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Picosecond Photophysics of Covalently Linked Pyrochlorophyllide *a* Dimer. Unique Kinetics within the Singlet Manifold

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Abstract: The excited state absorption and fluorescence characteristics of the folded configuration of bis(pyrochlorophyllide *a*) ethylene glycol diester were studied on a picosecond time scale. This model for the primary photochemical electron donor in photosystem I of green plants displayed photophysical properties that were found to depend strongly on both solvent and temperature. First, at 290 K the fluorescence lifetime and the lifetime of the excited state absorbance changes following an 8-ps flash at 528 nm varied dramatically with a change in solvent, CH_2Cl_2 vs. CCl_4. At 290 K the absorbance changes of the dimer in CCl_4 showed a large positive optical density change at 660 nm and bleaching at 700 nm. The 660-nm positive optical density change was absent for the folded dimer dissolved in CH_2Cl_2. Second, similar dramatic lifetime variations were observed for folded dimer in CH_2Cl_2 as a function of temperature. Both fluorescence lifetimes and absorption change decay rates were very similar with a lifetime at 290 K of 110 ps increasing to 4.6 ns at 200 K. However, the quantum yield for fluorescence of the dimer remained relatively constant in the 290–200 K temperature range. As the sample of folded pyrochlorophyll *a* dimer in CH_2Cl_2 was cooled from 290 to 270 K the bleaching centered at 700 nm broadened somewhat and a new positive optical density change appeared at 660 nm. This spectrum was completely analogous to that obtained for the folded dimer in CCl_4 at 290 K. These results were interpreted in terms of a dual excited singlet state model with one singlet state fluorescent while the other remains nonfluorescent. Initial excitation of the dimer into the nonfluorescent state followed by a kinetically controlled population distribution between both excited states was shown to account for the data.

The primary role of chlorophyll (Chl) in photosynthetic organisms is to convert incident solar radiation into chemical energy. Photons are captured by an extended array of chlorophyll molecules known as the antenna.[2-5] The electronic excitation produced by the light absorption is transmitted via the antenna chlorophyll to a "special pair" of chlorophyll molecules.[6-9] This pair of molecules traps the excitation energy and then rapidly undergoes one-electron oxidation. The resulting charge separation subsequently generates a chemical potential gradient within the organism. The special pair and its associated redox components are contained in a protein matrix and are collectively known as the reaction center.

Although reaction centers of photosystem I (P700) in green plants have not as yet been isolated completely free of antenna chlorophyll, a great deal of information exists regarding their spectroscopic properties.[10] P700 undergoes bleaching upon illumination with 700-nm light. The subsequent appearance of a photoinduced electron spin resonance (ESR) signal of about 7 G line width with $g = 2.0025$ results from the formation of $P700^{+}\cdot$. A comparison between the signal of monomeric Chl $a^{+}\cdot$ and $P700^{+}\cdot$ reveals that the line width of the signal due to the in vivo species is narrower by $\sqrt{2}$ than that of in vitro Chl $a^{+}\cdot$.[9] This result agrees with theory regarding delocalization of one electron spin equally over two chlorophyll molecules.[9] Further studies of in vivo systems by electron nuclear double resonance (ENDOR) spectroscopy support the special pair proposal for the structure of reaction center chlorophyll.[11] The delocalization of charge over two chlorophyll molecules in the special pair cation radical is reflected in a lowering of the oxidation potential of the special pair relative to that of monomeric chlorophyll.

Several models have been proposed for the structure of the chlorophyll (Chl_{sp}) special pair. In one model two chlorophyll *a* molecules are linked by coordinating the magnesium atom of one Chl *a* to a nucleophile such as ROH which in turn hydrogen bonds to the keto carbonyl group of the second Chl *a*. Two such interactions result in a structure possessing C_2 symmetry and a 3.6-Å interplanar distance (Figure 1).[12,13] A different model suggests that the structure of the special pair is based on the coordination of a water molecule to the magnesium of one Chl *a* which in turn hydrogen bonds to the carbonyl function of the carbomethoxy group of a second Chl *a*. In this model two such interactions also result in a C_2 symmetric structure with the carbomethoxy groups pointing into the interior of the structure (Figure 2). This results in a larger 5.6-Å interplanar distance.[14]

