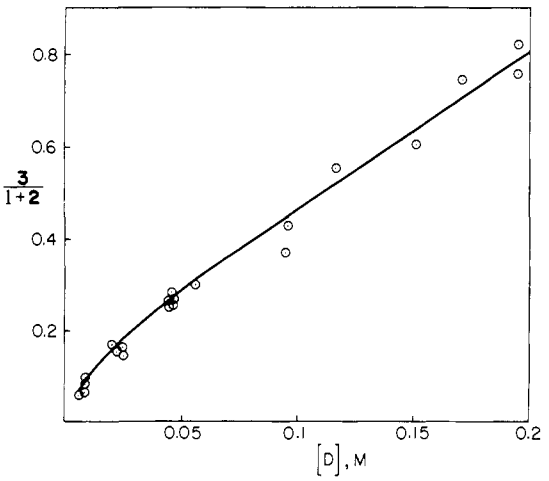

Figure 2. Plot of $3/(1 + 2)$ (analyzed by NMR) vs. [D]. The data were obtained from irradiations ($\lambda \geq 405$ nm) of saturated solutions of DCA or TCA under N_2. The concentration of D decreased by ca. 0.003 M during the irradiation. The points are placed at the average of the starting and final concentrations. The curve is calculated from $3/(1 + 2) = 3.3[D] + 0.15[D]/([D] + 0.015)$.

benzene, which has a lower oxidation potential than D, and correcting for its competing reaction with S^{*1}, we observed inefficient quenching of dimerization, which suggests that k_2 is almost diffusion controlled (ca. 10^{10} M^{-1} s^{-1}). This means that k_1 is $5–6 \times 10^8$ s^{-1}, which is well within the expected range.[7] Accordingly, the reaction constant for the reverse electron transfer, k_3, is $\sim 2.2 \times 10^9$ s^{-1} for $DCA^{-}\cdot/D^{+}\cdot$ and $\sim 1.5 \times 10^{10}$ s^{-1} for $TCA^{-}\cdot/D^{+}\cdot$.

According to the proposed mechanism, the ratio $3/(1 + 2)$ should increase with [D], as shown by eq 2,[8] and is likely to be

$$\frac{3}{1+2} = \frac{\alpha k_2}{k_1}[D] + \frac{k_4}{k_5}\frac{[D]}{k_{-4}/k_6 + [D]} \qquad (2)$$

similar with DCA and TCA. Indeed, similar dependence of the ratio on [D] was obtained with both sensitizers (Figure 2).

The reversible formation of the cyclobutane radical cation, $3^{+}\cdot$, and the proposed electron transfer from D to $3^{+}\cdot$ were incorporated into the mechanism in order to account for the deviation from linearity of the plot of $3/(1 + 2)$ vs. [D] at low concentrations of D.[9] The data in Figure 2 fit a curve obtained by use of the following sets of constants in eq 2: $\alpha k_2/k_1 = 3.3$; $k_4/k_5 = 0.15$; $k_{-4}/k_6 = 0.015$. From the value of 3.3 for $\alpha k_2/k_1$ and the ratio k_2/k_1, which was shown above to be ca. 18, α is estimated to be 0.18.[8] In spite of this low efficiency for cyclization, the quantum yield of cyclobutane formation increases steadily with increasing [D] because interception of the geminate pair competes with the energy-wasting reverse electron transfer, which is the major reaction path for the geminate pair. From the ratio k_{-4}/k_6, which can have considerable error, k_{-4} can be estimated by assuming that k_6 is diffusion controlled (1.5×10^{10} M^{-1} s^{-1}), i.e., k_{-4} is $2–3 \times 10^8$ s^{-1}.

