−0.14, −0.55). This shows that complex formation to give CuCl$_3^-$ and CuCl$_4^{2-}$ becomes important at increased chloride concentration. Since the activity coefficients are unknown at high chloride concentration, calculation of the free chloride ion concentration in our case is not straightforward. (b) L. G. Sillén and A. E. Martell, "Stability Constants", The Chemical Society, London, 1964. (c) D. F. C. Morris and E. L. Short, *J. Chem. Soc.*, 2672 (1962).

(28) W. Kitching, *Organomet. Chem. Rev.*, **3**, 61 (1968).

(29) (a) C. Agami, J. Levisalles, and F. Rose-Munch, *J. Organomet. Chem.*, **65**, 401 (1974); (b) K. Zetterberg, Dissertation, The Royal Institute of Technology, Stockholm, 1977.

(30) R. H. Grubbs, A. Miyashita, M. Liu, and P. L. Burk, *J. Am. Chem. Soc.*, **100**, 2418 (1978).

(31) (a) G. M. Whitesides, J. F. Gaasch, and E. R. Stedronskij, *J. Am. Chem. Soc.*, **94**, 5258 (1972); (b) J. X. McDermott, J. F. White, and G. M. Whitesides, *ibid.*, **98**, 6521 (1976).

(32) D. L. Thorn and R. Hoffmann, *J. Am. Chem. Soc.*, **100**, 2079 (1978).

(33) B. Åkermark, J. E. Bäckvall, L. S. Hegedus, K. Zetterberg, K. Siirala-Hansen, and K. Sjöberg, *J. Organomet. Chem.*, **72**, 127 (1974).

(34) Because of the different size of the nucleophiles (Me$_2$NH contra H$_2$O), a comparison between the ratios of rate for different olefins must, for steric reasons, be interpreted with great caution. This is particularly true for the comparison of the rate ratio of 1-olefin/2-olefin for the two reactions. However, the rate ratio of cis olefin/trans olefin should be less dependent on the size of the nucleophile and rather reflect the feature of the mechanism.

(35) J. E. Bäckvall and B. Akermark, *J. Organomet. Chem.*, **78**, 177 (1974).

(36) F. Pesa and M. Orchin, *J. Organomet. Chem.*, **108**, 135 (1976).

(37) B. Åkermark, J. E. Bäckvall, A. Löwenborg, and K. Zetterberg, *J. Organomet. Chem.*, **168**, C33 (1979).

(38) B. M. Trost and L. Weber, *J. Am. Chem. Soc.*, **97**, 1611 (1975).

(39) S. Numata and H. Kurosawa, *J. Organomet. Chem.*, **131**, 301 (1977).

(40) P. M. Henry, *J. Org. Chem.*, **39**, 387 (1974).

(41) J. K. Stille and P. K. Wong, *J. Organomet. Chem.*, **70**, 121 (1974).

(42) J. E. Bäckvall, unpublished results.

(43) There is no direct evidence that the intramolecular process is favored over the external attack in this case. However, the intramolecular reaction is known to take place: G. Calvin and G. E. Coates, *J. Chem. Soc.*, 2008 (1960); *Chem. Ind.* (*London*), 160 (1958).

(44) J. K. Stille and R. Morgan, *J. Am. Chem. Soc.*, **88**, 5135 (1966).

(45) R. B. Silverman and D. Dolphin, *J. Am. Chem. Soc.*, **98**, 5626 (1976).

(46) W. Hafner, R. Jira, J. Sedlmeier, and J. Smidt, *Chem. Ber.*, **95**, 1575 (1962).

(47) R. Jira, J. Sedlmeier, and J. Smidt, *Justus Liebigs Ann. Chem.*, **693**, 99 (1966).

(48) J. Hillis, J. Francis, M. Ori, and M. Tsutsui, *J. Am. Chem. Soc.*, **96**, 4800 (1974).

(49) J. K. Stille and R. Divakaruni, *J. Am. Chem. Soc.*, **100**, 1304 (1978).

(50) B. Åkermark, M. Almemark, J. Almlöf, J. E. Bäckvall, B. Roos, and Å. Støgård, *J. Am. Chem. Soc.*, **99**, 4617 (1977).

(51) C. Burgess, F. R. Hartley, and G. W. Searle, *J. Organomet. Chem.*, **76**, 247 (1974).

(52) J. A. Gladysz, J. C. Selover, and C. E. Strouse, *J. Am. Chem. Soc.*, **100**, 6766 (1978).

(53) H. B. Lee and P. M. Henry, *Can. J. Chem.*, **54**, 1726 (1976).

(54) P. P. Nicholas and R. T. Carrol, *J. Org. Chem.*, **33**, 2345 (1968).

(55) (a) K. H. Hoffmann and J. Sand, *Chem. Ber.*, 1340 (1900); (b) F. A. Cotton and J. R. Leto, *J. Am. Chem. Soc.*, **80**, 4823 (1958).

The Triplet State of the Chlorophyll Dimer[1]

Richard H. Clarke,* David R. Hobart, and Willem R. Leenstra

Contribution from the Department of Chemistry, Boston University, Boston, Massachusetts 02215. *Received July 28, 1978*

Abstract: The photoexcited triplet state properties of chlorophyll aggregate systems in vitro were investigated by zero-field optically detected magnetic resonance (ODMR) spectroscopy at 2 K. Measurements of the triplet state zero-field splittings, overall triplet lifetimes, and individual spin sublevel intersystem crossing rate constants were obtained for solutions of chlorophyll *a* and zinc-substituted chlorophyll *a* and for the covalently linked dimeric derivative of pyrochlorophyllide *a*. The triplet-state properties found for these systems are interpreted within the framework of the triplet exciton model to assess the applicability of the exciton approach in determining the geometry of chlorophyll aggregate systems and to evaluate structural features of chlorophyll systems proposed as models for in vivo chlorophyll.

I. Introduction

It has been recognized for some time that the chlorophyll molecule is uniquely suited for forming aggregate structures utilizing its magnesium metal center and carbonyl ring substituents. Such structures form the basis for most of the present models of the pigment complexes proposed for in vivo chlorophyll units in photosynthesis.[2-7] Recent work using both optical spectroscopy[8] and magnetic resonance[9,10] has shown that the reaction center in photosynthetic systems contains a pair of strongly interacting chlorophyll molecules, the so-called "special pair", which is involved in the initial photophysical processes of the reaction center.[2] Models for the geometry of such a reaction center dimer have been proposed by Fong[5] and by Katz and co-workers.[2,6] All the models considered for the reaction center structures of photosynthetic systems involve water-linked chlorophyll (or bacteriochlorophyll) dimers. Fong and his group first suggested that the chlorophyll dimer in the reaction center is held together by two water molecules coordinated and hydrogen bonded to provide a plane-parallel dimer structure with C_2 symmetry.[5] In his most recent work Fong postulates that such dimers may be formed from either the chlorophyll monohydrate (Chla·H$_2$O)$_2$ or the dihydrate (CHla·2H$_2$O)$_2$, which have been proposed respectively to be the P700 and water-splitting reaction centers in plant photosynthesis.[7,11] The two dimer structures differ in that the C10 ester carbonyl group is used for hydrogen bonding by the Mg-coordinated water molecule of the chlorophyll monohydrate, whereas in the chlorophyll dihydrate the C9 keto carbonyl unit is utilized.[7,11] Katz and co-workers also proposed a reaction center dimer containing one water molecule linking two plane-parallel chlorophylls (although this model does allow for freedom of motion away from the plane-parallel configuration).[2] In their most recent work, however, they also invoke a dimer locked into a plane-parallel arrangement by two water molecules coordinated to the central Mg atoms, but hydrogen bonded to the chlorin rings by the C9 keto carbonyl group.[6] Further, the Katz group proposes a structure for the antenna system requiring no water linkages but, rather, utilizing self-coordination among chlorophylls, with one chlorophyll acting as a donor via its carbonyl substituents to the Mg atom of another.[2] The carbonyl–Mg self-aggregation can lead to the formation of large networks (oligomers) of chlorophylls making up the antenna system.[2]

In the present paper we explore the use of the triplet state as a paramagnetic probe into the structural features of chlorophyll complexes, essentially using the triplet state of chlorophyll as a particularly convenient "spin label",[12,14,15] one which can be introduced nondestructively into the system of interest by photoexcitation.

