

LETTERS

Chlorophyll *a* Franck–Condon Factors and Excitation Energy Transfer

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The Franck–Condon factors for the $S_1(Q_v) \leftrightarrow S_0$ electronic transition of chlorophyll (Chl) molecules are important for understanding excitation energy transfer in photosynthetic complexes. Currently, there are two sets of Chl *a* Franck–Condon factors for over 40 modes, one determined by spectral hole burning and the other by fluorescence line narrowing. Those obtained by the latter spectroscopy are, on average, a factor of 30 times smaller than the hole burning values. Nonline-narrowed fluorescence results for the light-harvesting complex II of photosystem II at 4.2 K are presented that agree quite well with the hole burning but not the fluorescence line narrowing values.

Introduction

Excitation energy transfer (EET) processes in photosynthetic antenna are of longstanding and much current interest. The complexities associated with theoretically describing EET in such systems have recently been reviewed.¹ Fermi's golden rule has often been used to calculate EET rates between chlorophyll donor (D) and acceptor (A) states. Förster theory emerges when the electronic coupling between the D and A states is weak relative to their dephasing frequencies. In this limit, the operator in the electronic coupling matrix element of the golden rule is the static intermolecular potential energy. That is, the zero-order states in the matrix element are localized on D and A. In the case of strong coupling, the zero-order states in the matrix element are delocalized (excitonic) and defined by a Hamiltonian that contains the static intermolecular potential energy. Thus, relaxation between the delocalized exciton levels is triggered by the dependence of the intermolecular potential energy on, for example, librational motions of the chlorophyll (Chl) pigments that modulate the excitonic interactions.^{2–4} Such a mechanism for aromatic molecular crystals has been extensively

studied, both experimentally and theoretically.^{5–9} It appears to be important for the ultrafast (~ 100 fs) downward interexciton-level relaxations observed for the B850 and B875 BChl *a* rings of the LH1 and LH2 complexes of purple bacteria.¹⁰

Irrespective of the electronic coupling strength, characterization and determination of the electron–vibration (intramolecular) and electron–phonon couplings is required for calculation of transfer/relaxation rates since they enter into the spectral density. For what follows, it is instructive to consider the expression of Kolaczowski et al.¹¹ for the average Förster transfer rate

$$\langle k_{DA} \rangle(T) = 2\pi n^{-4} V^2 (1 - e^{-S}) [2\pi(\Gamma^2 + \Sigma^2)]^{-1/2} \times \sum_i (2FC_i) \exp[-(\Omega_o - \omega_i - S\omega_m)^2 / 2(\Gamma^2 + \Sigma^2)] \quad (1)$$

which includes all relevant parameters. They are the electronic coupling V , the Huang–Rhys factor S and mean frequency ω_m of the phonons, the Franck–Condon factor and frequency ω_i of intramolecular mode i , the average value Ω_o and variance Γ^2 of the D–A energy gap, and Σ^2 , the variance of the homogeneous broadening contribution to the spectral density, which is given by

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$$\Sigma^2(T) = \tilde{S}(T)(\sigma^2 + \omega_m^2) + (\Gamma_h/2)^2 \quad (2)$$

Here, $\tilde{S}(T) = S \coth(\hbar\omega_m/2kT)$, σ^2 is the variance of the one-phonon profile, and Γ_h represents homogeneous broadening other than that from linear electron–phonon coupling. Equation 1 is appropriate when $\Gamma^2 \leq \Sigma^2$, i.e., when the kinetics are nondispersive. (The case of dispersive kinetics is treated in ref 11.) The simple manner in which the electron–vibration coupling for mode i is incorporated is a consequence of the FC factors for the $S_1(Q_y) \leftarrow S_0$ transition of Chls being small, <0.1 , vide infra. It is important to note that the electron–phonon coupling in antenna protein complexes is generally weak, as reviewed in ref 1. Typically, $S \leq 0.8$ and $\omega_m \approx 20\text{--}30\text{ cm}^{-1}$ based on hole spectra. There were two motivations for the development of eq 1. First, values for all parameters except V are obtainable by hole burning spectroscopy. Second, application of the Förster spectral overlap criterion is problematic for inhomogeneously broadened spectral transitions and, moreover, can be difficult to apply (see, e.g., ref 12). Equation 1 has been successfully applied to the $\sim 1\text{ ps}$ B800 \rightarrow B850 EET process of the LH2 complex of purple bacteria.¹³ The calculated rates were found to be within a factor of 2 of the observed values, and moreover, the weak dependences of the rate on temperature and on significant variation of the B800–B850 energy gap ($\sim 450\text{--}1050\text{ cm}^{-1}$) were explained.

