Mimicking the Photosynthetic Triplet Energy-Transfer Relay

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Abstract: In the reaction centers of photosynthetic organisms, chlorophyll triplet states are sometimes formed by recombination of charge-separated intermediates. These triplets are excellent sensitizers for singlet oxygen formation. Carotenoid polyenes can provide photoprotection from singlet oxygen generation by rapidly quenching chlorophyll triplet states via triplet–triplet energy transfer. Because in bacteria the reaction center carotenoid is not located adjacent to the bacteriochlorophyll special pair, which is the origin of the charge separation, it has been postulated that quenching may occur via a triplet relay involving an intermediate chlorophyll monomer. We now report the synthesis and spectroscopic study of a covalently linked carotenoid (C)–porphyrin (P)–pyropheophorbide (Ppd) triad molecule which mimics this triplet relay. The pyropheophorbide singlet-state C–P–^{1}Ppd (generated by direct excitation or energy transfer from the attached porphyrin) undergoes intersystem crossing to the triplet C–P–^{3}Ppd. In oxygen-free solutions, this triplet decays to ^{3}C–P–Ppd through a triplet-transfer relay involving an intermediate C–^{3}P–Ppd species. In aerated solutions, quenching of C–P–^{3}Ppd by the attached carotenoid competes with singlet oxygen sensitization and thus provides a degree of photoprotection. In a similar triad containing a zinc porphyrin moiety, triplet transfer is slow due to the higher energy of the C–$^3P_{Zn}$–Ppd intermediate, and photoprotection via the relay is nonexistent. The triplet relay ceases to function at low temperatures in both the natural and biomimetic cases due to the endergonicity of the first step.

Introduction

The primary energy conversion step of photosynthesis is photoinitiated electron transfer to produce a charge-separated state whose stored energy can ultimately be used to meet the needs of the organism. In the photosynthetic bacterium *Rhodobacter sphaeroides*, for example, excitation of a special pair of bacteriochlorophyll molecules, $(Bchl)_2$, is followed by electron transfer from the excited singlet state to a nearby bacteriopheophytin to generate an energetic charge-separated intermediate. Under normal conditions, a subsequent series of electron-transfer steps yields long-lived, transmembrane charge separation. However, in some circumstances, such as when secondary electron carriers are reduced prior to charge separation, the intermediate charge-separated state recombines to give the triplet state of the special pair. This triplet can, in principle, migrate to other chlorophyll species. The chlorophyll triplet states are sensitizers for the generation of singlet oxygen via an energy-transfer process. The singlet oxygen thus produced is very reactive and harmful to the organism.[1-4]

Carotenoid polyenes provide photoprotection to photosynthetic organisms by rapidly quenching chlorophyll triplet states via triplet–triplet energy transfer so that they are not kinetically competent to produce singlet oxygen.[1-4] We have previously reported the synthesis and study of several dyads consisting of carotenoid polyenes covalently linked to porphyrins or other cyclic tetrapyrroles which mimic this photoprotective behavior.[5-8] In properly designed models, the carotenoid moiety rapidly quenches the triplet state of the attached cyclic tetrapyrrole via triplet–triplet energy transfer. This precludes singlet oxygen production because the carotenoid triplet state is incapable of sensitization and quickly decays to the ground state. The rapid triplet–triplet energy transfer occurs by an electron exchange mechanism, which in turn requires spatial overlap of the donor and acceptor orbitals. The model system studies have shown that the carotenoid and cyclic tetrapyrrole π-electron systems must be in virtual contact or must be well coupled through the linkage bonds if efficient photoprotection is to occur.

With these results in hand, the structure of the bacterial reaction center as deduced from X-ray structural investigations[9-11] presents a conundrum. The carotenoid polyene C is not located near the special pair but rather adjacent to, and in van der Waals contact with, an accessory bacteriochlorophyll ($Bchl_M$), which in turn lies near the special pair. This accessory bacteriochlorophyll has a triplet-state energy which is slightly higher than that of $(BChl)_2$.[12] How does the carotenoid effectively quench the triplet state of the special pair? It has been proposed that the accessory bacteriochlorophyll might act as a relay between the special pair and the carotenoid.[12-14] Thermally activated triplet transfer from $^3(Bchl)_2$ to $Bchl_M$ would yield $^3Bchl_M$, which could be rapidly quenched by the carotenoid to give ^{3}C. In order to investigate the viability of this proposal in a model system and explore the structural parameters required for efficient photoprotection, we have now prepared triad molecules **1** and **2**, which feature a