The finding that two chlorophyll molecules closely interact in vivo to form P700 has stimulated efforts to prepare an in vitro chlorophyll special pair. Chl *a* adducts with ethanol have been prepared that successfully mimic the optical and ESR properties of photosystem I reaction center chlorophyll.[13] These Chl_{sp} systems are assembled from two monomer units by cooling a mixture of Chl *a* and ethanol to temperatures near 100 K. The free energy of association of this in vitro analogue of P700 is greater than zero at room temperature. This is due to a large negative entropy of dimerization as is indicated by the fact that formation of in vitro special pairs requires high Chl *a* concentrations and low temperatures. Solutions of Chl *a* in hydrocarbons containing water also yield species possessing a 700-nm optical absorption.[15] The formation of the desired structure depends not only on the Chl *a* concentration, but also on the mole ratio of Chl *a* to nucleophiles in solution,

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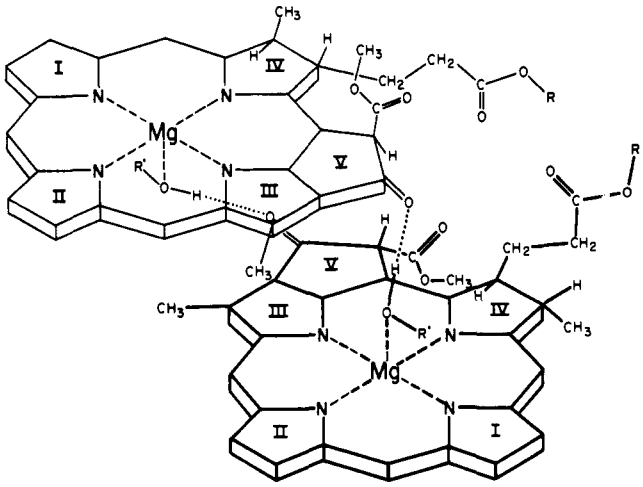

Figure 1. Proposed C_2 symmetric model of special pair Chl *a* utilizing hydrogen-bonding interactions involving the 9-keto carbonyl function of each macrocycle.

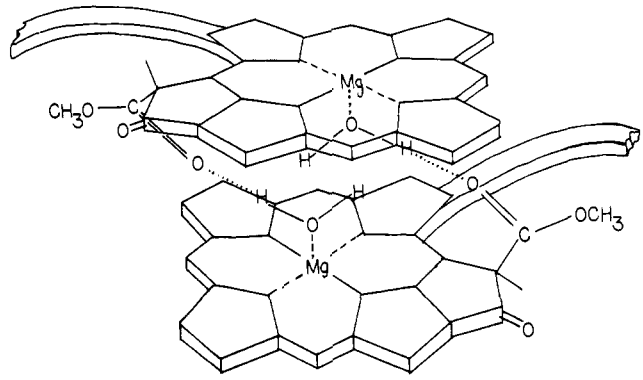

Figure 2. Proposed C_2 symmetric model of special pair Chl *a* utilizing hydrogen-bonding interactions involving the keto carbonyl function of the 10-carbomethoxy group of each macrocycle.

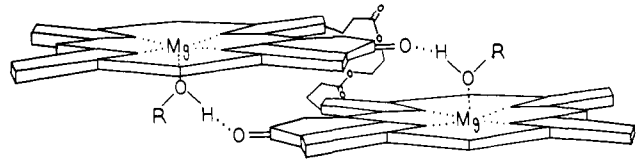

Figure 3. Average C_2 symmetric conformation of the folded PChl *a* dimer.

the solvent, the rate of cooling, etc. There is reason to suppose that mixtures of various species of poorly defined structure are present in even the best of these preparations. The uncertainties in composition and structure and the experimental problems and restrictions imposed by working at low temperature in organic glasses have limited the information that can be derived from such model systems. The solution to this problem is to provide a mode of physical attachment between two chlorophyll molecules so that the magnitude of the entropy of dimerization is lowered.

Covalently linked dimers of both Chl *a*[16] and pyrochlorophyll *a* (PChl *a*)[12] have been prepared which mimic the spectroscopic and redox properties of P700. The two chlorophylls are joined in each case at their propionic acid side chains via an ethylene glycol diester linkage. The orientation of the chlorophyll macrocycles with respect to one another and consequently their electronic properties depend strongly on the solvent. Treating solutions of these covalent dimers in dry nonnucleophilic solvents, e.g. toluene, CCl_4, or CH_2Cl_2 with 10–100-fold molar excesses of a hydrogen bonding nucleophile, e.g. water, primary alcohols, or primary thiols, results in a folded conformation which is shown in Figure 3. This folding is accompanied by shifts of both their longest wavelength absorption band (663 to 694 nm)[12,16-18] and of their fluorescence maxima (670 and 735 nm).[17,18] By studying the properties of a model for the Chl_{sp}, we hope to gain insight into the primary photosynthetic events.