The intramolecular FC factors used in the B800 \rightarrow B850 transfer rate calculations were those of Gillie et al.¹⁴ for Chl *a*.¹⁵ They employed vibronic satellite hole burning spectroscopy. Photosystem I particles were used in that study. The excited-state frequencies and FC factors of the active modes are given in the first two columns of Table 1. The FC factors of the two most intense modes at 746 and 1259 cm^{-1} are only about 0.04. An alternative method for determination of the FC factors is fluorescence line narrowing (FLN) spectroscopy. Prior to the work of Gillie et al., FLN and line-narrowed fluorescence excitation spectroscopy had been used to obtain high-resolution vibronic spectra of Chl and BChl molecules imbedded in glasses at liquid helium temperatures.¹⁶ However, it was not possible to determine FC factors, although the spectra provided relative vibronic intensities. The relative vibronic intensities for Chl *a* from ref 16 were shown in ref 14 to be in reasonable agreement with those of Gillie et al. Recently, Peterman et al.¹⁷ reported origin-excited FLN spectra of Chl *a* in the LHC II antenna complex of photosystem II. Their analysis of the spectra led to FC factors which are exceedingly small, *1 to 2 orders of magnitude smaller* than those of Gillie et al. (Their largest FC factor is 0.0019 for a mode with a ground-state frequency of 742 cm^{-1} .) Peterman et al. implied that the FC factors of Gillie et al. may be in error.

Given that the Chl FC factors are of critical importance for understanding D \rightarrow A transfer in antenna complexes in which the D–A energy gaps are large enough for intramolecular modes to play a role, it is important to determine which of the two sets of FC factors is more correct. This question is addressed here.

Results and Discussion

The Chl *a* vibrational frequencies and FC factors from Gillie et al. and Peterman et al. are compared in Table 1. An important observation is that the FC factors of Peterman et al. are generally well over an order of magnitude smaller than those of Gillie et al. The two sets correspond, respectively, to $S_1 \rightarrow S_0$ and $S_1 \leftarrow S_0$ vibronic transitions. The question arises then as to whether this difference can account for such a large discrepancy in the

TABLE 1: Chlorophyll *a* Vibrational Frequencies and Franck–Condon Factors

$\omega(\text{cm}^{-1})$, S_1^a	FC factors, S_1^a	$\omega(\text{cm}^{-1})$, S_0^b	FC factors, S_0^b
262	0.012	260	0.00010
283	0.004	298	0.00016
350	$\sim 0.02^*$	342*	0.0010
390	0.015*	388*	0.00076
425	0.007	425	0.00044
469	0.019*		
501	0.007		
521	0.017*	518*	0.0013
541	0.009	546	0.000089
574	0.025*	573	0.00028
588	0.005	585	0.00010
607	0.012	604	0.000064
714	0.010	722	0.00013
746	0.044*	742*	0.0019
		752*	0.0013
771	0.007		
791	0.014		
805	0.012		
819	0.005		
855	0.009		
864	0.007		
874	0.007		
896	0.013		
		916*	0.00074
932	0.025*	986	0.00034
994	0.028*	995*	0.00080
1009	0.005		
		1052	0.00040
1075	0.012	1069	0.00021
1114	0.009	1110	0.00015 ^c
		1143*	0.001 ^c
1178	0.018*	1181*	0.00058
		1190	0.00022
1203	0.012	1208*	0.00061
		1216*	0.00058
		1235	0.00023
1259	0.041*	1252	0.00021
		1260	0.00021
1285	0.011	1286	0.00015
		1304	0.00019
		1322*	0.0011 ^c
1340	0.011	1338	0.00013
1364	0.032	1354	0.00019
1390	0.018*	1382	0.00022
1411	0.005		
1433	0.009	1439	0.00022
1455	0.006		
1465	0.006		
		1487	0.00026
1504	0.010		
1524	0.032*	1524	0.00021
		1537*	0.00072
		1553*	0.00051

^a From ref 14. The FC factors are for $S_1 \leftarrow S_0$ vibronic transitions.