The triplet state of the chlorophyll molecule has been studied

0002-7863/79/1501-2416$01.00/0

extensively by a variety of EPR techniques in recent work.[16] The zero-field splittings have been reported for the commonly occurring forms of the chlorophyll and bacteriochlorophyll molecules, as well as most of the important derivative structures.[16] In addition, intersystem crossing rates have been measured for the individual triplet state spin sublevels of many of these chlorophylls.[16] There has emerged from these efforts a fairly comprehensive description of the electron distribution in the chlorophyll triplet state[16,17] and the mechanisms active in promoting intersystem crossing in the chlorophyll molecule.[16-18] In our previous work we have suggested that the triplet zero-field splitting and intersystem crossing rates could be utilized to provide structural information in chlorophyll aggregates.[19-21] Specifically, the structure of a chlorophyll dimer such as might exist in the reaction center "special pair"[2] could be inferred from the triplet properties as interpreted within the framework of a simple triplet exciton treatment.[19,21,22] If the assumptions of the triplet exciton approach, as applied successfully in the description of triplet exciton states in organic molecular solids,[23,24] are applicable to the chlorophyll aggregate, then both the values of the zero-field splitting and the intersystem crossing rate constants in the chlorophyll dimer are expected to be directly dependent on the relative orientation of the chlorophyll molecules in the pair.[19,21,22] The use of a triplet exciton treatment to interpret dimer structure in chlorophylls has also been discussed recently by Hägele, Schmid, and Wolfe[25] and by Bowman and Norris[26] and has been applied to chlorophyll solutions by Kooyman et al.[20b]

The ability to investigate the structural aspects of specific chlorophyll aggregate units within a given sample by triplet-state EPR is dependent on the ability to select and isolate the unit of interest from what may be a complex system. This is particularly important when several forms of the chlorophyll aggregate may exist simultaneously, as in a solution containing equilibrium mixtures of monomer, dimer, and higher aggregates, or in vivo, where several chlorophyll aggregate forms are expected in the reaction centers and antenna systems. In such cases optically detected magnetic resonance (ODMR) allows an optical screening of the system, monitoring the triplet-state EPR spectrum on fluorescence peaks which originate from the unit of interest.[27] And by varying the optical detection wavelength among the fluorescence peaks, structural features from different units within the same sample may be evaluated and compared under identical conditions.

We have investigated by fluorescence detected zero-field triplet state ODMR spectroscopy the zero-field splittings and intersystem crossing rates for several chlorophyll aggregate systems in hydrocarbon solution. The intention of this work is to evaluate the applicability of the triplet exciton model for investigating the makeup of interacting chlorophyll systems and to assess the structural features of in vitro chlorophyll dimer systems proposed as models for in vivo reaction center chlorophylls.[2,6,7]

II. Experimental Section

Chlorophyll *a* was used as obtained from Sigma Chemical Co. Preparation of zinc-substituted pheophytin *a* (referred to in this work as zinc chlorophyll *a*) followed the procedure described in previous work.[28] Solvents used in preparing chlorophyll aggregates, *n*-pentane (research grade, Phillips Petroleum Co.), and methylcyclohexane ("photorex" grade, J. T. Baker) were employed without further purification. Toluene (spectrographic grade, Fisher) was distilled before use.

Hydrocarbon mixtures containing chlorophyll aggregates were made up according to the preparations described by Fong and Koester[29] and by Shipman et al.[6] For the Fong-Koester systems a solvent of 1:1 methylcyclohexane-*n*-pentane and $\sim 10^{-2}$ M distilled water was prepared. To this was added chlorophyll *a* to make the chlorophyll concentration $\sim 2 \times 10^{-5}$ M. For the hydrocarbon solution described by Shipman et al., the toluene was distilled in order to remove water. For high concentrations of chlorophyll *a* (10^{-2} M), it was necessary to add the solvents, toluene and absolute ethanol, to the chlorophyll *a* in a capillary tube in microliter quantities; the ethanol concentration was approximately 1.5 times that of the chlorophyll.

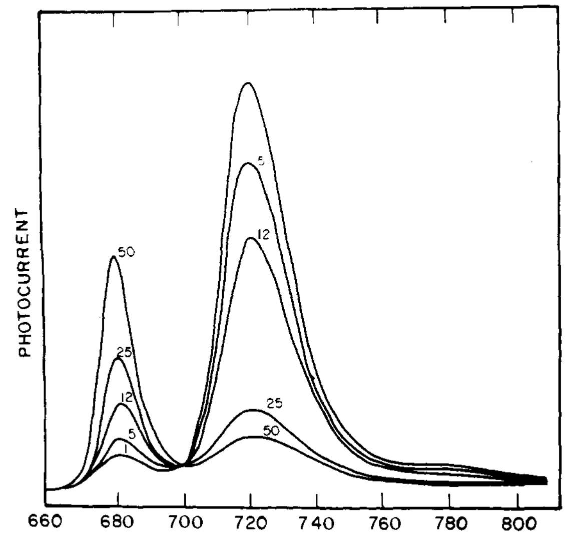

Figure 1. Fluorescence spectrum (uncorrected for phototube response) of the covalently bound dimeric derivative of zinc pyrochlorophyllide *a* in toluene solution at 77 K as a function of added percent methanol. The wavelength scale is in nanometers.

The covalently linked dimers of pyrochlorophyllide *a* and zinc pyrochlorophyllide *a* as well as the pyrochlorophyllide *a* molecules were generously made available to us by Professor G. L. Closs. The dimers were dissolved in a 3:1 (by volume) mixture of toluene and methanol. The ratio produces approximately equal amounts of folded (dimer) and unfolded (monomer) species at 2 K so that both could be studied in similar environments.

Figure 1 illustrates the 77 K dependence of the degree of unfolding on the percentage of methanol present. Whereas previous work on the covalently linked dimers[30] utilized solvents meticulously prepared free of hydrogen-bonding components (H_2O or methanol) to produce the unfolded dimer, we found it experimentally simpler to produce monomer-like species by adding substantial amounts of methanol. This behavior is interpreted as arising from steric factors; i.e., at high concentrations of methanol, a hydrogen-bonding "wedge" network causes the two halves of the linked dimer to be forced apart.

The optical and microwave equipment and the experimental arrangement utilized in obtaining the triplet-state ODMR data were described previously for chlorophylls.[27,28,31] Intersystem crossing rates were obtained by monitoring the buildup and decay of each observed ODMR transition,[28,31] and these dynamics curves were analyzed directly by an on-line computer system (Digital Equipment PDP 11/34) interfaced with the detection equipment.