In these studies we have employed the PChl *a* dimer for two reasons. First, the PChl *a* dimer is inherently more stable toward photodegradation than is the Chl *a* dimer.[19] Second, steric requirements imposed on the model special pair structure by the partial epimerization of the C-10 carbomethoxy groups in the Chl *a* dimer prevent approximately 25% of the Chl *a* dimer molecules from folding into the conformation depicted in Figure 3.[16] This isomerism serves to complicate the analysis of the properties of the Chl *a* dimer.

Experimental Section

The PChl *a* dimer was prepared from pheophytin *a* in five steps. Pheophytin *a* (extracted from partially dried alfalfa)[20] was decarbomethoxylated in refluxing pyridine to yield pyropheophytin *a*.[21] Selective hydrolysis of the phytyl ester with 80% aqueous trifluoroacetic acid yielded pyropheophorbide *a* (PPhide *a*), which was subsequently reesterified at 25 °C with ethylene glycol using benzotriazole *N*-methanesulfonate and Et_3N in dry THF.[22,23] The glycol ester was coupled with 1 equiv of PPhide *a* using the same esterification method, but substituting 4-dimethylaminopyridine as the base and CH_2Cl_2 as the solvent.[23] The resulting PPhide *a* ethylene glycol diester exhibits a mass spectrum (*m/e* 1094, M^+), ^1H NMR, and electronic spectrum consistent with the assigned structure. The reinsertion of the magnesium atoms was accomplished by a known method[24] to yield the PChl *a* dimer in 40% overall yield based on pheophytin *a*.

Methylene chloride, chloroform, and carbon tetrachloride were spectroscopic grade (Eastman). Methylene chloride and carbon tetrachloride were repeatedly vacuum distilled and stored over Linde 3A molecular sieves. Chloroform containing ethanol as a stabilizer was used as received. Absolute ethanol (U.S. Industries) was used as received. PChl *a* dimer solutions were prepared in the chlorocarbons containing 5×10^{-3} M ethanol.

Time-resolved fluorescence measurements were made using a streak camera (Hamamatsu).[25] In the fluorescence studies sample cells of 1, 2, and 10 mm path length were used. The fluorescence was observed with both 180 and 90° detection. For some of the measurements a polarizer was placed in front of the streak camera. The absorption apparatus was a standard double-beam picosecond spectrometer.[26] The absorption studies were carried out in a 2-mm path length cell. The use of an echelon allowed picosecond absorption measurements to be taken at 15 separate times over a 500-ps range in a single laser flash. Several such measurements with overlapping time ranges were correlated to obtain time-resolved absorption data up to 8 ns following an 8-ps flash at 528 nm.

Absolute fluorescence quantum yield measurements for the PChl *a* dimer at room temperature were calibrated against the fluorescence quantum yield of rhodamine B excited with 530-nm light.[27] The samples were dilute (10^{-6} M) and detection was accomplished by photon counting. The optical density of each sample at 530 nm was optimized to a 0.01 optical density. Quantum-yield changes as a function of temperature were measured by comparing the fluorescence spectrum of PChl *a* dimer at room temperature with those obtained at low temperature using a corrected fluorimeter (Perkin-Elmer). The data were not corrected for the effect of polarization.

Results

The fluorescence lifetime of folded PChl *a* dimer in methylene chloride varied from 110 ps at room temperature to 4.6 ns at 200 K (Figure 4). Below 200 K the lifetime lengthened slightly, reaching a limit of 5.8 ns. No change in this lifetime could be detected down to 4 K. The measured fluorescence lifetime of the folded PChl *a* dimer was identical both for observation along the axis of the excitation beam and perpendicular to the axis of the excitation beam in methylene chloride and all solvents studied. Moreover, the lifetime was indepen-

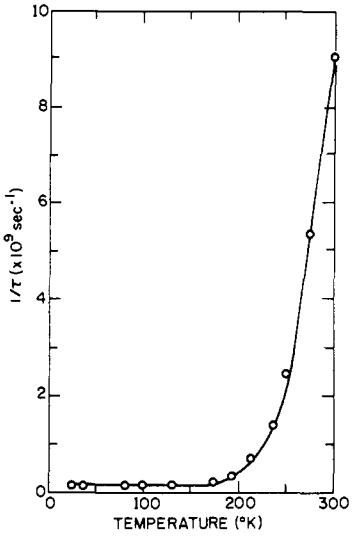

Figure 4. The temperature dependence of the fluorescence lifetime of 2 × 10^{-4} M bis(pyrochlorophyllide *a*) ethylene glycol diester folded with ethanol in methylene chloride.

