Energy Transfer in Photosynthesis

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I. Introduction

Time-resolved fluorescence spectroscopy provides a convenient probe of the kinetics of the light harvesting process; however, the absence of structural information on all but a few photosynthetic systems makes it difficult to obtain a full understanding of the mechanism of this process.

Recently we have been engaged in studies designed to improve our understanding of the transport and trapping of electronic excitations in both Photosystem I complexes of higher plants and reaction center complexes of photosynthetic bacteria. On the one hand, we wish to understand the overall organization of the light harvesting complex and specifically those structural features that make the conversion so remarkably efficient. On a more fundamental level, we are attempting to understand the microscopic transfer of energy between neighboring pigments and to obtain a detailed molecular view of the mechanism of transfer. In this paper we briefly review some recent experimental and theoretical approaches in both areas.

II. Light Harvesting Complexes of Higher Plants

Higher plants consist of two photosystems (PSI and PSII) each of which is connected to a pool of chlorophyll a/b protein complexes. The individual photosystem core complexes comprise tens of chlorophyll a pigments that serve to feed energy to the reaction centers. The overall time scale of the energy transport process can be monitored by time resolved fluorescence spectroscopy. The trapping of excitations by the reaction centers quenches the fluorescence of the chlorophyll donor molecules leading to lifetimes that are substantially shorter than that for chlorophyll in vitro.

Understanding the relation between fluorescence decay profiles and structural aspects of the chlorophyll antenna is a major goal of our studies. Recently we have carried out a series of fluorescence measurements on PSI core antenna complexes of both barley and detergent-isolated preparations and genetically altered mutants of the green alga Chlamydomonas reinhardtii using time-correlated single-photon counting (1-3). The ratio of chlorophyll a molecules to the reaction center pigment P700 was varied from approximately 20-70 by adjusting the isolation procedures. In each preparation the fluorescence decay profiles were accurately fit to a two exponential decay law. The longer time constant was 6 ns and is assigned to chlorophylls which are disconnected from the reaction center. The shorter time constant varied from 15 to 40 ps and varied linearly with the ratio of chl a to P700 in accord with predictions from a lattice model developed by Pearlstein (4). This model assumes: (a) that the antenna pigments form a regular lattice with a centrally located trap and with only nearest neighbor coupling, (b) there is no disorder in the energy of the donor excited states, and (c) the transfer of energy occurs through an incoherent, or random, process.

From an analysis of the experimental data using the above model, we arrived at a value of 0.21 ± 0.04 ps for the single step hopping time and 2.8 ± 0.7 ps for the charge separation time for P700. These results are model dependent though
it is interesting to note the similarity of the latter result to the experimentally measured electron transfer times of 2.6 ps for isolated PSII reaction center complexes (5) and 2.7 ps for the reaction center complexes of purple bacteria (6).

For preparations that contain peripheral chl a/b protein, the linear relationship between lifetime and antenna size no longer holds (see Figure 1) leading us to conclude that excitations originating in the peripheral part of the array are rapidly and irreversibly transferred to the core region.

![Figure 1. Lifetime of the fast decay component as a function of antenna size (chl a+b) for detergent-isolated PSI preparations and three strains of C. reinhardtii. The dashed line shows the relationship between lifetime and PSI core size for detergent-isolated preparations free of peripheral pigments. Predicted PSI core sizes (right y-axis) were determined for all samples from the relationship: antenna size = 1.67τ - 7.01. (O) Detergent-isolated PSI samples lacking chl b; (*) PSI samples containing chl b; (□) C. reinhardtii strain A4d; (■) strain LM3-4d1c; (♦) strain B1; (●) wild-type strain 137c; (*) relationship between average core size for strains A4d and B1 (~65 and 125 chl/a/P700, respectively) and average lifetime of excited states in each type of core antenna.

Our studies on mutants of C. reinhardtii lacking PSII have concentrated on two strains, one with a wild-type PSI core containing approximately 120 chlorophylls and the second containing only about 60 chlorophylls per P700. Both strains gave rise to decay curves with the major component having a time constant of 75 and 45 ps, respectively. These values agree well with those obtained from the detergent isolated preparations. The wild-type strain was found to have an intermediate component with a time constant of 300-800 ps with spectral properties of both the core pigments and the peripheral pigments. From a detailed study of the spectral and temporal properties of the decays, we concluded that excitations become distributed over all spectral forms of chlorophyll on a time scale that is fast compared to the trapping time, and that excitations, on the average, visit a representative sample of spectral forms regardless of excitation wavelength.

We conclude this section by briefly discussing the role of spectral disorder in the transport dynamics. It is well known that even in complexes containing a single type of chlorophyll that more than one spectral form exists. These different spectral forms presumably arise through pigment-protein interactions which vary depending on the nature of the various binding sites in the protein. Alternatively, pigment-pigment interactions could be responsible for the distribution of transition energies. In either case, the result is an absorption band for the light harvesting complex that is substantially broader than that for chlorophyll a in vitro.

An important observation of our PSI studies is that the spectral characteristics of the fluorescence decays and the absorption spectra appear to be independent of the core antenna size. While we have no direct structural information on our core complexes, we might naively assume that in the isolation procedures pigments remote from the reaction center are preferentially removed. If this is true and if there were any spatial correlation of the inhomogeneous broadening, we would expect to observe a wavelength shift in the absorption and fluorescence properties as we changed antenna size. The absence of these argue
against a funnel-type structure in the PSI core.