III. Results

The systems chosen for the study of chlorophyll aggregates were made up in solutions containing hydrogen-bonding ligands, ligands expected to effect linkage of the chlorophyll molecules into dimeric and higher order aggregate structures.[2,6,7,30] The chlorophyll systems on which our ODMR experiments focused were (a) 10^{-5} M chlorophyll *a*, 10^{-2} M water in 1:1 solvent mixture of methylcyclohexane-*n*-pentane;[29] (b) 10^{-5} M zinc chlorophyll *a*, 10^{-2} M water in 1:1 solvent mixture of methylcyclohexane-*n*-pentane; (c) 10^{-4} M chlorophyll *a* in ethanol; (d) 10^{-2} M chlorophyll *a*, 10^{-2} M ethanol in toluene;[6] (e) covalently linked dimer of pyrochlorophyllide *a* dissolved in 3:1 solvent mixture of toluene-methanol;[30] (f) covalently linked dimer of zinc pyrochlorophyllide *a* dissolved in 3:1 solvent mixture of toluene-methanol.

The presence of chlorophyll aggregate species in each of these systems was assessed through their characteristic fluorescence spectra at 2 K. The fluorescence typical of a low-

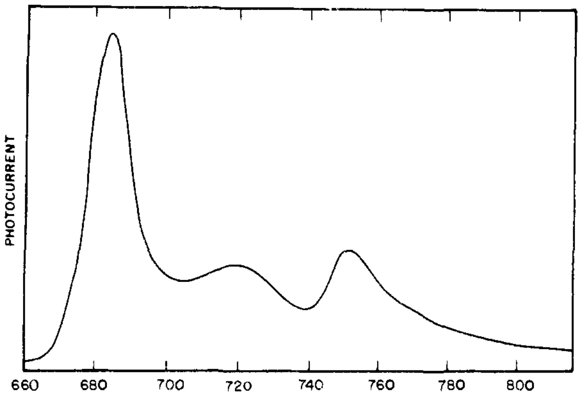

Figure 2. Fluorescence spectrum (uncorrected for phototube response) of chlorophyll *a* in a solution of methylcyclohexane-*n*-pentane (1:1) containing water at 77 K. The wavelength scale is in nanometers.

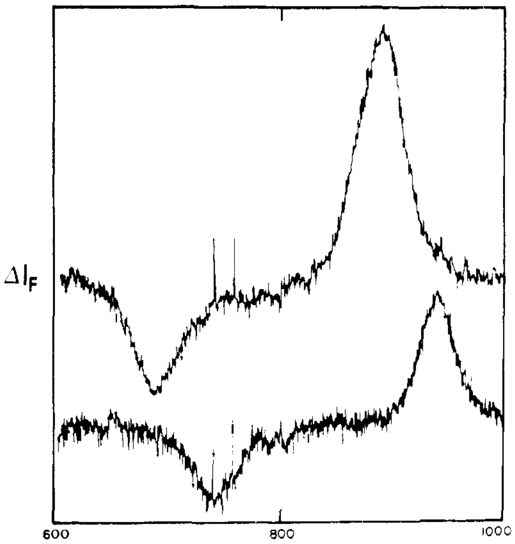

Figure 3. The fluorescence detected zero-field ODMR spectrum of the covalently bound dimeric derivative of zinc pyrochlorophyllide *a* in a 3:1 solvent mixture of toluene-methanol at 2 K. The lower trace was detected at a wavelength of 675 nm (unfolded form) and the upper trace was detected at 720 nm (folded form). The two sharp-line features near the center of each trace are frequency markers; the frequency scale is in megahertz. The scale of fluorescence intensity change, ΔI_F, is such that ODMR peaks pointing downward correspond to an increase in fluorescence.

temperature hydrocarbon solution containing chlorophyll and either alcohol or water is shown in Figures 1 and 2. The system whose fluorescence spectrum is depicted in Figure 2, a frozen hydrocarbon solution containing chlorophyll *a* and an excess of water, is that proposed as an in vitro model system for the reaction center pair by Fong and Koester.[29] This solution exhibits an absorbance band at 700 nm (A-700 in the Fong-Koester notation), which they conclude is due to a water-linked dimer of chlorophyll *a*. The solution also exhibits fluorescence bands at 680 and 720 nm (L-720 in their notation) at low temperatures.[29] The L-720 peak is identified with the water-linked chlorophyll dimer; the fluorescence band at 680 nm is most likely the fully ligated monomer.[29] These spectral assignments are supported by the recent work of Boxer and Closs, who synthesized a covalently linked dimeric derivative of pyrochlorophyllide *a* which can be folded over into a parallel dimer structure in hydrocarbon solvents by the addition of water.[30] In the presence of water the folded pyrochlorophyllide dimer (and zinc-substituted pyrochlorophyllide dimer) exhibits an absorbance at 700 nm and a long-wavelength fluorescence at 720-730 nm; in its unfolded form the system fluoresces at 680 nm (see Figure 1). We have utilized fluorescence detected ODMR to measure the triplet state zero-field splittings at 2 K for the monomer (680 nm) and dimer (720 nm) chlorophyll in the Fong-Koester hydrocarbon solution and the results are presented in Table I. Similarly, for the chlorophyll-ethanol solution ODMR transitions were detected on the fluorescence bands at 680 and at 730 nm, assumed to be the (fully ligated) monomeric and ethanol-linked dimeric forms of chlorophyll *a*, and these results are included in Table I. For the high-concentration chlorophyll *a*, ethanol in toluene system, the ODMR was first obtained at the fluorescence maximum, at 760 nm, and then after dilution with toluene, at the usual monomer fluorescence band at 680 nm; these results are also given in Table I.

Table I. ODMR Transitions of Chlorophyll *a* in Hydrocarbon Solution[a]

system	detection wavelength, nm	ν_1, MHz	ν_2, MHz
chlorophyll *a* (10^{-5} M) and H_2O (10^{-2} M) in methylcyclohexane-pentane (1:1)	680 720	728 707	947 925
chlorophyll *a* (10^{-4} M) in ethanol	680 730	707 639	1026 874
chlorophyll *a* (10^{-5} M) and H_2O (10^{-5} M) in toluene	680	765	1035
chlorophyll *a* (10^{-2} M) and EtOH (10^{-2} M) in toluene	760	698	894
zinc chlorophyll *a* (10^{-5} M) and H_2O (10^{-2} M) in methylcyclohexane-pentane (1:1)	680 720	774 724	1038 955

[a] All microwave frequencies reported are accurate to ±2 MHz.

Table II. ODMR Transitions of Covalently Linked Dimers of Pyrochlorophyllide *a* and Zinc Pyrochlorophyllide *a*[a]

system	detection wavelength, nm	ν_1, MHz	ν_2, MHz
pyrochlorophyllide *a* dimer in toluene-methanol (3:1)	682 733	722 701	940 909
zinc pyrochlorophyllide *a* dimer in toluene-methanol (3:1)	675 720	785 739	1015 943

[a] All microwave frequencies reported are accurate to ±2 MHz.

A typical series of ODMR spectra detected on the two fluorescence maxima usually observed in chlorophyll-hydrocarbon solutions at 2 K are shown in Figure 3. In all our ODMR spectra only two of the three zero-field EPR transitions are observed, the D + E and D − E transitions; the third, 2E, is presumably between zero-field spin sublevels of nearly identical steady-state population, and, therefore, difficult to detect.[18,27,28] The observed zero-field ODMR transition line widths are all relatively sharp and structureless on the order of 10-30 MHz. Occasionally, structure was observed on the ODMR transitions for the covalently linked dimer of pyrochlorophyllide *a* when the ODMR was detected at 680 nm, corresponding to the unfolded form of the dimer.[30] In this case the structure may arise from the two rings being frozen into inequivalent local environments at either ends of the covalently linked dimer in the hydrocarbon matrix.