^b From ref 17. The FC factors are for $S_1 \rightarrow S_0$ vibronic transitions.

^c Corrected for a misplaced decimal point in Table 1 of ref 17. *Modes that exhibit significant mirror symmetry between $S_1 \leftarrow S_0$ and $S_1 \rightarrow S_0$ vibronic transitions.

FC factors. This is most unlikely since the $S_1(Q_y) \leftarrow S_0$ is strongly allowed and there are no higher energy states lying sufficiently close to the S_1 state to warrant consideration of a breakdown of the Born–Oppenheimer approximation for the $S_1 \leftarrow S_0$ vibronic transitions.¹⁸ Thus, the sum of the FC factors for the $S_1 \leftarrow S_0$ and $S_1 \rightarrow S_0$ absorption and fluorescence transitions should be nearly the same.

This does not mean, however, that mirror symmetry need

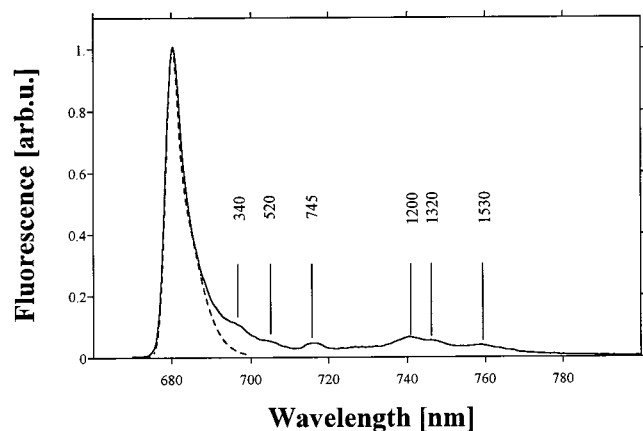


Figure 1. Nonlinear-narrowed fluorescence spectrum of the lowest energy Chl *a* state of the isolated LHC II trimer complex, 4.2 K. The dashed line is the calculated profile of the fluorescence origin.

exist between the absorption and fluorescence vibronic spectra. Even in the Condon approximation, the Duschinsky effect and anharmonicity can lead to significant differences between ground- and excited-state vibrational frequencies and between the relative intensities of vibronic transitions in absorption and fluorescence (see, e.g., refs 19, 20 and references therein). The Duschinsky and anharmonic effects become more important for large molecules. From Table 1 one sees that there is considerable mirror symmetry breakdown for Chl *a*. For example, the $S_1 \leftarrow S_0$ spectrum shows a rich, relatively weak structure in the 800–900 cm^{-1} region which is absent in the $S_1 \rightarrow S_0$ spectrum, while the latter is richer in structure between 1200 and 1300 cm^{-1} . There is, however, considerable mirror symmetry between the most intense vibronic bands. They are marked with an asterisk and have intensities that are ≥ 0.3 times that of the most intense band in each spectrum, 746 and 742 cm^{-1} in absorption and fluorescence, respectively.

The above discussion indicates that the striking difference between the two sets of FC factors is most likely due to a systematic error in their measurement by FLN or hole burning (or both). The FLN FC factors were determined using the phonon sideband (PSB) that builds on the zero-phonon line of the pure electronic transition because the latter transition was severely interfered with by laser light scatter. Such scatter is not present in persistent spectral hole burning. This is not to say that the hole burning values for the FC factors are not subject to some type of significant, systematic error, although we have not been able to identify any. Given that and that Peterman et al. do not present results that establish that the intensity of the above-mentioned PSB is not contaminated by light scatter, one need look to results of another type experiment.

