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*Proceedings of the National Academy of Sciences of the United States of America*,  
Volume 75, Issue 6 (Jun., 1978), 2563-2566.

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# Photoprocesses in covalently linked pyrochlorophyllide dimer: Triplet state formation and opening and closing of hydroxylic linkages

(photosynthesis/chlorophyll aggregation/active center/photochemistry of metal complexes)

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Contributed by Gerhard L. Closs, March 30, 1978

**ABSTRACT** Covalently linked pyrochlorophyllide *a* dimers exist in solution in either "pinned" or unfolded form, depending on the concentration of hydroxylic (H bonding) or non-H-bonding Lewis bases. Laser flash photolysis studies have been made on these dimer forms and on pyrochlorophyllide monomer at 30-nsec resolution. Spectra and recovery kinetics, including observation of both the triplet decay and ground state folding reactions, show that the flash transient obtained from the pinned form consists of a triplet and ground state moiety in the unpinned configuration. A charge-transfer excited state is not seen.

Various lines of evidence indicate that the site of primary electron transfer in green plant or bacterial photosynthesis, the "active center," contains chlorophyll or bacteriochlorophyll in an associated, probably dimeric, form (1-3). In attempts to prepare structures simulating the naturally occurring entity, pyrochlorophyllide *a* (pchl) (4) and chlorophyllide *a* (5) molecules have been covalently linked as glycol diesters, thereby joining two macrocycles by a flexible 10-atom chain. Like monomeric chlorophyll, these "dimers" can exist in solution in various aggregated or complexed structures, depending on the nature and concentration of polar and H-bonding substances. In particular, in benzene containing small amounts of hydroxylic components (water, methanol), the chlorin peak of the dimer appears at a position (695 nm) close to that of the natural center, P<sub>700</sub>. On the basis of NMR, infrared spectrum, and other data (4-7), the red-shifted absorption is attributed to a folded configuration in which the two halves of the dimer are pinned by two Mg...OH...O=C— linkages, each binding the Mg of one macrocycle to the keto carbonyl (ring V) group of the other. It is this structure (denoted here by D<sub>p</sub>) which is suggested as a model for the natural "special pair" (4-6).

It is clearly of interest to study photoprocesses in such systems. As a step in this direction, we have carried out flash photolysis experiments on the pchl dimer and related compounds. Excitation leads to a transient consisting of a linked but unpinned triplet and ground state pair. Under suitable conditions, two recovery processes can be observed corresponding to decay of the triplet and to regeneration of the locked structure, respectively.

## METHODS AND MATERIALS

A Q-switched, frequency-doubled ruby laser (Holobeam, model 600) provided 30-nsec excitation flashes at 694 nm (up to 2 J) or 347 nm (0.2 J). Excitation homogeneity was improved by spreading the beam horizontally with a cylindrical lens (over-

lapping the 6- or 10-mm sample cell) and placing a ground-glass scattering screen and Al reflector before and behind the cell, respectively. Sample absorbance was measured perpendicular to the laser beam by means of a collimated beam from a xenon arc (intensified by a capacitor discharge), monochromator (~3-nm slit width), photomultiplier, and fast preamplifier. Transmitted light profiles were displayed either on a storage oscilloscope (Tektronix 7633) or, at somewhat lower bandpass, on a digital transient recorder (Biomation, 8100). Intensity variations (±10%) of each laser flash were monitored by sampling the beam with a Pyrex flat and photodiode and measuring the integrated photocurrent. Because data were taken at close to saturation conversion intensities, such variations were not important. Exposure of the sample to light was minimized by a rotating sector shutter and filters, placed in the analyzing beam.

Test solutions were deoxygenated by bubbling with high-purity argon. Concentrations were obtained spectrophotometrically before and after flashing.

pchl dimer was prepared as described (4). Chloranil was repeatedly crystallized from benzene. Methanol (Fisher, Analar) and pyridine (M.C.B. spectroquality) were used as received. Benzene (E.K. spectrograde) was dried over CaH<sub>2</sub>.