The ODMR frequencies listed in Tables I and II show a trend consistently observed for all the chlorophyll (and substituted chlorophyll) systems investigated. The D + E and D − E transitions always shifted to lower frequencies as the fluorescence was monitored on peak maxima to higher wavelengths. The lowest frequencies observed in our ODMR experiments were those observed in the high-concentration

Table III. $T_1 \rightarrow S_0$ Intersystem Crossing Rates for the Individual Spin Sublevels of Chlorophyll Systems (s^{-1})

system	detection wavelength nm	k_x	k_y	k_z	K_{calcd}[d]	K_{obsd}
chlorophyll *a* in *n*-octane[a]	680	661 ± 89	1255 ± 91	241 ± 15	719	
chlorophyll *a* in toluene-pyridine[c]	n.a.					585 ± 70
chlorophyll *a* in M THF[b]	n.a.	620 ± 80	1120 ± 20	140 ± 40	628	630 ± 60
chlorophyll *a* (10^{-5} M) and H_2O (10^{-2} M) in methylcyclohexane-pentane (1:1)	680	564 ± 100	1330 ± 200	176 ± 50	690	690 ± 30
	720	732 ± 110	980 ± 170	180 ± 30	630	690 ± 50
pyrochlorophyllide *a* dimer in toluene-methanol (3:1)	733	830 ± 100	070 ± 80	170 ± 20	690	
pyrochlorophyllide *a* molecule in toluene	680					720 ± 20

[a] Reference 27. [b] Reference 44. [c] H. A. Frank, unpublished results. Lifetime determined by T-T absorption. [d] $K_{calcd} = \frac{1}{3}(k_x + k_y + k_z)$.

Table IV. $T_1 \rightarrow S_0$ Intersystem Crossing Rates for the Individual Spin Sublevels of Zinc Chlorophyll Systems (s^{-1})

system	detection wavelength, nm	k_x	k_y	k_z	K_{calcd}	k_{obsd}
zinc chlorophyll *a* in *n*-octane[a]	680	340	330	660	445	
zinc chlorophyll *a* (10^{-5} M) and H_2O (10^{-2} M) in methylcyclohexane-pentane (1:1)	680					
	720	525 ± 25	585 ± 25	1220 ± 50	770	800 ± 50
zinc pyrochlorophyllide *a* in toluene-methanol (3:1)	675	340 ± 20	380 ± 20	660 ± 40	460	470 ± 50
	720	600 ± 30	620 ± 30	1490 ± 60	903	980 ± 50

[a] Reference 17.

chlorophyll *a* in ethanol-toluene solution (Table I), detected on the strong peak in the fluorescence spectrum at 760 nm.

Intersystem crossing dynamics for the individual triplet spin sublevels of the chlorophyll-hydrocarbon systems were obtained by monitoring the ODMR intensity buildup and decay curves for each zero-field transition as the microwaves were turned on and off.[27,31] Since two zero-field transitions are observed, this procedure provides four measurements for the three triplet decay rates listed in Tables III and IV. In addition, the overall (average) triplet lifetime (listed in the tables as K_T) was obtained at 77 K on each fluorescence peak for which individual triplet spin sublevel decay rates were determined by ODMR. These overall lifetimes were obtained by the method of Avarmaa[32] in which, essentially, the rate at which the fluorescence intensity at a chosen wavelength reaches its steady-state value after turning on the exciting light is monitored as a function of light intensity.[32] Comparison of the overall triplet rate constant and the average of the spin sublevel decay rates provides a further check for consistency in the measured triplet dynamics. All these data are presented in Tables III and IV.

In the case of the pyrochlorophyllide *a* dimer system in its folded configuration an ambiguity exists in the interpretation of the ODMR kinetics curves for the two triplet zero-field transitions observed at 701 and 909 MHz (detected at 733 nm). For this system the actual observed microwaves-on (k_{on})-microwaves-off (k_{off}) dynamics curves[31] gave rate constants of $k_{on}^{701} = 620 \pm 30$, $k_{off}^{701} = 300 \pm 40$, and $k_{on}^{909} = 500 \pm 40$, and $k_{off}^{909} = 170 \pm 20\ s^{-1}$. The values appearing in Table III were obtained from these data by choosing the set of values which gave a set of individual spin sublevels consistent with the overall triplet lifetime of the pyrochlorophyllide *a* molecule (also in Table III), which meant determining the value for k_z from k_{off}^{909}, rather than k_{off}^{701}. Although the procedure resulted in consistency among the triplet dynamics measurements and with the dynamics observed for the other systems in Table III (as might be expected for systems with similar structural features), the reason for the approximate factor of 2 difference in the values for k_{off}^{701} and k_{off}^{909} is not clear at present.

IV. Discussion

A. The Triplet Exciton Model. As a first approximation the triplet states associated with a pair of identical chlorophyll molecules may be conveniently described through the simple exciton formalism, a formalism which has been successfully employed in the description of triplet levels in molecular crystals.[23,24,33,34] If necessary, additional corrections to the exciton state functions may be included,[35] depending on the adequacy of prediction of physical observables derived from the simple exciton description, to take into account charge transfer, localized, or inequivalent environmental interactions, or other terms important to the representation of a specific chlorophyll pair.

Sternlicht and McConnell[33] first formulated the exciton description for identical interacting organic molecules excited into a triplet state in which the intermolecular interaction between molecules is larger than the zero-field splitting. Following their approach, diagonalization of the matrix for the interacting dimer system in a molecular product basis leads to two sets of (triply degenerate) triplet dimer levels.[24,33] The spin Hamiltonian for the triplet states of the system can then be represented as the average of the spin Hamiltonians for the isolated molecules.[33,34] In this limit the intermolecular interactions force quantization of the dimer spin system about a new set of principal axes whose orientations are essentially the average of the relative positions of the two monomer principal axis systems.[23,24,34] If the dimer system possesses a symmetry axis, one of the "averaged" directions will necessarily fall along the symmetry axis.[24,33,34] As discussed previously,[20] for a symmetrical pair of interacting chlorophyll molecules displaying the relatively small zero-field splitting characteristic of extensively delocalized π-electron systems, we may expect that the chlorophyll dimer is adequately described in the strong spin coupling limit of Sternlicht and McConnell.[33] Diagonalization of the dimer matrix, as described by Hochstrasser and Lin,[24] leads to the state functions for the triplet dimer levels of the form

$$ {}^3\Psi_i{}^0(\pm) = 2^{-1/2}(T_A{}^0 t_A{}^i S_B{}^0 \pm S_A{}^0 T_B{}^0 t_B{}^i) \qquad (1) $$

where the subscripts A, B refer to the two molecules of the pair, $i = x^*, y^*, z^*$ designates a specific dimer spin sublevel in the "averaged" principal axis system, $T_{A,B}{}^0$ refers to the triplet state space function in the zeroth vibrational level localized on molecule A or B, $t_{A,B}{}^i$ is the appropriate spin function for the ith triplet spin sublevel (on molecule A or B), and the $S_{A,B}{}^0$ are the complete ground-state functions, also in their lowest energy vibrational level. The $T_{A,B}{}^0$ are most conveniently represented by pure spin adiabatic Born–Oppenheimer wave functions for manipulation in expressions for the intramolecular radiationless transitions of molecule A or B as described by Siebrand et al.[36-38] The triplet spin functions $t_{A,B}{}^i$ contain directly information on the geometrical features of the dimer, since they can be expressed in the form

$$ t_A{}^i = \xi^i{}_{Ax} t_{Ax} + \xi^i{}_{Ay} t_{Ay} + \xi^i{}_{Az} t_{Az} \qquad (2) $$

where the $\xi^i{}_A$ are the set of direction cosines which express the "averaged" principal axis system for the symmetrical dimer triplet in the molecular-based principal axis system centered on molecule A, with a similar expression for $t_B{}^i$ for the symmetrical dimer. Essentially, the dimer state functions in the strong spin coupling limit are the usual product functions of one molecule excited, the second in its ground electronic state, but with the triplet spin functions now quantized along a new axis system determined by the geometry of the pair.[24]

