer along the D1–branch probably is the low site energy of ChlD0, which allows this pigment to function as primary electron donor in PSII. Secondary electron transfer, which involves CarD2 leads to a controlled reduction of P680 by Cytb559, that may be completed by a subsequent charge recombination with the reduced Qb [106]. A second photoprotective function of CarD2 discussed [21,107] is the quenching of singlet oxygen, that is generated by reaction of triplet oxygen with triplet states of Chl.

A third building principle is the use of a unique mechanism for the quenching of triplet states of Chl, which cannot be quenched by carotenoids, as in the antennae. The new mechanism is the quenching by the reduced plastoquinone Qa−. The Chl with the lowest triplet energy is therefore the one closest to Qa−, i.e. ChlD1 (Figs. 2 and 13).

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