## RESULTS AND DISCUSSION

**Spectra of Dimeric Forms.** For the purpose of this paper, we will be concerned with two forms of the pchl dimer in solution, as shown in Fig. 1. Curve A, which extends data given previously (4), is for the D<sub>p</sub>, found in benzene solution containing moderate concentrations of methanol (0.005-0.5 M). In addition to the shifted red peak, the Soret band was broadened by an intense high-energy shoulder. D<sub>p</sub> is converted reversibly to the unfolded form, (D<sub>u</sub>) (curve B, peak at 670 nm), simply by bubbling dry argon through the solution and thereby removing the methanol or by replacing the solvent with fairly dry benzene on the vacuum line. Infrared absorption at 1.42 μm (8), followed during the argon bubbling, showed that the conversion D<sub>p</sub> → D<sub>u</sub> occurred at methanol concentrations less than 5 mM (D<sub>p</sub> ~ 10 μM). The red peak of monomeric pchl in benzene/0.5 M methanol was at 666 nm and its Soret band (434 nm) was narrow and closely similar in form to that of D<sub>u</sub> and

Abbreviations: pchl, pyrochlorophyllide *a*; pchl monomer, methyl pchl; pchl dimer, bis(pchl) ethylene glycol diester; D<sub>p</sub>, pinned form of pchl dimer; D<sub>u</sub>, unfolded form of pchl dimer.

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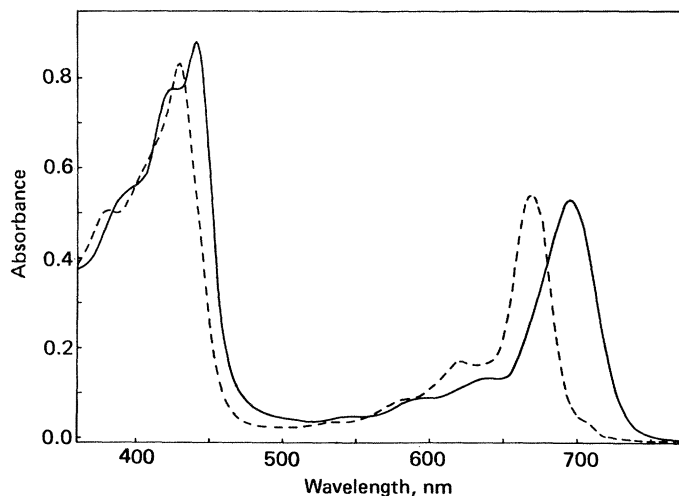


FIG. 1. Absorption spectra of pchl dimer ( $7 \mu\text{M}$ , 1 cm). Curve A (solid line), in benzene/0.5 M methanol; curve B (dashed line), after replacing solvent with "dry" benzene (see text).

to the unfolded dimer (4) in pyridine. Thus, although a precise structure cannot as yet be given for  $D_u$ , the rings are evidently unpinned. The degree of methanol solvation is probably less than maximal. For further comparison, we noted that in carefully dried, methanol-free benzene, the pchl dimer showed yet another spectrum, with the red peak at 678 nm and a broad Soret band. The location (9) and form of this spectrum and the remarkably diffuse proton NMR spectrum (4) indicate that this "dry dimer" is folded and held together by magnesium-keto carbonyl interactions.<sup>§</sup>

**Flash Transients.** Fig. 2 shows spectral changes after flash excitation of  $D_p$  in benzene/0.5 M methanol. Absorbance was increased in the 500-nm region and at 670 nm, and bleaching was seen at 695 nm and in the Soret band. These changes were reversible; a concentration loss of only about 10% was observed after 50 flashes. There was no evidence of multiple-photon processes, even at flash energies far above those necessary to obtain maximum (saturation) conversion. The decay kinetics of the transient were independent of wavelength (compare spectra in Fig. 2 and see below). Thus, in benzene/0.5 M methanol, only one intermediate state is observed. The total absorption spectrum of the solution, immediately after flashing, is given in Fig. 3.

To identify this transient, we note first that the absorption in the 500-nm region closely resembles that obtained by flashing pchl monomer (Fig. 4) or chlorophyll *a* itself (10). The spectrum, oxygen-sensitivity, and decay kinetics (see below) establish this species as a triplet. The peak near 670 nm corresponds to an unpinned pchl ring (Figs. 1 and 3). In agreement with this, the narrow Soret band of  $D_u$  appears clearly in the flash transient (Fig. 2, spectrum A), superimposed on the broader bleached Soret region of  $D_p$ . We conclude that the photoreaction corresponds to the conversion of  $D_p$  to a half-excited species containing covalently linked triplet and ground state units. The magnitudes of the transient absorbance changes confirm this, at least semiquantitatively.

