On Electron Spin Polarization Created in the Excited Triplet State of Accessory Chlorophyll via Photoinduced Charge-Recombination of the Photosystem II Reaction Center

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Abstract

We present a theoretical approach to investigate the electron spin polarization (ESP) of the excited triplet state that has been detected by using the time-resolved EPR (TREPR) method in the Photosystem II reaction center (PS II RC) of the plants. We show, by using the stochastic-Liouville equation, that the ESP pattern created in the accessory chlorophyll ($\text{Chl}_{\text{accD1}}$) which reside near the P_{D1} chlorophyll of the active branch is explained by one-step, concerted double electron transfer model initiating from the singlet-triplet conversion of the light-induced charge-separated (CS) state composed of P_{D1} radical cation and pheophytin radical anion. We also considered the sequential electron spin polarization transfer (ESPT) model via 1) the triplet charge-recombination (CR) and 2) the triplet-triplet energy transfer processes. It has been clearly shown that the ESP created in the $^3\text{Chl}_{\text{accD1}}^*$ is dependent on the rate constant (k_{TT}) of the triplet-triplet energy transfer from the

intermediate triplet state created by the CR. Also we show that relative orientation of the principal axes of the spin-dipolar interaction in the intermediate triplet state ($^3P_{DI}^*$, as an example) may play a role on the ESP pattern when the k_{TT} is smaller than the angular frequency of the Zeeman energy. We have theoretically shown that the TREPR measurement of the ESP is very powerful to investigate the primary chemical process and to characterize the intermediate as a signature of the stepwise ESPT.

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Introduction

In photosystem II (PS II) of plants, light energy is used to drive the charge separation in the reaction center. The electron moves along the active cofactor branch, consisting of chlorophyll P_{D1} , accessory chlorophyll Chl_{accD1} , pheophytin $Pheo_{D1}$ (Figure 1) and two quinone molecules, Q_A and Q_B . The primary charge separation is known to take place from the excited singlet state of Chl_{accD1} to $Pheo_{D1}$ ¹. The electron is subsequently transferred from $Pheo_{D1}^{\cdot-}$ to the primary (Q_A) and then to the secondary (Q_B) quinone acceptors. On the electron donor side, the hole moves from $Chl_{accD1}^{+\cdot}$ to P_{D1} and then to the tyrosine YZ. Finally the Mn cluster is oxidized by the tyrosine radical and molecular oxygen is evolved². The PS II RC proteins contain six chlorophylls, two pheophytins, and two carotenes, but the Mn cluster and the Q_A and Q_B quinone acceptors are absent². Light absorption of the PS II RC complex leads to the primary charge separation, and the radical pair $Chl_{accD1}^{+\cdot} Pheo_{D1}^{\cdot-}$ is formed upon the photo-excitation. Subsequently, the distant radical pair of $P_{D1}^{+\cdot} Pheo_{D1}^{\cdot-}$ is created via the hole transfer from $Chl_{accD1}^{+\cdot}$ as shown in A) in Figure 2^{1,2}. Because of the absence of Q_A , however, further electron transfer is blocked, and the $P_{D1}^{+\cdot} Pheo_{D1}^{\cdot-}$ recombines with a lifetime of 55 ns at 5 K after the singlet (S)-triplet (T) interconversion in the radical pair state, forming an excited triplet state of chlorophyll^{2,3}. This chlorophyll triplet decays with a lifetime of 0.9 ms at 5-120 K³. The similar triplet formation of chlorophyll by radical pair recombination has also been observed in PS II membrane preparations when Q_A is doubly reduced

or depleted^{2,4}. Until recently, the triplet state produced in the PS II RC was thought to be located on P_{D1} (or $P_{D1}P_{D2}$ pair) by considerations analogous to the bacterial RC and PS I, in which the triplet state resides on the special pairs of the chlorophylls. Recently, Lubitz and his coworkers⁵ unambiguously demonstrated that the excited triplet state is located at the accessory chlorophyll Chl_{accD1} by using the time-resolved EPR investigation of the single crystal sample of the PS II RC at 10 K.

Figure 1. X-ray crystal structure of the chlorophyll of P_{D1} , the accessory chlorophyll (Chl_{accD1}) and the pheophytin ($Pheo_{D1}$) in the PS II reaction center. The principal Z axis of the spin dipolar interaction in the radical pair state of $P_{D1}^{+\bullet} Pheo_{D1}^{-\bullet}$ is indicated by an arrow. The Z'' axis denotes the one of the principal axes in the zero-field splitting interaction in the excited triplet state of $^3Chl_{accD1}^*$ detected by the TREPR.