Strong support for the proposed mechanism of interception of the geminate radical ion pair was obtained by irradiation in the presence of biphenyl (BP). Electron transfer to TCA^{*1} from both D and BP is exothermic and proceeds, as judged by fluorescence quenching, at the diffusion-controlled rate. Thus, both compounds compete for the excited sensitizer. BP has higher oxidation potential than D, so electron transfer from D to $BP^{+}\cdot$, being exothermic, should be an efficient reaction leading to $D^{+}\cdot$. Since this process circumvents the geminate pair $S^{-}\cdot/D^{+}\cdot$ and leads directly to $D^{+}\cdot$, the ratio $3/(1 + 2)$ would be expected to decrease with increasing [BP] and to level off at a value corresponding to the products formed from the out-of-cage reactions.

The results agree well with this prediction. For example, the ratio $3/(1 + 2)$ at [D] = 0.05 M decreases from ca. 0.29 in the absence of BP to ca. 0.23 at 0.05 M, 0.20 at 0.1 M, and 0.15 at 0.4 M BP.[12]

The data presented here show that the interception of the geminate pair leads to the biradical Ḋ–Ḋ, which cyclizes predominantly or exclusively to the cyclobutane, whereas the radical cation Ḋ–D^{+}, from the out-of-cage reaction, undergoes 1,4- and 1,6-cyclization in the ratio of only 0.15:1.0.[13] This study has also shown that the reaction constant for the reverse electron transfer in the geminate radical ion pair seems to *decrease* with increasing energy content stored in the radical ion pair, i.e., with *increasing* exothermicity of the reaction. We are evaluating this phenomenon in related reactions and also the structure of the interceptable geminate pair. These points will be discussed in a future publication.

Acknowledgment. We thank Professor Jack Kampmeier of the University of Rochester for helpful discussions.

Registry No. 1, 41977-31-5; **2**, 72805-46-0; **3**, 84537-61-1; D, 530-48-3.

(7) Schulten, K.; Staerk, H.; Weller, A.; Werner, H. J.; Nickel, B. *Z. Phys. Chem. Wiesbaden* **1976**, *101*, 371. Weller, A. *Ibid.* **1982**, *130*, 129. See also ref 2.

(8) α is the overall efficiency of dimerization via the intercepted geminate pair. The energy-wasting steps along this path are the cleavage of the biradical and/or reverse electron transfer in the intermediate $S^{-}\cdot/D^{+}\cdot\cdots D$. The latter process is not shown in the scheme.

(9) The involvement of similar chain propagation steps is frequently encountered in cyclobutadimerization whenever the oxidation potential of the dimer is higher than that of the monomer.[10] The cyclobutane **3** does not quench the fluorescence of DCA, which shows that this condition applies for the present system. There are also several examples of reversible cyclizations[11] analogous to $D–D^{+} \rightleftharpoons 3^{+}\cdot$.

(10) Ledwith, A. *Acc. Chem. Res.* **1972**, *5*, 133.

(11) Ledwith, A. *J. Chem. Soc. C* **1970**, 2508. Evans, T. R.; Wake, R. W.; Jaenicke, O. In "The Exciplex"; Gordon, M.; Ware, W. R., Eds.; Academic Press: New York, 1975; p 345. Majima, T.; Pac, C.; Sakurai, H. *J. Am. Chem. Soc.* **1980**, *102*, 5265 and references therein.

(12) The efficiency of ion separation from the pair $TCA^{-}\cdot/BP^{+}\cdot$ is ca. 1.6 times higher than that of the $TCA^{-}\cdot/D^{+}\cdot$ pair, as judged by an increase in ϕ_{1+2} with increasing [BP]/[D].

(13) In the electron-transfer-sensitized photooxygenation of D, the biradical Ḋ–Ḋ and the radical cation Ḋ–D^{+} are intercepted by O_2 forming 3,3,6,6-tetraphenyl-1,2-dioxane.[14]

(14) This dioxane was reported first by Haynes: Haynes, R. K.; Probert, M. K. S.; Wilmot, J. D. *Aust. J. Chem.* **1978**, *31*, 1737.