Table I. Quantum Yield of 2 × 10^{-4} M Bis(pyrochlorophyll *a*) Ethylene Glycol Diester

solvent	temp, K	quantum yield	fluorescence lifetime, ns
CCl_4	298	0.32	3.8
CH_2Cl_2	298	0.03	0.11
CH_2Cl_2	269	0.03	0.19
CH_2Cl_2	234	0.03	0.71
CH_2Cl_2	208	0.04	2.3
CH_2Cl_2	194	0.06	4.6
CH_2Cl_2	178	0.10	5.4

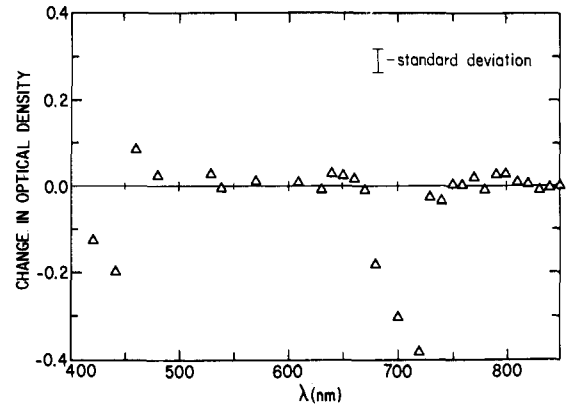

Figure 5. Excited state difference spectrum of 2 × 10^{-4} M bis(pyrochlorophyllide *a*) ethylene glycol diester with ethanol in methylene chloride. Spectrum was recorded 6 ps after excitation.

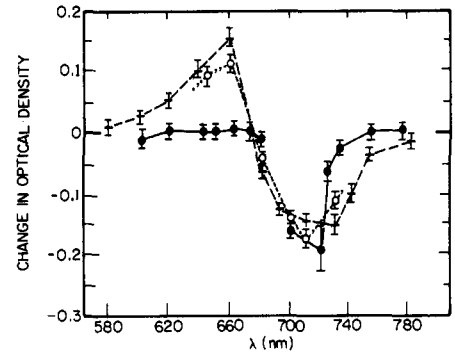

Figure 6. Excited state difference spectrum of bis(pyrochlorophyllide *a*) ethylene glycol diester (2 × 10^{-4} M) folded with ethanol. At 298 K the solvents were methylene chloride (—Φ—) and carbon tetrachloride (- - Φ - -). At 273 K the solvent was methylene chloride (· · Φ · ·). Spectra were recorded 6 ps after excitation.

dent of concentration from 10^{-6} to 10^{-3} M. The path length of the cell was varied from 1 to 10 mm without affecting the fluorescence lifetime. In addition, observation of the fluorescence at polarizations parallel and perpendicular with respect to that of the excitation beam showed no variation in fluorescence lifetime. The quantum yield for fluorescence of these samples remained relatively constant in the 290–200 K temperature range (Table I). Since the fluorescence quantum yields were not corrected for the effect of polarization, it is possible that the increase in viscosity of the solvent as the temperature was lowered could be influencing the quantum yields. Since monomeric chlorophyll *a* is nearly depolarized in this region and the fluorescence lifetimes are insensitive to the polarization of the emission, polarization effects on the quantum yield should be minimal. The quantum-yield data obtained at room temperature is in close agreement with that measured previously at higher concentrations.[17]

The excited state difference spectrum of the folded PChl *a* dimer taken 6 ps after excitation is shown in Figure 5. It shows a narrow band of bleaching at 700 nm. A bleaching of the red-most absorption band has also been seen in the excited singlet state of monomeric chlorophyll *a*.[28] An additional negative absorbance change is also seen at 440 nm. A small positive optical density change was observed at 460 nm. As the sample was cooled from 290 to 270 K the bleaching centered at 700 nm became somewhat broader and a new positive optical density change appeared at 660 nm (Figure 6).