The above view that the triplet state in the PS II RC resides on the accessory chlorophyll has raised questions about the mechanism of the triplet formation². Is the triplet excitation formed first on P_{D1} through the triplet charge recombination (CR) of P_{D1}⁺⁺ Pheo_{D1}^{-•} and then transferred to Chl_{accD1} via the triplet-triplet energy transfer process as shown by pathway (2) in Figure 2? Are there other mechanisms, like triplet-state transfer using unknown intermediates? Noguchi et al.² proposed alternative mechanism of the ³Chl_{accD1}* formation directly from the radical pair of P_{D1}⁺⁺ Pheo_{D1}^{-•}; concerted, double electron transfers 1) from Pheo_{D1}^{-•} to LUMO of Chl_{accD1} and 2) from HOMO of Chl_{accD1} to P_{D1}⁺⁺ may produce the ³Chl_{accD1}* as shown by pathway (1) in Figure 2. So far, the charge-recombination mechanism leading to the ³Chl_{accD1}* is still unknown from the CS state of the PS II RC.

By using the TREPR, electron spin polarization (ESP) has been observed at the nanosecond regions on radicals, radical pairs, excited triplet states, and so on⁶. Basically, the ESP is a signature of the primary photochemical processes⁷ and the several ESP mechanisms have been established to account for non-Boltzmann distributions in the reaction intermediates observed by the TREPR technique^{8,9,10}. The ESP mechanisms have been utilized to characterize several primary photochemical processes in details^{11,12}. In the PSII RC systems, the ESP pattern of A/E/E/A/A/E

(where A and E denote microwave absorption and emission, respectively) has been observed by the TREPR method at cryogenic temperature (Figure 3)^{13,5,14}. This pattern is typical of so-called S-T₀ mechanism¹⁵; among the three spin sublevels in the excited triplet state under the applied magnetic field B_0 , the middle-energy level (level 2'' in Figure 2C) is selectively populated via the CR process from the T₀ level (which also denotes the middle-energy level of 2 in Figure 2A) generated by the singlet-triplet interconversion (S-T₀ mixing) in the CS state. However, much remains to be resolved regarding how the ESP is transferred when we consider both 1) the concerted, double electron transfers as described above and 2) the sequential processes of CR and the energy transfer reaction.

Here, basic theoretical treatments for the analysis of the ESP on the excited triplet state are considered on the basis of the stochastic-Liouville equation to account for the S-T₀ polarization in the photosynthetic reaction centers. We show that the ESP can be affected 1) by the rate constant of the electron spin polarization transfer (ESPT) processes and 2) by the relative orientations of the intermediate triplet state with respect to the principal axes of the spin dipolar interaction in the triplet state of Chl_{accD1}, suggesting that the ESP can be utilized, in principle, to characterize the mechanism of the triplet formation processes in the PS II RC.

Figure 2. Schematic representation of pathways to ${}^3Chl_{accD1}^*$ initiating from the triplet CS state generated by the $S-T_0$ mixing in the PS II reaction center in the presence of the external magnetic field. Direct formation from the triplet radical pair of $P_{D1}^{+\bullet}$ $Pheo_{D1}^{\bullet-}$ by concerted, double electron transfers is shown by solid arrows of (1) in A) resulting in C). Stepwise reaction model with the triplet charge recombination of $P_{D1}^{+\bullet}$ $Pheo_{D1}^{\bullet-}$ creating ${}^3P_{D1}^*$ in B) and with the triplet-triplet energy transfer is represented by the pathways of (2) and (3). Corresponding triplet spin-levels in the presence of magnetic field are shown within squares together with their sublevel populations. Density matrix of each triplet state is denoted by ρ . In the stepwise reaction model, the case a) $k_{TT} > g\beta B_0/\hbar$ and the case b) $k_{TT} < g\beta B_0/\hbar$ are considered in this study.

Figure 3. Calculated X-band TREPR spectrum of $^3\text{Chl}_{\text{accD1}}^*$ according to the S- T_0 model.

Theory

1) One step charge recombination model.

In this section, the one-step concerted double electron transfer process in Figure 2 is considered for generating the ESP on the $\text{Chl}_{\text{accD1}}$ starting from the radical pair state of $\text{P}_{\text{D1}}^{+\bullet} \text{Pheo}_{\text{D1}}^{\bullet-}$. According to the process (1) in Figure 2, the density matrix (ρ^{RP}) for the spin state of the radical pair and that (ρ^{Chl}) of the excited triplet state $^3\text{Chl}_{\text{accD1}}^*$ obey the stochastic Liouville equation, as follows,

$$\dot{\rho}_{123}^{\text{RP}} = -i \left[\hat{\mathbf{H}}_{123}^{\text{RP}}, \rho_{123}^{\text{RP}} \right] - k_{\text{CCR}} \rho_{123}^{\text{RP}}, \quad (1)$$

$$\dot{\rho}_{123}^{\text{Chl}} = -i \left[\hat{\mathbf{H}}_{123}^{\text{Chl}}, \rho_{123}^{\text{Chl}} \right] + k_{\text{CCR}} \rho_{123}^{\text{RP}}, \quad (2)$$

where subscript 123 denotes the basis spin wavefunctions of 1, 2 and 3 that diagonalize the spin Hamiltonian of the triplet radical pair in the presence of the magnetic field B_0 as shown in Figure

