Nature of Disorder and Inter-Complex Energy Transfer in LH2 at Room Temperature: A Three Pulse Photon Echo Peak Shift Study†

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Received: November 5, 2001; In Final Form: January 18, 2002

Three Pulse Photon Echo Peak Shift data are reported for the B850 band of the peripheral light-harvesting complex (LH2) of *Rb. sphaeroides* at room temperature. The data are obtained for both the detergent solubilized and purified complex, and the native membrane samples containing LH2 as the sole bacteriochlorophyll-protein complex. Both samples reveal an ultrafast decay on a 100−200 fs time scale that is attributed to exciton relaxation dynamics within individual aggregates. The samples in native membranes also contain a ∼5 ps decay component which is assigned to inter-complex, LH2−LH2 energy transfer. The observation of the 5 ps component in the membrane samples strongly suggests the presence of two levels of energetic disorder in the system.

1. Introduction

The process of transduction of light energy into chemical energy is initiated at the reaction center (RC) of a photosynthetic system.[1−5] The RC pigments absorb photons at a very low rate of 10 Hz compared to a maximum turnover rate of ∼1000 Hz for the optimum functioning of the RC.[6,7] To fulfill metabolic requirements for sustenance, the photosynthetic systems are left with two options; (i) synthesize multiple copies of RC complexes or (ii) supply the RC with a very high rate of excitation energy. Option (i) is impractical as the RC and its associated proteins are large[8] and have large energy requirements for their synthesis. Nature has adopted an elegant version of the second strategy; the RC is surrounded with pigment−protein complexes called light-harvesting complexes (LHC).[9−13] In purple bacteria, the RC is surrounded by a core antenna light-harvesting complex called LH1[9,14] The LH1−RC complexes are in turn surrounded by the peripheral light harvesting complexes called LH2.[11,15] Therefore, the number of chlorophyll molecules per RC and consequently the absorption cross-section of photon absorption increase dramatically. To supply the RC with excitation energy, the whole assembly of RC-LHC must be optimally connected to eventually trap any excitation energy available in the entire apparatus. Remarkably, the trapping efficiency of excitation energy in purple bacteria is greater than 95%.[16]

Much is known about the rates and the mechanism of excitation energy transfer that occur within the individual light-harvesting complexes.[2,17,18] The LH2 complex of *Rhodobacter acidophila* is composed of two concentric rings of membrane spanning polypeptides, α and *β*, which enclose a ring of 18 overlapping BChls (B850) and a second ring of 9 monomeric BChls (B800).[19] The electronic coupling between the B800 pigments is weak ($J \approx 30$ cm^{-1}) and results in Förster-like energy migration within the B800 pigments on a 500 fs time scale.[18,20,21] The B800 pigments also transfer their excitation energy to the neighboring B850 ring, containing 18 rather strongly coupled BChls ($J \approx 300$ cm^{-1}), on subpicosecond time scales.[2,17,18] The electronic structure of the B850 pigments can be described via a disordered exciton model.[17,18] Upon excitation of the B850 band, ultrafast excitonic relaxation on a 100−200 fs time scale occurs.[18] The thermalized exciton state in the B850 band is believed to be delocalized over 3−4 pigments, although the issue is still contentious.[18,21−23] The LH2 complex of *Rb. sphaeroides* is believed to adopt an arrangement of polypeptides and chromophores which is very similar to that of the *Rps. acidophila* LH2, on the basis of cryo-electron microscopy data.[24] The LH1 complex of *Rb. sphaeroides* surrounds the RC,[24] absorbs at 875 nm and has 26 ± 3 strongly coupled BChls.[25] The energy migration process within the LH1 complex is believed to be very similar to the dynamics occurring in the B850 ring.

