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Discussion Letter

Applications of a CP-II model to excitation transfer experiments on light-harvesting chlorophyll *a*/*b*-protein complexes

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A theoretical study of energy-transfer rates between chlorophyll *a* molecules within light-harvesting chlorophyll *a*/*b* complexes of photosystem II (LHC-II) based on transient absorption recovery data [(1985) FEBS Lett. 193, 267–270] has been made using a trimer model of interacting chromophores [(1977) Biochim. Biophys. Acta 462, 642–658]. It provides a kinetic explanation of the data and shows that the experimental results on LHC-II are consistent with and therefore support the model.

Light-harvesting chlorophyll; Chlorophyll-protein; Linear dichroism; Transient absorption

1. INTRODUCTION

Studies on energy transfer among different types of chlorophyll (Chl) *a* and Chl *b* molecules within the light-harvesting Chl *a*/*b*-protein complex of photosystem II may enable us to understand nature's primary physical apparatus for capturing solar energy. Recently, there has been much work done both theoretically and experimentally [1,2]. An atomic-resolution crystal structure is certain to become available soon [3]. Among the features of LHC recently discovered are its trimer structure and predominance of C_3 symmetry [3c].

LHC-II is a Chl *a*/*b*-protein complex containing about half of the total Chl *a* of the thylakoid, and is known to be mostly associated with photosystem II as an antenna system. Our definition of CP-II is the solubilized form of LHC obtained with SDS-polyacrylamide gel electrophoresis. It is believed to contain three Chl *a*, three Chl *b*, and one carotenoid molecule [4] having a total molecular mass of 30 kDa [1,5]. Because of its small number of chromophores, CP-II has received more attention theoretically, and a model involving a trimer of Chl *b* has been developed based on spectral studies [6–8]. Although LHC-II particles are more complicated than CP-II, they have many similar features. The study of optical spectra of CP-II [9–11] shows that there is little or no change in the state of the Chl *a* upon SDS treatment. Therefore, we use the CP-II model in a first approach to study the energy-transfer processes within LHC-II.

2. STATEMENT OF THE PROBLEM

On the basis of absorption, circular dichroism, and fluorescence polarization, a model of the relative chromophore orientations in the Chl *a*/*b*-protein has been developed [6–8,12] in which the Chl *b* molecules are strongly coupled into states α,β,γ in which two at 1.902 eV (652 nm) are degenerate (β,γ) and split from the third at

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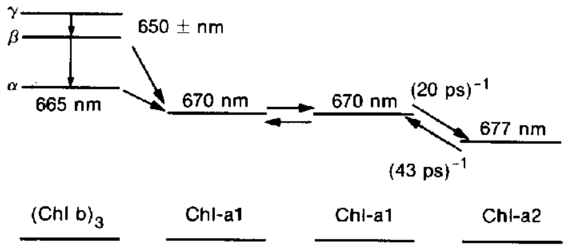

Fig.1. The proposed energy levels of the smallest chlorophyll-protein complex CP-II [6–8]. There are also transfer pathways between the leftmost Chl *a* and the rightmost, omitted here for clarity (see fig.2).

1.864 eV (665 nm). The three Chl *a* molecules assumed to exist in the complex are weakly coupled with one being shifted to 1.833 eV (677 nm) from the other two at 1.851 eV (670 nm). The energy levels of the distinct energy-transferring species appearing in the model of the Chl *a/b*-protein discussed above are shown in fig.1.

A picosecond absorption spectroscopy experiment on LHC-II was performed by Gillbro et al. [13]. It was interesting that when the sample was excited at 665 nm (the Chl *a* region) there appeared a very short recovery component (~20 ps) which did not take the recovery curve back to the original level of absorption. After a 20 ps period, the curve stayed at a certain level, recovering very slowly. Gillbro et al. argued that the long component had to be explained in terms of a thermal equilibrium between two spectrally different species of Chl *a*. Fluorescence anisotropy under these same excitation conditions also showed a 20 ps decay from $r(t) = 0.4$ down to only 0.28, staying at that fairly high value on the same time scale as the slow absorption recovery. Here we present a theoretical study showing that these experimental data on LHC-II are consistent with and therefore support the Van Metter-Shepanski-Knox (VSK) model. Earlier [14] we presented arguments showing that the data in [13] also support the strong-coupling Chl *b* aspect of the CP-II model even though, at first sight, they contradict it.