2A. Therefore, the spin Hamiltonian in the CS state is represented as follows,

$$\hat{\mathbf{H}}_{123}^{\text{RP}} = \mathbf{U}_{\text{RP}}^{-1} \hat{\mathbf{H}}_{\text{XYZ}}^{\text{RP}} \mathbf{U}_{\text{RP}} = \begin{pmatrix} \omega_1 & & \\ & \omega_2 & \\ & & \omega_3 \end{pmatrix}, \quad (3)$$

where $\mathbf{U}_{\text{RP}}$ denotes unitary matrix composed of eigenvectors as,

$$\mathbf{U}_{\text{RP}} = \begin{pmatrix} c_{1X} & c_{2X} & c_{3X} \\ c_{1Y} & c_{2Y} & c_{3Y} \\ c_{1Z} & c_{2Z} & c_{3Z} \end{pmatrix} \quad (4)$$

for the eigenfunctions of $|i\rangle = c_{iX}|X\rangle + c_{iY}|Y\rangle + c_{iZ}|Z\rangle$ (for $i = 1, 2$ and 3) in which

$|X\rangle = |\beta\beta - \alpha\alpha\rangle/\sqrt{2}$, $|Y\rangle = i|\beta\beta + \alpha\alpha\rangle/\sqrt{2}$ and $|Z\rangle = |\alpha\beta + \beta\alpha\rangle/\sqrt{2}$ that diagonalize the spin dipolar interaction with the eigenvalues of X , Y and Z in the spin state of the radical pair. $\hat{\mathbf{H}}_{XYZ}^{RP}$ is the spin Hamiltonian of the radical pair represented using the basis functions of $|X\rangle$, $|Y\rangle$ and $|Z\rangle$. {See eq.(18).} ω_i represents eigenvalues for the energy levels of 1, 2 and 3. By the Laplace transform of eqs. (1) and (2), following relation can be obtained,

$$s\tilde{\rho}_{123}^{RP} - \rho_{123}^{RP}(0) = -i\left[\hat{\mathbf{H}}_{123}^{RP}, \tilde{\rho}_{123}^{RP}\right] - k_{CCR}\tilde{\rho}_{123}^{RP}, \quad (5)$$

$$s\tilde{\rho}_{123}^{Chl} = -i\left[\hat{\mathbf{H}}_{123}^{Chl}, \tilde{\rho}_{123}^{Chl}\right] + k_{CCR}\tilde{\rho}_{123}^{RP}. \quad (6)$$

where

$$\tilde{\rho} \equiv \int_0^\infty e^{-st} \rho dt \quad (7)$$

Since the S-T₀ mixing creates the triplet CS state, the initial condition of the RP state can be described as,

$$\rho_{123}^{RP}(0) = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix}. \quad (8)$$

Since there exists no off-diagonal elements in eq (8), eq. (5) can easily be solved to obtain $\tilde{\rho}_{123}^{RP}$ in eq. (6). Since the off-diagonal elements are not generated in $\rho_{123}^{RP}(t)$, only the middle-level element (ρ_{22}) is transferred by the reaction on the 123 eigenbasis. Thus, at the time when the reaction is completed ($s \approx 0$), $k_{CCR}\tilde{\rho}_{123}^{RP}$ can be represented as,

$$k_{CCR}\tilde{\boldsymbol{\rho}}_{123}^{RP} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix}, \quad (9)$$

and can be treated as the initial population in ${}^3\text{Chl}_{\text{accD1}}^*$. To compute the EPR transition intensities in ${}^3\text{Chl}_{\text{accD1}}^*$, we need to obtain the density matrix from eq. (9) for the ${}^3\text{Chl}_{\text{accD1}}^*$ on the basis of the $1''$, $2''$ and $3''$ levels that diagonalize the spin Hamiltonian in ${}^3\text{Chl}_{\text{accD1}}^*$. Therefore, we need to determine a transformation matrix of $\mathbf{U}_{\text{CCR}}$ shown below:

$$\begin{pmatrix} |1''\rangle \\ |2''\rangle \\ |3''\rangle \end{pmatrix} = \mathbf{U}_{\text{CCR}}^{-1} \begin{pmatrix} |1\rangle \\ |2\rangle \\ |3\rangle \end{pmatrix}. \quad (10)$$