Aggregation of the Bacteriochlorophylls *c*, *d*, and *e*. Models for the Antenna Chlorophylls of Green and Brown Photosynthetic Bacteria

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Chlorophylls and bacteriochlorophylls function *in vivo* as antenna and phototraps that harvest light and provide the source of chemical oxidants and reductants that drive the biochemistry of photosynthetic organisms.[1] The visible absorption spectra of (bacterio)chlorophylls in vivo are often significantly red shifted relative to those of monomeric species in vitro.[2] A considerable

(1) (a) Clayton, R. K., Sistrom, W. R., Eds. "The Photosynthetic Bacteria"; Plenum Press: New York, 1978. (b) Govindjee, Ed. "Bioenergetics of Photosynthesis"; Academic Press: New York, 1975. (c) Olson, J. M.; Hind, G. *Brookhaven Symp. Biol.* **1976**, *28*.

(2) Katz, J. J.; Norris, J. R.; Shipman, L. L., ref 1c, pp 15–66. Cogdell, R. J.; Thornber, J. P. *Ciba Found. Symp.* **1979**, *61*, 61–79. Thornber, J. P.; Trosper, T. L.; Strouse, C. E., ref 1a, pp 133–160.

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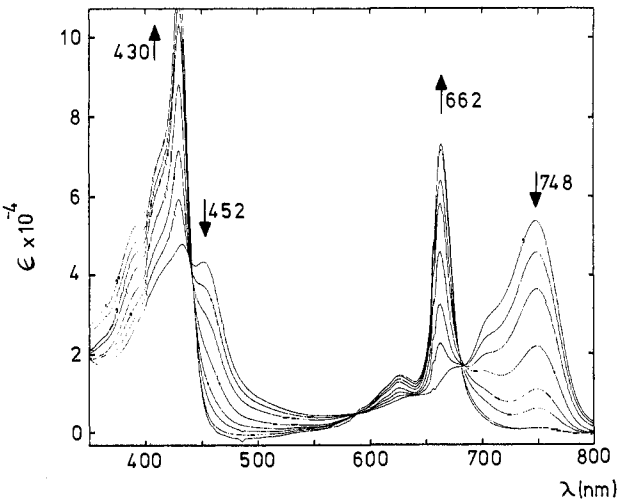


Figure 1. Spectrophotometric titration of a 1.95×10^{-5} M solution of bacteriochlorophylls *c* (**1**) in hexane–methylene chloride (200:1). During the titration, 1-μL aliquots of methanol were added to a 1-cm cell containing 4 mL of solution.

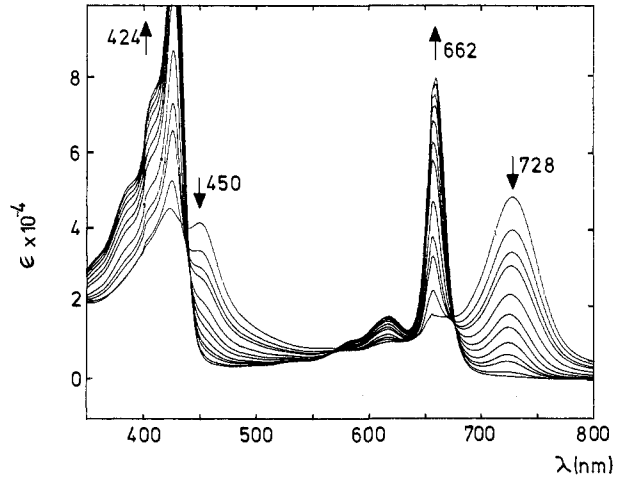


Figure 2. Spectrophotometric titration of a 1.65×10^{-5} M solution of zinc(II) methyl bacteriopheorbides *c* in hexane–methylene chloride (200:1). During the titration, 1-μL aliquots of methanol were added to a 1-cm cell containing 4 mL of solution.