The three spin sublevels in each of the symmetric (${}^3\Psi_i{}^0(+)$) and antisymmetric (${}^3\Psi_i{}^0(-)$) dimer states are initially degenerate. Inclusion of the spin-spin interaction leads to expressions for the dimer zero-field energies, I, identical for both the symmetric and antisymmetric dimer levels, of the form given by several authors[21,25,26] for each of the three dimer spin sublevels i:

$$ I = \tfrac{1}{2}(\xi_{Ax}{}^{i2} + \xi_{Bx}{}^{i2})X + \tfrac{1}{2}(\xi_{Ay}{}^{i2} + \xi_{By}{}^{i2})Y + \tfrac{1}{2}(\xi_{Az}{}^{i2} + \xi_{Bz}{}^{i2})Z \qquad (3) $$

where, again the $\xi_{A,B}{}^i$ are the coefficients found in the expressions for the dimer triplet spin functions, the direction cosines for the transformation from monomer to dimer principal axes, and the X, Y, Z are the triplet zero-field energies of the monomer. The applicability of these expressions has been verified by EPR studies of triplet excitons in organic crystals,[23,34,39,40] and the expressions have been utilized in the study of the geometry of organic aggregates in solution.[41-43]

B. Dimer Zero Field Splitting. It can be seen immediately from the form of eq 3 that for a chlorophyll dimer of the type suggested by Fong for the chlorophyll dihydrate[7,11] and by Shipman et al. for a symmetrical dimer[6] in which the molecular axes are all parallel the zero-field splittings expressions for the dimer reduce to the values of the monomer.[22] For these dimer structures one expects zero-field ODMR transitions at the same frequencies for monomer and dimer. Two cases provide particularly appropriate tests of this application of the simple exciton approach to the chlorophyll dimer triplet—the Fong–Koester water-saturated chlorophyll hydrocarbon solution (chlorophyll dihydrate dimer) and the Boxer–Closs pyrochlorophyllide *a* dimer, both of which have been interpreted as containing a symmetric dimer with all axes parallel.[7,11,22,30]

From Table I, for the Fong–Koester system the two sets of ODMR zero-field transitions, detected on the monomer and dimer fluorescence bands, are indeed within ~20 MHz, suggesting that the dimeric species must be very close to an all-axes parallel (or antiparallel) configuration. As has been discussed previously,[22] the zero-field splittings alone do not determine uniquely all direction cosines in eq 3 fixing the orientation of the dimer molecules relative to one another, but the range of angles fitting the dimer zero-field energies from eq 3 requires that the monomer principal axes in the pair all be within 10–14° of parallel. The small calculated deviation from exactly parallel (calculated from the small difference in monomer and dimer zero-field transitions) is most likely a reflection of the approximations inherent in the triplet exciton approach rather than a real geometrical feature of the dimer.[22]

Table II provides the zero-field transition frequencies for the triplet states of the covalently bound dimeric derivative of pyrochlorophyllide *a* and zinc pyrochlorophyllide *a*, as prepared by Boxer and Closs,[30] in frozen toluene solutions at 2 K. In hydrocarbon solutions containing water (or methanol) a structural assignment has been made for these systems, consistent with NMR data,[30] in which the two pyrochlorophyllide ring systems fold over and are locked into a plane-antiparallel dimer structure with two water molecules stabilizing the configuration by coordination to the metal (magnesium or zinc) of one chromatophore and hydrogen bonding to the keto carbonyl of the second.[30] The covalently linked dimer systems can be converted reversibly between the folded (dimer) and unfolded (monomer) configurations by varying the amount of hydrogen-bonding ligand (methanol, in our experiments) added to the toluene solution and monitored through the fluorescence spectrum (Figure 1). We detect the ODMR transitions for both configurations in the same solution, toluene with 25% methanol, at the peak maxima at 682 and 733 nm, corresponding respectively to the unfolded and folded configuration. Again, as was found for the Fong–Koester solutions, we observe zero-field transitions in the magnesium-containing system within ~30 MHz in the unfolded (monomer) and folded (dimer) forms. For the zinc-containing covalently linked derivative the ODMR transitions are at higher frequencies than for the magnesium-containing dimer, consistent with the previously noted fact[17] that zinc substitution raises the zero-field splitting of chlorophylls. But, again, both the folded and unfolded forms of the zinc dimer display similar transition frequencies, as is expected for a chlorophyll pair in the configuration in which all principal molecular axes are close to parallel (or antiparallel).

In the cases of the chlorophyll–ethanol and high concentration chlorophyll–toluene solutions differences, comparable in both systems, in zero-field ODMR transition frequencies on what are expected to be monomer and dimer fluorescence peaks are larger than observed in the Fong–Koester hydrocarbon solution, although the ODMR frequencies for the aggregates are still within 50–100 MHz of the monomer values. We calculate from the zero-field splittings and eq 3 that the normal to the molecular planes is in a range of 30–36° in these two systems, considerably outside the range calculated for the Fong–Koester dimer or the pyrochlorophyllide *a* dimer systems. The greater difference in monomer and dimer zero-field splittings in these two cases suggests the possibility of solvent-induced distortion of the dimer complex in ethanol. Such distortion might be due to saturation of hydrogen-bonding positions around the chlorophyll rings by the solvent, or to the possibility of self-complexing of the chlorophyll molecules in the high concentration chlorophyll solution, resulting in a non-plane-parallel dimer geometry, as has been suggested for antenna chlorophyll aggregates by Katz et al.[2]

C. Intersystem Crossing Rates. Since the coefficients of the spin functions in the triplet dimer state expressions written in eq 1 and 2 are determined by the geometry of the complex, it is clear that expressions for the probability of transitions from the spin sublevels of the metastable triplet level to the ground state will also reflect the geometry of the pair. Triplet to ground state transitions in chlorophylls are predominantly nonradiative.[17,27,44] Expressions for nonradiative transition probabilities from any of the six triplet dimer levels ${}^3\Psi_i{}^0(\pm)$ into an isoenergetic vibronic level of the ground state may be straightforwardly expressed using the state functions of eq 1. Within our exciton description the ground-state vibronic levels can be formally expressed as

$$ {}^1\Psi^\nu(\pm) = 2^{-1/2}(S_A{}^\nu S_B{}^0 \pm S_A{}^0 S_B{}^\nu) \qquad (4)$$

where the quantum number ν refers to a molecular vibrational state of appropriate energy for intersystem crossing. The rate of intersystem crossing from dimer spin sublevel i is determined by the probability of triplet energy conversion to both the symmetric and antisymmetric ground state vibrational exciton levels, i.e.[21]

$$\begin{aligned} k^i{}_{\mathrm{isc}}(\pm) &= k_{\mathrm{isc}}[{}^3\Psi_i{}^0(\pm) \rightarrow {}^1\Psi^\nu(+)] + k_{\mathrm{isc}}[{}^3\Psi_i{}^0(\pm) \\ &\qquad \rightarrow {}^1\Psi^\nu(-)] \\ &= 2\pi/h|\langle {}^3\Psi_i{}^0(\pm)|\Omega|{}^1\Psi^\nu(+)\rangle|^2\rho(\mathrm{E}) \\ &\quad + 2\pi/h|\langle {}^3\Psi_i{}^0(\pm)|\Omega|{}^1\Psi^\nu(-)\rangle|^2\rho(\mathrm{E}) \qquad (5) \end{aligned}$$