The spin Hamiltonian in ${}^3\text{Chl}_{\text{accD1}}^*$ is represented as follows,

$$\hat{\mathbf{H}}_{1''2''3''}^{\text{Chl}} = \mathbf{U}_{\text{Chl}}^{-1} \hat{\mathbf{H}}_{X''Y''Z''}^{\text{Chl}} \mathbf{U}_{\text{Chl}} = \begin{pmatrix} \omega_{1''} & & \\ & \omega_{2''} & \\ & & \omega_{3''} \end{pmatrix}, \quad (11)$$

where $\mathbf{U}_{\text{Chl}}$ denotes unitary matrix composed of eigenvectors as,

$$\mathbf{U}_{\text{Chl}} = \begin{pmatrix} c_{1''X''} & c_{2''X''} & c_{3''X''} \\ c_{1''Y''} & c_{2''Y''} & c_{3''Y''} \\ c_{1''Z''} & c_{2''Z''} & c_{3''Z''} \end{pmatrix} \quad (12)$$

for the eigenfunctions of $|i''\rangle = c_{i''X''}|X''\rangle + c_{i''Y''}|Y''\rangle + c_{i''Z''}|Z''\rangle$ (for $i'' = 1'', 2''$ and $3''$) of the ${}^3\text{Chl}_{\text{accD1}}^*$, that is:

$$\begin{pmatrix} |1''\rangle \\ |2''\rangle \\ |3''\rangle \end{pmatrix} = \mathbf{U}_{\text{Chl}}^{-1} \begin{pmatrix} |X''\rangle \\ |Y''\rangle \\ |Z''\rangle \end{pmatrix}. \quad (13)$$

In eq. (11), $\hat{\mathbf{H}}_{X''Y''Z''}^{Chl}$ is represented as,

$$\hat{\mathbf{H}}_{X''Y''Z''}^{Chl} = \begin{pmatrix} X'' & -ig\beta B_0 \cos \theta_B & ig\beta B_0 \sin \phi_B \sin \theta_B \\ ig\beta B_0 \cos \theta_B & Y'' & -ig\beta B_0 \cos \phi_B \sin \theta_B \\ -ig\beta B_0 \sin \phi_B \sin \theta_B & ig\beta B_0 \cos \phi_B \sin \theta_B & Z'' \end{pmatrix}, \quad (14)$$

where $X'' = D/3 + E$, $Y'' = D/3 - E$, and $Z'' = -(2/3)D$. θ_B and ϕ_B are the polar angles of the external magnetic field B_0 with respect to the Z'' and X'' principal axes, respectively, in the zero-field splitting interaction. From eq. (4), the following relation is obtained:

$$\begin{pmatrix} |1\rangle \\ |2\rangle \\ |3\rangle \end{pmatrix} = \mathbf{U}_{RP}^{-1} \begin{pmatrix} |X\rangle \\ |Y\rangle \\ |Z\rangle \end{pmatrix}. \quad (15)$$

The zero-field basis functions are related as follows,

$$\begin{pmatrix} |X''\rangle \\ |Y''\rangle \\ |Z''\rangle \end{pmatrix} = \mathbf{U}_{M''}^{-1} \begin{pmatrix} |X\rangle \\ |Y\rangle \\ |Z\rangle \end{pmatrix}, \quad (16)$$

where

$$\mathbf{U}_{M''} = \begin{pmatrix} a_{X''X} & a_{Y''X} & a_{Z''X} \\ a_{X''Y} & a_{Y''Y} & a_{Z''Y} \\ a_{X''Z} & a_{Y''Z} & a_{Z''Z} \end{pmatrix} \quad (17)$$

where each element a_{KL} denotes direction cosine between the principal axes of the spin dipolar interactions in the triplet state $^3\text{Chl}_{\text{accD1}}^*$ and the principal axes in the radical pair. In eq. (3), $\hat{\mathbf{H}}_{XYZ}^{RP}$ is obtained as,

$$\hat{\mathbf{H}}_{XYZ}^{RP} = \begin{pmatrix} X & 0 & 0 \\ 0 & Y & 0 \\ 0 & 0 & Z \end{pmatrix} + \mathbf{U}_{M''} \begin{pmatrix} 0 & -ig\beta B_0 \cos \theta_B & ig\beta B_0 \sin \phi_B \sin \theta_B \\ ig\beta B_0 \cos \theta_B & 0 & -ig\beta B_0 \cos \phi_B \sin \theta_B \\ -ig\beta B_0 \sin \phi_B \sin \theta_B & ig\beta B_0 \cos \phi_B \sin \theta_B & 0 \end{pmatrix} \mathbf{U}_{M''}^{-1} \quad (18)$$

From eqs. (13), (15) and (16), $\mathbf{U}_{CCR}$ in eq. (10) is represented as,

$$\mathbf{U}_{CCR} = \mathbf{U}_{RP}^{-1} \mathbf{U}_{M''} \mathbf{U}_{Chl} . \quad (19)$$

Unitary transform of eq. (6) yields the density matrix for the $^3\text{Chl}_{\text{accD1}}^*$ on the basis of the 1'', 2'' and 3'' levels as,

$$\rho_{1''2''3''}^{Chl} \equiv \begin{pmatrix} \rho_{1''1''} & \rho_{1''2''} & \rho_{1''3''} \\ \rho_{2''1''} & \rho_{2''2''} & \rho_{2''3''} \\ \rho_{3''1''} & \rho_{3''2''} & \rho_{3''3''} \end{pmatrix} = \mathbf{U}_{CCR}^{-1} \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix} \mathbf{U}_{CCR} . \quad (20)$$

From eqs. (19) and (20), the density matrix for the $^3\text{Chl}_{\text{accD1}}^*$ is formulated as follows,

$$\rho_{1''2''3''}^{Chl,CCR} = \mathbf{U}_{Chl}^{-1} \mathbf{U}_{M''}^{-1} \mathbf{U}_{RP} \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix} \mathbf{U}_{RP}^{-1} \mathbf{U}_{M''} \mathbf{U}_{Chl} . \quad (21)$$

The diagonal terms in eq. (21) are used as sublevel populations in $^3\text{Chl}_{\text{accD1}}^*$ to compute the TREPR spectrum in Figure 3.

