Conf-780741--1

EXCITED AND IONIC STATES OF DIMERIC CHLOROPHYLL DERIVATIVES. BIOMIMETIC MODELLING OF THE PRIMARY EVENTS OF PHOTOSYNTHESIS

Michael R. Wasielewski

Prepared for

Frontiers of Biological Energetics; Electrons to Tissues
July 19-21, 1978
Univ. of PA
Philadelphia, PA

U of C-AUA-USDOE

ARGONNE NATIONAL LABORATORY, ARGONNE, ILLINOIS

Operated under Contract W-31-109-Eng-38 for the
U. S. DEPARTMENT OF ENERGY

The facilities of Argonne National Laboratory are owned by the United States Government. Under the terms of a contract (W-31-109-Eng-38) between the U. S. Department of Energy, Argonne Universities Association and The University of Chicago, the University employs the staff and operates the Laboratory in accordance with policies and programs formulated, approved and reviewed by the Association.

MEMBERS OF ARGONNE UNIVERSITIES ASSOCIATION

The University of Arizona
Carnegie-Mellon University
Case Western Reserve University
The University of Chicago
University of Cincinnati
Illinois Institute of Technology
University of Illinois
Indiana University
Iowa State University
The University of Iowa
Kansas State University
The University of Kansas
Loyola University
Marquette University
Michigan State University
The University of Michigan
University of Minnesota
University of Missouri
Northwestern University
University of Notre Dame
The Ohio State University
Ohio University
The Pennsylvania State University
Purdue University
Saint Louis University
Southern Illinois University
The University of Texas at Austin
Washington University
Wayne State University
The University of Wisconsin

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately-owned rights. Mention of commercial products, their manufacturers, or their suppliers in this publication does not imply or connote approval or disapproval of the product by Argonne National Laboratory or the U. S. Department of Energy.

EXCITED AND IONIC STATES OF DIMERIC CHLOROPHYLL DERIVATIVES. BIOMIMETIC MODELLING OF THE PRIMARY EVENTS OF PHOTOSYNTHESIS

Michael R. Wasielewski

Chemistry Division
Argonne National Laboratory
Argonne, Illinois

I. INTRODUCTION

A clear understanding of the primary events of photosynthesis ultimately relies on acquiring detailed knowledge of the structurally dependent photoredox properties of special pair chlorophylls and their associated electron transport components. Employing a biomimetic modelling approach we have prepared a series of dimeric chlorophylls which not only mimic the key photochemistry and redox properties of reaction center chlorophyll, but more importantly allow us to make predictions concerning the structural and environmental constraints necessary to achieve this chemistry in vivo.

Although porphyrin dimer models have shown that interannular interactions are a strong function of the conformation of one macrocycle relative to the other, they remain limited in their ability to model special pair chlorophyll function in photosynthesis because they do not possess the optical, redox, or donor-acceptor properties unique to the chlorophylls (Schwarz et al., 1972; Anton et al., 1976; Ogoshi et al., 1977; Collman et al., 1977; Kagan et al., 1977).

Based on the previous porphyrin work the solution to this problem is to provide a mode of physical attachment between the two chlorophyll macrocycles. However, the reactive functional groups of the chlorophyll molecule, most notably the β keto ester in ring V, make the chemistry somewhat more challenging. Noting this fact, Boxer and Closs employed the more stable pyrochlorophyll a (PChl a) as the starting point in their synthesis of a model for P700 photoreaction center dimers (Boxer and Closs, 1976). Although the PChl a covalent dimer exhibits several biomimetic properties (vide infra), it alone cannot serve to assess the steric requirements imposed on special pair structure by the presence of the C-10 carbomethoxy group in the chlorophylls of the a and b series and in BChl a (Boxer and Closs, 1976; Shipman et al., 1975; Fong, 1974).

II. CHLOROPHYLL DIMER MODELS OF P700 AND P865

We have developed synthetic methods that have resulted not only in the preparation of covalently bound dimeric species of both Chl a and BChl a, but have general utility in the preparation of PChl a dimer and many other chlorophyll species requiring high yield esterification at the propionic acid side chain (Wasielewski et al., 1976, 1977b).