All light-induced absorption changes for folded PChl *a* dimer in CH_2Cl_2 decay with a lifetime of 110 ± 40 ps at room temperature. This decay rate is the same as the fluorescence lifetime of the folded PChl *a* dimer under identical conditions. When the temperature was lowered the lifetime of the excited-state absorbance increased while the magnitude of the initial bleaching at 700 nm remained largely unchanged. The 700-nm bleaching and the positive optical density change at 660 nm each possess a decay rate which is identical once again with the fluorescence lifetime of the dimer.

In CCl_4 the excited state difference spectrum had a broad bleaching around 700 nm and a positive absorbance change at 660 nm (Figure 6). This spectrum resembles that seen in CH_2Cl_2 at temperatures below 270 K. Unlike the excited state difference spectra of the dimer in CH_2Cl_2, those taken in CCl_4 were temperature independent.

Discussion

The two most striking aspects of the data involve the strong dependence of the excited singlet state lifetime of the folded PChl *a* dimer on the solvent and the temperature. Before the strange temperature and solvent effects on the lifetimes can be ascribed to any new phenomena, other well-known mechanisms for shortening the fluorescence lifetime must be discounted. The artifacts which can influence the data are stimulated emission, energy transfer, rotational relaxation, and photochemical reactions of the solvent.

Rapid excited singlet state decay rates have been observed in systems undergoing coherent processes such as stimulated emission. In these cases the fluorescence lifetime is strongly dependent on concentration, cell path length, and excitation intensity.[29,30] The shortening of a fluorescence lifetime due to stimulated emission using a relatively long path length cell is often more dramatic when the viewing angle with respect to the excitation beam is 180° rather than 90°.[30] This is due to

the fact that the emitted photons sample more of the excited-state population in the direction of the excitation than in the orthogonal direction. Moreover, in systems undergoing stimulated emission the decay of the excited singlet state is nonexponential. For example, these effects have been observed for 3,3′-diethyloxdicarbocyanine iodide (DODCI). The decay rates for DODCI measured using excited-state absorption as a probe depend strongly on concentration, path length, and excitation intensity.[29] On the other hand, the experimental results show that neither changes in concentration, path length, viewing angle, nor excitation intensity (over two orders of magnitude) affect the excited-state behavior of folded pyrochlorophyll *a* dimer in all the chlorocarbon solvents we examined.

Aggregation of the pyrochlorophyll *a* dimer molecules could also be responsible for the large variation of lifetime with solvent and temperature. The degree of aggregation of chlorophyll *a* is sensitive to concentration.[31] The excited singlet state lifetime of folded PChl *a* dimer in each chlorocarbon solvent used in this study does not depend on concentration in the range 10^{-6}–10^{-3} M.

Energy transfer from the fluorescent dimer to a nonfluorescent aggregate would also shorten the singlet lifetime.[32] The rate of energy transfer is very sensitive to concentration; however, the lifetime at every temperature examined appeared to be free of any concentration effects. Moreover, an estimate of the Förster transfer rate at 10^{-6} M for even the most favorable conditions indicated that transfer would occur at a much slower rate than the observed quenching.

The fluorescence lifetime of the folded PChl *a* dimer is independent of the orientation of the emission polarization with respect to the excitation polarization. Therefore, rotational reorientation does not influence the lifetimes or the quantum-yield data. Fluorescence from chlorophyll *a* monomer is almost completely depolarized when 528-nm excitation is used.[32] One would expect the polarization of the dimer to be similar. Thus, in any case the observed excited state singlet lifetime should not be influenced by rotational reorientation.

Chlorinated hydrocarbons often undergo photochemical reactions. These usually involve free-radical intermediates. The relative reactivity of the chlorinated hydrocarbons in these reactions is $CCl_4 > CHCl_3 > CH_2Cl_2$. The fluorescence lifetime of folded pyrochlorophyll *a* dimer in carbon tetrachloride, hexane, and toluene is identical. Therefore, it is unlikely that the CCl_4 is participating in a photochemical reaction with the PChl *a* dimer since this would result in a change in the observed lifetime in CCl_4. Since the relative reactivity of $CHCl_3$ and CH_2Cl_2 is less than that of CCl_4, the likelihood of their participation in photochemical reactions leading to the observed reduction in lifetime in these solvents is even further reduced. Therefore, the usual explanations for extremely rapid fluorescence decay rates cannot be utilized to explain the data. It is necessary to examine the effect of temperature on the absorption and fluorescence data in both CCl_4 and CH_2Cl_2 to obtain a model that describes the experimental observations.

Changes in temperature alter three quantities for the folded PChl *a* dimer in CH_2Cl_2.