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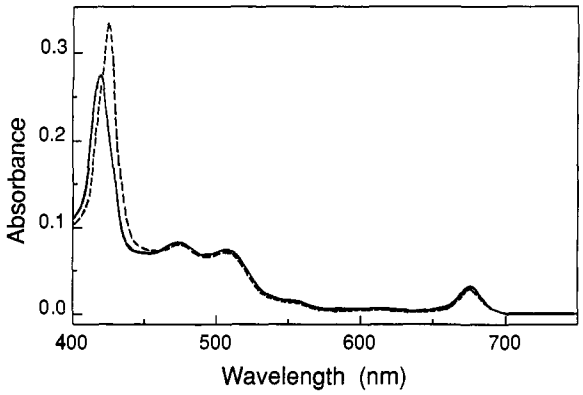
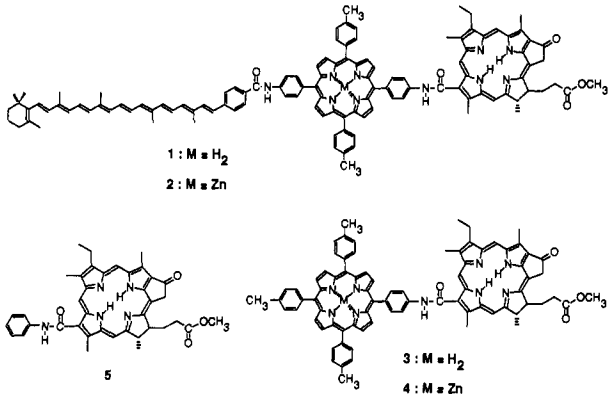


Figure 1. Absorption spectra of free-base triad **1** (—) and zinc triad **2** (- - -) in acetone solution.

carotenoid polyene (C) covalently linked to a pyropheophorbide (Ppd) through a bridging porphyrin moiety (P). The relevant spectroscopic and photophysical properties of these triads have been investigated.



Results

The triads, model porphyrin–pyropheophorbide dyads **3** and **4**, and pyropheophorbide derivative **5** were prepared from pyropheophorbide-*a* (synthesized from natural chlorophyll-*a*) and previously reported porphyrin and carotenoid precursors. The syntheses of the required compounds and their characterization with UV–vis, NMR, and mass spectrometric techniques are described in the Experimental Section, as are the details of the spectroscopic studies.

Absorption Spectra. Figure 1 shows the absorption spectra of **1** and **2** in acetone solution. In both molecules, the long-wavelength band at ~672 nm is due only to the pyropheophorbide moiety. Triad **1** features a Soret absorption at 415 nm, whereas the corresponding maximum in **2** is 423 nm. These maxima are close to those of *meso*-tetraphenylporphyrin and its zinc analog, respectively. The Soret band of **5** occurs at 406 nm, and the absorption of the pyropheophorbide moiety contributes ≤10% at the Soret maxima of **1** and **2**. The strong absorbances in the 450–510 nm region are due mostly to the carotenoid polyene. In short, the spectra of **1–4** are similar to the sum of the absorption spectra of the component chromophores and suggest that in absorption the chromophores act as discrete entities rather than one large, delocalized π-electron system.

Fluorescence Spectra. The fluorescence emission spectrum of **1** in acetone, with excitation at 420 nm, is shown in Figure 2. The spectral shapes, with maxima at 675 and 722 nm, were identical, within experimental error, for **1–5**. This result demonstrates that significant emission comes only from the Ppd moiety, as zinc and free-base tetraarylporphyrin model systems for the porphyrin moieties of **1–4** have quite different emission characteristics. The lack of emission from the porphyrin moieties suggests the possibility of singlet–singlet energy transfer from these chromophores to the pyropheophorbide, which has a lower-lying first excited singlet state. This prospect was confirmed by the fact that the corrected, normalized excitation and absorption spectra of dyad **3** were coincident, within the accuracy of the fluorimeter calibration, as were those for **4**. Thus, the porphyrin-to-pyropheophorbide singlet–singlet energy-transfer efficiency in these two molecules is essentially 100%.