In a typical example BChl a, obtained from R. spheroides, was treated with trifluoroacetic acid to obtain bacteriopheophorbide a (BPhide a), which was esterified at 25° with ethylene glycol using benzotriazole-N-methanesulfonate and Et_3N in dry THF. The glycol monoester was coupled with an equivalent of BPhide a using the same esterification method, but substituting 4-dimethylaminopyridine as the base and CH_2Cl_2 as the solvent. Re-insertion of the magnesium (Wasielewski, 1977a) results in the structure illustrated in Figure 1. Each bis(chlorophyllide) ethylene glycol diester is prepared in an analogous fashion and is obtained in 30-50% overall yield based on the starting Chl.

The structure of each covalently linked dimer under differing solvation conditions is based upon ^1H nmr spectral evidence. The ^1H nmr spectrum of each compound dissolved in nucleophilic solvents, i.e., those solvents which provide the Mg atoms with a 5th or 6th ligand, e.g. pyridine, is identical with that of the corresponding monomer under these same conditions, except for an additional singlet at about δ 4.00 ppm due to the protons of the glycol. This is illustrated in Figure 2A for BChl a covalent dimer. Similarily, the electronic transition spectrum of each dimer dissolved in nucleophilic solvents is identical to that of its corresponding monomer.

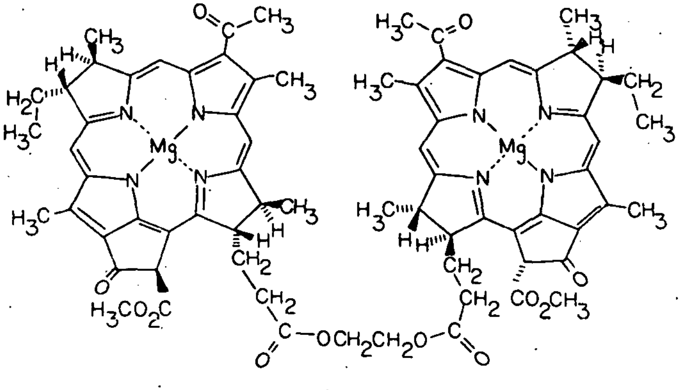

Fig. 1. Structure of bis(bacteriochlorophyllide a) ethylene glycol diester.

Dissolving each dimer in dry benzene or carbon tetrachloride results in broad, ill-defined proton nmr spectra as illustrated in Figure 2B. This change is accompanied in each case by a shift in the electronic transition spectrum to longer wavelength, 667 nm in the case of Chl a and PChl a dimers and 780 nm in the case of BChl a dimer. These spectra are consistent with the formation of aggregates in which the oxygen atom of C-9 keto carbonyl of one chlorophyll coordinates to the Mg atom of another chlorophyll in either an intra- or intermolecular fashion (Closs et al., 1963).

However, treating these dry solutions with a large (10-100 x) molar excess of water, primary alcohols, or primary thiols results in a dramatic sharpening of the proton resonances. This is illustrated in Figure 2C. A comparison between the spectra in Figures 2A and 2C reveals that several resonances of the dimer in the water saturated solution are substantially shifted from their respective positions in the fully disaggregated species. These chemical shift changes are closely paralleled in both the PChl a and Chl a dimers. Since all three dimers display only one set of resonances in the wet benzene solution, the two rings of each dimer must be equivalent on the ^{1}H nmr time scale. Interestingly, the methyl resonance of the acetyl groups shifts very little from its position in the disaggregated dimer in Figure 2A. As the changes in chemical shift are due to the influence of the diamagnetic ring current of one macrocycle on its dimeric partner (Abraham et al., 1977), the ^{1}H nmr data suggest that the macrocycles are on the average, parallel to each other with the III and V rings of each macrocycle experiencing the greatest interannular overlap, and that the acetyl groups do not participate in folding the dimer through hydrogen bonding interactions. Thus, the evidence suggests that the BChlide a covalent dimer and indeed the PChl a and Chl a dimers fold into the C_2 symmetric structure depicted in Figure 3.

