The origin of unidirectional charge separation in photosynthetic reaction centers: nonadiabatic quantum dynamics of exciton and charge in pigment–protein complexes†

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Exciton charge separation in photosynthetic reaction centers from purple bacteria (PbRC) and photosystem II (PSII) occurs exclusively along one of the two pseudo-symmetric branches (active branch) of pigment–protein complexes. The microscopic origin of unidirectional charge separation in photosynthesis remains controversial. Here we elucidate the essential factors leading to unidirectional charge separation in PbRC and PSII, using nonadiabatic quantum dynamics calculations in conjunction with time-dependent density functional theory (TDDFT) with the quantum mechanics/molecular mechanics/polarizable continuum model (QM/MM/PCM) method. This approach accounts for energetics, electronic coupling, and vibronic coupling of the pigment excited states under electrostatic interactions and polarization of whole protein environments. The calculated time constants of charge separation along the active branches of PbRC and PSII are similar to those observed in time-resolved spectroscopic experiments. In PbRC, Tyr-M210 near the accessory bacteriochlorophyll reduces the energy of the intermediate state and drastically accelerates charge separation overcoming the electron–hole interaction. Remarkably, even though both the active and inactive branches in PSII can accept excitons from light-harvesting complexes, charge separation in the inactive branch is prevented by a weak electronic coupling due to symmetry-breaking of the chlorophyll configurations. The exciton in the inactive branch in PSII can be transferred to the active branch via direct and indirect pathways. Subsequently, the ultrafast electron transfer to pheophytin in the active branch prevents exciton back transfer to the inactive branch, thereby achieving unidirectional charge separation.

1. Introduction

Light reactions of photosynthesis achieve an extremely high internal quantum efficiency from photosorption to separated electrons and holes through ingeniously regulated pathways of energy and charge transfers in pigment–protein complexes. Light-harvesting (antenna) complexes, which contain a number of pigments, absorb a photon to create an electronically excited state characterized as a bound electron–hole pair, i.e. exciton. Exciton charge separation necessitates a sufficient potential difference between the donor and acceptor of electrons for overcoming the electron–hole Coulomb binding energy. Photosystem II (PSII) consists of core antenna complexes (CP43 and CP47) and a reaction center (RC). Chlorophyll a (Chl) molecules in CP43 and CP47 mediate exciton transfers to the RC consisting of Chls (P$_{D1}$, P$_{D2}$, Chl$_{D1}$, and Chl$_{D2}$), pheophytin a (Pheo$_{D1}$ and Pheo$_{D2}$), and plastoquinone (Q$_A$ and Q$_B$) (Fig. 1). Charge separation occurs in the RC, where the electron reduces plastoquinone and the hole eventually oxidizes water at the Mn$_4$CaO$_5$ cluster. Similarly, bacteriochlorophyll a (BChl) molecules in the light harvesting complex I (LHI) from purple bacteria, Rhodobacter sphaeroides, transfer an exciton to the RC (PbRC) consisting of BChls (P$_A$, P$_M$, B$_{L}$, and B$_{M}$), bacteriopheophytin a (Bpheo, H$_L$ and H$_M$), and ubiquinone (Q$_A$ and Q$_B$) (Fig. 1). Charge separation in PSII and PbRC occurs only along the active branch of the pseudo-C$_2$ symmetric pigment–protein complexes, i.e., D1- and L-branches, respectively (Fig. 1). The D2- and M-branches are referred to as inactive branches. PSII and PbRC may have been evolved from a common ancestor and classified as type II RCs. In type II RCs, Q$_A$ in the inactive branch accepts an electron from QA in the active branch while it does not directly accept an electron from (B) Pheo in the inactive branch (Fig. 1).

In PbRC, the strong electronic coupling between the special pair BCHls, P$_L$ and P$_M$, leads to stabilization of the delocalized...
exciton, ($P_lP_m$)$^\pm$. The $P_lP_m$ can accept an exciton from LHI, which absorbs a near infrared photon. Time-resolved spectroscopic measurements indicated that the excited electron in ($P_lP_m$)$^\pm$ is transferred to H$_2$ via B$_i$ along the L-branchn on a time scale of a few ps. Despite the pseudo-C$_2$ symmetric cofactor arrangement, the difference in the amino acid sequences between the L- and M-branches leads to the difference in the redox potentials of the pigments via electrostatic interactions and polarization.