2) Two-step reaction model

Here, the stepwise reaction model 1) with the triplet charge recombination of $\text{P}_{\text{D1}}^{+\bullet} \text{Pheo}_{\text{D1}}^{-\bullet}$

creating $^3P_{D1}^*$ (Figure 2B) and 2) with the triplet-triplet energy transfer is considered. According to the processes of (1) and (2) in Figure 2, the Laplace transforms of the density matrices (ρ^{RP} , ρ^P and ρ^{Chl}) of the radical pair, the charge-recombined triplet state $^3P_{D1}^*$ and the excited triplet state $^3Chl_{accD1}^*$ obey the following relations, respectively:

$$s\tilde{\rho}_{123}^{RP} - \rho_{123}^{RP}(0) = -i\left[\hat{H}_{123}^{RP}, \tilde{\rho}_{123}^{RP}\right] - k_{CR}\tilde{\rho}_{123}^{RP}, \quad (22)$$

$$s\tilde{\rho}_{123}^P = -i\left[\hat{H}_{123}^P, \tilde{\rho}_{123}^P\right] + k_{CR}\tilde{\rho}_{123}^{RP} - k_{TT}\tilde{\rho}_{123}^P, \quad (23)$$

$$s\tilde{\rho}_{123}^{Chl} = -i\left[\hat{H}_{123}^{Chl}, \tilde{\rho}_{123}^{Chl}\right] + k_{TT}\tilde{\rho}_{123}^P. \quad (24)$$

As shown in eq. (9), since the off-diagonal elements are not produced in $\rho_{123}^{RP}(t)$, the initial population in $^3P_{D1}^*$ is treated in eq. (23) as follows,

$$k_{CR}\tilde{\rho}_{123}^{RP} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix}. \quad (25)$$

Therefore, the unitary transform of eq. (25) yields the initial condition of the density matrix of $^3P_{D1}^*$

on the basis of the 1', 2' and 3' levels in Figure 2B as follows,

$$\rho_{1'2'3'}^P(0) \equiv \begin{pmatrix} \rho_{1'1'}^0 & \rho_{1'2'}^0 & \rho_{1'3'}^0 \\ \rho_{2'1'}^0 & \rho_{2'2'}^0 & \rho_{2'3'}^0 \\ \rho_{3'1'}^0 & \rho_{3'2'}^0 & \rho_{3'3'}^0 \end{pmatrix} = U_{CR}^{-1} \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix} U_{CR}, \quad (26)$$

where

$$\begin{pmatrix} |1'\rangle \\ |2'\rangle \\ |3'\rangle \end{pmatrix} = U_{CR}^{-1} \begin{pmatrix} |1\rangle \\ |2\rangle \\ |3\rangle \end{pmatrix}. \quad (27)$$

The spin Hamiltonian in $^3P_{D1}^*$ is represented as follows,

$$\hat{H}_{1'2'3'}^P = U_P^{-1} \hat{H}_{XYZ}^P U_P = \begin{pmatrix} \omega_{1'} & & \\ & \omega_{2'} & \\ & & \omega_{3'} \end{pmatrix}, \quad (28)$$

where U_P denotes unitary matrix composed of eigenvectors as,

$$U_P = \begin{pmatrix} c_{1'X'} & c_{2'X'} & c_{3'X'} \\ c_{1'Y'} & c_{2'Y'} & c_{3'Y'} \\ c_{1'Z'} & c_{2'Z'} & c_{3'Z'} \end{pmatrix} \quad (29)$$

for the eigenfunctions of $|i'\rangle = c_{i'X'}|X'\rangle + c_{i'Y'}|Y'\rangle + c_{i'Z'}|Z'\rangle$ (for $i' = 1', 2'$ and $3'$) of the $^3P_{D1}^*$,

that is:

$$\begin{pmatrix} |1'\rangle \\ |2'\rangle \\ |3'\rangle \end{pmatrix} = U_P^{-1} \begin{pmatrix} |X'\rangle \\ |Y'\rangle \\ |Z'\rangle \end{pmatrix}. \quad (30)$$