The folding of the PChl a and Chl a dimers into the configuration depicted in Figure 3 is accompanied by wavelength shifts of the longest wavelength absorption in their electronic transition spectra to 694 nm. The Chl a linked dimer retains a shoulder at 667 nm of approximately one-third the oscillator strength of the 694 nm band (Figure 4). At equilibrium in vitro the carbomethoxy group at C-10 is partially epimerized to form about 15% of the a' isomer. Because the special pair model is dimeric, the stereoisomerism at C-10 results in a distribution that consists of about 73% a-a isomer, 25% a-a' isomer, and 2% a'-a' isomer. The a-a and a'-a' isomers are both able to fold into the closely spaced C_2 symmetric, stacked structure proposed for the folded species (Fig. 3). In this structure both carbomethoxy groups point away from the interior of the structure. However, space-filling models predict that the a-a' isomer, which comprises about 25% of the equilibrium mixture should have difficulty folding into a similar structure because of steric compression of one carbomethoxy group between the macrocycles.

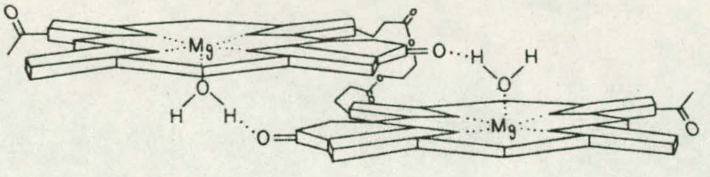

Fig. 3. Covalently linked BChl a dimer in the C_2 symmetric folded configuration.

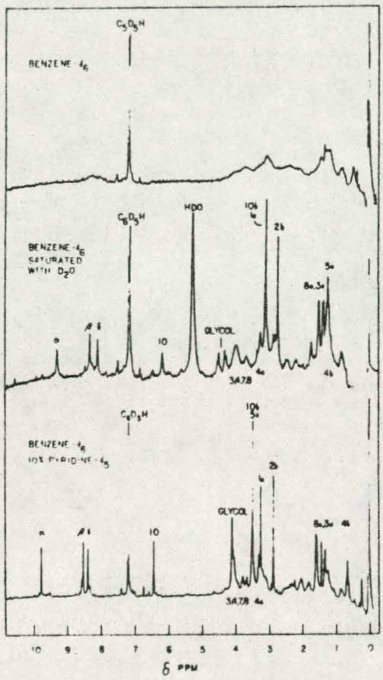

Fig. 2. ^1H nmr spectra of the BChl a covalent dimer dissolved in the indicated solvents.

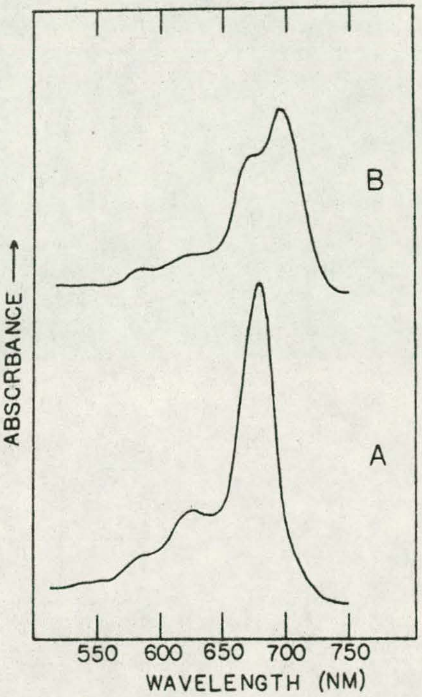

Fig. 4. The electronic transition spectrum of 10 μM Chl a covalent dimer in (A) dry CCl_4; and (B) water-saturated CCl_4.