(1) The fluorescence lifetime increases monotonically by a factor of 50 over a temperature range of 298–115 K.

(2) Over the same temperature range the quantum yield for fluorescence increases by only a factor of 2.

(3) As the temperature is changed the lifetime of the absorption changes closely follows the lifetime of the fluorescence changes. However, the size of the initial 660-nm absorption change following excitation has a different temperature dependence. In the range of 298–270 K the initial 660-nm absorption change attains its largest magnitude and remains essentially unchanged as the temperature is lowered further. This is in contrast to the dimer dissolved in CCl_4, for which the excited state absorption spectrum was essentially independent of temperature.

Several models for the excited singlet state of the PChl *a* dimer must be examined to determine which one can best explain observations (1) and (2). A single excited level would require that both the radiative rate (k_{rad}) and the sum of the nonradiative (k_{nrad}) rates would each change by a factor of 30 as the temperature is varied from 298 to 200 K. While such a change in the nonradiative rate has been previously seen,[34] there is no precedent to support a radiative rate change by such an amount. While the radiative rate could change somewhat with temperature due to changes in population of various vibronic levels, drastic changes in Franck–Condon factors from one vibrational level to another would then be necessary to explain a very large change in the radiative rate with temperature. Such a dramatic change in the Franck–Condon factors would almost certainly result in a change in the fluorescence spectrum. Only a slight temperature dependence of the emission spectrum is observed. It is also hard to imagine that the radiative rate would depend so strongly on temperature when the solvent was CH_2Cl_2 and be relatively temperature independent in CCl_4. Thus a single excited state cannot account for the observations.

In order to explain the data one needs at least a two-level scheme. These two states may reside on the same molecule or be representative of two distinct excited species in solution. Consider the case in which two distinct excited species exist in solution. In this context, the appearance of the 660-nm absorption change between 290 and 270 K would indicate a shift in the relative equilibrium concentration of the two species. If both species were fluorescent, the emission spectrum from each species would almost certainly be different. Thus one would expect that the luminescence spectrum would change with temperature. Moreover, unless the fluorescence lifetimes of the two species were identical the observed emission lifetime would exhibit a wavelength dependence. No changes in the fluorescence spectra were observed as the temperature was changed; in fact, identical fluorescence lifetimes were measured throughout the fluorescence spectrum. Two states should also have different excited state absorption spectra. The temperature dependence of the excited state difference spectrum indicates that, if there was an equilibrium, it would favor the excited species which does not absorb at 660 nm at 293 K and be shifted in favor of the 660-nm absorbing species at temperatures below 270 K. Thus, below 270 K the system would behave as a simple single level system, yet between 270 and 200 K the lifetime changes by more than a factor of 20. As we have argued above, such large changes in lifetime without parallel changes in quantum yield cannot be explained by a single-level scheme. Hence, two different ground-state conformations in equilibrium cannot give rise to the observed excited-state behavior.

It is necessary to require that two levels are present in the same molecule. The observation of a 660-nm excited-state absorption for the folded PChl *a* dimer dissolved in CCl_4 and in cold CH_2Cl_2 is indicative of only one excited state. Call this state S_1. Similarly call the excited state observed in CH_2Cl_2 at room temperature S_2 (i.e., *no* 660-nm absorption). From the previous discussion it must be assumed that both states S_1 and S_2 must be excited states of the same molecule. In this view the effect of solvent is to shift the population of the excited PChl *a* dimer molecules from one excited state to a second excited state.

If an excited-state equilibrium between S_1 and S_2 exists, the number of molecules in state S_1 as measured by the initial size of the 660-nm transient absorbance would depend on temperature. This is not the case. The excited-state absorption

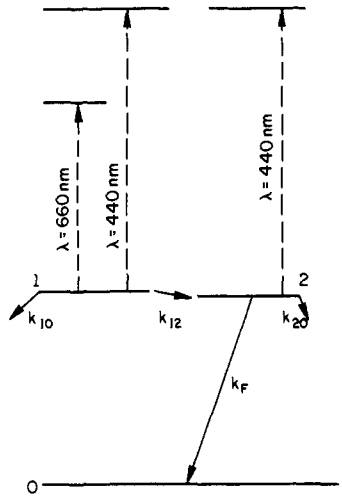

Figure 7. A model for the excited state dynamics of bis(pyrochlorophyllide *a*) ethylene glycol diester. S_1 is the state originally populated. Transfer out of S_1 proceeds by two nonradiative paths. k_{12} represents transfer to the fluorescent state S_2. k_{10} represents all other decay rates. k_f is the radiative rate and k_{20} represents all nonradiative paths from S_1. The vertical arrows represent the absorptions observed when the excited dimer is in S_1 or S_2.

attributed to S_2 disappears in large part between 290 and 270 K (Figure 6), yet the fluorescence lifetime is still strongly dependent on temperature. Therefore, a kinetic rather than a thermodynamic relationship determines the relative population distribution between S_1 and S_2.