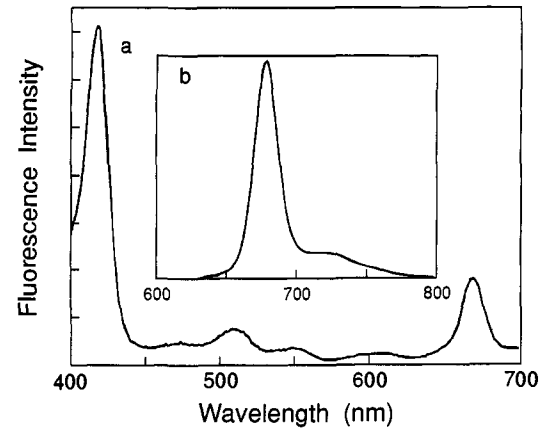


Figure 2. Corrected fluorescence excitation spectrum of ca. 1×10^{-7} M free-base triad **1** in acetone solution with emission detected at 730 nm (a) and corrected emission spectrum of the same solution with excitation in the Soret band at 420 nm (b).

Table I. Photophysical and Photochemical Parameters for Pyropheophorbide **5** and Related Dyads and Triads in Acetone Solution

compd	ϕ_Δ[a]	$\phi_{\Delta r}$[b]	ϕ_{tr}[c]	ϕ_{fr}[d]	τ_f[e] (ns)	ϕ_{fr}[f]
5	0.80	1.00	1.00	1.00	6.4	1.00
3	0.80	1.00	1.00	0.95	6.0	0.94
1	0.36	0.45		0.85	5.1	0.80
4	0.23	0.29	0.30	0.26	1.6	0.25
2	0.20	0.25		0.24	1.4	0.22

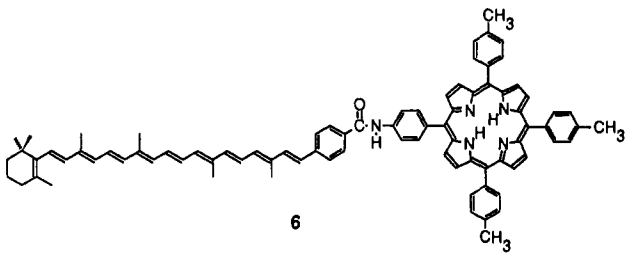
[a] Absolute singlet oxygen quantum yield, based on a yield of 0.80 for pyropheophorbide **5**. [b] Relative singlet oxygen quantum yield, based on a relative yield of 1.00 for pyropheophorbide **5**. [c] Relative triplet quantum yield, based on $\phi_{tr} = 1.00$ for pyropheophorbide **5**. [d] Relative fluorescence quantum yield, based on $\phi_{fr} = 1.00$ for pyropheophorbide **5**. [e] Fluorescence lifetime of the pyropheophorbide moiety in acetone solution. [f] Relative fluorescence quantum yield based on $\phi_{fr} = 1.00$ for **5**, as calculated from the fluorescence lifetime data, assuming that the radiative rate constant for the pyropheophorbide moiety is unchanged.

The corrected fluorescence excitation spectrum of triad **1**, with emission monitored at 730 nm, is shown in Figure 2. It is clear that although absorption by the porphyrin moiety contributes strongly to the emission of the pyropheophorbide, as in **3**, the carotenoid polyene contributes only slightly, as evidenced by the small peak at about 475 nm. This is consistent with previous studies of carotenoporphyrin dyad **6**, with a structure similar to the carotenoporphyrin portion of **1**, where carotenoid-to-porphyrin singlet energy-transfer efficiency was ~10%.[7] In addition, little carotenoid contribution to the fluorescence excitation spectrum of **2** was observed. Thus, the porphyrin moieties of **1–4** are excellent antenna chromophores for the attached pyropheophorbides, whereas the carotenoid moieties of **1** and **2** are poor antennas.