A previous study using time-dependent density functional theory (TDDFT) with the quantum mechanics/molecular mechanics/polarizable continuum model (QM/MM/PCM) method indicated that the intermediate states of charge separation along the L- and M-branches, i.e., $[P_lP_m]^\pm$B$_i^\mp$ and $[P_lP_m]^\mp$B$_m^\pm$, are lower and higher in energy than that of ($P_lP_m$)$^\pm$, respectively.

In contrast to PBR, the excitation energies of $P_{D1}$ and $P_{D2}$ in PSII are higher than those of ChlD$_1$ and ChlD$_2$, where the exciton tends to be localized on a single pigment owing to a weak excitonic coupling. Charge separation in PSII creates a hole localized on $P_{D1}^\mp$, which is the nearest pigment to the Mn$_4$Ca$_3$O$_5$ cluster located on the D$_1$ side. The localized nature of a hole on $P_{D1}^\mp$ is important for PSII to keep a high oxidation potential.

In PSII, CP43 and CP47 transfer an exciton to the RC, presumably, via the peripheral Chls on the D$_1$ (ChlD$_1$) and D$_2$ (ChlD$_2$) sides. Time-resolved spectroscopic measurements on PSII suggested that the primary electron transfer occurs from an exciton on ChlD$_1^\pm$ to Pheo$_{D1}$ on a time scale of a few hundred fs. The hole on ChlD$_1^\mp$ is, in turn, transferred to $P_{D1}$ on a time scale of a few ps.

Because the potential for electron transfer is energetically downhill along both the D$_1$- and D$_2$-branches toward Pheo$_{D1}$ and Pheo$_{D2}$, respectively, the energetics alone cannot explain unidirectional charge separation in PSII. Given that both ChlD$_1$ and ChlD$_2$ can accept an exciton from the core antenna complexes, the mechanism that leads to charge separation exclusively along the D$_1$-branch is of particular interest. The charge separation pathways in pigment–protein complexes can be determined by various factors including energetics, electronic coupling, vibronic coupling, and quantum effects.

In this study, we address the long-standing open question as to how PBR and PSII achieve unidirectional charge separation exclusively along the active branch, by means of nonadiabatic quantum dynamics calculations parametrized on the basis of TDDFT in the framework of the QM/MM/PCM method.

First, we show that the experimentally observed kinetics of charge separation along the active branches of PBR and PSII are fairly well reproduced by nonadiabatic quantum dynamics calculations, which is based on the energetics and electronic coupling of the pigments, accounting for electrostatic interactions and polarization of whole protein environments from the X-ray crystal structures. On this basis, we clarify the essential factors which regulate the charge separation pathways in the reaction centers.

2. Methods

The energetics and electronic couplings in PBR and PSII are analyzed by means of the polarizable QM/MM/PCM method, using the QuanPol code implemented in the GAMESS code. The electronic states in the QM regions are calculated using DFT and TDDFT with the CAMB3LYP functional with the range separation parameter $\mu$ of 0.14, $\alpha$ of 0.19, and $\beta$ of 0.46, which is well suited for the present systems including charge separated states. The quantitative values of excitation energies may depend on functionals and parameters. The 6-31G(d) basis set is used for all the QM calculations.

The QM region comprises pigments, ligands, hydrogen bonded water, and residues which interact directly with pigments as detailed in a previous report. A polarizable amber-02 force field is applied for proteins in the MM region, where induced dipoles of the MM atoms are taken into account to reproduce the dielectric screening. The PCM with a dielectric constant of 80 is applied to reproduce the polarization of water, which surrounds the proteins and fills the cavities. The PCM in the QuanPol code is based on a conductor-like screening model, where the polarization points are put on spheres of radius 3.0 Å from the atom positions. All atoms from the X-ray crystal structures are explicitly considered, where each MM atom contains an induced dipole in addition to the permanent charge. The induced dipole of each MM atom is determined iteratively together with the self-consistent field calculation of
electronic states, considering the electrostatic interactions with the electrons and nuclei in the QM region as well as the permanent charges and induced dipoles of other MM atoms.\textsuperscript{53} The molecular orbital levels of the cofactors calculated using QM/MM reproduce the redox potential values calculated solving the Poisson–Boltzmann equation.\textsuperscript{33,34,57,69} While the dielectric constant for the membrane region may be lower than 80 (e.g. 20),\textsuperscript{70} a small dielectric constant makes the electrostatic interactions with the charged groups in the membrane-extrinsic region overestimated for membrane proteins. The optimal values for the dielectric constant depend on the protein model used.\textsuperscript{71,72} The dielectric constant of 80 for the bulk region appears to be optimal for the present models, as suggested previously.\textsuperscript{33,34}