The intra-complex energy migration processes occur on very short time scales and equilibration is typically reached within a few ps.[18] The trapping time of the excitation energy is however ∼100 ps,[26] implying that the excitation spends most of the time undergoing inter-complex energy transfer processes, namely LH2 → LH2, LH2 → LH1 and LH1 → RC transfer. The LH2 → LH1 transfer has been measured at 296 K to be ∼3 ps[27,28] and LH1 → RC is ∼30−50 ps.[29−31] However, not much is known about the other inter-complex energy transfer processes especially LH2−LH2 energy transfer. This is mostly a result of the use of techniques that are sensitive to changes in population, making inter-complex transfer between similar molecules hard to detect as the population remains in the same spectral region. Information about inter-complex transfer is crucial to our understanding of energy transfer and trapping in the photosynthetic apparatus. Recently, Freiberg and co-workers measured the LH2 → LH2 energy transfer time to be between 1 and 10 ps at cryogenic temperatures.[32] However, it is desirable

† Part of the special issue "G. Wilse Robinson Festschrift".

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10.1021/jp014054m CCC: $22.00
Published on Web 03/22/2002

to study the photosynthetic system at physiological temperatures to fully understand the design principles nature adopts and possibly to mimic these strategies in the design of synthetic devices.

Three Pulse Photon Echo Peak Shift spectroscopy (3PEPS) has been shown to be insensitive to energy transfer outside the laser spectral window.[33−35] On the other hand, in a disordered system, it provides an incisive probe of energy transfer processes occurring between similar species.[33] It has been successfully applied to study energy transfer within the B800,[20] B850,[21,36] and the B875 bands[21,36] of the LH2 and LH1 complexes, respectively. The success of 3PEPS on these systems makes it a good choice to probe energy transfer between LH2 rings. It has also been shown that 3PEPS data are sensitive to the nature of disorder in these complexes, i.e., if any correlation exists in the disorder.[37] Previously, from low-temperature linear and nonlinear absorption,[38] single molecule spectroscopy,[39−42] and 3PEPS data of B800 band of LH2,[20,21] it was proposed that two-levels of disorder exist in these circular aggregates. The first level of disorder arises from the distribution of site energies within the rings and the second level is due to the distribution of the mean energy of each ring compared to the other rings. In other words, the 3PEPS technique allows determination of whether individual rings of the LH2 complex display in full or in part the total disorder observed in the ensemble averaged absorption spectrum. The use of a mutant strain containing only LH2 complexes in intact membranes provides a convenient means to study the nature of disorder and dynamics of energy transfer in situations that should be close to physiological conditions for the antenna system. In this paper, we clearly show the presence of two-levels of disorder in the LH2-only membrane samples. In addition, we determined that energy transfer between the LH2 complexes occurs on ∼5 ps time scale at room temperature.

2. Experimental Section

The membrane samples were prepared as previously described.[43] The purified complexes were made by solubilizing units of membranes in 50 mM NaCl, 50 mM Tris pH 8 (final volume 10 mL) using LDAO to a final concentration of 0.15%. The LDAO (as a 1% solution) was added dropwise to the membranes with gentle stirring in a cooled cabinet at 10 °C. The membranes were then gently stirred for a further 30 min at 10 °C. The solubilized complexes were loaded onto a preequilibrated DEAE column, bed volume 15 mL, through a 0.2 micron syringe filter to exclude any unsolubilized material, at 1 mL/min, at 10 °C. The column was washed with buffer (50 mM NaCl/50 mM TRIS pH 8/0.1% LDAO) for 1 h at 1 mL/min at 10 °C. The complexes were then eluted using a 50−400 mM NaCl salt gradient and 4 mL fractions collected. Each highly colored fraction was assessed for yield and purity by measuring the OD at 280 and 850 nm. The fractions having a 850/280 ratio of better than 2 were retained and frozen at −20 °C. Size exclusion chromatography was conducted on the detergent isolated samples and it was found that the sample contains mainly aggregates that are likely to be tetramers with a minor population of dimers of the nonameric LH2 rings (Olsen & Hunter, unpublished results).