body of data, based on infrared,[3] visible,[2] resonance Raman,[4] fluorescence,[5] circular dichroism,[6] magnetic resonance,[3,7] and X-ray techniques,[8] has provided evidence for dimerization, aggregation, and chlorophyll–protein interactions to account for the various (bacterio)chlorophyll forms observed in vivo. The vast majority of models proposed have invoked either direct or hydrogen-bonded nucleophilic interactions between the metal and the 9-keto and/or the carbomethoxy groups of ring V of the chlorophylls.[2,3,8a,9-11] We describe here aggregates of bacterio-

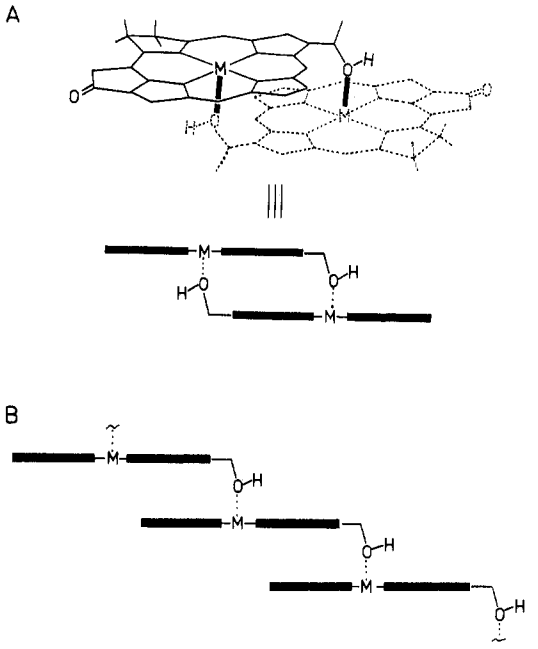
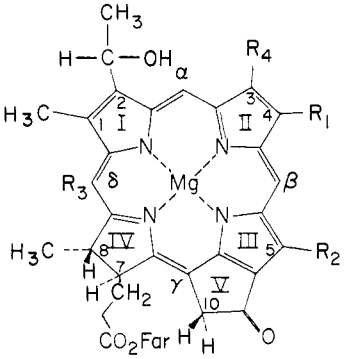


Figure 3. Schematic representation of aggregates responsible for red-shifted optical spectra: (A) possible dimeric species; (B) more likely oligomeric aggregate.

chlorophylls *c*, *d*, and *e* and of zinc(II) methyl bacteriopheophorbides *c* and *d*, which exhibit large red shifts in hexane *without* the essential intervention of any carbonyl function on ring V.[12] The aggregates appear to provide viable in vitro models for the antenna chlorophylls of green and brown photosynthetic bacteria[13] and a rationale for the presence of the 2-(1-hydroxyethyl) group in bacteriochlorophylls *c*, *d*, and *e*.

When bacteriochlorophyll *c* (**1**)[13] was dissolved in a minimum



1, R^1 = Et, *n*-Pr, or *i*-Bu; R^2 = Me or Et; R^3 = R^4 = Me
2, R^1 = Et, *n*-Pr, *i*-Bu, or *neo*-Pn; R^2 = Me or Et; R^3 = H; R^4 = Me
3, R^1 = Et, *n*-Pr, or *i*-Bu; R^2 = Et; R^3 = Me; R^4 = CHO

of methylene chloride and diluted with a large excess of hexane, a pronounced change from green to yellow was observed (Figure 1).[14] The formation of an aggregate was confirmed by addition

(3) Katz, J. J.; Shipman, L. L.; Cotton, T. M.; Janson, T. R. "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. 5, pp 401–458.

(4) Lutz, M.; Brown, J. S.; Remy, R. *Ciba Found. Symp.* **1979**, *61*, 105–125. Lutz, M. *Biochim. Biophys. Acta* **1977**, *460*, 408–430. Lutz, M.; Kleo, J. *Ibid.* **1979**, *546*, 365–369.

(5) Papageorgiou, G., in ref 1b, pp 320–371.