This leads us to examine what, if any, role spectral disorder plays in the migration of excitations to the trap region. To gain some understanding of how this might affect fluorescence properties of light harvesting arrays, we have performed exact numerical simulations of excitation hopping on two-dimensional lattices containing two or more spectral forms of chlorophyll (7). The individual site-site transfer rates were calculated from the spectral overlap in the manner prescribed by Förster (8). An important conclusion from our calculation of the time-resolved excitation and emission spectra of these models are that excitations are rapidly randomized among all spectral forms regardless of whether any spatial correlation of spectral forms exists. It was found that the time evolution of the total fluorescence, while slightly different for different models, were essentially the same on time scales accessible by single photon counting, pointing out the limitation of this technique in obtaining certain types of specific structural information.

Another important result from our hopping simulations concerns the relation between excitation wavelength dependence of the amplitudes of the various decay components (9). It is commonly assumed that the excitation spectrum of a decay component is a direct measure of the number of pigments that contribute to that component. Our results, however, have shown that this depends on the assumption that there is a rapid equilibration of the excitations among all the spectral forms. If bottlenecks, either spectral or spatial, exist in the transport path to the trap, then there is no simple relation between amplitude spectra and antenna size.

III. Excitation Transfer in the Chl a/b Protein and in Bacterial Reaction Centers

We focus now on the microscopic aspects of energy transfer in photosynthetic systems by considering relatively simple systems for which some structural information exists. The three we discuss are the LHC II chl a/b protein complex and the reaction center complexes of the purple bacteria Rps. viridis and Rb. sphaeroides.

The overall stoichiometry of the chl a/b complex is uncertain, however, circular dichroism (10,11) and electron microscopy studies (12) suggest that three chlorophyll b molecules are arranged in a strongly coupled trimer. This forms the basis of the Van-Metter-Knox-Shepanski model (10,11), which we adopt to analyze our experimental results. By exciting into the chl b bands, we have been able to the risetime of the chl a fluorescence using subpicosecond fluorescence upconversion (13). We obtain a value of 0.5 ± 0.2 ps for the chl b to chl a transfer time, which is consistent with our estimates based on the lattice model. The result is shown in Figure 2.

A standard assumption in photosynthesis research is that the transfer of energy between individual pigments occurs through a Förster (dipole-dipole) mechanism. The validity of the weak coupling theory of Förster depends on the assumption that the fluctuations in the site energies are uncorrelated, that is there are no "shared" vibrational modes, and that a separation of time scales exists between vibrational relaxation and energy transfer. These approximations allow the transfer rate to be expressed in terms of steady state optical properties of the individual pigments. The observation of very rapid energy transfer in the chl a/b protein and, as we shall see later, in bacterial reaction centers, calls into question the validity of the weak coupling approach.

A clearer understanding of this can be gained by studying energy transfer in systems for which detailed structural information exists. Such an example is found in the reaction center complexes of Rps. viridis and Rb. sphaeroides, which have recently been crystallized and studied by x-ray crystallography (14,15). The complexes of both species consist of a special pair(P) of bacteriochlorophyll (BCh)
molecules which form the primary electron transfer donor. The special pair is surrounded by two bacteriochlorophyll molecules and two bacteriopheophytin (BPh) molecules. The six pigments are strongly coupled as evidenced by their close proximity to one another. Time-resolved studies of energy transfer to the special pair from the neighboring pigments have been performed recently by Breton, et al. (6). The results show that energy transfer from any pigment to the pair occurs in less than 100 fs. Using the crystallographic distances and spectral information such as bandwidths and transition dipole strengths for the pigments, we have calculated the population of each excited state after exciting into various pigments by adopting a Förster hopping model and numerically solving the resulting set of coupled kinetic equations (16). Our results predict risetimes of the special pair states to be on the order of a picosecond or longer. An example is shown in Figure 3. We thus conclude that a weak coupling theory is grossly inadequate to account for the observed rates in these complexes. Extrapolation of these results to other systems is dubious until further structural information exists, however, it is clear that Förster theory may not be as universally valid as previously thought. Similar conclusions have recently been reached by Sauer and co-workers from studies on C-phycoerythrin (17).

To gain a more thorough understanding of the fundamental process in light harvesting, it would seem important to address the following questions:

(1) What is the nature of the electronic states? Are they delocalized?
(2) What is the role, if any, of the protein in the transfer process? If the electronic states are appreciably coupled to protein vibrations, are these motions localized or delocalized on distance scales comparable to pigment-pigment separations?
(3) What are the time scales of the relevant vibrational relaxation rates?
(4) In a strongly coupled system, what effect does the nature of the initially prepared state have on the observed dynamics?

We are currently developing a microscopic theoretical approach based on Redfield relaxation theory (18,19) to address these issues.

Fig. 2. (a) Time-resolved isotropic (magic angle) fluorescence of LHC II containing thylakoids. Excitation at 650 nm; emission at 690 nm. The solid line is a best fit of the data to a prompt exponential decay convoluted with the instrument response function. The residuals of the fit are displayed above the decay. (b) Fit of the same data including an exponential rise time of 0.67 ps.
IV. Concluding Remarks

Energy transfer in photosynthesis has relevant time scales ranging from tens of femtoseconds to hundreds of picoseconds. It is clear that a thorough understanding of this process requires a synthesis of techniques from molecular biology, experimental chemical physics and condensed matter theory.

V. References

Fig. 3. Population of various excited states in Rps. viridis after exciting into the BPhL band.

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