The 694 nm absorption of the Pyrochl a dimer does not exhibit a short wavelength shoulder. This result is consistent with the proposed greater degree of conformational freedom in the Pyrochl a dimer. Similarly, the BChl a dimer does not exhibit a short wavelength shoulder due to the >99% stereochemical (natural isomer) purity at C-10.

Both the PChl a and Chl a dimers in their folded configuration exhibit electronic absorption maxima at 694 nm and thus mimic the P700 absorption fairly well. Exciton calculations based on the structure of these dimers suggested by the ^1H nmr spectra predict an absorbance at 693 nm (Shipman and Katz, 1977).

Illumination of a 10 μM solution of PChl a or Chl a dimer in water saturated CCl_4 or toluene containing an equivalent of I_2 with red light (λ>648 nm) results in complete bleaching of the 694 nm band in <30 seconds. The bleaching is accompanied by the formation of a Gaussian photo-EPR signal with 7.54 G linewidth at g = 2 (Wasielewski et al., 1976).

This linewidth corresponds well to that predicted by theory for an electron delocalized equally over two chlorophyll a macrocycles and compares favorably with the EPR linewidth obtained via photooxidation of P700 in Chlorella vulgaris (Norris et al., 1971).

The BChl a dimer absorbs light at 803 nm nanometers in its folded configuration (Figure 5). Clearly this value is nowhere near the 865 nm absorption of the phototrap in R. spheroides (Parson and Cogdell, 1975; Clayton, 1973). Yet, the 803 nm signal photobleaches and the EPR linewidth of the cation radical produced upon one electron oxidation of the BChl dimer is line narrowed by the Norris $1/\sqrt{2}$ relationship. Moreover, the cation radical of the BChl a dimer displays a strong absorption at 1150 nm, not very far removed energetically from the 1250 nm absorption observed in vivo (Dutton et al., 1975).

Thus, the covalently linked BChl a dimer mimics the properties of oxidized bacterial photoreaction center chlorophyll quite well, yet absorbs light in the ground state at 803 nm instead of 865 nm. At this point it is well to remember that we are probing the properties of an isolated pair of BChl a molecules in a given average structure and that therein lies the strength of the model. Interaction of the in vivo bacteriochlorophyll special pair with the additional two BChl a and two BPheo a molecules present in the bacterial reaction center may shift the special pair optical transition to longer wavelengths. The important point to be made is that these additional chromophores need not be involved in the delocalization of the unpaired spin in the oxidized special pair.

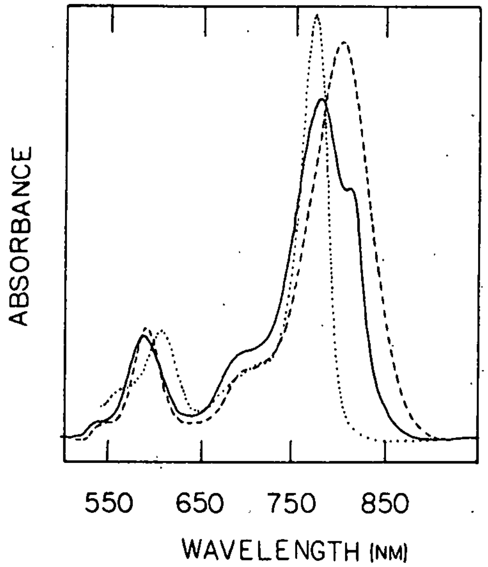

Fig. 5. The electronic transition spectrum of 10 μM BChl a covalent dimer in 10% pyridine in benzene (···), in dry benzene (——), and in water-saturated benzene (---).

III. BIS(CHLOROPHYLL) CYCLOPHANES: SPECIAL PAIR MODELS WITH STRUCTURAL CONSTRAINTS

Since the configuration of the singly linked dimers responsible for their biomimetic properties is both dynamic and highly solvent dependent, studies of special pair properties as a function of structure are somewhat restricted. Thus, in order to test our hypotheses regarding the relationship between optical data and dimer redox properties we decided to prepare a special pair model in which the Q_y transition dipole of one macrocycle is held fixed at a 90° orientation relative to that of the second macrocycle. The result of this effort is a bis(chlorophyll) cyclophane which is currently under intensive study and is proving to be an intruiging molecule.