The relative extinction coefficients of the triplet (at 500 nm) and ground states (red peak) of pchl and chlorophyll *a* are close to 1:4 (Fig. 4 and ref. 10). Also, the extinction coefficients in the red of  $D_p$  and  $D_u$  are similar (Fig. 1). Thus, allowing for slight overlap of absorptions at 670 nm and not quite complete flash

<sup>§</sup> A more detailed report on the several forms of the dimer in solution will be given elsewhere.

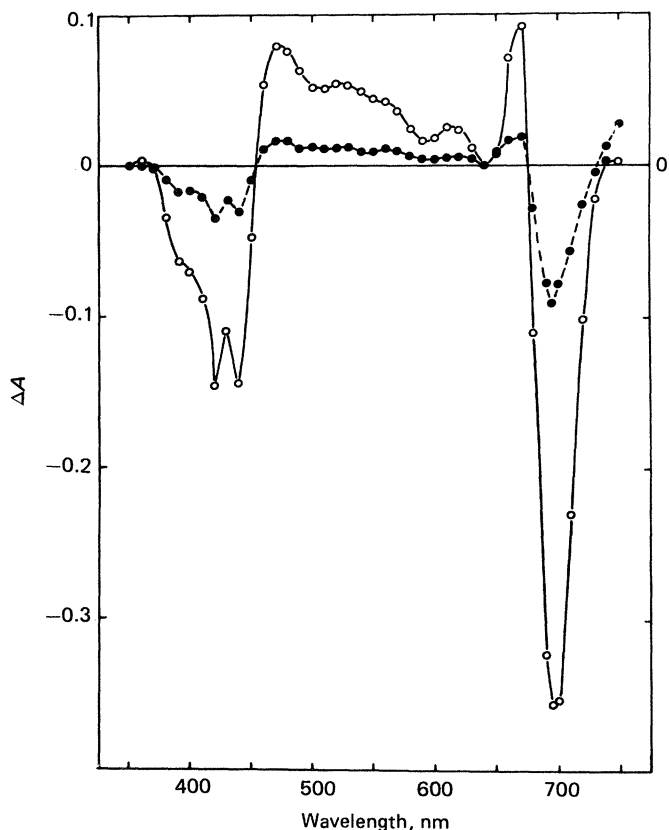


FIG. 2. Difference spectra of pchl dimer ( $6 \mu\text{M}$ ) in benzene/0.5 M methanol. Curve A, immediately (O) after flash; curve B, 20  $\mu\text{sec}$  (●) after flash.

conversion, Fig. 3 shows an equivalent concentration of triplet and ground state units in the transient and approximately half-conversion of  $D_p$  macrocycles to the unpinned (670-nm) state. Such a result is reasonable because absorption of a single 694-nm photon should bleach the pinned dimer at that wavelength and remove it from further excitation. However, similar behavior (including saturation) was observed on flashing at 347 nm. This may possibly be attributed, at least in part, to rapid T-T annihilation (10), eliminating doubly excited dimers. The

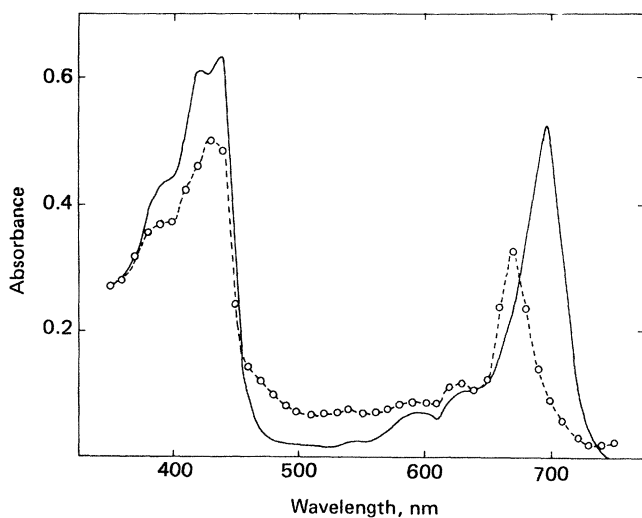


FIG. 3. Total absorption spectra of pchl dimer ( $6 \mu\text{M}$ , 1 cm) in benzene/0.5 M methanol. Solid line, before flash; dashed line, immediately after flash.