Fluorescence quantum yields for **1–4** relative to pyropheophorbide **5** (ϕ_{fr}), were determined in acetone solution with excitation of the pyropheophorbide at 670 nm. The results are presented in Table I. In the zinc-containing compounds **2** and **4**, the fluorescence quantum yield is reduced by a factor of about 4, relative to **5**. This quenching is not due to energy transfer because, as mentioned above, the first excited singlet-state energy of the pyropheophorbide is much lower than that of the porphyrin. We tentatively ascribe the quenching to electron transfer from the

zinc porphyrin to the singlet excited pyropheophorbide to yield a $P_{Zn}^{\bullet+}$–$Ppd^{\bullet-}$ charge-separated state. Similar electron-transfer phenomena have been observed in other porphyrin–pyropheophorbide dyads.[15] In contrast, the relative fluorescence quantum yields for free-base molecules **1** and **3** are nearly 1.00; minimal quenching of the pyropheophorbide first excited singlet state is observed. The lack of significant electron transfer in these molecules is consistent with the fact that the oxidation potential of a free-base porphyrin related to that in **1** and **3**, ~0.95 V vs SCE, is substantially higher than that of the corresponding zinc porphyrin moiety (~0.77 V).[16]

The results of time-resolved fluorescence studies are consistent with the steady-state results. Excitation of **5** in acetone solution at 600 nm yielded a single exponential decay at 675 nm with a lifetime of 6.4 ns ($\chi^2 = 1.08$). Emission from the free-base dyad **3** was monitored at eight wavelengths in the 660–730 nm region. Global analysis of the decays gave a single lifetime of 6.0 ns ($\chi^2 = 1.11$). Triad **1** yielded a single fluorescence lifetime of 5.1 ns when excited at 610 nm, where essentially all of the light is absorbed by the pyropheophorbide moiety, and monitored at 675 and 720 nm (average $\chi^2 = 1.21$). Thus, as noted above, the quenching of the pyropheophorbide excited singlet state by the attached free-base porphyrin is minimal. The slight reduction in the lifetime of the pyropheophorbide singlet state of **1** relative to **3** may be due to endergonic energy transfer from the pyropheophorbide singlet-state C–P–^{1}Ppd to the adjacent porphyrin to give C–^{1}P–Ppd followed by quenching of the porphyrin singlet state by the carotenoid. Such quenching has been observed in the related carotenoporphyrin **6**. In the case of the zinc-



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containing materials **2** and **4**, shortening of the fluorescence lifetimes to about 25% of that of pyropheophorbide **5** was noted (Table I), as expected from the steady-state results.

Triplet–Triplet Absorption Spectra. The triplet–triplet absorption spectrum (proportional to $\epsilon_T - \epsilon_G$) obtained 30 ns after excitation of an ~7×10^{-6} M solution of **5** in air-saturated acetone with a 670-nm laser pulse is shown in Figure 3. Depletion of the ground state was less than 5% in these experiments. Essentially identical spectra were observed following laser flash excitation of dyads **3** and **4**. Thus, excitation of all three molecules leads to formation of the pyropheophorbide triplet state. This result is consistent with the singlet energy-transfer results discussed above and the fact that the energy of the pyropheophorbide triplet state is below that of the porphyrin moieties (see below). It is interesting to note that in all of these molecules, the transient absorption (ΔA) at 540 nm is nearly 0. Thus, the extinction coefficients for the triplet and ground states must be essentially equal at this wavelength.

In an air-saturated acetone solution of **5**, the transient absorption at 470 nm decayed as a single exponential with a lifetime of 200 ns (Figure 4). This triplet lifetime is similar to that observed for pheophytin-*a* in air-saturated solutions.[17] After

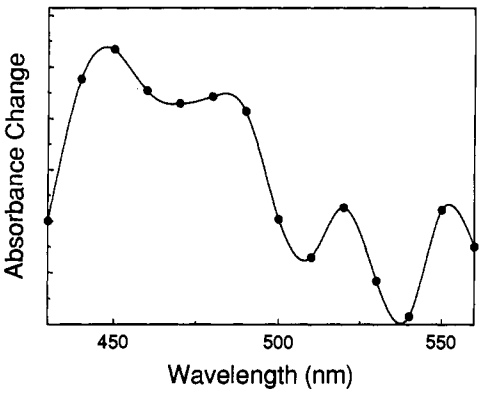

Figure 3. Triplet–Triplet transient absorption spectrum ($\epsilon_T - \epsilon_G$) obtained 30 ns after excitation of a ca. 7×10^{-6} M solution of pyropheophorbide **5** in air-saturated acetone with a 670-nm, 15-ns laser pulse.