The preparation of this molecule is depicted schematically in Figure 6 (Wasielewski et al., 1978).

The proton nmr spectra of 7 and 8 indicate that both macrocycles in each cyclophane are equivalent and therefore support a symmetric structure. The protons at the periphery of the macrocycles in 7 and 8 experience small chemical shift changes relative to monomeric meso-methyl pyropheophorbide a (9) and its magnesium complex (10), respectively (Table I). Since these shifts result from the influence of the diamagnetic ring current of the adjacent macrocycle, their small magnitude suggests a structure for 7 and 8 in which the macrocycles are stacked

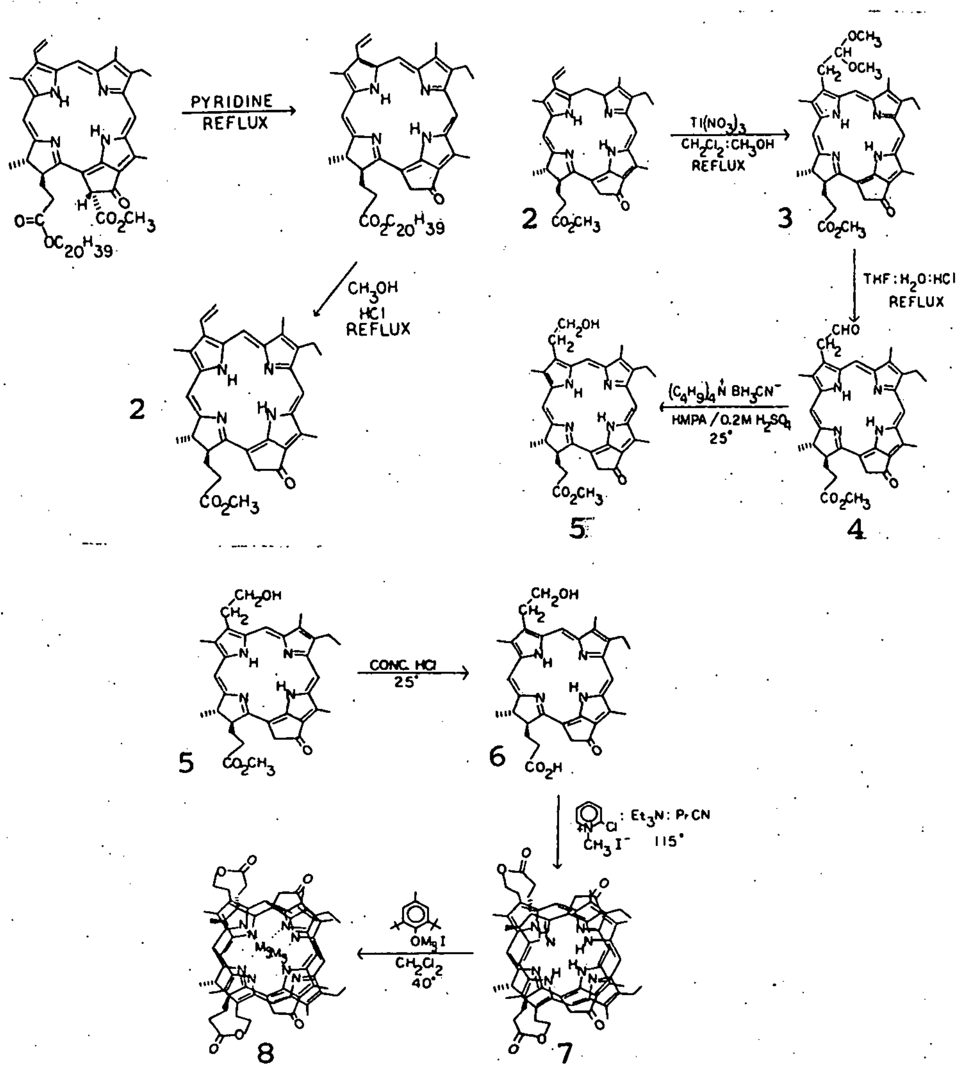

Fig. 6. Synthetic pathway for the preparation of bis(chlorophyll) cyclophane 8.