The zero-field basis functions in $^3P_{D1}^*$ are now related as follows,

$$\begin{pmatrix} |X''\rangle \\ |Y''\rangle \\ |Z''\rangle \end{pmatrix} = U_{MM''}^{-1} \begin{pmatrix} |X'\rangle \\ |Y'\rangle \\ |Z'\rangle \end{pmatrix}, \quad (31)$$

where

$$U_{MM''} = \begin{pmatrix} a_{X''X'} & a_{Y''X'} & a_{Z''X'} \\ a_{X''Y'} & a_{Y''Y'} & a_{Z''Y'} \\ a_{X''Z'} & a_{Y''Z'} & a_{Z''Z'} \end{pmatrix} \quad (32)$$

where each element a_{KL} denotes direction cosine between the principal axes of the spin dipolar

interactions in the triplet state $^3Chl_{accD1}^*$ and the principal axes in $^3P_{D1}^*$. $\hat{H}_{XYZ}^P$ is obtained as,

$$\hat{\mathbf{H}}_{\text{XYZ}}^P = \begin{pmatrix} X' & 0 & 0 \\ 0 & Y' & 0 \\ 0 & 0 & Z' \end{pmatrix} + \mathbf{U}_{\text{MM}''} \begin{pmatrix} 0 & -ig\beta B_0 \cos \theta_B & ig\beta B_0 \sin \phi_B \sin \theta_B \\ ig\beta B_0 \cos \theta_B & 0 & -ig\beta B_0 \cos \phi_B \sin \theta_B \\ -ig\beta B_0 \sin \phi_B \sin \theta_B & ig\beta B_0 \cos \phi_B \sin \theta_B & 0 \end{pmatrix} \mathbf{U}_{\text{MM}''}^{-1} \quad (33)$$

and is used to determine $\mathbf{U}_P$ in eq. (28). From eqs. (15), (16), (27), (30) and (31) $\mathbf{U}_{\text{CR}}$ in eq. (26) is obtained as,

$$\mathbf{U}_{\text{CR}} = \mathbf{U}_{\text{RP}}^{-1} \mathbf{U}_{\text{M}''} \mathbf{U}_{\text{MM}''}^{-1} \mathbf{U}_P. \quad (34)$$

This relation is used to obtain the density matrix in the 1', 2' and 3' levels in Figure 2B from eq.

(26). Now, in $\rho_{1'2'3'}^P(0)$ the off-diagonal terms are generated by the matrix transformations in eq.

(26). Therefore, depending on the rate constant (k_{TT}) of the triplet-triplet energy transfer in Figure 2,

situation of the off-diagonal terms in the primary charge-recombined triplet state of $^3\text{P}_{\text{D1}}^*$ should

vary before the spin polarization is transferred to the $^3\text{Chl}_{\text{accD1}}^*$. From the unitary transforms of eqs.

(23) and (24) with $\mathbf{U}_{\text{CR}}$, the following approximation can be made at the time when the first-step CR

reaction is completed to create the $^3\text{P}_{\text{D1}}^*$,

$$s\tilde{\rho}_{1'2'3'}^P - \rho_{1'2'3'}^P(0) = -i \left[\hat{\mathbf{H}}_{1'2'3'}^P, \tilde{\rho}_{1'2'3'}^P \right] - k_{\text{TT}} \tilde{\rho}_{1'2'3'}^P, \quad (35)$$

$$s\tilde{\rho}_{1'2'3'}^{\text{Chl}} = -i \left[\hat{\mathbf{H}}_{1'2'3'}^{\text{Chl}}, \tilde{\rho}_{1'2'3'}^{\text{Chl}} \right] + k_{\text{TT}} \tilde{\rho}_{1'2'3'}^P. \quad (36)$$

By the inverse Laplace transform of eq. (35), the following time dependence of the diagonal (ρ_{rr})

and the off-diagonal (ρ_{rj}) terms are obtained for $\rho_{1'2'3'}^P$:

$$\rho_{1'2'3'}^P(t)_{i'i'} = \rho_{1'2'3'}^P(0)_{i'i'} \exp(-k_{TT}t), \quad (37)$$

$$\rho_{1'2'3'}^P(t)_{i'j'} = \rho_{1'2'3'}^P(0)_{i'j'} \exp(-k_{TT}t) \exp\{-i(\omega_{i'} - \omega_{j'})t\}, \quad (38)$$

with $i' \neq j'$. In the followings, two limiting cases a) and b) are considered to compute the ESP of $^3\text{Chl}_{\text{accD1}}^*$.