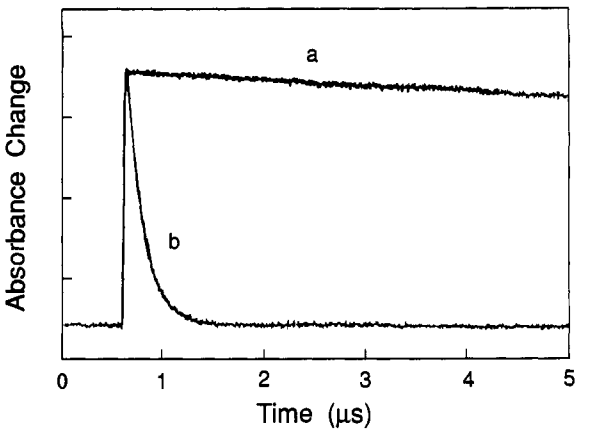

Figure 4. Decay of the triplet transient absorption at 470 nm of a ca. 7×10^{-6} M solution of pyropheophorbide **5** in nitrogen-purged (a) and air-saturated (b) acetone following excitation with a 670-nm, 15-ns laser pulse.

purging the solution for 30 min with nitrogen, the transient decayed much more slowly (Figure 4). In general, the transient absorption under these conditions decayed nonexponentially over a period of several hundred microseconds. At high laser powers, a significant contribution from a short-lived decay phase was noted, but this was greatly reduced as the laser flash energy was decreased. These kinetics may be ascribed to a combination of triplet–triplet annihilation (especially at high laser powers), spontaneous monomolecular deactivation of the triplet state, and quenching by ground-state pigment molecules. Decay kinetics similar to those found for **5** were observed for dyads **3** and **4** under air-saturated and nitrogen-purged conditions.

It is clear from Figure 4 that in air-saturated solutions the decay of the pyropheophorbide triplet state is dominated by quenching of the triplet by oxygen (to yield singlet oxygen). Under these conditions, the lifetime of the triplet state will be given by eq 1, where τ_t is the observed lifetime of the triplet state (200 ns),

$$\tau_t = 1/(k_{tq}[O_2]) \quad (1)$$

k_{tq} is the rate constant for triplet state quenching by oxygen, and $[O_2]$ is the concentration of oxygen in air-saturated acetone (~2×10^{-3} M). Thus, k_{tq} is about 2.5×10^9 M^{-1} s^{-1}. This value is about one-ninth of the rate constant for diffusion-controlled reactions in acetone (~2×10^{10} M^{-1} s^{-1}), as expected from similar studies of other cyclic tetrapyrroles.[17]

Quantum yields of pyropheophorbide triplet-state formation in the dyads relative to that for pyropheophorbide **5**, ϕ_{tr}, were determined by measuring absorption changes at 470 nm following excitation of acetone solutions at 670 nm. The results are presented in Table I. The triplet quantum yield for free-base

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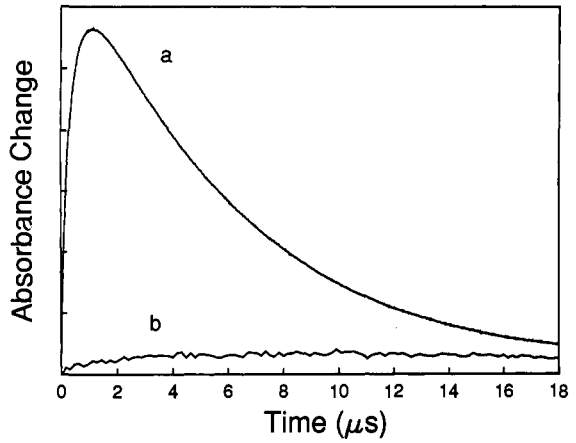

Figure 5. Rise and decay of the carotenoid triplet-state transient absorption at 540 nm following excitation of nitrogen-purged acetone solutions of the triads with 670-nm, 15-ns laser pulses. Curve a was obtained from a solution of free-base triad **1** with an absorbance of 0.2 at 670 nm and was measured at 20 ns per data point. Curve b is from a solution of zinc triad **2** whose absorbance at 670 nm is normalized to that of **1** and was obtained at 160 ns per data point. The difference in signal-to-noise ratio is due to differences in signal averaging times.

dyad **3** is identical to that found for **5**. In the case of zinc dyad **4**, the yield is reduced by a factor of about 4. These results parallel the relative fluorescence quantum yield data reported above and show that the decrease in ϕ_{tr} for **4** is due to quenching of the first excited singlet state of the pyropheophorbide by the zinc porphyrin moiety, as discussed above.