3. ABSORPTION RECOVERY NEAR 665 nm

The absorption recovery data near 665 nm (wavelength of both pump and probe) showed double-exponential decay – a fast component with 20 ps lifetime and a slow component of approx. 2 ns. From the energy levels and the deconvoluted absorption spectra associated with the VSK model [6–8], we can infer that the 665 nm pulse mostly excited those Chl *a* having the higher excited-state energy level (Chl *a*1). Chl *a*2 will refer to those with the lower energy level. It is also possible that Chl *b* (665 nm) can be excited and transfer its excitation energy to Chl *a*, but considering its relatively small absorption cross-section [8] and its likelihood of extremely rapid transfer, its contribution to the recovery curve will be negligible. Therefore, the only energy-transfer process considered here is that from Chl *a*1 to Chl *a*2, taking place with a rate constant of $(20\ \mathrm{ps})^{-1}$. If Chl *a*2 absorption is negligible as well, the absorption will recover its original value with the same time constant, as in the case of transfer from Chl *b* to Chl *a* [13,14]. The existing slow component implies that some excitation remains in Chl *a*1.

Gillbro et al. concluded qualitatively that the spectrally different Chl *a* components reach a quasi-thermal equilibrium after 20 ps. We wish to show that this effect may be related directly to the VSK model, on which one may make specific predictions about these kinetic processes. The energy difference between the two Chl *a* species is 0.019 eV, so the principle of detailed balance predicts that the forward and reverse rate constants at room temperature will have a ratio of $\exp(-0.77) = 0.47$. Therefore, back-transfer establishing fairly rapid equilibrium is possible but the kinetics need not necessarily be simple. Fig.2 shows the energy-transfer pathways we use in analyzing the kinetic model. They are appropriate to CP-II in terms of the original Van Metter model but can also be considered as a basis of a de novo analysis of the data of Gillbro et al. The major assumption we make is that there are twice as many molecules in the higher (670 nm) pool than in the lower (677 nm) pool. The higher-energy pool is considered to have two parts with equal numbers of Chl *a*, N_{10} and $N_{10'}$, of which $N_1(t)$ and $N_{1'}(t)$ may be excited at time t and the lower energy pool has an again equal number N_{20} of which $N_2(t)$ may be excited at time t.

We make the common assumption that excited molecules contribute nothing to absorption. The isotropic transient change in absorption at 665 nm is then taken to be proportional to

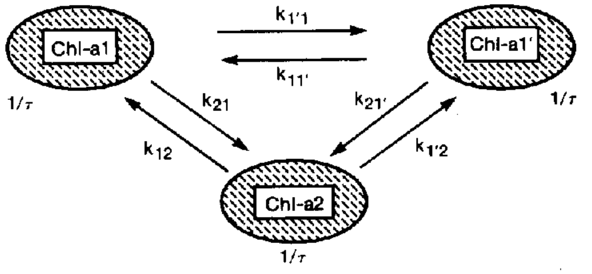

Fig.2. Rate constants for excitation transfer among the chlorophylls (see eqns 2).

$$C(t) = 0.475N_1(t) + 0.475N_{1'}(t) + 0.050N_2(t), \quad (1)$$

where the coefficients account for the relative absorption of the two spectral species of Chl *a*1 at 665 nm. A ratio of 95:5 is obtained from Van Metter's deconvolution; the 95% is assumed to be distributed over the two degenerate Chl *a*1. Simple first-order kinetics were used:

$$\begin{aligned} \mathrm{d}N_1(t)/\mathrm{d}t &= I(t)\sigma_1 N_{10} - (k_{1'1} + k_{21} + \tau_1^{-1})N_1(t) + \\ &\quad k_{12}N_2(t) + k_{11'}N_{1'}(t) \\ \mathrm{d}N_{1'}(t)/\mathrm{d}t &= I(t)\sigma_{1'}N_{1'0} - (k_{11'} + k_{21'} + \tau_{1'}^{-1})\cdot \\ &\quad N_{1'}(t) + k_{1'2}N_2(t) + k_{1'1}N_1(t) \qquad (2) \\ \mathrm{d}N_2(t)/\mathrm{d}t &= I(t)\sigma_2 N_{20} - (k_{12} + k_{1'2} + \tau_2^{-1})N_2(t) + \\ &\quad k_{21}N_1(t) + k_{21'}N_{1'}(t) \end{aligned}$$

where (relative absorption cross-sections) $\sigma_1 = \sigma_{1'} = 0.475$, $\sigma_2 = 0.050$, $\Sigma\sigma_i = 1$. Here $I(t)$ is the intensity of the exciting light, the τ denotes lifetimes, and energy-transfer rate constants k_{ij} refer to transfer from j to i. Since the N_{i0} are assumed equal, and since the equations are linear, only the relative values of the absorption cross-sections are needed. As with the coefficients in eqn 1, the values are taken from the CP-II spectrum of [7]. Since by symmetry the kinetics are not affected by it, we made the arbitrary but reasonable choice $k_{11'} = k_{1'1} = (33\ \mathrm{ps})^{-1}$.