Figure 1 shows a 4.2 K nonlinear-narrowed fluorescence spectrum due to the lowest energy Chl *a* state at 679.8 nm of solubilized LHC II trimer complexes. (This state is the same as that studied by Peterman et al.) Experimental details including the preparation of solubilized LHC II trimers are given in Pieper et al.²¹ Briefly, excitation was provided by a N_2 -laser-pumped dye laser operating at 430 nm with pulse energies sufficiently low to eliminate nonlinear effects. Reabsorption of the fluorescence origin was shown to be negligible (the optical density of the sample at 4.2 K was 0.02 at 676 nm in a 0.01 mm cuvette). The most prominent features of the fluorescence spectrum are labeled with cm^{-1} values corresponding to ground-state vibrations of Chl *a*. Comparison of these frequencies with those of the most intense FLN modes given in Table 1 reveals a close correspondence. However, the FC factors of Peterman

et al. are far too small to account for the intensities of the vibronic bands of Figure 1. To be quantitative, we use the well-known Debye–Waller factor

$$\frac{I_{(0,0)}}{I_{(0,0)} + \sum_j I_j} = \exp(-S_{\text{total}}) \quad (3)$$

where $I_{(0,0)}$ is the intensity of the fluorescence origin, I_j is the intensity of the j th vibronic band, and S_{total} is the total Huang–Rhys factor equal to $\sum_j S_j$. All of the vibronic structure seen in Figure 1 is due to 1-quantum transitions originating from the zero-point level of the S_1 state. The FC factor for such a transition is $S_j \exp(-S_j)$ which for small S_j (< 0.1) is well approximated by S_j .²² Thus, S_{total} can be replaced by $\sum_j (\text{FC})_j$. The value of this sum for the Peterman et al. and Gillie et al. FC factors is 0.018 and 0.58, respectively, which lead to $\exp(-S_{\text{total}}) = 0.98$ and 0.56. The value of the left-hand side of eq 3 determined from the spectrum shown in Figure 1 is 0.65 ± 0.05 , which is in reasonable agreement with the value of 0.56. The value of 0.65 for the Debye–Waller factor corresponds to $S_{\text{total}} = 0.43 \pm 0.07$, which should be compared with the value of 0.58 based on the FC factors of Gillie et al. which we estimate carry an uncertainty of $\pm 10\%$. The experimental value of 0.65 for the Debye–Waller factor²⁵ was obtained by dividing the integrated intensity of the (0,0) or origin band by the integrated intensity of the entire fluorescence spectrum between 670 and 800 nm. The profile used for the fluorescence origin includes the dashed line extension on its low-energy side, which was calculated using the linear electron–phonon coupling and inhomogeneous broadening parameters for the LHC II trimer reported in ref 21 and the theory of Hayes et al.²³ Details will be given elsewhere.²⁴ Suffice it to say that the calculated (dashed) profile provides an adequate fit on the high- and low-energy sides of the fluorescence origin band. Reasonable deviations from the dashed line extension would not significantly affect the experimental Debye–Waller factor.

Although the relative values of the FC factors of Peterman et al. appear to be reliable,¹⁷ it is apparent that their absolute values are well over an order of magnitude too small, so small that vibronic transitions on the scale of the fluorescence spectrum shown in Figure 1 would be unobservable. On the other hand, the FC factors of Gillie et al. are consistent with the spectrum. (We note that the fluorescence spectrum of Figure 1 is very similar to that shown in Figure 2 of Peterman et al., although they did not recognize that it is incompatible with their FC factors.) The same problem with the FC factors of Peterman et al. arises with the $S_1(Q_y) \leftarrow S_0$ absorption spectrum of monomer Chl *a* at cryogenic temperatures (see, e.g., ref 26).

Concluding Remarks

The Chl *a* Franck–Condon factors of Gillie et al.¹⁴ were shown to be sufficiently accurate to justify their use in quantum mechanical calculations of excitation energy transfer rates. Gillie et al. also determined the FC factors for 15 Chl *b* modes of photosystem I particles with frequencies between 255 and 1143 cm^{-1} , which are quite well correlated with the mode frequencies of Chl *a*. The Chl *b* FC factors are, on average, about a factor of 2 smaller than those of Chl *a*. This might be due to a systematic error, a possibility that can be checked by vibronic satellite hole burning of Chl *b* monomer in glasses or polymers. Such experiments are planned. It will be interesting to use reliable Chl *a/b* FC factors with eq 1 to calculate the Chl *b* \rightarrow

Chl *a* transfer rates of the LHC II and other complexes of photosystem II.

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