In order to investigate the possible formation of the carotenoid triplet state in triads **1** and **2**, the laser flash-induced absorption changes at 540 nm were determined. It will be recalled that this wavelength corresponds to $\Delta A \cong 0$ for **3**–**5**. It also corresponds approximately to the maximum of the triplet–triplet absorption spectrum of carotenoids.[18] The results of these studies are shown in Figure 5. Excitation of a 5×10^{-6} M solution of free-base triad **1** in nitrogen-purged acetone solution with a 670-nm laser pulse resulted in a strong transient absorption which rose in a few hundred nanoseconds and decayed on the microsecond time scale. Kinetic studies on several samples at 540 and 560 nm yielded a rate constant for formation of 2.9×10^6 s^{-1} and a decay rate constant of 1.9×10^5 s^{-1}. The decay time is similar to the lifetime of the carotenoid triplet state in the absence of oxygen.[5] Thus, these data are consistent with the relatively slow formation and decay of the carotenoid triplet following excitation of the pyropheophorbide moiety and signal triplet–triplet energy transfer from the initially formed pyropheophorbide triplet to the carotenoid by some mechanism. As the rise time was not a function of concentration, the transfer process must be intramolecular in nature.

In contrast, the zinc-containing triad **2** showed only a very weak transient absorption at 540 nm following excitation under the same conditions (Figure 5). Even though the yield of pyropheophorbide triplet state in the zinc-containing dyad **4** is only 4 times lower than that in free-base dyad **3**, the amplitude of the transient at 540 nm in **2** is *ca.* one-sixteenth that in **1**. Indeed, the time course of this transient is also quite different from that observed in **1**. The absorption rises in about 2 μs and decays in about 30 μs. The *rise* time is on the order of the lifetime of the carotenoid triplet state, mentioned above, and the *decay* time is so long that it must be associated with a pyropheophorbide triplet state. These data, coupled with the low transient absorbance of the carotenoid triplet, can be rationalized by assuming "inverted" kinetics: the carotenoid triplet is formed from the pyropheophorbide triplet on the 30-μs time scale and

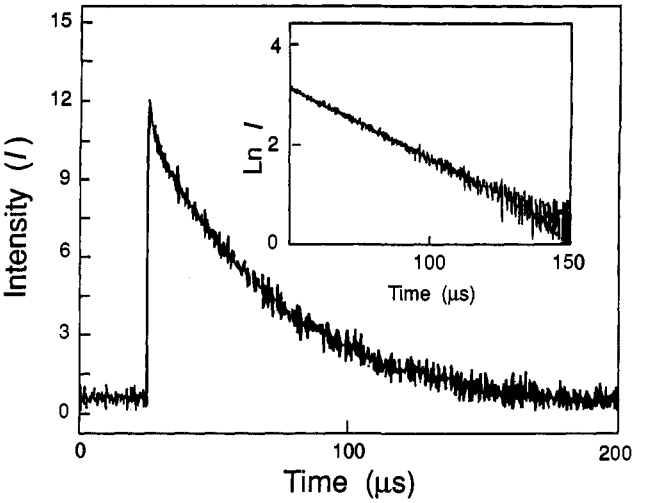

Figure 6. Singlet oxygen luminescence at 1270 nm following excitation of an air-saturated, ca. 10^{-6} M solution of pyropheophorbide **5** in acetone with a 670-nm, 15-ns laser pulse. The inset shows the same data plotted in logarithmic form on the intensity axis. The lifetime is 51 μs.

decays with its usual lifetime of a few microseconds.[5] Indeed, the data for **2** in Figure 5, including the relative magnitudes, can be fit satisfactorily using a rate constant for formation of the carotenoid triplet of 3.7×10^4 s^{-1} and a decay rate constant of 1.9×10^5 s^{-1} (the lifetime of the carotenoid triplet state) and assuming a minor (1.5%) contribution from triad **1** that can be formed by loss of zinc due to trace amounts of acid.

In the presence of air, the carotenoid triplet transient absorption of **1** is still observed. It rises and decays within a few hundred nanoseconds. Thus, triplet–triplet energy transfer to the carotenoid competes with oxygen quenching of the pyropheophorbide triplet. Quenching of the β-carotene triplet state by oxygen on this time scale is well known.[19] A very small residual absorption with a lifetime of 7 μs was also observed and is assigned to the carotenoid triplet state sensitized by $^1O_2$. Indeed, the lifetime of singlet oxygen under these conditions was determined to be 7 μs by luminescence measurements.