Case a): Fast triplet-triplet energy transfer

If the k_{TT} value is much larger than the angular frequency determined mostly by the Zeemann energy ($g\beta B_0$), since the coherence terms (off-diagonal terms) created in $\rho_{1'2'3'}^P$ are not modulated by the energy difference between the levels $1', 2'$ and $3'$, the initial condition in $^3\text{Chl}_{\text{accD1}}^*$ is treated as follows in eq. (36),

$$k_{TT} \tilde{\rho}_{1'2'3'}^{P, \text{fast}} = \rho_{1'2'3'}^P(0). \quad (39)$$

Thus, as has been treated in the one-step model, the density matrix for the $^3\text{Chl}_{\text{accD1}}^*$ on the basis of the $1'', 2''$ and $3''$ levels is formulated as follows,

$$\rho_{1''2''3''}^{\text{Chl}} = U_{\text{TT}}^{-1} \rho_{1'2'3'}^P(0) U_{\text{TT}}, \quad (40)$$

where

$$U_{\text{TT}} = U_{\text{P}}^{-1} U_{\text{MM}} U_{\text{Chl}}. \quad (41)$$

From eqs. (26), (34), (40) and (41), the density matrix for the $^3\text{Chl}_{\text{accD1}}^*$ is represented as follows,

$$\rho_{1''2''3''}^{Chl,fast} = U_{Chl}^{-1} U_{M''}^{-1} U_{RP} \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{pmatrix} U_{RP}^{-1} U_{M''} U_{Chl} . \quad (42)$$

This result is exactly the same as the sublevel populations in $^3\text{Chl}_{accD1}^*$ considered in the one-step CR model of eq. (21), meaning that, if the coherence terms in the density matrix are conserved on the secondary spin polarization transfer process, we cannot differentiate the direct, concerted CR mechanism and the stepwise reaction mechanism. Kinetic condition to satisfy this situation is detailed below.

Case b): Slow triplet-triplet energy transfer

When the k_{TT} value is much smaller than the angular frequency of $g\beta B_0$, the coherence terms (off-diagonal terms) created in $\rho_{1'2'3'}^P$ undergo the quantum oscillations effectively because of $k_{TT} \ll |\omega_{1'} - \omega_{j'}|$ ($\approx g\beta B_0$) in eq. (38). In this case, one can easily see from eqs. (7), (36) and (38) that the off-diagonal terms in the transferred density matrix of $k_{TT} \tilde{\rho}_{1'2'3'}^P$ are averaged to be vanished at the time ($s \ll k_{TT}$) when the T-T energy transfer is finished.

$$k_{TT} \tilde{\rho}_{1'2'3'}^{P,slow}(s)_{i'j'} = 0 , \quad (43)$$

with $i' \neq j'$. In the diagonal terms, one can obtain the following populations from eq. (37),

$$k_{TT} \tilde{\rho}_{1'2'3'}^{P,slow}(s)_{i'i'} = \rho_{1'2'3'}^P(0)_{i'i'} \quad (44)$$

Thus, the transferred density matrix in eq. (36) can be described at the time when the T-T energy transfer is completed as,

$$k_{TT} \tilde{\rho}_{1'2'3'}^{P,slow} = \begin{pmatrix} \rho_{1'1'}^0 & 0 & 0 \\ 0 & \rho_{2'2'}^0 & 0 \\ 0 & 0 & \rho_{3'3'}^0 \end{pmatrix}. \quad (45)$$

From eq. (41), the density matrix for the $^3\text{Chl}_{\text{accDl}}^*$ on the basis of the 1'', 2'' and 3'' levels is then formulated as follows,

$$\rho_{1''2''3''}^{Chl,slow} = U_{\text{Chl}}^{-1} U_{\text{MM''}}^{-1} U_{\text{P}} \begin{pmatrix} \rho_{1'1'}^0 & 0 & 0 \\ 0 & \rho_{2'2'}^0 & 0 \\ 0 & 0 & \rho_{3'3'}^0 \end{pmatrix} U_{\text{P}}^{-1} U_{\text{MM''}} U_{\text{Chl}}. \quad (46)$$

In principle, this electron spin polarization should be distinguished from the polarization obtained in the fast energy transfer case of a). {See eq. (42).} It is also noted that, in the stepwise model of eq. (46) the ESP is also dependent of relative orientation $U_{\text{MM''}}$ of the principal axes of the spin-dipolar interaction in the triplet state that is primary formed as an intermediate state by the CR process.

The diagonal terms in $\rho_{1''2''3''}^{Chl}$ together with eigenvalues of $\omega_{1''}$ are used to compute the TREPR spectrum SP of $^3\text{Chl}_{\text{accDl}}^*$ using the powder pattern averaging as follows,

$$SP = \int_0^{2\pi} \int_0^\pi \sum_{i'',j''} \text{Im} \frac{\rho_{i''i''}^{Chl} - \rho_{j''j''}^{Chl}}{\omega_{i''} - \omega_{j''} + \omega_0 - \frac{i}{T_2}} \sin \theta_B d\varphi_B d\theta_B, \quad (47)$$

where ω_0 and T_2 are the angular frequency of the microwave and the phase memory time for the EPR transition, respectively.