The atomic coordinates of PSII and PbRC are obtained from the X-ray crystal structures from \textit{Thermosynechococcus vulcanus} at 1.9 Å resolution (PDB code, 3ARC)\textsuperscript{33} and from \textit{Rhodobacter sphaeroides} at 2.01 Å resolution (PDB code, 314D),\textsuperscript{74} respectively. The intramolecular reorganization energies of pigments are calculated through geometry optimization with QM/MM, where DFT with the CAMB3LYP functional plus Grimme’s dispersion correction\textsuperscript{75} is used for the QM region. The atomic coordinates of the MM region are fixed to the X-ray crystal structures. The reorganization energy of the MM region is not taken into account.

The electronic coupling between excited states is evaluated on the basis of the diabatization scheme for TDDFT\textsuperscript{76} in the framework of the QM/MM/PCM method.\textsuperscript{34,77} The protocol of diabatization is summarized below.

1. We prepare a set of reference wavefunctions, \( \Phi_i \), that possess pure characters of the excited states such as an exciton on a single molecule (i.e., Frenkel exciton) and charge separated states for decoupled molecules.

2. We calculate adiabatic electronic states in the pigment–protein complexes using TDDFT-QM/MM/PCM.

3. The diabatic wavefunctions are expressed as a linear combination of the adiabatic wavefunctions, \( \Psi_j \), by evaluating the overlap integrals between the reference and adiabatic wavefunctions:

\[
\Phi_i = \sum_j C_{ij} \Psi_j, \quad C_{ij} = \langle \Psi_j | \Phi_i \rangle \quad (1)
\]

That is, the adiabatic states from the TDDFT-QM/MM/PCM calculations are considered as basis functions for expanding the diabatic states. We consider 10 adiabatic states for expanding the diabatic states. The diabatic coupling is then evaluated as follows:

\[
H_{ij} = \langle \Phi_i | H | \Phi_j \rangle, \quad (2)
\]

where \( H \) is the electronic Hamiltonian. The excitonic coupling in the present scheme includes both the Coulomb (Förster) and electron exchange (Dexter) contributions.\textsuperscript{76}

For the nonadiabatic quantum dynamics calculations, we consider the following linear vibronic coupling Hamiltonian in the diabatic representation:

\[
H = \sum_j h_j(x)|I_j\rangle\langle I_j| + \sum_{j<k} H_{ij}(|I_j\rangle\langle J_j| + |J_j\rangle\langle I_j|) \quad (3)
\]

\[
h_j(x) = \sum_{i,j} \frac{\hbar}{2} (p_i^2 + x_i^2) + \sum \kappa_i x_i + H_I \quad (4)
\]

\( H_I \) is the diabatic coupling (electronic coupling) between the states \( I \) and \( J \). \( H_I \) is the vertical excitation energy of the ith electronic state. \( \omega_i, x_i, \) and \( p_i \) are the frequency, position, and momentum of the ith vibrational mode (harmonic oscillator) in the dimensionless coordinate. \( \kappa_i \) is the vibronic coupling of the \( i \)th vibrational mode in the \( i \)th electronic state.

The exciton on the special pair, \( \{ P_1 P_2 \}^* \), is considered for the initial conditions of the quantum dynamics calculations of charge separation in PbRC. For PSII, in addition to the exciton localized on Chl\(_{D1}\) in the D1-branch, Chl\(_{D2}\) in the D2-branch is also considered for the initial conditions of the quantum dynamics calculations of charge separation. For the exciton transfer between Chl\(_{D2}\) and Chl\(_{D1}\), the direct pathway and the indirect pathway via \( P_{D1}^* \) and \( P_{D2}^* \) are considered, where the quantum dynamics calculations account for the interference of the phase factors from several pathways. The initial vibrational wave packet is put on the Franck–Condon region of the initial electronic state.