3PEPS experiments were performed using the idler of a commercial Optical Parametric Amplifier (Coherent OPA 9450) tuned to 850 nm at a repetition rate of 2 kHz. The OPA was pumped by the output of the partially home-built Ti:sapphire regenerative amplifier system producing 45 fs pulses centered at 800 nm. The regenerative amplifier used a Kapteyn−Murnane Labs stretcher-compressor kit, and was pumped by a Positive Light Evolution II at 2 kHz. The seed source was a home-built Ti:sapphire oscillator producing 16 fs (fwhm, E-squared) pulses. After prism compression, the OPA idler pulses were 55 fs (fwhm, E-Squared) in duration. The samples were flowed rapidly in a 100 μm path length quartz cell and cooled to 4 °C. The optical density of the samples was ∼0.15 in a 100 μm, and the pulse energy was ∼1 nJ per pulse. The 3PEPS data did not reveal any discernible differences at lower pulse energies (0.5 nJ).

3PEPS experiments were performed by splitting the 850 nm beam into three beams of roughly equal intensities in the three armed interferometer.[44] The beams were arranged in an equilateral triangle geometry and focused on the sample with a 20 cm focal length lens. The signals were simultaneously measured in $\mathbf{k_3} \pm (\mathbf{k_2} - \mathbf{k_1})$ phase matching directions where $\mathbf{k_3}$, $\mathbf{k_2}$, and $\mathbf{k_1}$ are the wave vectors associated with the three pulses. Two of the arms of the interferometer contained computer controlled optical delays and the third arm was fixed. The time integrated echo signals were detected using photomultiplier tubes and processed by the lock-in amplifiers. The data collection procedure for the membrane sample required greater signal averaging over many laser pulses (100 000 pulses for each data point as compared to 25 000 pulses for the solubilized sample) to give an adequate signal-to-noise ratio. In addition, each echo profile was averaged over 8−10 scans for the membrane sample (compared to 2 scans for the solubilized sample) for the accurate determination of the peak maximum.

3. Background for 3PEPS Experiment

3.1 3PEPS on Isolated Two-Level Systems Coupled to Solvent Bath. The decay of peak shift of isolated two-level optical chromophores dissolved in a condensed phase environment reflects the transition frequency correlation function of the ensemble of such systems.[44,45] This memory function, or $M(t)$, is given by

$$M(t) = \frac{\langle \delta\omega(t)\delta\omega(0)\rangle}{\langle \delta\omega^2\rangle} \tag{1}$$

where $\delta\omega(t)$ represents the fluctuations in the transition frequency. The main objective of a 3PEPS experiment is to obtain the $M(t)$ of the system, which contains relevant information about the time scales and amplitudes of solvent motions that couple to the solute's electronic transition. 3PEPS data has also been shown to be very sensitive to the presence of static disorder (dynamics much slower than the experimental time scale) in the observed system. Static disorder appears as a nonzero value of the peak shift at long times.[45]

3.2 3PEPS on Electronically Coupled Systems. In complex systems such as photosynthetic light-harvesting complexes and J-aggregates, the optical chromophores are in close proximity to each other, and their electronic eigenstates cannot be described by isolated two-level systems coupled to a solvent bath. The electronic interaction between the chromophores leads to very interesting phenomena such as energy transfer (for weak electronic coupling) and exciton relaxation for stronger electronic coupling. Yang and Fleming have developed a theory for 3PEPS for both weakly[33] (Forster energy transfer) and strongly coupled[21,46,47] (exciton relaxation) systems. The peak shift decay is shown to clearly reflect these two processes. The reason being that 3PEPS experiments in general are sensitive to the memory of the transition frequency of the initially created states and follow the time scale on which the initially created

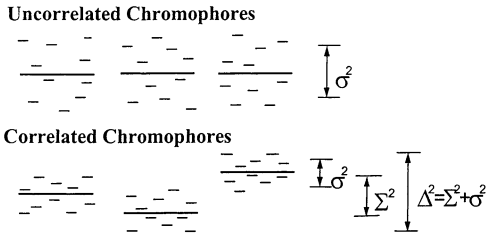

Figure 1. Illustration of one- (uncorrelated) and two-levels (partially correlated) of disorder in a multi-chromophore system.