(6) Olson, J. M., in ref 1a, pp 161–178. Sauer, K., in ref 1b, pp 116–181.

(7) Katz, J. J.; Norris, J. R.; Shipman, L. L.; Thurnauer, M. C.; Wasielewski, M. R. *Ann. Rev. Biophys. Bioeng.* **1978**, *7*, 393–434. Feher, G.; Hoff, A. J.; Isaacson, R. A.; Ackerson, L. C. *Ann. N.Y. Acad. Sci.* **1975**, *244*, 239–259. Hoff, A. J. *Biophys. Struct. Mech.* **1982**, *8*, 107–150. Fajer, J.; Fujita, I.; Davis, M. S.; Forman, A.; Hanson, L. K.; Smith, K. M. *Adv. Chem. Ser.* **1982**, *No. 201*, 489–514.

(8) (a) Chow, H. C.; Serlin, R.; Strouse, C. E. *J. Am. Chem. Soc.* **1975**, *97*, 7230–7237. (b) Mathews, B. W.; Fenna, R. E. *Acc. Chem. Res.* **1980**, *13*, 309–317. (c) Barkigia, K. M.; Fajer, J.; Smith, K. M.; Williams, G. J. B. *J. Am. Chem. Soc.* **1981**, *103*, 5890–5893. (d) Smith, K. M.; Goff, D. A.; Fajer, J.; Barkigia, K. M. *J. Am. Chem. Soc.* **1982**, *104*, 3747–3749.

(9) Boxer, S. G.; Closs, G. L. *J. Am. Chem. Soc.* **1976**, *98*, 5406–5408. Fong, F. K.; Koester, V. J. *Ibid.* **1975**, *97*, 6888–6890.

(10) Bystrova, M. I.; Mal'gosheva, I. N.; Krasnovskii, A. A. *Mol. Biol.* **1979**, *13*, 440–451. Krasnovakii, A. A.; Bystrova, M. I. *Biosystems* **1980**, *12*, 181–194.

(11) Brockmann, H., Jr.; Trowitzsch, W.; Wray, V. *Org. Magn. Reson.* **1976**, *8*, 380–381.

(12) Red-shifted aggregates of bacteriochlorophylls *c* absorbing at 710 nm in CCl_4 and 710–745 nm in hydrocarbon–CCl_4 mixtures were first noted by Bystrova et al.[10] and attributed to interactions of the Mg atom with both the 9-keto and the 2-(1-hydroxyethyl) substituents.

(13) Bacteriochlorophylls *c* were obtained from *Prosthecochloris aestuarii* grown in conjunction with *Desulfuromonas acetoxidans*; the chlorophylls were isolated by using standard procedures: Smith, K. M.; Bushell, M. J.; Rimmer, J.; Unsworth, J. F. *J. Am. Chem. Soc.* **1980**, *102*, 2437–2448.

(14) Even in pure methylene chloride, a broadening on the long-wavelength side of the 662-nm absorption was observed, which could be readily removed by addition of methanol.