\( \omega_i \) and \( \kappa_i \) in eqn (4), i.e., spectral density, are determined on the basis of the normal mode analysis and the geometry optimization of the pigments using the QM/MM/PCM method, where the atomic displacements from the Franck–Condon region to the potential bottom on the respective electronic states are projected onto the normal modes. The present model explicitly considers the vibronic couplings of the pigments and axial ligands, which are relevant to the dynamics of charge separation on a time scale of a few ps, whereas slow vibrational modes from surrounding proteins are neglected. The vibrational modes are reduced to a limited number of effective modes which reproduce the short-time dynamics and the reorganization energy of the system (see ESI).\textsuperscript{60-62} We consider 25 effective modes for each pigment, unless otherwise noted. For charge separation in PSII via indirect exciton transfer from Chl\(_{D1}\) to Chl\(_{D2}\), 10 effective modes are considered for the respective intermediate states, \( P_{D1}^* \) and \( P_{D2}^* \). The multi-configuration time-dependent Hartree (MCTDH) method\textsuperscript{79} is used for the nonadiabatic quantum dynamics calculations, which properly consider correlations among the nuclear degrees of freedom, the Franck–Condon factor of vibrational wavefunctions, and vibrational energy redistribution along with electronic state transitions.

For analyzing the time constants of the first (\( \tau_1 \)) and second (\( \tau_2 \)) charge transfers along the active branches, \( \tau_1 \) and \( \tau_2 \) in the following rate equations are determined via curve fitting against the populations of the exciton (\( P_{EX} \)), and the first (\( P_{CS1} \)) and second (\( P_{CS2} \)) charge separated states in the quantum dynamics calculations:

\[
\frac{dP_{EX}}{dt} = \frac{P_{EX}}{\tau_1}, \quad \frac{dP_{CS1}}{dt} = \frac{P_{EX} - P_{CS1}}{\tau_1}, \quad \frac{dP_{CS2}}{dt} = \frac{P_{CS1}}{\tau_2}, \quad (5)
\]
where the corresponding kinetic scheme is expressed as follows:

\[ P_{EX} \xrightarrow{\tau_1} P_{CS1} \xrightarrow{\tau_2} P_{CS2}. \]  

(6)

3. Results and discussion

3.1. Charge separation in PbRC

\((P_{i}P_{M})\) in PbRC can be regarded as a single molecular site owing to the strong electronic coupling.\(^{14}\) The electron transfers from \((P_{i}P_{M})^*\) to \((P_{i}P_{M})^{-}B_{i}^{-}\) and \((P_{i}P_{M})^{-}B_{i}^{-}\) are exothermic (downhill) and endothermic (uphill), respectively (Fig. 2a).\(^{14}\) As a benchmark, we first compare the calculated time constants of charge separation along the L-branch with the corresponding experimental values. The quantum dynamics calculations indicate that \((P_{i}P_{M})^*\) initially transfers the excited electron to \(B_{i}\) on a time scale of \(\tau_1 \approx 3.2\) ps. \(B_{i}^{-}\), in turn, transfers the electron to \(H_{i}\) on a time scale of \(\tau_2 \approx 1.8\) ps (Fig. 2c and f). A similar order of time constants was observed in time-resolved spectroscopic measurements on charge separation in PbRC (\(\tau_1 = 3.5 \pm 0.4\) ps and \(\tau_2 = 1.2 \pm 0.3\) ps).\(^{41}\)

The electronic coupling of the \(B_{i}^{-}\) → \(H_{i}^{-}\) transfer (16 meV) is stronger than that of the \((P_{i}P_{M})^*\) → \((P_{i}P_{M})^{-}B_{i}^{-}\) transfer (5 meV) (Fig. 2e). Thus, the population of the intermediate \((P_{i}P_{M})^{-}B_{i}^{-}\) state is kept small (Fig. 2c). The fast electron transfer from \(B_{i}^{-}\) to \(H_{i}\) is advantageous for preventing charge recombination, because \((P_{i}P_{M})^{-}H_{i}^{-}\) is difficult to decay to the ground state owing to a negligibly small orbital overlap between \((P_{i}P_{M})^{-}\) and \(H_{i}^{-}\). Charge separation along the M-branch is negligibly slow, because the intermediate \((P_{i}P_{M})^{-}B_{M}^{-}\) state is substantially higher in energy than