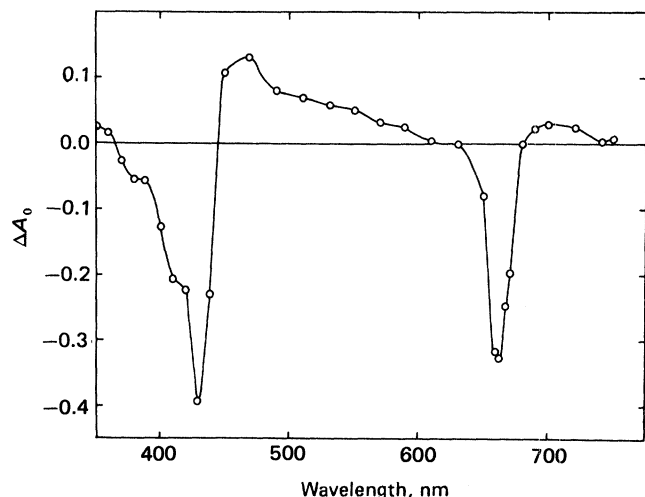


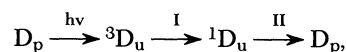
FIG. 4. Difference spectrum of pchl monomer ( $12 \mu\text{M}$ ,  $0.6 \text{ cm}$ ) in benzene/ $0.5 \text{ M}$  methanol, immediately after flash ( $347 \text{ nm}$ ).

close resemblance of the components of the transient spectrum to the separated species indicates that there is no strong interaction between the triplet and ground state components of the excited dimer.

In benzene/ $5\%$  pyridine, the dimer exists in open form, with peak absorptions at  $665$  and  $438 \text{ nm}$  (narrow). Flash excitation of such solutions ( $1 \mu\text{M}$ ) at  $347 \text{ nm}$  gave reversible absorption changes corresponding simply to conversion of  $665\text{-nm}$  to triplet ( $500 \text{ nm}$ ) species, with no shifts in red or blue peaks. Recovery kinetics were independent of wavelength (one observable intermediate) and saturation conversion was approached at close to  $50\%$  bleaching of the original red absorption. Thus, the transient in this case is also a half-excited species, containing one triplet and one ground state moiety. In this system we start and end with open dimer, and half-bleached bands are in their monomer location. We conclude that the excited species is also in open configuration.

**Kinetics of Transient Decay.** Fig. 2 shows that the transient absorptions at  $500$  and  $670 \text{ nm}$  decay together to form  $D_p$  at  $695 \text{ nm}$ . This process follows mixed first- and second-order kinetics characteristic of triplet decay (10):  $-d[T]/dt = k_1[T] + k_2[T]^2$ . Analysis of the decay and growing-in data by methods described previously (10) gives  $k_1 = 5 \times 10^3 \text{ sec}^{-1}$  and  $k_2 = 3 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$  at all peak wavelengths ( $\pm 20\%$ ). The constant  $k_1$  represents as usual only an upper limit to the intrinsic rates of radiationless and radiative decay. Self-quenching interactions between the excited and ground state halves of the dimer appear to be small, at best.<sup>¶</sup> We note, in addition, that the value of the T-T annihilation rate constant,  $k_2$ , is close to that measured for monomeric chlorophyll triplets (10). This result, together with the spectra, essentially rules out any interpretation of the transient as a free radical species.

We write for the excitation and recovery processes:



in which  ${}^3D_u$  represents the half-excited, unpinned dimer. The constancy of the  $k$ s for both disappearance of  $500\text{-nm}$  and  $670\text{-nm}$  absorption and recovery at  $695 \text{ nm}$  implies that triplet decay (I) is rate-limiting in the overall back reaction and the

<sup>¶</sup> If the equivalent "concentration" of the dimer component is taken to be  $\sim 0.1 \text{ M}$ , the value of  $k_1$  means that the bimolecular self-quenching rate constant must be substantially less than  $10^5 \text{ M}^{-1} \text{ sec}^{-1}$ .