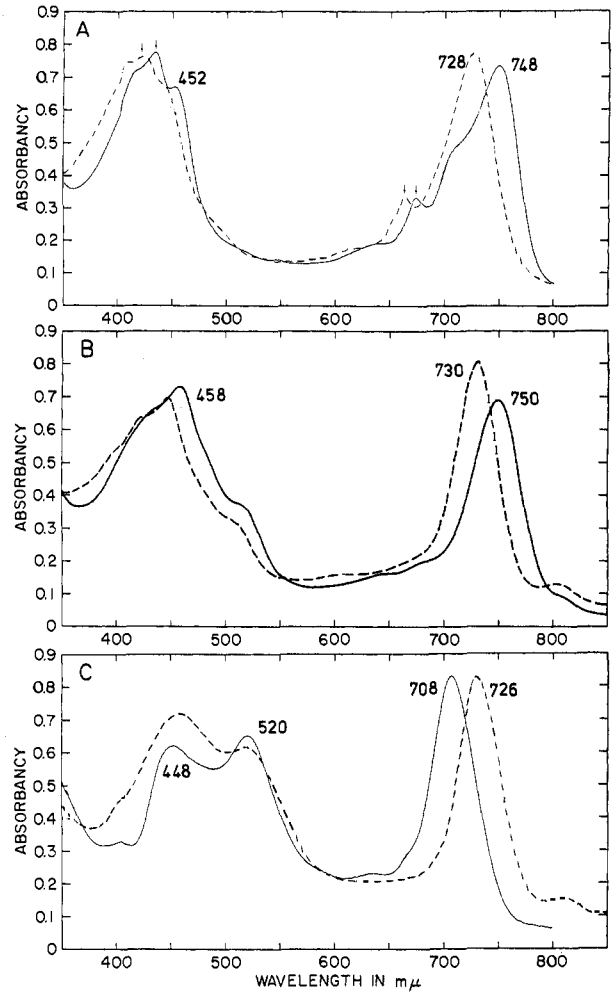


Figure 4. Electronic absorption spectra of (A) bacteriochlorophylls *c* (**1**) (full line) and bacteriochlorophylls *d* (**2**, broken line; both are in hexane–methylene chloride (200:1); arrowed peaks are due to contamination with unaggregated material), (B) cell-free extracts of green sulfur bacteria containing bacteriochlorophylls *c* (full line), and bacteriochlorophylls *d* (broken line;[19] the peak around 810 nm is due to bacteriochlorophyll *a*), (C) bacteriochlorophylls *e* (**3**) in hexane–methylene chloride (200:1, full line), and in living cells (broken line; both traces in this spectrum used bacteriochlorophylls *e* from *Chlorobium phaeovibrioides* (NCIB No. 2631), and the broken line spectrum is adapted from Gloe et al.[15] These authors have shown that the long-wavelength maximum in living cells of brown bacteria that produce bacteriochlorophylls *e* (**3**) can vary between 715 and 725 nm normally, and even down to 708 nm[22]).

of methanol; the long-wavelength peak at 748 nm gave way to the expected monomeric absorption at 662 nm, with observation of clean isosbestic points.

Bacteriochlorophylls *d*[8d] (**2**) and *e*[15] (**3**) also showed evidence of aggregation,[16] as did the zinc(II) complexes of methyl bacteriopheophorbides *c* (Figure 2) and *d*.[16] However, the optical spectra of the metal-free bacteriopheophorbides provided no evidence of aggregation, thereby clearly implicating the metal[17] in the formation of the aggregates.

The red shifts observed are reminiscent of those described[3,8a,9] for hydrated forms of chlorophyll *a* in which aggregation is proposed to be mediated by water molecules complexed to the magnesium and hydrogen-bonded to either the 9-keto or the carbomethoxy groups of ring V. Since the bacteriochlorophylls *c*, *d*, and *e* do not possess a carbomethoxy group on that ring, the aggregation obviously does not involve that group. Under the conditions described above, the zinc(II) complexes of methyl pyropheophorbide *a* (vinyl group at position 2) and methyl mesopyropheophorbide *a* (ethyl group at 2) showed no evidence of aggregation. Therefore, the 2-(1-hydroxyethyl) substituent is clearly involved in the aggregation. (These results also argue against a role by adventitious water.)

Although X-ray data[8d] provide evidence of cofacial dimer formation in methyl bacteriopheophorbide *d* in which the 2-(1-hydroxyethyl) groups hydrogen-bond to the 9-keto groups, a dominant role for the latter can be excluded in the case of the metallo complexes. Reduction of the carbonyl group of methyl bacteriopheophorbides *c* with sodium borohydride yielded a diastereomeric mixture of diols; the zinc(II) complexes of these diols, however, still showed spectra shifts similar to those displayed in Figures 1 and 2.[18]

We therefore propose either a dimer of the type illustrated in Figure 3A for the red-shifted species or (more likely) a larger aggregate (Figure 3B). NMR spectra (in cyclohexane-d_{12}, 360 and 500 MHz) reveal only broad featureless spectra from which no geometric information can be obtained but that tend to suggest an oligomeric (Figure 3B) rather than dimeric (Figure 3A) structure.