where Ω is an operator which contains all terms, intramolecular and intermolecular, which can promote mixing of triplet and singlet dimer functions. Substitution of dimer state functions 1 and 4 into eq 5 leads to

$$\begin{aligned} k^i{}_{\mathrm{isc}}(\pm) &= 2\pi/h|\tfrac{1}{2}[\langle T_A{}^0 t_A{}^i|\Omega|S_A{}^\nu\rangle \pm \langle T_B{}^0 t_B{}^i|\Omega|S_B{}^\nu\rangle \\ &\quad + \langle T_A{}^0 t_A{}^i S_B{}^0|\Omega|S_A{}^0 S_B{}^\nu\rangle \pm \langle S_A{}^0 T_B t_B{}^i|\Omega|S_A{}^\nu S_B{}^0\rangle]|^2 \\ &\quad \times \rho(\mathrm{E}) + 2\pi/h|\tfrac{1}{2}[\langle T_A{}^0 t_A{}^i|\Omega|S_A{}^\nu\rangle \\ &\quad \mp \langle T_B{}^0 t_B{}^i|\Omega|S_B{}^\nu\rangle - \langle T_A{}^0 t_A{}^i S_B{}^0|\Omega|S_A{}^0 S_B{}^\nu\rangle \\ &\quad \pm \langle S_A{}^0 T_B{}^0 t_B{}^i|\Omega|S_A{}^\nu S_B{}^0\rangle]|^2\rho(\mathrm{E}) \qquad (6) \end{aligned}$$

In each of the two squared expressions in eq 6 the first two terms are essentially monomer intersystem crossing rate expressions centered on either molecule A or B, while the last two terms represent *intermolecular* conversion with the triplet-state energy on one molecule being converted into vibrational energy on the second.

If one neglects the intermolecular terms, eq 6 may be simplified and rewritten in terms of only intramolecular spin sublevel intersystem crossing rate expressions for the monomeric nonradiative conversion of triplet energy centered on either molecule A or B.[21,25,26] This can be accomplished straightforwardly by expansion of the dimer spin functions $t_{A,B}{}^i$ in the molecular framework (eq 2) and reducing eq 6 to expressions involving the individual monomer spin sublevel intersystem crossing rates k_x, k_y, k_z.[21,25,26] Such a procedure has been worked out in detail for the case in which the dimer is essentially undergoing incoherent energy conversion from the triplet to the ground state.[25,26] However, since our experiments were carried out at low temperatures (2 K), it is necessary to consider the possibility that the energy conversion originates from coherent dimer levels. In this case the random phase approximation is not appropriate and the expansion of $t_{A,B}{}^i$ in eq 2, substituted into eq 6, again neglecting the intermolecular terms, leads to the result

$$\begin{aligned} k^i{}_{\mathrm{isc}}(\pm) &= \tfrac{1}{4}|(\xi^i{}_{xA} \pm \xi^i{}_{xB})k_x{}^{1/2} + (\xi^i{}_{yA} \pm \xi^i{}_{yB})k_y{}^{1/2} \\ &\quad + (\xi^i{}_{zA} \pm \xi^i{}_{zB})k_z{}^{1/2}|^2 + \tfrac{1}{4}|(\xi^i{}_{xA} \mp \xi^i{}_{xB})k_x{}^{1/2} \\ &\quad + (\xi^i{}_{yA} \mp \xi^i{}_{yB})k_y{}^{1/2} + (\xi^i{}_{zA} \mp \xi^i{}_{zB})k_z{}^{1/2}|^2 \qquad (7) \end{aligned}$$

Again, the result demonstrates that the intersystem crossing rate constants for the individual spin sublevels i of the triplet dimer states are expressible as functions of the dimer geometry through the set of direction cosines $\xi^i{}_{lA,B}$ ($l = x, y, z$) relating monomer and dimer principal axes and measurable properties of the molecular units making up the dimer.[21] If one assumes a twofold symmetry axis and the random phase approximation, this result reduces immediately to that of Bowman and Norris,[26] i.e., $k^i{}_{\mathrm{isc}}(\pm) = \sum_{l=x,y,z} \xi^{i2}{}_l k_l$. A similar result was also obtained by Hagele, Schmid, and Wolf.[25]

The full expression (6) for the rate of intersystem crossing for a triplet dimer spin sublevel demonstrates clearly that the interpretation of triplet dimer rate data for chlorophyll pair geometries can be a nontrivial problem. Apart from the question of whether the appropriate dimer dynamics description is coherent or incoherent, the presence of the intermolecular terms in eq 6 makes the dimer intersystem crossing expressions less simply interpretable when attempting to utilize these results in the determination of dimer geometries. One might expect that since the necessary intermolecular form of Ω would involve derivatives of the spin-orbit coupling operator centered on one molecule with respect to nuclear motions of the second, such terms would be small. However, there are, potentially, a large number of such terms, especially if one considers the possibility that the triplet energy is distributed among the vibrational levels of *both* molecules simultaneously on intersystem crossing, and in some systems where there exist centers of large spin-orbit coupling activity their cumulative effect may sum to a nonnegligible result.[21]

For the chlorophyll dimer systems the triplet state zero-field measurements have indicated that a symmetrical dimer with all axes close to parallel is a reasonable model. First neglecting intermolecular effects on the intersystem crossing rate constants, eq 7 provides the result that, for a dimer in which the molecules are plane parallel and the in-plane axes aligned either parallel or antiparallel, the dimer spin sublevel intersystem crossing rate constants are equal to the monomer spin sublevel rates. As the molecules are tipped away from the parallel configuration the monomer rates become mixed among the dimer spin sublevels. Note that for this choice of geometry, viz., all axes parallel, the coherent result described in eq 6 and that calculated from an incoherent model[25,26] predict the same result—monomer and dimer rate constants the same. Further, both models predict that the dimer rates are the same in both the symmetric (${}^3\Psi(+)$) and antisymmetric (${}^3\Psi(-)$) dimer levels.[21,45]

First considering the pigment systems listed in Table III, one sees that all chlorophyll systems, including pyrochlorophyllide *a* (structurally similar to chlorophyll *a*), provide essentially the same overall lifetime, whether monomeric or dimeric, as expected from the triplet exciton model. Comparison of the results for the Fong-Koester solutions (chlorophyll *a*, H_2O, methylcyclohexane-pentane) detected on the monomer and dimer fluorescence bands shows that the prediction of eq 7 for an all-axes-parallel dimer is qualitatively verified—the order of the rate constants in both monomer and dimer is $k_y > k_x > k_z$. The near equal value of k_z (z refers to an out of plane direction in the molecular axis system) for both monomer and dimer clearly indicates consistency with a plane-parallel dimer structure. Further, the in-plane spin sublevel rate constants, k_x, k_y, for the dimer are reasonably close, considering the experimental error associated with the rate measurements, to those for the monomer values measured in several solvent environments. For the pyrochlorophyllide *a* dimer in its folded form the dynamics are again reasonably similar to those for the parent chlorophyll monomer, as is its overall lifetime. For these systems, therefore, considering both the triplet zero-field splittings and intersystem crossing rate constants, the triplet exciton model provides a description of triplet dimer properties consistent with a symmetrical dimer structure as proposed by Fong[7] and by Boxer and Closs.[30]