We take $k_{21} = k_{21'} = (1/20\ \mathrm{ps})$ directly from [13] and the parameters τ_i and $k_{12} = k_{1'2}$ were varied to obtain the best fit. An autocorrelation trace of the pulse (Gillbro, T., personal communication) was used as an effective source function in the theory. Therefore the calculated quantities $N_i(t)$ are also system-convoluted, corresponding to the experimental results. The goal was to make $C(t)$ have the same shape as the experimental curve. Comparison of the two curves is shown in fig.3. We believe that our calculation is in good agreement with the experiment, using a forward rate of $(20\ \mathrm{ps})^{-1}$ and a back-transfer rate of $(43\ \mathrm{ps})^{-1} = 0.47(20\ \mathrm{ps})^{-1}$ [note that $\exp(-0.77) = 0.47$]. We tested the sensitivity of the model to including the back-transfer rate, as shown in fig.4a, where two cases are shown – it is taken equal to the forward rate, and equal to zero. The agreement is poor in both cases. Fig.4b shows, similarly, that the somewhat less decisive value of the time constant of the slow component (1300 ps vs 1500 and 1100 ps, and indicating that 1300 ps is probably the best) does not affect the fitting at short times. The fit is not sensitive at all to τ_i.

4. ANISOTROPY OF Chl *a*

We have also made calculation of the absorption anisotropy based on the VSK model of Chl *a* orientations in order to compare with the corresponding experimental result [13]. It can be shown that the absorption anisotropy is proportional to the fluorescence polarization anisotropy under the simplifying assumptions mentioned above. A general theory for incoherent transfer of excitation, i.e. one based on simple master equations,

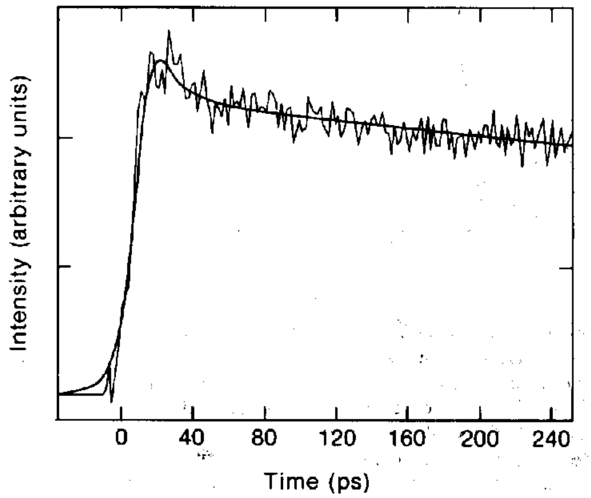

Fig.3. Fit of the solutions of eqns 2 (smooth line) to measured Chl *a*1 transient absorption (broken line) [13].

has been given earlier [15]. One writes eqns 2 in the compact form

$$\mathrm{d}N_i(t)/\mathrm{d}t = E_i - \Sigma G_{ij}N_j(t), \qquad (3)$$

where the sum runs from $j = 1$ to $j = N$, G_{ij} comprises the set of rate coefficients and E_i is the rate of external excitation of molecule i. As discussed below, we will avoid a tedious time-dependent treatment of this problem by working with the steady-state solutions of eqns 3, which are

$$[N_j] = E_i - \Sigma(G^{-1})_{jk}E_k \qquad (4)$$

Here [ ] denotes a steady-state value. The excitation function now involves more detail than just $[I]\sigma_k N_{k0}$ since we wish to include polarization effects. Thus we take E_k to be proportional to $(\mu_k \cdot z)^2$, where μ_k is the transition dipole moment of molecule k and z is a unit vector in the direction of polarization of the pulse or probe light. By analogy with [15] for the case of fluorescence, the transient absorption signals for probe light polarization parallel and perpendicular to that of the pulse will be given by

$$\begin{aligned} I_{\parallel} &= I_z \sim \Sigma_j\Sigma_k\sigma_j(\mu_j \cdot z)^2(G^{-1})_{jk}\sigma_k(\mu_k \cdot z)^2, \\ I_{\perp} &= I_y \text{ or } I_x \sim \Sigma_j\Sigma_k\sigma_j(\mu_j \cdot y)^2(G^{-1})_{jk}\sigma_k(\mu_k \cdot z)^2. \end{aligned} \qquad (5)$$

Here σ_k is taken as the relative amount of light absorbed by molecule k in the pump pulse and σ_j is the relative amount absorbed by j during the probe pulse. (In the fluorescence case [15], σ_j is replaced by f_j, which is proportional to the rate of the fluorescence of molecule j.) The absorption anisotropy is given by

$$r = \{\langle I_{\parallel}\rangle - \langle I_{\perp}\rangle\}/\{\langle I_{\parallel}\rangle + 2\langle I_{\perp}\rangle\}, \qquad (6)$$

where ⟨ ⟩ denotes the average over all configurations of the complexes. Because the rate constants are fixed within the complexes, the rate matrix G is not subject to this average and only the transition moments in eqn 5 must be considered.