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**Fig. 2** Bottom-to-bottom (adiabatic) excitation energies of the electronic states considering the intramolecular reorganization energies in (a) wild type and (b) Y(M210)F mutant PbRC. Dotted lines indicate the destabilized charge separated states in Y(M210)F mutant PbRC. Population of electronic states during quantum dynamics calculations of charge separation in (c) wild type and (d) Y(M210)F mutant PbRC, where the \((P_{i}P_{M})^*\), \((P_{i}P_{M})^{-}B_{i}^{-}\), and \((P_{i}P_{M})^{-}H_{i}^{-}\) states along the L-branch are considered. Dotted lines indicate curve fitting by using eqn (5). (e) Diagram of charge transfer pathways (red lines) with electronic coupling (meV). Tyr-M210 is shown in yellow. (f) Diagram of the electron and hole locations in the \((P_{i}P_{M})^{-}B_{i}^{-}\) and \((P_{i}P_{M})^{-}H_{i}^{-}\) states with \(\tau_1\) and \(\tau_2\) (ps) for wild type PbRC (\(\approx 5\) ps in total). \(\tau_1\) and \(\tau_2\) for the mutant PbRC are 110 and 8 ps, respectively (\(\approx 118\) ps in total).
(P_lP_M)*, even though (P_lP_M)*H_M is lower in energy than (P_lP_M)* (Fig. 2a).

The previous time-resolved spectroscopic measurements of mutant PbRC suggested that some specific residues especially contribute to unidirectional charge separation.\textsuperscript{47-50,52} We have extensively analyzed the contribution of each residue to the potential shift on the pigments one by one, and concluded that Tyr-M210 near B_L has the largest contribution to the stabilization of B_L + /C_0,\textsuperscript{33,34} where Phe-L181 is located at the counterpart position near B_M.

To verify the essential role of Tyr-M210, we consider the mutation of Tyr-M210 to phenylalanine, Y(M210)F, and investigate charge separation in the mutant PbRC by means of quantum dynamics calculations. The present TDDFT-QM/MM/PCM calculations indicate that the Y(M210)F mutation, in which the hydroxyl group is replaced with hydrogen, makes the intermediate (P_lP_M)*B_L + /C_0 state energetically uphill with respect to (P_lP_M)*, even though the final (P_lP_M)*H_L + /C_0 state remains downhill (Fig. 2b). The quantum dynamics calculation indicates that the destabilization of the intermediate (P_lP_M)*B_L + /C_0 state drastically slows charge separation along the L-branch through the superexchange mechanism (Fig. 2d). This trend is qualitatively consistent with the experimental observations for the mutant PbRC,\textsuperscript{47-50,52} where the calculated time constant (\approx 118 ps) is quantitatively larger than the experimental values (\approx 16 ps).\textsuperscript{17}

3.2. Charge separation in PSII

In PSII, Chl_{D1} and Chl_{D2} are supposed to accept an exciton from CP43 and CP47 via Chl*_{D1} and Chl*_{D2}, respectively (Fig. 3a).\textsuperscript{9,10} The present calculations indicate that the bottom-to-bottom excitation energy of Chl*_{D1} (1991 meV) lies between those of Chl*_{D1} (1965 meV) and P*_{D1} (2032 meV, Fig. 3b). Similarly, the Chl*_{D2} energy (2015 meV) lies between those of Chl*_{D2} (1992 meV) and P*_{D2} (2038 meV, Fig. 3b). Thus, Chl*_{D1} and Chl*_{D2} can accept an exciton from Chl*_{D1} and Chl*_{D2}, respectively, in terms of energetics.

The absolute values of the calculated excitonic couplings in PSII are in the range of 7 to 15 meV (Table 1). The lowest and second lowest excitons obtained by diagonalizing the coupling matrix are localized on Chl*_{D1} and Chl*_{D2}, respectively (Fig. S2\textsuperscript{†}), which can be regarded as Frenkel excitons. The quantitative values of the exciton energies in PSII calculated using TDDFT-QM/MM/PCM with the CAMB3LYP functional tend to be blueshifted as compared to the experimental values,\textsuperscript{25-27} where the