In the case of the zinc-substituted chlorophyll systems, however, a quite different result is obtained, as seen in Table IV. For the zinc-chlorophyll dimer (and zinc-pyrochlorophyllide *a* dimer) the triplet zero-field splittings were observed to be close to what is expected for a symmetrical all-axes-parallel dimer, as was the case for the magnesium-containing chlorophyll dimer systems. Therefore, one expects from the previous discussion on intersystem crossing rates that the zinc-chlorophyll dimer will also display intersystem crossing rate constants similar to those for the monomer. However, in Table IV, one finds that the overall triplet lifetime is increased substantially on aggregation in the zinc-chlorophyll, water, and methylcyclohexane-pentane system and in the folded form of the zinc-pyrochlorophyllide *a* dimer. For the individual spin sublevels, as well, the values in the dimer solution are consistently larger than those observed previously for the zinc chlorophyll *a* monomer.[17] For the zinc pyrochlorophyllide *a* covalently linked dimer, the intersystem crossing rates measured in the unfolded configuration agree quite well with those for zinc chlorophyll *a*, an expected result, since the small substituent differences between the chlorophyll and pyrochlorophyllide ring systems should not substantially alter the intersystem crossing mechanisms.[17,38] When the dimer is folded, the rates increase for each spin sublevel, all values larger by approximately a factor of 2 over those found in the unfolded form. In both systems, the zinc chlorophyll *a* solution and the zinc pyrochlorophyllide *a*, the measured triplet lifetime agrees well with the average of the individual spin sublevel rates, indicating reliability in the measurements. We interpret these increases in intersystem crossing efficiency upon aggregation, when the zero-field splittings remain about the same, as due to the additional intermolecular terms in the rate expression given in eq 6, now important owing to the presence of the heavy atom metal centers in the dimer system. In the case of magnesium-containing chlorophyll systems the relatively light metal center did not enhance the nonradiative transition probability over that for the monomer, but with zinc atom substitution the intermolecular terms become important and increase the intersystem crossing efficiency in the complex compared to the isolated monomer units. It is important to emphasize that this substantial increase in intersystem crossing rate upon aggregation of the zinc-containing chlorophyll and pyrochlorophyllide *a* is accompanied by no major change in zero-field splitting. These two results seem consistently explained by invoking an additional metal-induced mechanism for triplet energy conversion in the zinc-containing dimer, and further, suggest that eq 7 without intermolecular terms is indeed adequate to explain normal magnesium-containing chlorophyll aggregation dynamics within the triplet exciton model.

V. Conclusions

The value of ODMR spectroscopy to the investigation of structural features of chlorophyll aggregates is evident in the results of the previous sections. Using optical selectivity it is possible to measure triplet-state properties of monomeric and dimeric chlorophyll systems under identical conditions in the same solution. This situation minimizes difficulties in applying results of the triplet exciton description when comparing monomer and dimer triplet state properties to evaluate geometrical features of the dimer. Further, ODMR provides the possibility of identifying, in terms of their molecular or aggregate makeup, the origin of features in chlorophyll optical spectra from their distinctive triplet-state ODMR properties.[19,46,47]

The results of triplet-state ODMR measurements for the chlorophyll aggregate systems studied in this paper establish the applicability of the simple triplet exciton model to the study of the chlorophyll dimer. Both the zero-field splittings and individual spin sublevel intersystem crossing rate constants for the water-linked chlorophyll dimer and the pyrochlorophyllide *a* dimer are reasonably predicted by the exciton relationships, in terms of the monomer properties and the geometry of the dimer.[21,25,26] In particular eq 3 provides an adequate description of the zero-field splittings and eq 7 gives a reasonable representation of the intersystem crossing rates, suggesting that no new intermolecular terms are introduced into the intersystem crossing mechanism upon dimerization of ordinary chlorophylls. It is not, however, possible to distinguish between a coherent or an incoherent description for the intersystem crossing on the basis of the chlorophyll systems studied here. Heavy metal substitution may complicate the simple exciton description of intersystem crossing, but from the present results such effects are not expected to be important for in vivo chlorophyll.

It is evident from an inspection of the triplet state data in Tables I-IV that the accuracy in measurements of triplet state zero-field splittings is greater than that for the measurement of triplet-state dynamics. In a zero-field ODMR experiment transition frequencies may be typically measured for chlorophyll systems with standard deviations of less than 1%; for measurement of triplet intersystem crossing rates unusually high signal-to-noise ratios are required in the ODMR spectrum to achieve standard deviations less than 10% (only in the zinc-substituted chlorophyll systems have such experimental conditions been realized). Although the zero-field splitting parameters alone do not fix completely the dimer geometry, they do allow a direct overall assessment of the orientation of the dimer molecules. In the case of the symmetrical chlorophyll dimer, however, because of the smaller standard deviations associated with the zero-field measurements the geometries consistent with these frequencies, calculated using eq 3, are within a narrow range of values and attempting to include the intersystem crossing rates and their standard deviations in the sets of eq 3 and 7 simultaneously for the direction cosines ξ^i leads to no improvement in their determination over that obtained from the zero-field splitting alone.

Finally, the results of the ODMR investigations of solvent-linked chlorophyll dimer systems provide data consistent with the model proposed by Fong for the chlorophyll dihydrate dimer[7,11] and by Boxer and Closs for the covalently linked dimer.[30] The results of this study support the formation of chlorophyll dimers in vitro in which the solvent linkages provide a plane antiparallel configuration. And it seems clear that zero-field triplet state ODMR investigations—measurements of both zero-field splittings and intersystem crossing dynamics—will be of value in assessing the structural features of chlorophyll aggregates in the photosynthetic apparatus in vivo, a subject currently under investigation in our laboratory.

Acknowledgments. We are grateful to Professors G. L. Closs and H. Linschitz for making samples of the pyrochlorophyllide dimer available to us for these experiments and to Professors T. J. Schaafsma and R. Silbey for helpful discussions.

References and Notes

(1) Research program supported in part by the U.S. Department of Energy under Contract No. EY-76-S-02-2570 and by the donors of the Petroleum Research Fund, administered by the American Chemical Society.
(2) J. J. Katz and J. R. Norris, *Curr. Top. Bioenerg.*, **5**, 41 (1973).
(3) Govindjee, "Bioenergetics of Photosynthesis", Academic Press, New York, 1975.
(4) G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, **85**, 3809 (1963).
(5) F. K. Fong, *Appl. Phys.*, **6**, 151 (1975).
(6) L. L. Shipman, T. M. Cotton, J. R. Norris, and J. J. Katz, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1791 (1976).
(7) F. K. Fong, V. J. Koester, and L. Galloway, *J. Am. Chem. Soc.*, **99**, 2372 (1977).
(8) W. W. Parson and R. J. Cogdell, *Biochim. Biophys. Acta*, **416**, 105 (1975).
(9) J. R. Norris, M. E. Druyan, and J. J. Katz, *J. Am. Chem. Soc.*, **95**, 1680 (1973).
(10) J. R. Norris, R. A. Uphaus, H. L. Crespi, and J. J. Katz, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 625 (1976).
(11) F. K. Fong and V. J. Koester, *J. Am. Chem. Soc.*, **97**, 6888 (1975).
(12) M. T. Jones and W. D. Phillips, *Annu. Rev. Phys. Chem.*, 17, 323 (1966).
(13) A. Abragam and B. Bleaney, "Electron Paramagnetic Resonance of