Finally, we note that a comparison of the absorption spectra reported here for the bacteriochlorophylls *c*, *d*, and *e* with those observed for green[15,19,20] and brown[15] bacteria containing the same chromophores reveals a close analogy between the aggregates in vitro and the pigments in vivo (Figure 4). It seems likely, therefore, that the red shifts observed in vivo are not caused by protein interactions but rather by aggregation.[21] Thus, one likely function of the 2-(1-hydroxyethyl) variation in the bacteriochlorophylls *c*, *d*, and *e* is to promote such aggregation and its concomitant modulation of the light-absorbing properties of the pigments in the antenna network of green and brown bacteria.

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Registry No. Bacterichlorophyll *c*, 53986-51-9; bacteriochlorophyll *d*, 8067-29-6; bacteriochlorophyll *e*, 55963-87-6; zinc(II) methyl bacteriopheophorbide *c*, 84623-13-2; zinc(II) methyl bacteriopheophorbide *d*, 84623-14-3.

(15) Bacteriochlorophylls *e* were obtained from *Chlorobium pheovibrioides* (NCIB No. 2631): Gloe, A.; Pfennig, N.; Brockmann, H., Jr.; Trowitzsch, W. *Arch. Microbiol.* **1975**, *102*, 103–109.

(16) See Figure 4A (broken line) for the electronic absorption spectrum of the bacteriochlorophylls *d* in hexane and Figure 4C (solid line) for that of the bacteriochlorophylls *e*. Zinc(II) bacteriopheophorbide *d* showed a hypsochromic shift (not shown) from 716 to 648 nm (long wavelength band) upon addition of methanol.

(17) Other metals such as Cu and Pd, which do not readily form pentacoordinate complexes, did not yield red-shifted species in hexane.

(18) The zinc(II) diol showed a hypsochromic shift of its long wavelength peak from 670 to 628 nm upon addition of methanol. Although a role in the aggregation for the hydroxyl group resulting from the reduction of the 9-keto group cannot be excluded on the basis of the present data, an intact 9-keto group is not required. This is not to say that the carbonyl group does not assist in the aggregation process both in vitro and in vivo (note the proximity of the carbonyl group to the Mg in Figure 3B), but rather that the carbonyl is not an absolutely essential structural requirement in vitro at least. Note also that the zinc(II) complex of the alcohol obtained by borohydride reduction of methyl pyropheophorbide *a* did not yield a red-shifted species in hexane.

(19) Olson, J. M. *Biochim. Biophys. Acta* **1980**, *594*, 33–51.

(20) Betti, J. A.; Blankenship, R. E.; Natarajan, L. V.; Dickinson, L. C.; Fuller, R. C. *Biochim. Biophys. Acta* **1982**, *680*, 194–201.

(21) Betti et al.[20] report that the ESR line width of oxidized bacteriochlorophylls *c* antenna in *Chloroflexus auranticus* is 2.3 G. When compared to the line width of 8.4 G found for the cation radical of bacteriochlorophylls *c* in methylene chloride–methanol (J. Fajer, I. Fujita, unpublished results), these results suggest that oligomeric rather than dimeric species exist in vivo. If the unpaired electron of the radical is equally shared among *n* molecules, then the line width of the oxidized aggregate is narrowed by $\sqrt{n}$ relative to that of a monomer.[7] Since the narrowing factor is ~3.6, the number of bacteriochlorophylls in the in vivo aggregate is estimated at ~13. Twelve to fourteen bacteriochlorophylls are believed to be associated with the 15 kdalton protein subunits that comprise the antenna rod elements of *Chlorobiaceae*.[19]

(22) N. Pfennig, personal communication.