Singlet Oxygen Generation. Excitation of aerated acetone solutions of each of the five compounds with a laser pulse at 670 nm resulted in light emission at 1270 nm (Figure 6). Luminescence at this wavelength is characteristic of singlet oxygen ($^1O_2$).[4] Removal of oxygen from solution by nitrogen purging resulted in the disappearance of this emission. At low pigment concentrations, a luminescence lifetime of 51 μs was obtained, which coincides with the lifetime of singlet oxygen in acetone. The lifetime decreased with increasing pigment concentration. Addition of β-carotene (1×10^{-4} M) led to complete quenching of this luminescence on the microsecond time scale. All of these data confirm that the luminescence represents singlet oxygen emission. The intensity of the singlet oxygen luminescence was a linear function of the intensity of the laser flash at all the intensity ranges available for the exciting dye laser system.

The quantum yield of $^1O_2$ (ϕ_Δ) for pyropheophorbide **5** was determined by comparison with that of tetrakis(4-methylphenyl)porphyrin (**7**). The absorbance of the two solutions at 650 nm was 0.1 in 1-cm cuvettes. This corresponds to a porphyrin concentration of $\sim 2 \times 10^{-5}$ M and a concentration of **5** of $\sim 2 \times 10^{-6}$ M. In order to exclude irreversible photobleaching of the photosensitizers and deviation from linearity, the measurements were made with very weak excitation pulses. The ratio of singlet oxygen quantum yields for **7** and **5** is simply the ratio of the initial intensities of the singlet oxygen luminescence (obtained by extrapolation), normalized for any differences in the absorbance of the solutions at the excitation wavelength. The ϕ_Δ value for

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5 was found to be higher than that for 7 by a factor of 1.25 ± 0.06. The ϕ_Δ for 7 in organic solvents is 65 ± 5%.[17,20,21] Thus, ϕ_Δ for 5 equals 80 ± 10%. This result is close to the ϕ_Δ value reported for pheophorbide-*a*.[22] If the fraction of triplet states of 5 which yield singlet oxygen (S_Δ) is close to 1, as is typical for porphyrins and chlorophylls, then ϕ_t for 5 is also about 80%. This is reasonable, as the triplet quantum yield (ϕ_t) for the closely related pheophorbide-*a* is 80%.[22]

Turning to the dyad molecules, the quantum yields of singlet oxygen were measured as described above using 5 (ϕ_Δ = 0.80) as a standard. The results appear in Table I. The ϕ_Δ value for free-base dyad 3 is identical to that obtained with pyropheophorbide 5. This is in accord with the finding that both molecules have the same triplet quantum yield. In the case of zinc dyad 4, the ϕ_Δ value is reduced by a factor of approximately 4, which mirrors the reduction of triplet and fluorescence quantum yields in this molecule because of competition from electron-transfer quenching at the singlet level. Zinc-containing triad 2 behaves essentially identically to dyad 4; the 4-fold reduction in the ϕ_Δ value relative to 5 again parallels the similar reduction in relative fluorescence quantum yield due to electron transfer.

Free-base triad 1, on the other hand, has a ϕ_Δ value of only 0.36. This substantial reduction compared with the values of 0.80 for 3 and 5 is ascribed to the fact that the pyropheophorbide triplet state is quenched by triplet–triplet energy transfer to the carotenoid moiety on a time scale comparable with that for singlet oxygen sensitization. Quantitatively, it can be shown that if $S_\Delta \approx 1$,

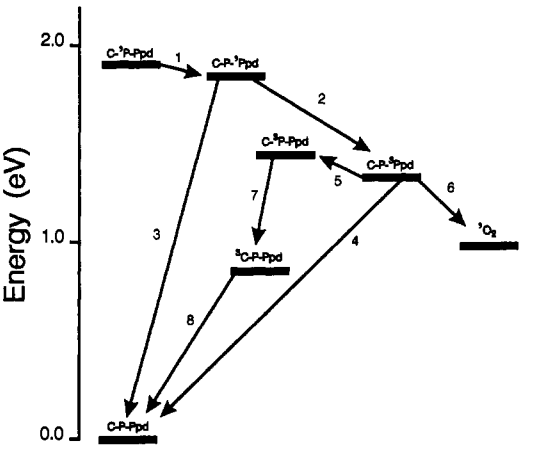
$$\phi_\Delta = \phi_t k_{tq}[O_2]\tau_t/(1 + k_{tq}[O_2]\tau_t) \qquad (2)$$