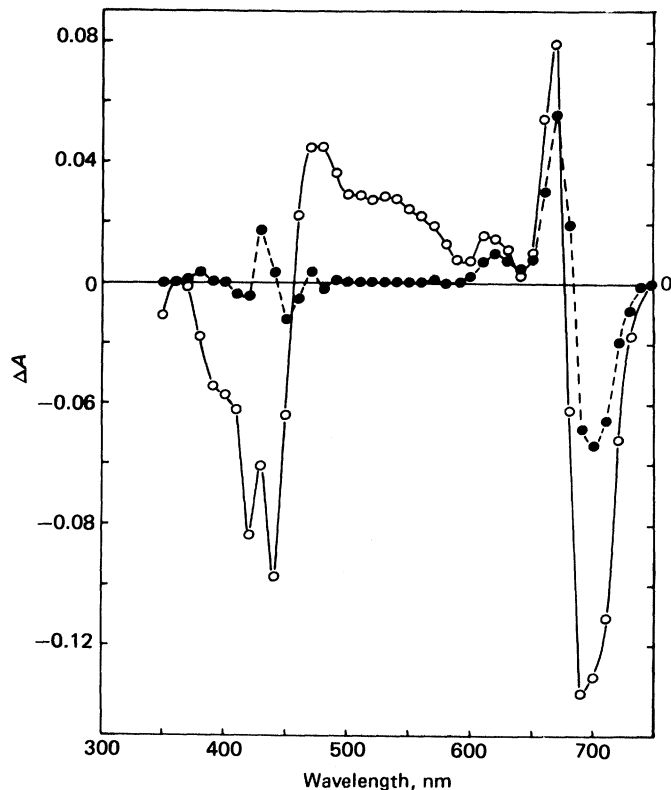


FIG. 5. Difference spectra of pchl dimer ( $3.8 \mu\text{M}$ ,  $1 \text{ cm}$ ) in benzene/methanol ( $5 \text{ mM}$ ) containing chloranil ( $0.16 \text{ mM}$ ) immediately after flash (O) and  $4 \mu\text{sec}$  after flash (●).

refolding reaction (II) is too fast to be observed in this solvent.

**Effects of Chloranil and Methanol Concentration.** In order to confirm by direct observation both steps in the reaction, we shortened the triplet lifetime (by adding chloranil as quencher) and slowed down step II by lowering the methanol concentration.

Chloranil quenched  ${}^3D_u$  efficiently and reversibly, and in nonpolar media no radical formation was observed by flash photolysis. At sufficiently high quencher concentration, the decay became pseudo-first-order. From the effect of chloranil concentration on lifetime, the bimolecular quenching constant is found to be  $9 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$  (independent of wavelength) in benzene/ $0.5 \text{ M}$  methanol.

The effect of lowering methanol concentration in such quenched solutions is shown in Fig. 5. In  $5 \text{ mM}$  methanol containing  $0.16 \text{ mM}$  chloranil, the decay of the triplet was complete after  $4 \mu\text{sec}$ , whereas the  $670\text{-}$  and  $430\text{-nm}$  peaks of  ${}^1D_u$  were still prominent, in contrast to the situation in  $0.5 \text{ M}$  methanol (Fig. 2). Fig. 6 gives more detailed absorption-time profiles at several characteristic wavelengths in  $0.5 \text{ M}$  and  $5 \text{ mM}$  methanol. At  $520 \text{ nm}$ , where the ground state macrocycles do not absorb, the decay of the triplet alone is seen and is independent of methanol concentration. In  $0.5 \text{ M}$  methanol, no intermediate stage in the recovery of  $D_p$  could be detected. However, in dilute methanol, the decay of triplet led to an increase in the ground state ( ${}^1D_u$ ) absorptions at  $430$  and  $670 \text{ nm}$ , and the subsequent much slower pinning reaction to  ${}^1D_p$ , including growing-in at  $710 \text{ nm}$ , was evident.