states lose memory of their transition frequency. For isolated chromophores dissolved in a condensed phase, the loss of memory is due to spectral fluctuations and spectral diffusion. For electronically coupled systems, energy transfer or exciton relaxation processes lead to additional loss of memory of the transition frequency of the initially created states resulting in further decay of the peak shift data. The 3PEPS technique has been successfully applied to extract detailed dynamical information for various light-harvesting complexes of purple bacteria,[20,21] green plants,[35] and J-aggregates in solution.[47]

3.3 Effect of the Magnitude and Nature of Disorder on 3PEPS Data. The presence of energetic (diagonal) disorder has a pronounced effect on peak shift data. For isolated two-level systems, the terminal peak shift value strongly depends on the magnitude of disorder present in a system.[45] For energy transfer in weakly coupled systems, disorder determines the distribution of energy transfer rates leading to nonexponential kinetics, an effect clearly observed in peak shift data.[20] For strongly coupled systems, disorder determines the electronic structure of the excitonic states[21,46] and, therefore, affects the dynamics of exciton relaxation. At present, rather little is known about the magnitude of off-diagonal (coupling) disorder. A recent study by Jang et al. looked at the effect of off-diagonal disorder on the distributions of the lowest exciton levels.[48] It was found that the off-diagonal disorder caused broader distributions of the excitonic energies than the same amount of diagonal disorder. It was also found that if the diagonal disorder was limited to 100 cm^{-1}, a comparable amount of off-diagonal disorder, 130 cm^{-1}, was needed to simulate low-temperature single molecule distributions.[48] Off-diagonal disorder has not yet been explicitly included in simulations of 3PEPS signals however, we do not expect that the 3 PEPS signal would change substantially if a portion of what we currently assign to diagonal disorder is in fact a slightly different amount of off-diagonal disorder. We therefore restrict the following discussion to energetic (diagonal) disorder only.

In an ensemble of molecular aggregates containing similar chromophores, two models for distribution of site energies have been previously proposed.[37] They are illustrated in Figure 1.[20,21,37] In the first model, the mean energy of each aggregate is assumed to be the same. The disorder in the molecular sites within the aggregates have no correlation with each other, i.e., the site energies are within the variance, σ. In the second model, the mean energy of individual rings is allowed to have another distribution given by Σ in addition to the intra-ring distribution given by σ. The total disorder is then given by

$$\Delta^2 = \sigma^2 + \Sigma^2 \qquad (2)$$

where Δ is the total disorder value. This model implies that static site energy distribution within the rings does not cover the entire distribution and is restricted to sample values only from a sub portion of the total disorder. The presence of second level of disorder may be due to the structural fluctuations of the rings on a very slow time scale. It may also be due to incomplete statistical sampling of the site energies as the individual rings contain only a finite number of pigments, *N*. The ratio, $\Sigma/\sigma = (\sqrt{1/(N-1)})$, for $N > 1$, decreases for increasing values of *N*, and the value of Σ approaches zero for very large values of N.[21] Clearly, LH2, which contains 9 and 18 pigments in the B800 and the B850 rings respectively, will have a finite contribution from the incomplete sampling effect. The absorption spectrum is only sensitive to the value of total disorder, Δ, and not on the way it is partitioned between σ and Σ. Energy transfer or exciton relaxation processes within individual rings depend only on the value of σ, whereas Σ influences the energy transfer rate between the aggregates. Therefore, if energy transfer occurs *within* the aggregate, the excitation samples over a distribution of σ values and loses the memory of σ on the time scale of energy transfer (see section 4). In the absence of energy transfer *between* the aggregates as in the case of dilute, solubilized samples, the memory of Σ is retained leading to finite long time peak shift values.[37]

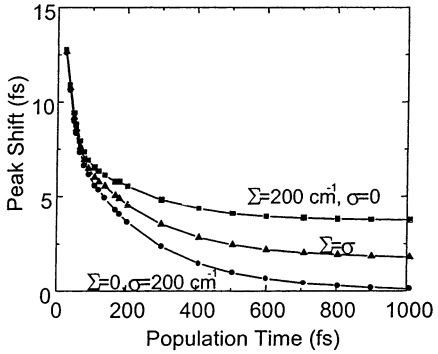

Figure 2. Model peak shift calculations for different values of σ and Σ with Δ ($(\sigma^2 + \Sigma^2)^{1/2}$) fixed at 200 cm^{-1}. The energy transfer rate is fixed at (300 fs)$^{-1}$.