Results and Discussion

To compute the TREPR spectrum, we have calculated the direction cosines in eq. (17) between the principal axes (X'' , Y'' and Z'') in $^3\text{Chl}_{\text{accD1}}^*$ and the principal axes of X , Y and Z in $\text{P}_{\text{D1}}^{++} \text{Pheo}_{\text{D1}}^*$ by using the X-ray crystallography data (PDB code: 1FE1) reported for the PSII. (Figure 1) The Z axis was set to the center-to-center direction between P_{D1} and Pheo_{D1} as shown in Figure 1. Directions of the X'' , Y'' and Z'' (Figure 1) in $^3\text{Chl}_{\text{accD1}}^*$ were taken from the reported principal axes in $^3\text{Chl}_{\text{accD1}}^*$ together with the zero-field splitting parameters ($D = 30.7$ mT and $E = 4.60$ mT). The point-dipole approximation was utilized to obtain $D = -0.73$ mT (with $E = 0$) for the radical pair from the center-to-center separation of 1.56 nm between P_{D1} and Pheo_{D1} . As in Figure 3, we have obtained the A/E/E/A/A/E-pattern spectrum on $^3\text{Chl}_{\text{accD1}}^*$ by using the one-step CCR model calculation by eq. (21). This result is coincident with the reported TREPR spectrum in the PSII RC^{13,5}.

Even when the two-step reaction shown in Figure 2 is assumed to take place to create the $^3\text{Chl}_{\text{accD1}}^*$, the ESP spectrum pattern of the A/E/E/A/A/E (in Figure 3) can be explained by the case a) in the stepwise reaction model, as eq. (42) is exactly the same as the one-step model. However, eq. (42) is applicable only when the triplet-triplet energy transfer process (determined by k_{TT}) is fast enough to conserve the coherence terms (in the primary triplet state $^3\text{P}_{\text{D1}}^*$) to be transferred to the $^3\text{Chl}_{\text{accD1}}^*$. This situation is fulfilled if k_{TT} is larger than the angular frequency of the Zeemann splitting; i. e. $k_{\text{TT}} > |\omega_1 - \omega_2|$ ($\approx g\beta B_0/\hbar$) in eq. (38). Thus, in the X-band frequency region, the T-T

energy transfer process needs to be faster than ~ 20 ps to memorize the coherence terms created in the primary triplet state by the CR process. Recent experimental studies on the excited triplet state dynamics in light-harvesting antenna proteins of peridinin-chlorophyll *a*-protein (PCP) have shown that the T-T excitation energy transfer from chlorophyll *a* to carotenoid compounds is dominated by the one-step process from one chlorophyll to a specific peridinin pigment which resides in close proximity to the donor chlorophyll¹⁶. The triplet energy transfer dynamics was measured to be 17 ns¹⁷ for the center-to-center separation of 0.54 nm¹⁶. This relatively slow kinetics compared with the singlet excitation energy transfer processes occurring at picoseconds in the antenna complexes has been explained by the Dexter type electron exchange mechanism (not by the Föster type energy transfer mechanism¹⁸): since the transition dipole moments associated with the spin-forbidden $S_0 - T_1$ transition is vanishingly small, close contact between the active molecular orbitals in the two pigments is required for the efficient electron exchange between the chromophores¹⁶. In this respect, the center-to-center distance of 0.98 nm between P_{D1} and Chl_{accD1} (in Figure 1) is too long to be attributable to the 20 ps dynamics of the T-T energy transfer process from P_{D1} to Chl_{accD1} , excluding the case a) of the sequential ESP mechanism of eq.(42).

From the above arguments, the excited triplet state formation of ${}^3Chl_{accD1}^*$ might proceed via the concerted, double electron transfer directly from the triplet CS state of $P_{D1}^{++} Pheo_{D1}^{\cdot-}$. However, from eq.(46), since the ESP is also dependent on the principal axes orientation U_{MM} of the

intermediate state when the T-T energy transfer is slower than 20 ps, the ESP calculations based on the stepwise reaction model of the case b) is required with considering several candidates of the intermediate triplet states. The ESP calculations based on eq. (46) are now in progress to see how the ESP pattern is dependent on the intermediate state orientation.

Conclusion

Theoretical treatments for the analysis of the ESP are proposed on the basis of the stochastic-Liouville equation to account for the S-T₀ polarization in the excited triplet state of ³Chl_{accD1}* observed by the TREPR method in the PS II RC. The A/E/E/A/A/E-pattern spectrum on ³Chl_{accD1}* was reproduced by using the concerted, one-step double electron transfer model. Contrarily, according to the Dexter-type triplet energy transfer mechanism, the sequential ESPT model (model A) with the fast T-T energy transfer process followed by the radical pair CR is excluded as an origin to create the S-T₀ polarization of the ³Chl_{accD1}*. Since the T-T energy transfer kinetics should be nanosecond timescale, it has been revealed that, when the stepwise model is assumed, the ESP is also dependent on the principal axes orientation of the intermediate triplet state. This shows that the further detailed ESP analyses are required to clarify the mechanism of the triplet excitation pathways in plants and that, in principle, the TREPR measurements of the ESP is very powerful tool to investigate the primary chemical process as a signature of the stepwise ESPT.

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