![Fig. 3](a) Arrangement of Chl molecules in PSII. Calculated bottom-to-bottom (adiabatic) excitation energies considering the intramolecular reorganization energies of the (b) exciton and (c) exciton and charge separated states in PSII. The intermediate and final charge separated states are indicated in red and blue, respectively.
calculated lowest exciton energy of 632 nm is blue-shifted as compared to the experimental value of 680 nm.24,25

We analyze charge separation from an exciton on ChlD1 by means of quantum dynamics calculations. The initial electron transfer from ChlD1 to ChlD1′−PheoD1′− occurs on an ultrafast time scale (τ1 ≈ 0.15 ps) (Fig. 4a and c) owing to a strong electronic coupling (~22 meV, Fig. 4c and Table 1). The subsequent hole transfer to P680−PheoD1− occurs on a time scale of τ2 ≈ 3.7 ps (Fig. 4a and c). Thus, once ChlD1 accepts an exciton, charge separation occurs efficiently along the D1branch. Similar time constants of charge separation in PSI were observed in the time-resolved spectroscopy measurements.14,22 Another charge separation pathway, ChlD1 → P680−ChlD1′−, is endothermic (Fig. 3c) and thus cannot compete with ChlD1 → ChlD1′−PheoD1′−. Although other charge separation pathways from an exciton on P680 and P680′ were also proposed,23,24 the quantum dynamical analysis for these pathways is beyond the scope of the present study. Overall, we can conclude that charge separation along the D1branch proceeds via two-step ChlD1 → ChlD1′−PheoD1′− and ChlD1′− → P680 transfers, considering the quantum dynamical analysis based on the energetics and electronic couplings from the QM/MM/PCM method.

The electronic coupling of the ChlD1 → ChlD1′−PheoD1′− transfer (~22 meV) is stronger than that of the ChlD1′− → P680 transfers (~6 meV, Fig. 4c and Table 1). The strong electronic coupling between the accessory ChlB/Chl and the PheoBPheo is a common feature of PSI/PbRC. Nevertheless, the Be− → He− electron transfer (~1.8 ps) in PbRC is slower than the ChlD1′− → ChlD1′−PheoD1′− transfer (~0.15 ps) in PSI, because the population of the (P680P680′)Be− intermediate state in PbRC is kept small.

Because ChlD2 can also accept an exciton form CP47 on the D2 side,18,19,38 the question arises as to how the exciton on ChlD2 eventually undergoes charge separation in the D1branch. To analyze charge separation mechanisms from an exciton in the D2branch, we carried out quantum dynamics calculations considering the initial exciton localized on ChlD2. The charge separated state in the D2branch, P680−PheoD2−, is less stable than that in the D1branch, P680−PheoD1− (Fig. 3c), owing mainly to a difference in the potentials between PheoD1− and PheoD2−.33,34,40

The most stable charge separated state in the D2branch is P680−ChlD2− (Fig. 3c). However, PSIII can avoid charge separation from ChlD2 to P680−ChlD2− (Fig. 4b) because of a weak electronic coupling (~0.3 meV, Fig. 4d and Table 1), which is significantly weaker than that between ChlD1′− and P680−ChlD1′− (~5.4 meV) on the counterpart side (Fig. 5a and Table 1). The difference originates from the difference in the vinyl-group orientation between P680 and P680′ (Fig. 5). The vinyl group is rather in plane for P680 and out of plane for P680′ (Fig. 6).27 The present results indicate that the in-plane P680 vinyl group interferes with the π−π interaction between P680 and ChlD2 (Fig. 5b), thereby preventing the charge transfer to form PD1−ChlD2−.

The out-of-plane orientation of the P680 vinyl group is caused by the relatively large steric hindrance from the P680 phytol chain as compared with that between the P680 vinyl group and the P680′ phytol chain (Fig. 5c and 6). The potential curves calculated using QM/MM indicate that the rotations of the P680 and P680′ vinyl groups are hindered in the protein environments (Fig. 6 and S1). Umema et al. reported that the conformations of vinyl groups were determined unambiguously from the corresponding electron density distributions and most of the vinyl groups are located in or near the same plane of the chlorine rings,23 which suggests that the out-of-plane vinyl orientation for P680 is exceptional. The same conformations of the P680 and P680′ vinyl groups have also been observed in the X-ray free electron laser (XFEL) structure.26 Thus, the observed vinyl orientations of P680 and P680′ are considered to be robust in the protein environments. The phytol chains of P680 and P680′ are less flexible due to the presence of the highly packed protein environment of D1/D2/CP43/CP47, as compared to those exposed to the protein surface in antenna proteins (e.g., LH1 and the Fenna–Matthews–Olson protein). Umema et al. also confirmed that all of the C8 and C13 positions in the phytol chains have a (RR) configuration as indicated by the low B-factor values.27 Notably, the difference in the phytol-chain conformation also contributes to the asymmetric hole distribution on P680 and P680′, i.e., P680′+ > P680+.27 These results suggest that the symmetry-breaking of the P680P680 geometry not only increases the P680′+ population, which facilitates water oxidation at the Mn4CaO5 moiety on the D1 side, but also prevents charge separation along the D2branch via a weak electronic coupling between ChlD2 and P680−ChlD2−.