In Figure 2, we show the results of simulations for a system with weak electronic coupling and a fixed energy transfer time constant of 300 fs.[20,37] The simulations are presented for three different values of σ and Σ but with a fixed value of Δ. The simulations show that, as expected, if $\Sigma = 0$, the long time peak shift goes to zero. However, if $\Sigma \neq 0$, the long time peak shift is always nonzero as the memory of Σ is retained. At room temperature, conventional time-resolved techniques such as pump−probe or transient grating spectroscopy are not sensitive to the nature of disorder in the system. The 3PEPS method has been previously applied to elucidate the nature of disorder in the B800 band of the LH2 complex of *Rps. acidophila*,[20,21] resulting in an estimate of $\sigma = 90$ cm^{-1} and $\Sigma = 50$ cm^{-1} for the system.[20] A substantial part of Σ(30−40 cm^{-1}) was estimated to originate from incomplete statistical sampling of site energies within a ring containing only nine pigments.[20,21]

The above discussion would also hold for systems with stronger electronic coupling that undergo ultrafast excitonic relaxation. If $\Sigma = 0$, the peak shift should decay to near zero owing to exciton dynamics within the individual rings and if $\Sigma \neq 0$ for a sample of isolated aggregates, the long time peak shift should not decay to zero.[21]

We conclude this section by mentioning once again that the presence of a second level of disorder (Σ) in molecular aggregates leads to a finite value of the terminal peak shift. However, if the different rings come in close contact with each other, energy transfer between the rings can occur leading to a

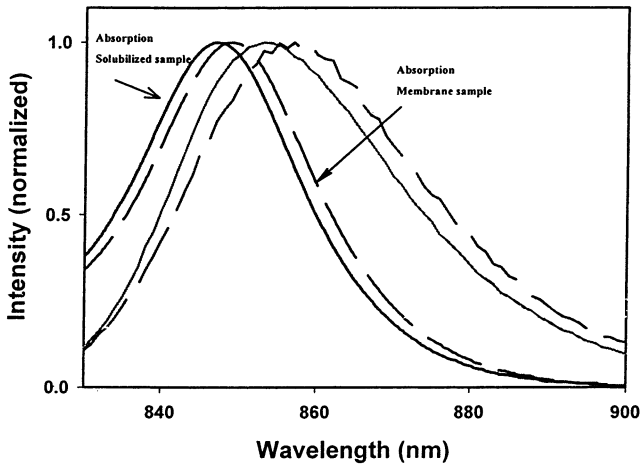

Figure 3. Absorption and emission spectra of solubilized (solid line) and membrane embedded (dashed line) samples.

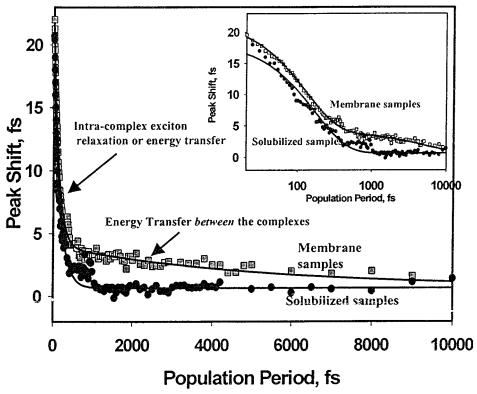

Figure 4. 3PEPS data for solubilized (circles) and membrane (squares) samples and the fitted results. The inset shows the same data on a logarithmic scale.

loss of memory of Σ on a time scale of inter-ring energy transfer. If $\Sigma = 0$, the information about energy transfer between the rings will not be present in the peak shift data as the peak shift would have already decayed to zero due to intra-ring dynamics. The main objective of this paper is to explore whether two-levels of disorder exist in membrane embedded LH2 samples at room temperature. We proceed with the assumption that if $\Sigma = 0$, the peak shift data for the membrane and the solubilized samples will not be very different. Only if $\Sigma \neq 0$, should the 3PEPS data clearly reflect the inter-ring energy transfer process.