In connection with the formation of  ${}^3D_u$  it is of interest to examine the charge distributions in the ground and excited states of chlorins. Extensive SCMO computations (ref. 11, see figure 18) and earlier work (ref. 12, see figure 5) indicate that

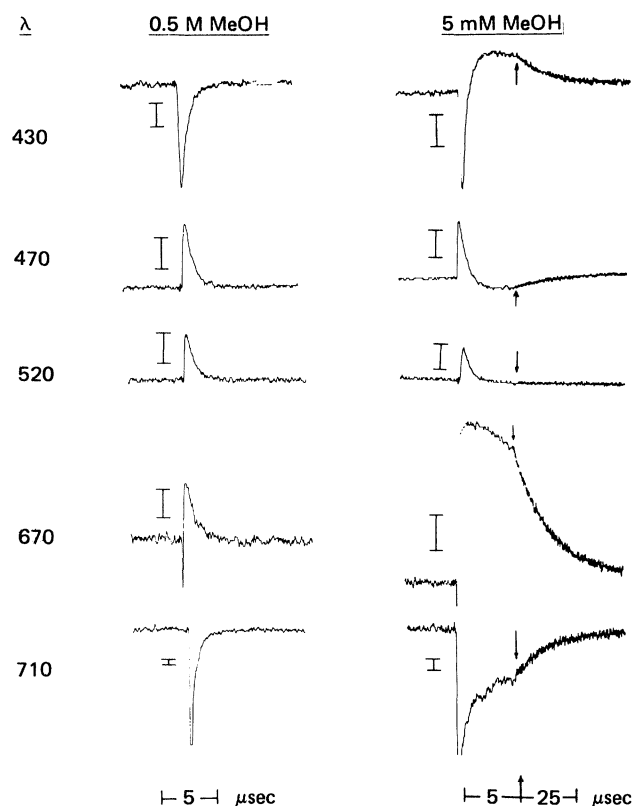
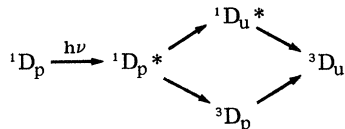


FIG. 6. Flash profiles of pchl dimer ( $3.8 \mu\text{M}$ ) at 430, 470, 520, 670, and 710 nm in the presence of chloranil (0.16 mM). Solvent, benzene/methanol (at 0.5 M and 5 mM). I represents 5% change in transmission; downward deflection means increased transmission; composite time base for right column, as indicated. Initial large deflections at 670 and 710 nm are caused by scattered light and fluorescence.

the lowest-lying excitation corresponds to strong displacement of charge toward the central nitrogens of the chlorin ring. This would decrease the acidity of the complexed  $\text{Mg}^{2+}$  and weaken the bond to the ROH unit holding the dimer in the pinned configuration. However, from the standpoint of the photochemistry of the dimer, it is significant that the lifetime of  $^1\text{D}_p^*$  is sufficiently long to permit a fluorescence to be observed (4).

The transitions leading from  $^1\text{D}_p$  to the unpinned triplet may follow alternative pathways:



The energy of the ground state pinned dimer,  $^1\text{D}_p$ , is presumably lower than that of the unpinned form,  $^1\text{D}_u$ , and its absorption (695 nm, leading to  $^1\text{D}_p^*$ ) lies also at lower energies than that of  $^1\text{D}_u$  (670 nm, leading to  $^1\text{D}_u^*$ ). Thus, the process  $^1\text{D}_p^* \rightarrow ^1\text{D}_u^*$  should be energetically unfavorable and  $^1\text{D}_p^* \rightarrow ^3\text{D}_p$  appears more likely. Indeed, the time lag during which fluorescence from  $^1\text{D}_p^*$  occurs may depend heavily on the spin restriction of the prescribed pathway.

In view of the charge-separation function of the active center in photosynthesis, it is natural to think of dimer excited states with an internal charge-transfer structure,  $\text{M}^+\text{M}^-$  (13, 14). Such states have also been suggested as intermediates in the triplet annihilation reaction (10). However, the transient spectra found here can be quantitatively accounted for as stoichiometrically equivalent triplet and ground state units. On the time scale of our experiments, charge-transfer states are not observed. This, of course, is not inconsistent with the possible occurrence of such short-lived structures *in vivo*, where the dimer may exist in highly unsymmetrical environment.

We thank Professor M. Gouterman, University of Washington, for helpful comments, and E. V. Sitzmann for the preparation of a sample of methyl pyrochlorophyllide *a*. We are pleased to acknowledge support of this work by the Energy Research and Development Administration (Grant EY-76-S-02-3117, to Brandeis University) and The National Science Foundation (Grant CHE 76-01745 A04, to The University of Chicago).

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