The quantum dynamics calculations indicate that the exciton on ChlD1 can be transferred to ChlD1 via the direct pathway and the indirect pathway mediated by P680 and P680′, owing to adequately strong excitonic couplings (Table 1 and Fig. 4g) and small energy differences (Fig. 4h). Note that among the residues near ChlD1 and ChlD2, D1-Met172 adjacent to ChlD1 contributes to the difference in excitation energy between ChlD1′− and ChlD2−.28 Previous calculations by Sirohiwal et al.28 using other DFT functionals and an equation-of-motion coupled cluster method also indicated that ChlD1 exhibits the lowest excitation energy in the protein environment of PSI. Once the exciton is transferred to ChlD1, subsequent charge separation to ChlD1′−PheoD1′− occurs rapidly. The present analysis indicates that an overall time scale of charge separation from ChlD1′− to ChlD1′−PheoD1′− is in the order of a few tens ps (Fig. 4e and f), where exponential fitting indicates a τ of ~50 ps. It is highly likely that the exciton on ChlD2 can eventually undergo charge separation in the D1branch without charge separation in the D2branch.
D2-branch. Even though the energy difference between Chl$_{D1}^*$ and Chl$_{D2}^*$ is small (Fig. 4h), the ultrafast charge separation from Chl$_{D1}^*$ to Chl$_{D2}^*$ prevents exciton back transfer to Chl$_{D2}$, enhancing the robustness of unidirectional charge separation along the D1-branch. The charge separation pathway via the exciton transfer from the D2- to D1-branches may...
The direct excitonic coupling between Chl$^{*}_{D1}$ and Chl$^{*}_{D2}$ is relatively weak (~2 meV) owing to the long distance (~20 Å) as compared with the coupling between neighboring Chls, i.e., P$^{*}_{D1}$−Chl$^{*}_{D1}$, P$^{*}_{D1}$−Chl$^{*}_{D2}$, P$^{*}_{D2}$−Chl$^{*}_{D1}$ and P$^{*}_{D2}$−Chl$^{*}_{D2}$ pairs (Fig. 4g and Table 1). Consequently, charge separation considering only the direct Chl$^{*}_{D2}$→Chl$^{*}_{D1}$→Chl$^{*}_{D1}$Pheo$^{*}_{D1}$ pathway is slower than charge separation considering only the indirect Chl$^{*}_{D2}$→(P$^{*}_{D1}$, P$^{*}_{D2}$)→Chl$^{*}_{D1}$→Chl$^{*}_{D1}$Pheo$^{*}_{D1}$ pathway in the quantum dynamics calculations (Fig. 4f). The excitonic coupling between P$^{*}_{D1}$ and Chl$^{*}_{D2}$ (~14 meV) is stronger than that between P$^{*}_{D1}$ and Chl$^{*}_{D1}$ (7 meV, Table 1). The former and latter are characterized as J- and H-aggregates (minus and plus signs), respectively, considering the directions of the transition dipole moments (Fig. 4g). The excitonic coupling is relatively insensitive to the orbital overlap as compared with the case of the charge transfer coupling. The direct and indirect Chl$^{*}_{D2}$→Chl$^{*}_{D1}$ exciton transfers exhibit the destructive interference of the quantum phase factor, which is dictated by the signs of excitonic couplings, i.e., relative orientation of the transition dipole moments. Consequently, the exciton transfer rate considering all pathways is slower than the rate considering only the indirect pathway (Fig. 4f). Thus, in terms of the phase factor, the configuration of Chls in PSII is not necessarily optimal for accelerating the Chl$^{*}_{D2}$→Chl$^{*}_{D1}$ transfer, while the configuration is optimal for charge separation along the D1-branch.