Given this relationship only one of the two states may be fluorescent. If both were fluorescent, we would observe a change in the emission spectrum with temperature and a wavelength dependence for the fluorescence lifetime. These effects were not observed. This leaves only four possible kinetic relationships between S_1 and S_2: (1) $S_1 \rightarrow S_2$, and S_1 is fluorescent; (2) $S_1 \rightarrow S_2$, and S_2 is fluorescent; (3) $S_2 \rightarrow S_1$, and S_1 is fluorescent; (4) $S_2 \rightarrow S_1$, and S_2 is fluorescent. The two possibilities (1) and (4), may be quickly rejected. These situations would behave essentially as a single-level system because depletion of excited molecules from the fluorescent state is simply another nonradiative pathway.

Consider the data for the folded pyrochlorophyll *a* dimer in methylene chloride at room temperature. If relationship (3) is correct, then the lack of a 660-nm transient absorption at 298 K (in CH_2Cl_2) indicates that the lifetime of S_1 must be so short that it cannot be observed with picosecond resolution. This would imply that the sum of radiative and nonradiative decay rates out of S_1 must be much faster than the transfer rate (k_{21}). The fluorescence lifetime would then be determined by k_{21}. This yields a k_{21} of about 10^{10} s^{-1}.

Since $k_{21} \ll k_{rad} + k_{nrad}$ and the fluorescence quantum yield, which is given by

$$\phi = \frac{k_{rad}}{k_{nrad} + k_{rad}} \tag{1}$$

is about 0.03, the radiative rate (k_{rad}) would have to be much greater than 3×10^8 s^{-1}, which is much larger than one would expect.

We are left with relationship (2) as the only viable two-level scheme. This scheme is illustrated in Figure 7. The rate equations for this scheme are

$$dN_1/dt = -(k_{10} + k_{12})N_1 \tag{2}$$

$$dN_2/dt = -(k_{20} + k_f)N_2 + k_{12}N_1 \tag{3}$$

where k_{12}, k_{10}, and k_{20} are rate constants for the processes illustrated in Figure 7. The nonradiative decay rate from level 1 (660-nm absorbing state) is k_{10}. The nonradiative rate from level 2 (no 660-nm absorption) is k_{20}, while the radiative rate from this level is k_f. The transition rate from level 1 to 2 is k_{12}. Their solution is given as

$$N_1(t) = N_1(0) \exp[-(k_{10} + k_{12})t] \tag{4}$$

$$N_2(t) = N_1(0)k_{12}(k_{20} + k_f - k_{10} - k_{12})^{-1} \times (\exp[-(k_{10} + k_{12})t] - \exp[-(k_{20} + k_f)t]) \tag{5}$$

This solution to the rate equations assumes that the molecules are all initially in state 1. The quantum yield can also be readily calculated from the model and is given by

$$Q_y = \left(\frac{k_{12}}{k_{12} + k_{10}}\right)\left(\frac{k_f}{k_f + k_{20}}\right) \tag{6}$$

By choosing appropriate values of the rate constants these equations can be utilized to fit all the data presented for this system. Rate constant k_{12} determines the presence of the 660-nm excited-state absorption. For the folded PChl *a* dimer in CH_2Cl_2 at room temperature k_{12} must be faster than 10^{11} s^{-1} or, perhaps, S_2 is directly populated. Thus the population of molecules in S_1 is so small even when the excited dimer is observed 6 ps after excitation no 660-nm absorption is detected in the difference spectrum. As the temperature is lowered k_{12} decreases. The population of S_1 is then observable 6 ps after excitation. This explains the results presented in Figure 6.

Rate constant k_{12} is solvent dependent as well as temperature dependent. In carbon tetrachloride k_{12} is small. The measured fluorescence lifetime is a probe of $k_{10} + k_{12}$. Therefore the fluorescence lifetime is identical with the lifetime of S_1.