4. Results and Discussion

The room temperature absorption and emission spectra of solubilized and membrane samples are shown in Figure 3. The absorption spectrum for the membrane sample is slightly broader and red shifted in comparison to the solubilized sample. The Stokes shift between the absorption and emission spectra is very small (~4 nm) for both samples.

3PEPS data for solubilized and membrane samples are shown in Figure 4. The initial peak shift value for the membrane sample is slightly larger than for the solubilized sample. The initial decay time scale of the peak shift is similar for both the samples, although the solubilized sample has a larger amplitude of this ultrafast decay component. The peak shift for the solubilized complex decays rapidly with apparently a single decay constant to a near zero value (<1 fs) within 1 ps. Our results for the solubilized sample are very similar to the 3PEPS data previously reported for the same species at room temperature by Jimenez et al.[36] The peak shift data for the membrane sample decay on at least two time scales; the peak shift decays from an initial value of ~25 fs to ~5 fs within 1 ps, followed by a much slower decay on a 4−5 ps time scale to reach a near zero value (<1 fs) within 10 ps. Direct comparison of the peak shift data for these two samples clearly reveals an additional slower decay component in the membrane sample that is absent in the solubilized sample.

The peak shift data were fit to simple sums of exponential decay components. The data for the solubilized samples were best described by a single-exponential decay component (95%, 160 fs), whereas the membrane samples were best fit by two exponential components (80%, 180 fs, 16%, 5 ps). Adding more exponential components to either data set did not improve the quality of the fits, and we believe that the above fitting procedure has captured the essential features of the decay of the peak shift data. The results of the fitting procedure are shown in Figure 4 along with the experimental data.

Given the nature of the 3PEPS experiment and its unique sensitivity to intraband energy transfer or exciton relaxation processes, it seems appropriate to assign the 5 ps decay component to energy transfer *between* the LH2 complexes in the membrane sample. This component is absent in the solubilized sample. The membrane and the solubilized samples differ in the spatial separation of the LH2 complexes. In the solubilized samples, a detergent layer surrounds each LH2 complex and at low concentrations the separation of two LH2 rings can be much larger than the Forster radius. In the samples in the native membranes, each LH2 ring has multiple neighbors in close proximity thereby enabling transfer of excitation energy between the rings. Our data strongly suggests that energy transfer between the LH2 rings takes place on a 5 ps time scale. On the basis of the discussion in section 3.3, the presence of the 5 ps inter-complex energy transfer component strongly suggests that the mean energy of each ring has its own distribution in the ensemble.

The peak shift data for the B850 ring of the solubilized LH2 complex was analyzed using a disordered exciton theory.[21,46] The number of pigments in the B850 ring is 18 and the statistical sampling effect is small in comparison to the B800 ring. Therefore, we expect and indeed observe that the long time peak shift for the solubilized B850 ring is very close to zero (<1 fs). In the simulation model, two levels of disorder were not used explicitly because the disordered exciton theory properly incorporates the statistical sampling owing to the limited (18) number of pigments in the ring. The theory reproduces the absorption spectrum and the 3PEPS data satisfactorily and it is not necessary to invoke the concept Σ arising from the structural fluctuations of the whole LH2 ring.

A rough estimate of the magnitude of Σ for the 3PEPS data from the membrane sample can be made by using an approximate expression derived by Yang et al. for excitonic systems[21]

$$\tau^*(\infty) \sim \frac{\Sigma^2 + (\sigma^2/N_{\mathrm{del}})f}{\Delta_{\mathrm{el-ph}}{}^3/N_{\mathrm{del}}{}^{3/2}} \qquad (3)$$

where $\tau^*(\infty)$ is the terminal peak shift, N_{del} is the delocalization length, $\Delta_{\mathrm{el-ph}}$ is the exciton phonon coupling strength and f is the fraction of the exciton population remaining on the initial states (f is 1 if the exciton remains in the initial state and 0 if it moves completely). Assuming that $\Sigma = 0$ for the solubilized

sample, the ratio of the terminal peak shift for the membrane and the solubilized samples will be

$$\frac{\tau_{\text{men}}^*(\infty)}{\tau_{\text{sol}}^*(\infty)} = \frac{\Sigma^2 + (\sigma^2/N_{\text{del}})f}{(\sigma^2/N_{\text{del}})f} \tag{4}$$