Overall, it can be concluded that the Chl$^{*}_{D2}$→Chl$^{*}_{D1}$ exciton transfer followed by charge separation to Chl$^{*}_{D1}$Pheo$^{*}_{D1}$ in the D1-branch is overwhelmingly faster than charge separation in the D2-branch (Fig. 4f). The irreversible Chl$^{*}_{D2}$→Chl$^{*}_{D1}$ exciton transfer allows PSII to utilize the excitation energy from both the CP43 and CP47 antenna complexes for charge separation in the active branch.

3.3. Role of Mn$_4$CaO$_5$ in the charge separation pathway in PSII

The localized electronic states on P$^{*}_{D1}$ in PSII are advantageous to maintain a high oxidation potential for water splitting in contrast to the strongly coupled (P$^{*}_{D1}$P$^{*}_{M}$) and (P$^{*}_{M}$P$^{*}_{M}$) in PbRC. The hole on P$^{*}_{D1}$ is largely stabilized by acidic residues near the Mn$_4$CaO$_5$ cluster, namely D1-Asp61, D1-Glu189, and D1-Asp170. This may explain why the Mn$_4$CaO$_5$ cluster is located on the D1 side, because the electrostatic potential, which attracts a hole toward the D1 side, also enhances charge separation to P$^{*}_{D1}$Pheo$^{*}_{D1}$. Because the difference in the redox potential between P$^{*}_{D1}$ and Chl$^{*}_{D1}$ is small, P$^{*}_{D1}$−Chl$^{*}_{D1}$ is substantially higher in energy than Chl$^{*}_{D1}$Pheo$^{*}_{D1}$ (Fig. 3c). Thus, the exciton funneling to Chl$^{*}_{D1}$ rather than P$^{*}_{D1}$ is a reasonable design principle for efficient charge separation to use excitons from the antenna complexes in PSII.

4. Conclusion

Quantum dynamics calculations indicated that two-step (P$^{*}_{D1}$P$^{*}_{M}$)→(P$^{*}_{M}$P$^{*}_{M}$)→B$_{D1}$→H$_{D1}$ electron transfers occur on a time scale of ~2.2 and ~1.8 ps, respectively (Fig. 2c). The population of the intermediate (P$^{*}_{M}$P$^{*}_{M}$)B$_{D1}$ state is kept...
small, owing to a strong $B_{c}^{+}\rightarrow H_{c}^{+}$ coupling ($\sim$16 meV, Fig. 2e). The rapid electron transfer to $H_{c}$ is advantageous for preventing charge recombination, because the orbital overlap between $(P_{D1}P_{M})^{+}$ and $H_{c}^{+}$ is negligibly small owing to a long molecular distance. The electrostatic interaction with the hydroxyl group of Tyr-M210 near BL stabilizes the intermediate $(PLPM)_{C24}$ 3.7 ps (Fig. 4a), as suggested by time-resolved spectroscopic measurements.** Charge separation in the D2-branch is unlikely to occur despite the relatively stable $P_{D1}^{+}$-$ChlD2^{+}$ state, because the in-plane $P_{D1}$ vinyl group interferes with the $\pi$-$\pi$ interaction between $P_{D1}$ and ChlD2, thereby weakening the electronic coupling. The exciton on ChlD2 can be transferred to ChlD1 via the direct and indirect pathways. Subsequently, the ultrafast $ChlD1^{+}\rightarrow ChlD1^{+}PheoD1^{+}$ charge separation prevents exciton back transfer to ChlD2, thereby enhancing the robustness of unidirectional charge separation in the D1-branch. Thus, PSI efficiently utilizes excitons not only from CP43 (D1 side) but also from CP47 (D2 side) for charge separation in the D1-branch, which leads to electron transfer to $Q_{b}$ via $Q_{A}$ and hole transfer to the Mn$_{4}$CaO$_{5}$ cluster on the D1 side.

**Author contributions**

H. T. designed the research. H. T., K. S., and H. I. performed the research. H. T. wrote the main part of the manuscript. All the authors were involved in the discussion of the results and contributed to the final version of the manuscript.

**Conflicts of interest**

There are no conflicts to declare.

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