approximately center to center (Abraham et al., 1977). Moreover, the pyrrolic NH protons of 7 occur 0.75 ppm upfield of those in 9 consistent with the positioning of these protons directly above the π system of the adjacent macrocycle. The data do not preclude the possibility that there may be a small angle of tilt between the planes which the macrocycles occupy. Space filling models indicate that the angle of twist between the ring I - ring III axes of the macrocycles may oscillate about 40° either side of the 90° average configuration. An x-ray crystallographic study is in progress to define the structure in more detail.

The Q_y transition in the optical spectra of 7 and 8 occurs at 658 nm and 650 nm respectively both at room temperature and at 77°K. These transitions do not exhibit the solvent dependency

Table I. Proton Magnetic Resonance Data

Position	Chemical shift, δ (ppm)			
	7^a	9^a	8^b	10^b
4b	1.27	1.64	1.47	1.59
8a	1.53	1.72	1.49	1.62
7a, 7b	1.6–2.3	1.6–2.3	1.8–2.4	1.8–2.4
1	3.05	3.18	2.87	3.05
3	3.10	3.22	3.23	3.07
4a	3.70	3.81	3.50	3.62
5	3.57	3.54	3.57	3.15
7d CO_2CH_3		3.59		3.67
$-\mathrm{C}H_2\mathrm{CH_2O}-$	3.75		3.76	
$\mathrm{CH_2C}H_2\mathrm{O}-$	3.90		3.86	
7	4.19	4.24	4.28	3.90
8	4.45	4.46	4.28	4.05
10	4.67	5.09	4.81	4.97
δ	7.97	8.38	8.38	8.27
α	8.79	9.13	8.90	9.29
β	9.22	9.40	9.62	9.73
NH	−2.42	−1.67		

[a] In chloroform-*d*. [b] In 10% pyridine-d_5 in benzene-d_6.

characteristic of structural changes in the singly linked chlorophyll dimers. Moreover, the spectra of 7 and 8 are virtually identical with those of their monomeric counterparts 9 and 10 respectively both at 297°K and 77°K. This result is consistent with a rigid structure for 7 and 8 in which the Q_y transition dipoles are completely uncoupled.

The fluorescence maxima of 7 and 8 are unchanged relative to monomeric 9 and 10 with maxima at 666 nm and 657 nm (420 nm excitation), respectively in acetone. Despite the proximity (∿4-6 Å) of the macrocycles in the cyclophane structure there is no evidence of exciton splitting.

Thus, the cyclophane structure is sufficiently rigid so that optical spectroscopy of the singlet manifold over a wide range of temperatures reveals virtually no electronic interaction between the macrocycles. However, an examination of the doublet state properties of 8 indicates quite the opposite.

Cyclophane 8 undergoes photooxidation when irradiated with 650 nm light in the presence of electron acceptors, e.g., iodine, quinone, etc. and chemical oxidation by $ZnTPP^{\dagger}$ (Borg et al., 1970) to yield a cation radical possessing a Gaussian EPR signal with 6.44 G linewidth. Based on the treatment of Norris et al., (1971) the narrowing of this line relative to the 9 G linewidth of monomeric Chl a indicates that once again spin is shared equally by the two macrocycles analogous to special pair chlorophyll in vivo.