The value of N_{del} was estimated to be ~4 from the simulation of the solubilized sample data,[21] and we assume that it is the same for the membrane complex. The terminal peak shift value obtained for the membrane sample after removing the 5 ps component from our peak shift data is ~3.6 fs, whereas the value for the solubilized sample is 0.8 fs. The value of σ that best fit the solubilized data[21] was estimated to be 150 cm^{-1}, and we assume that it is same for the membrane complex. The only parameter not known is f and we estimate its value to be ~4/18. (Because N_{del} is estimated to be 4 and there are 18 pigments in the complex, after the exciton equilibrates in the complex, the probability of the exciton to be found on the initial state is approximately given by the ratio of N_{del}/N (N being the total number of pigments)). Using these values we obtain a value of $\Sigma = 65$ cm^{-1} which compares well with the value of 54 cm^{-1} estimated by Freiberg et al.[38] Single molecule fluorescence excitation spectroscopy of the B850 band also revealed two-levels of disorder and the estimated value ($\Sigma = 65$ cm^{-1}) compares well with that of Freiberg et al.[41,42,49] Bopp et al. also measured the single molecule spectrum of the B850 band and observed structural fluctuations on a very slow time scale of a few seconds.[42] The slow fluctuation dynamics leads to a distribution of the mean energy of the aggregate of rings.

On inspecting the absorption spectrum, we find the spectral widths of both the samples are similar (fwhm $\approx$ 370 cm^{-1}). This is not inconsistent with the concept of a second level of disorder. From eq 2, the total inhomogeneous width of the absorption spectrum is given by the sums of squares of the two levels of disorder. Using the value of $\sigma = 150$ cm^{-1} for the solubilized LH2 complex estimated by Yang et al. in eq 2,[21] we find that the total disorder width (Δ) changes from 150 cm^{-1} ($\Sigma = 0$) to only 180 cm^{-1} (for $\Sigma = 100$ cm^{-1}). The increase of Δ from 150 to 180 cm^{-1} corresponds to only a 2−3 nm difference in fwhm of the spectral width and may not be observable at room temperature. We expect that the value of $\Sigma = 100$ cm^{-1}, compared to a value of 150 cm^{-1} for σ, can only be an upper limit for the estimated value of Σ. From our estimate of Σ(65 cm^{-1}) the total inhomogeneous width obtained is ~160 cm^{-1} and the increase from 150 cm^{-1} is negligible.

Implicit in the above discussion is the notion that the rings are more disordered in the membrane than in the solubilized sample. It is likely that most of the LH2 rings in detergent solution experience very similar environments around them. The individual pigments in the ring have different static environments due to their local protein contacts but different rings will not have greatly varying interactions with their immediate surroundings. Therefore, the estimated value of Σ (due to structural fluctuations) from the simulations of the B800 and B850 band was small or not required at all. The solubilized samples contain large amounts of tetramers (and dimers) of the LH2 rings (section 2) and energy transfer may occur within these aggregates. However, our 3PEPS data does not exhibit any relaxation components on time scales longer that 1 ps and the peak shift decays to a near zero value. From earlier studies of 2D crystals, created by gentle dialysis of solubilized LH2 samples to remove the detergent,[24] it was speculated that these extensively aggregated LH2 rings do not have an identical orientation, i.e., all C-termini on the same side. Under these circumstances, the rings of B850 BChls do not lie in the same plane in a dimer arrangement although within a tetramer two B850 rings can be in the same plane. However, the individual complexes within the much smaller aggregates studied in the present work are very likely to be surrounded by sufficient detergent molecules to prevent any close contact and hence preclude significant energy transfer. Such a mechanism could explain the absence of 5 ps component in the peak shift decay of the solubilized sample.