Transition Ions'', Oxford University Press, London, 1970.
(14) J. R. Bolton, D. Borg, and H. Schwartz, "Biological Applications of Electron Spin Resonance Spectroscopy", Wiley-Interscience, New York, 1972.
(15) H. M. McConnell and B. G. McFarland, *Q. Rev. Biophys.*, **3**, 91 (1970).
(16) For a thorough, recent review article on the chlorophyll triplet state, see H. Levanon and J. R. Norris, *Chem. Rev.*, **78**, 185 (1978).
(17) R. H. Clarke, R. E. Connors, T. J. Schaafsma, J. F. Kleibeuker, and R. J. Platenkamp, *J. Am. Chem. Soc.*, **98**, 3674 (1976).
(18) R. H. Clarke and H. A. Frank, *Chem. Phys. Lett.*, **51**, 13 (1977).
(19) R. H. Clarke, R. E. Connors, and H. A. Frank, *Biochem. Biophys. Res. Commun.*, **71**, 671 (1976).
(20) (a) R. H. Clarke, R. E. Connors, H. A. Frank, and J. C. Hoch, *Chem. Phys. Lett.*, **45**, 523 (1977); (b) R. P. H. Kooyman, T. J. Schaafsma, and J. F. Kleibeuker, *Photochem. Photobiol.*, **26**, 235 (1977).
(21) R. H. Clarke, D. R. Hobart, and W. R. Leenstra, *Semicond. Insul.*, in press.
(22) R. H. Clarke and D. R. Hobart, *FEBS Lett.*, **82**, 155 (1977).
(23) D. Haarer, D. Schmid, and H. C. Wolf, *Phys. Status Solidi*, **23**, 633 (1967).
(24) R. M. Hochstrasser and T.-S. Lin, *J. Chem. Phys.*, **49**, 4929 (1968).
(25) W. Hägele, D. Schmid, and H. C. Wolf, *Z. Naturforsch. A*, **33**, 94 (1978).
(26) M. K. Bowman and J. R. Norris, *Chem. Phys. Lett.*, **54**, 45 (1978).
(27) R. H. Clarke and R. H. Hofeldt, *J. Chem. Phys.*, **61**, 4582 (1974).
(28) R. H. Clarke and R. E. Connors, *Chem. Phys. Lett.*, **33**, 365 (1975).
(29) F. K. Fong and V. J. Koester, *Biochim. Biophys. Acta*, **423**, 52 (1976).
(30) S. G. Boxer and G. L. Closs, *J. Am. Chem. Soc.*, **98**, 5406 (1976).
(31) P. A. Chiha and R. H. Clarke, *J. Magn. Reson.*, **29**, 535 (1978).
(32) R. Avarmaa, *Chem. Phys. Lett.*, **46**, 279 (1977).
(33) H. Sternlicht and H. M. McConnell, *J. Chem. Phys.*, **35**, 1793 (1961).
(34) D. Haarer and H. C. Wolf, *Mol. Cryst.*, **10**, 359 (1970).
(35) M. C. Thurnauer, J. J. Katz, and J. R. Norris, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 3270 (1975).
(36) B. R. Henry and W. Siebrand, *J. Chem. Phys.*, **54**, 1072 (1971).
(37) V. Lawetz, G. Orlandi, and W. Siebrand, *J. Chem. Phys.*, **56**, 4058 (1972).
(38) R. H. Clarke and H. A. Frank, *J. Chem. Phys.*, **65**, 39 (1976).
(39) M. Schwoerer and H. C. Wolf, *Mol. Cryst.*, **3**, 177 (1967).
(40) L. Yarmus, J. Rosenthal, and M. Chopp, *Chem. Phys. Lett.*, **16**, 477 (1972).
(41) H. Schmidt, *Z. Naturforsch. A*, **26**, 763 (1971).
(42) H. Schmidt and R. Zellhofer, *Z. Phys. Chem.* (*Frankfurt am Main*), **91**, 204 (1974).
(43) D. Schweitzer, K. M. Hanser, R. G. H. Kirrstetter, and H. A. Staub, *Z. Naturforsch. A*, **21**, 1189 (1976).
(44) J. K. Kleibeuker, Thesis, Agricultural University, Wageningen, 1977.
(45) Note that these results, as well as the form of the dimer intersystem rate expression in eq 7, differ from those previously published in ref 18 and 19. In the previous work[18,19] an oversimplification of the ground vibrational state description resulted in terms being omitted in the rate expressions of eq 6, leading to predictions of differences for the symmetrical dimer in spin sublevel rate constants in the plus and minus exciton state,[18,19] predictions which are no longer supported by the complete expressions given in this work.
(46) S. J. van der Bent, Thesis, Agricultural University, Wageningen, 1977.
(47) S. J. van der Bent, T. J. Schaafsma, and J. C. Goedheer, *Biochem. Biophys. Res. Commun.*, **71**, 1147 (1976).

Lactim/Lactam Tautomeric Interconversion Mechanism in Water/Polar Aprotic Solvent Water Systems. 2. Hydration of 2-Hydroxypyridines. Evidence for a Bifunctional Water-Catalyzed Proton Transfer

Olivier Bensaude, Marianne Chevrier, and Jacques-Emile Dubois*

Contribution from the Institut de Topologie et de Dynamique des Systèmes de l'Université Paris VII, associé au C.N.R.S., 75005 Paris, France. *Received September 20, 1978.*

Abstract: In previous work, lactim/lactam tautomeric interconversion of 2-hydroxypyridines has been shown to partly involve in aqueous solution a nondissociative proton-transfer mechanism, whereas, in aprotic solvents, the interconversion occurs totally within a cyclic dimer. Therefore, in order to determine whether water could also catalyze the tautomeric interconversion within a water/substrate association, temperature-jump experiments were performed in the water/propylene carbonate solvent system. Indeed, evidence is presented which suggests that the tautomeric interconversion partly involves a bifunctional water-catalyzed proton transfer. A combination of kinetic and UV spectral data indicates the formation of stoichiometric hydrates which inhibit the substrate dimerization. A deuterium kinetic isotope effect confirms the dimerization step to be rate-encounter controlled.

Introduction

Understanding the tautomerism of nitrogen heterocycles is important in view of the significant role it might play in many biochemical processes such as proton transport, enzymatic catalysis, and spontaneous or induced mutations. Previous works[1,2] have shown that, in aprotic media, tautomeric interconversion involves intermolecular proton transfers within the self-associated substrate species. By contrast, in aqueous solutions, two successive and distinct intermolecular proton transfers are usually[3-5] required between the substrate and another acid or base belonging to the media. In this mechanism, which we call the dissociative mechanism, both the protonation and the deprotonation steps behave separately like "normal" acid–base reactions.[6,7] However, tautomeric interconversions do not proceed exclusively via this latter mechanism. In the case of aqueous 2-hydroxypyridines and 2-aminopyridines, it is necessary to postulate the existence of an additional interconversion pathway, which we call the nondissociative mechanism. Its contribution to the rate law is independent of substrate concentration, which excludes a self-association mechanism like that observed in aprotic media.[2] Such a nondissociative mechanism has been exclusively observed for the tautomeric interconversion of 2-substituted pyridines, but not for pyrazoles;[8,9] this fact, together with some recent CNDO/2[10] and ab initio[11] calculations, suggests the participation of water molecules acting as bifunctional catalysts. Indeed, NMR experiments[12] have tended to indicate that the participation of hydrogen-bonded intermediates in internal proton exchange reactions is rather common in hydroxylic solvents.[13-16] However, results obtained by this method[17,18] differ from those obtained by other techniques.[19,20]

By contrast, in both aprotic[2] and aqueous[4,5] media, temperature-jump relaxation has proved to be particularly reliable when applied to the study of tautomeric interconversion. Kinetic measurements are accurate; the relaxation signals are related to UV spectra and are easily and unambiguously[21] attributed to the tautomeric reactions. Using this technique, we have shown that in "dry" aprotic solvents, the interconversion process implies[2] the intermediate dimerization of the substrate. Adding water to the media should reveal the hypothetical water-catalyzed mechanism. Although experiments

0002-7863/79/1501-2423$01.00/0