To make use of the steady-state solutions, we assume that near $t = 0$ no transfer has taken place, so that the matrix G can be taken diagonal and constant. The theory produces $r = 0.4$, as it should. After the 20 ps transient has occurred, the system can in fact be considered to be essentially in a steady state, so the entire kinetic matrix G is inserted into the theory. Specifically

$$G = \begin{pmatrix} (k_{1'1} + k_{21} + \tau_1^{-1}) & -k_{11'} & -0.47k_{21} \\ -k_{1'1} & (k_{11'} + k_{21'} + \tau\tau_{1'}^{-1}) & -0.47k_{21'} \\ -k_{21} & -k_{21'} & (k_{12} + k_{1'2} + \tau_2^{-1}) \end{pmatrix}$$

Again we take $k_{11'} = k_{1'1} = 1/(33\text{ ps})$ and $k_{21} = k_{21'} = 1/(20\text{ ps})$. The principal angle we varied in

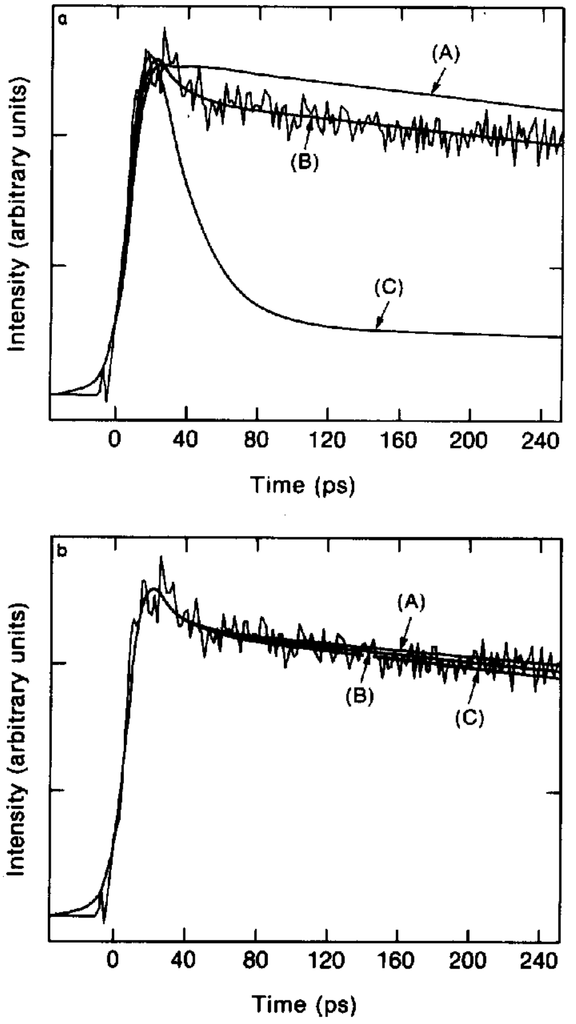

Fig.4. (a) Illustration of the sensitivity of the fit of fig.3 to the value chosen for back-transfer (k_{12} and $k_{1'2}$). Curve A corresponds to the case of maximum back-transfer ($k_{12} = k_{21}$, $k_{1'2} = k_{21'}$), while curve C corresponds to the minimum, $k_{12} = k_{1'2} = 0$. Curve B is the fit shown in fig.3 and discussed in the text. (b) Illustrating the sensitivity of the fit of fig.3 to the value of the lifetime of the long component. Curves A and C correspond to $\tau = 1500$ and 1100 ps, respectively (curve B is the fit shown in fig.3 and discussed in the text, with $\tau = 1300$ ps).

the model was Θ_2, the one between the two Chl *a*1 molecules. Earlier we had found $\Theta_2 \sim 70°$ from the steady-state fluorescence polarization [8]. We find here that the absorption anisotropy is equal to 0.28 when Θ_2 is chosen to be 40°. The difference between these two angles could result from a conformational change when CP-II is built into LHC-II, or from the development of an asymmetry between the *a*1-*a*2 and *a*1′-*a*2 transfer pathways. The essential point is that the data of Gillbro et al. can be explained by a model of this type.

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