The situation is different if the LH2 complexes are embedded in the native membrane, as the B850 rings lie in the same plane to facilitate rapid energy transfer in the photosynthetic assembly. In this case, each ring may have a unique environment dependent on how many other LH2 complexes it is in contact with and the packing arrangement they adopt. This would result in a structural and energetic distribution of complexes. Inter-ring excitonic interactions between a certain number of rings in a well-defined geometry[50] (which may be regarded as a "super-complex") may lead to a net absorption of such a "super-complex" that is red- or blue-shifted with respect to the ensemble average thereby giving rise to the second level of disorder. Work by Vos et al.[51] on the *Rb. sphaeroides* LH2-only mutant NF57 at RT revealed that ~350 BChls were connected together to form a domain in the membrane. Then, energy transfer within or between the domains will lead to a further decay of the peak shift, perhaps responsible for the 5 ps component.

The origin of the 5 ps component could also be due to structural or dielectric relaxation of the system. The absence of the 5 ps component in isolated complexes implies that the structural relaxation of individual rings does not take place on this time scale. Even though such relaxation cannot be completely ruled out, it is highly unlikely that collective motion of multiple rings can occur in the native membrane embedded complex on a 4−5 ps time scale.

Dielectric relaxation has been observed at low temperature transient absorption (TA) studies of the LH2-only mutant of *Rb. sphaeroides* in native membranes by Sundstrom and co-workers[52] The low-temperature results displayed large spectral evolution to the red on time scales ranging from 600 fs to tens of picoseconds. The room-temperature spectra, however, showed no evolution after 2 ps. The low-temperature results were explained on the basis of relaxation to states with charge-transfer character followed by a slow dielectric relaxation from these states. At room temperature, the thermal activation is enough to remove the population from the charge transfer states and therefore spectral evolution on slow time scales was not observed. At room temperature, inter-complex energy transfer was not observed with the TA technique.[52]

Our results for energy transfer between the complexes are in accord with those of Freiberg and co-workers who compared TA spectra of the solubilized and the membrane samples at cryogenic temperatures.[32] Their conclusion about energy transfer between the rings was based on the red shift of the bleaching maximum of the TA spectrum for membrane samples on a single to a few picosecond time scales and its absence in the solubilized samples. Because their experiment was performed at low temperatures, a broader distribution of energy transfer time scales is expected. They also observed a large value of Stokes shift for both the solubilized (20.8 nm) and the membrane samples (32.7 nm) at low temperatures compared to the values at room temperature reported in this work (~4 nm). This observation suggests that at low temperatures emission from charge transfer states occur. Also, the difference in Stokes shift between the solubilized and the membrane samples at room

temperature is negligible compared to that at low temperatures (~12 nm). This is possible as in membrane samples the excitation can travel over a large number of rings before it is trapped at a low energy site. Upon reaching the low energy site, uphill energy transfer is less likely and hence emission is biased toward such sites. At room temperature, the thermal energy allows for uphill energy transfer and the excitation is not trapped in an energy minimum leading to a smaller value of Stokes shift.

5. Concluding Remarks

3PEPS data are shown to reveal inter-complex energy transfer in the LH2-only complex of *Rb. sphaeroides* in native membranes on a 5 ps time scale at room temperature. Energy transfer between LH2 complexes has not been observed before at room temperature to the best of our knowledge. The presence of an additional 5 ps decay component in the membrane sample is argued to be indicative of the presence of two-levels of disorder in the sample. In other words, our results imply that the total disorder present in the LH2 complex is not entirely contained in the individual rings, but is partitioned to a distribution of the mean energy of the rings in the ensemble. We believe our results will aid in modeling and understanding energy transfer and trapping in the whole photosynthetic unit in greater detail.[31,50,52,53]

Acknowledgment. We thank John Kennis, Rienk van Grondelle, Jennifer Herek, Arvi Freiberg, and Mino Yang for helpful suggestions. This work was supported by the Director, Office of Science, office of Basic Energy Sciences, Chemical Sciences Division, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098

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