Since the structure of the cyclophane is primarily solvent independent unlike that of the singly linked covalent chlorophyll dimers, we were able to carry out a series of electrochemical experiments on this molecule in polar media. The redox potentials of 8 were determined accurately by ac voltammetry and are listed in Table II. Cyclophane 8 undergoes reversible one electron oxidation 70 mV more easily than chlorophyll a. This result is consistent with the delocalization of unpaired electron density over both macrocycles in the cation radical of 8. Characteristically chlorophyll special pairs in vivo are more readily oxidized than bulk chlorophyll in the organisms. Controlled potential oxidation of 8 with removal of one coulombic equivalent in a cell designed for optical observation of the electrolyzed species yielded the electronic transition spectrum of the cation radical. The spectrum is characterized by a broad maximum at 800 nm slightly blue shifted from typical chlorophyll radical cations in the a series.

Table II. Redox Potentials vs. SCE

	Oxidation[a,b]		Reduction[b,c]	
Compd	$E^1_{1/2}$	$E^2_{1/2}$	$E^1_{1/2}$	$E^2_{1/2}$
8	0.54	0.77	−1.29	−1.77
Chlorophyll a	0.61	0.84	−1.14	−1.61

[a] In $CH_3CH_2CH_2CN$. [b] 0.1 M tetra-*n*-butylammonium perchlorate supporting electrolyte. [c] In DMF.

The reversible one electron reduction of 8 is more difficult than that of Chl a by 150 mV. This is indeed a surprising result. Cyclophanes of aromatic molecules characteristically are both oxidized and reduced more readily than their monomeric constituents. This effect is due once again to delocalization of charge over both macrocycles for both radical cations and anions. Interestingly, EPR experiments on the radical anion of 8 generated via potassium reduction of HMPA show that the anion is in fact localized on one macrocycle.

The photoexcited triplet states of 7 and 8 have also been examined and present yet another surprising set of results. The triplets were generated by irradiation of 10^{-3} M compound in 10% pyridine-toluene glass at the temperatures indicated in Table III. The surprising feature of the data is the equivalence of the zero field splitting parameters for each cyclophane and its corresponding monomer. If, as we initially expected, the triplet excitation is rapidly exchanging between both macrocycles, the C_2 symmetric structure of 7 and 8 with the Q_y transition dipoles at 90° to one another requires that $E=0$. However, this is not the case and it thus appears that even the triplet excitation is confined to one macrocycle. Varying solvation conditions, e.g. 2-methyl-tetrahydrofuran, do not change the result.

Several workers are currently engaged in attempts to determine the relative orientation of the macrocycles with respect to one another in vivo by comparing in vitro chlorophyll triplet data with that obtained by generating triplets of the special pair (Clarke et al., 1976; Kleibeuker and Schaafsma, 1974; Levanon and Sherz, 1975; Hagele et al., 1976; Thurnauer and

Norris, 1977). It is assumed in all cases that the excitation is exchanging between the macrocycles of the pair. Changes in the zero field splitting parameter D in vivo relative to monomeric BChl a suggest that the triplet excitation in the reaction center is exchanging between two or more sites. Yet, there is no evidence in vivo based on triplet hyperfine interactions to confirm either the existence or extent of this exchange. In vitro studies of chlorophyll dimer triplets face similar uncertainties. The fact that our cyclophane model special pair yields a localized triplet should at the very least be regarded as a cautionary note in further conclusions concerning special pair structure derived via triplet state EPR.

TABLE III. Zero Field Splittings for Selected Compounds at 4.2 in 10% Pyridine-Toluene Glass

Compound	|D|	|E|
7	0.0345	0.0027
9	0.0345	0.0024
8	0.0300	0.0036
8*	0.0301	0.0038
8**	0.0302	0.0038
10	0.0301	0.0036

*65 K.

**100 K.

The cyclophane models show that the optical and EPR properties of special pairs in vivo may be indicative of very different features of the special pair. The only evidence that photoreaction center chlorophyll in both purple photosynthetic bacteria and in green plants consists of a pair of chlorophyll molecules is derived from magnetic resonance experiments. On the other hand, all known reaction center chlorophylls possess a long wavelength optical transition which is red shifted relative both to that of bulk antenna chlorophyll in the organisms and to that of the corresponding monomeric chlorophyll in vitro.