Equation 5 predicts that the appearance of the fluorescence from state 2 should not be instantaneous. When methylene chloride is used as a solvent, the fluorescence does have a finite rise time. To show that the state 2 indeed has a finite rise time slower than the apparatus resolution, rhodamine B fluorescence is compared to that from the PChl *a* dimer in carbon tetrachloride in Figure 8. A finite rise time would not occur from different ground-state molecules in equilibrium. This is additional evidence against two distinct ground-state molecules giving rise to the observed phenomena.

The observations just described strongly suggest that dual excited states exist in the dimers of PChl *a*. The relative populations of these levels are controlled by kinetics and not by thermodynamics. This is a unique situation which to the best of our knowledge has not been previously observed. The possibility exists that the switching between two excited states may be a feature which allows the primary photochemical electron donor in reaction centers to store excitation energy for a brief period of time while waiting for an oxidized acceptor or a reduced donor to be formed. If the reaction center is unable to

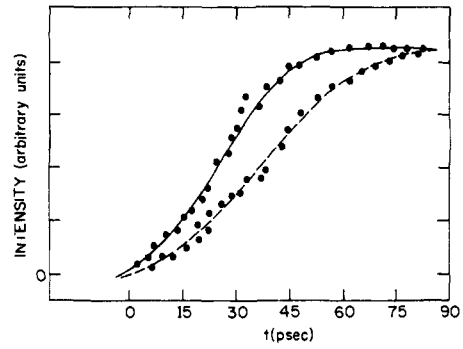

Figure 8. Rise time for the fluorescence of bis(pyrochlorophyllide *a*) ethylene glycol diester (2×10^{-4} M) folded with ethanol in carbon tetrachloride (- -), and the instrumental rise time measured by monitoring the fluorescence of rhodamine B (2×10^{-4} M) in methanol (——).

perform photochemistry, the molecule could then rapidly quench the excitation.

This sensitivity to the environment might explain the interesting results observed when *Rhodopseudomonas viridis* reaction centers were poised at a potential low enough to reduce the intermediate acceptor before being excited. Absorption features that correspond to the excited bacteriochlorophyll *a* special pair decayed in 20 ps.[35] Based on the lifetimes measured for the monomer in solution, the lifetime of the bacterial dimer should have been much longer. Also the dimer singlet decay rate must be much slower than the rate of oxidation of the dimer (<5 ps)[36] because the quantum yield for photochemistry approaches unity.[37] The authors argued that a series of electron-transfer reactions between the reduced bacteriopheophytin *a* and the singlet excited dimer could lead to a quenching of the dimer excited state. An alternate explanation would be that the presence of the reduced intermediate acceptor alters the environment in a manner analogous to the change observed in going from CCl_4 to CH_2Cl_2 as the solvent.

Recently, the dynamics of the triplet states of pyrochlorophyll *a* dimer have been reported.[38] It was found that the triplet state of folded pyrochlorophyll *a* dimer opens into isolated triplet and ground-state molecules. The main evidence for this is the appearance of a positive optical density change near 660 nm and a decrease in optical density at 700 nm. The bleaching at 700 nm was interpreted as being caused by opening of the folded dimer. The "unpinned" monomer results in an increase in absorbance at 660 nm. In the presence of triplet quenchers and at low alcohol concentrations these workers were able to observe re-formation of the folded dimer within a few microseconds. The appearance of a positive optical density change at 660 nm in Figure 6 would be indicative of just such an opening of the dimer in the singlet state. However, the rapid decay of the 700-nm bleaching indicates that the ground state of the folded dimer is being repopulated at the same rate the excited state is decaying. Thus, on the picosecond time scale the bleaching at 660 nm does not result from the opening of the folded dimer. Small changes in the excited-state geometry could destroy the electronic interaction leading to an excited state difference spectrum which indicates the formation of two isolated molecules. However, during the triplet lifetime, the two macrocycles could diffuse apart resulting in the true dissociation observed in the microsecond experiments.[38]

Conclusions

Our data strongly suggest that dual excited states exist in the dimers of pyrochlorophyll *a*. The relative populations of these levels are controlled by kinetics and not by thermodynamics. This is a unique situation which to the best of our knowledge has not been previously observed. The possibility exists that the switching between two excited states may be a feature which allows the primary photochemical electron donor in reaction centers to store excitation energy for a brief period of time while waiting for an oxidized acceptor or a reduced donor to be formed. If the reaction center is unable to perform photochemistry, the molecule could then rapidly quench the excitation. Further work is necessary to understand the effect of solvent on the barrier between the two levels.

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References and Notes

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