The results of our cyclophane studies support the proposal that special pair geometries exist which adequately account for both the redox and spin delocalization properties of chlorophyll special pairs in vivo, yet do not give rise to unusually red-shifted optical spectra. Thus, we have shown that the optical spectra exhibited by chlorophyll special pairs in vivo need not be exclusively an intrinsic property of the pair itself but may be influence strongly both by the presence of additional chromophores and by important chlorophyll-protein interactions.

ACKNOWLEDGEMENTS

This work was performed under the auspices of the Division of Basic Energy Sciences of the Department of Energy.

REFERENCES

Abraham, R. J., Fell, S. C. M., and Smith, K. M. (1977). Org. Magn. Res. 9:367.

Anton, J. A., Kwong, J., and Loach, P. A. (1976). J. Heterocyclic Chem. 13:717.

Borg, D. C., Fajer, J., Felton, R. H., and Dolphin, D. (1970). Proc. Natl. Acad. Sci. USA 67:813.

Boxer, S. G., and Closs, G. L. (1976). J. Am. Chem. Soc. 98:5406.

Clarke, R. H., Connors, R. E., Schaafsma, T. J., Kleibeuker, J. F., and Platenkamp, R. J. (1976). J. Am. Chem. Soc. 98:3674.

Clayton, R. K. (1973). Ann. Rev. Biophys. Bioeng. 2:131.

Closs, G. L., Katz, J. J., Pennington, F. C., Thomas, M. R., and Strain, H. H. (1963). J. Am. Chem. Soc. 85:3809.

Collman, J. P., Elliot, C. M., Halbert, T. R., and Tovrog, B. S. (1977). Proc. Natl. Acad. Sci. USA 74:18.

Dutton, P. L., Kaufmann, K. J., Chance, B., and Rentzepis, P. M. (1975). FEBS Lett. 60:275.

Fong, F. K. (1974). Proc. Natl. Acad. Sci. USA 71:3692.

Hagele, W., Drissler, F., Schmid, D., and Wolf, H. C. (1976). "ESR Studies of the Photoexcited Triplet States of Chlorophyll a and Chlorophyll b in Poly(methyl methacrylate) and Methyl Tetrahydrofuran at 4.2 K." Presented at Third Int. Seminar on Excitation Energy Transfer in Condensed Matter, Prague, Czechoslovakia.

Kagan, N. E., Mauzerall, D., and Merrifield, R. B. (1977). J. Am. Chem. Soc. 99:5484.

Kleibeuker, J. F. and Schaafsma, T. J. (1974). Chem. Phys. Lett. 29:116.

Levanon, H. and Scherz, A. (1975). Chem. Phys. Lett. 31:119.

Norris, J. R., Uphaus, R. A., Crespi, H., and Katz, J. J. (1971). Proc. Natl. Acad. Sci. USA 68:625.

Ogoshi, H., Sugimoto, H., and Yoshida, Z. (1977). Tetrahedron Lett. 169.

Parson, W. W. and Cogdell R. J. (1975). Biochim. Biophys. Acta 416:105.

Schwarz, F. P., Goutermann, M., Muljiani, Z., and Dolphin, D. H. (1972). Bioinorg. Chem. 2:1.

Shipman, L. L., Janson, T. R., Ray, G. T., and Katz, J. J. (1975). Proc. Natl. Acad. Sci. USA 72:2873.

Shipman, L. L., and Katz, J. J. (1977). J. Phys. Chem. 81:577.

Thurnauer, M. C., and Norris, J. R. (1977). Chem. Phys. Lett. 47:100.

Wasielewski, M. R., Studier, M. H., and Katz, J. J. (1976). Proc. Natl. Acad. Sci. USA 73:4282.

Wasielewski, M. R. (1977a). Tetrahedron Lett. 1373.

Wasielewski, M. R., Smith, U. H., Cope, B. T., and Katz, J. J. (1977b). J. Am. Chem. Soc. 99:4172.

Wasielewski, M. R., Svec, W. A., and Cope, B. T. (1978). J. Am. Chem. Soc. 100:1961.