where τ_t is the lifetime of the pyropheophorbide triplet state of 1 in anaerobic conditions and the other quantities are as defined above. Taking τ_t equal to the 200-ns rise time for the carotenoid triplet state in 1 and the values for k_{tq} and $[O_2]$ given earlier, this equation yields ϕ_Δ equal to 0.5 × ϕ_t. If one assumes that the pyropheophorbide ϕ_t values for 1, 3, and 5 are essentially identical to that for pheophorbide-*a* (80%),[22] then eq 2 gives a ϕ_Δ value of 0.4. Thus, the observed ϕ_Δ value for 1 (0.36) is consistent with the data for ϕ_Δ, ϕ_{tr}, and ϕ_{fr} for these molecules and the observed triplet–triplet transfer to the carotenoid.

Singlet Oxygen Quenching. The singlet oxygen lifetime in the solutions of 1–5 was strongly dependent on the concentration of the pigment, decreasing as the concentration was increased. This effect was particularly strong for carotenoid-containing pigments 1 and 2. Thus, the dyads and triads quench $^1O_2$. For the dyads, the quenching rate constant is about 10^7 M^{-1} s^{-1}, which is similar to the corresponding value for pheophytin-*a*.[4] For the triads, this rate constant was close to diffusion controlled.

Discussion. The results for pyropheophorbide 5, free-base dyad 3, and free-base triad 1 may be conveniently discussed with reference to Scheme I. In the case of 5, excitation yields the pyropheophorbide first excited singlet state at 1.84 eV. This state decays by fluorescence, internal conversion (step 3), and intersystem crossing (step 2) with a lifetime of 6.4 ns. The resulting pyropheophorbide triplet state is estimated to have an energy of ~1.33 eV.[22] In the absence of oxygen, the pyropheophorbide triplet decays back to the ground state by various processes (step 4) with a lifetime of ~300 μs. In aerated solution, the lifetime of the triplet state is decreased dramatically by triplet–triplet energy transfer to oxygen to yield $^1O_2$ (step 6). The rate constant for this process is about 2.5 × 10^9 M^{-1} s^{-1}, and the quantum yield of singlet oxygen is about 0.80.

Scheme I



In the case of dyad 3, excitation into the porphyrin moiety yields the first excited singlet state at 1.90 eV. Singlet–singlet energy transfer (step 1 in Scheme I) yields the pyropheophorbide singlet state with a quantum yield of essentially 1.00. From this point on, the photochemistry is identical to that observed for 5. The porphyrin moiety plays no additional role.

For triad 1, the singlet excitation sink is again the pyropheophorbide. This moiety may be excited directly or via singlet–singlet energy transfer from the attached porphyrin with nearly 100% efficiency. The porphyrin first excited singlet state may be created by direct excitation or rather inefficiently via energy transfer from the carotenoid (quantum yield ≅ 10%). Intersystem crossing of the pyropheophorbide presumably yields the triplet state with a quantum yield similar to those for 3 and 5. As is the case with these other molecules, the triplet state can decay to the ground state by step 4 or can sensitize singlet oxygen formation in the presence of air (step 6) with a rate constant of about 2.5 × 10^9 M^{-1} s^{-1}. The yield of singlet oxygen is substantially reduced, however, by competing triplet–triplet energy transfer to give the carotenoid triplet state, which is formed with a rate constant of 2.9 × 10^6 s^{-1}. In air, this triplet state decays within a few hundred nanoseconds due to quenching by oxygen (that does not involve singlet oxygen sensitization). In nitrogen-purged solutions, however, the lifetime for decay by the usual relaxation processes (step 8) is 5.3 μs.

The results for 1 raise the question of the mechanism of triplet–triplet energy transfer from the pyropheophorbide to the carotenoid. As triplet–triplet transfer generally occurs by electron exchange, it requires orbital overlap between the donor and acceptor moieties. The essentially linear structure of the triad precludes conformations in which the carotenoid and pyropheophorbide are in orbital contact. Thus, we propose that the energy transfer is a two-step process. An endergonic energy transfer from the pyropheophorbide at 1.33 eV to the directly attached free-base porphyrin yields the porphyrin triplet state at 1.44 eV (step 5).[23-25] This triplet is rapidly quenched by the attached carotenoid. Previous studies of carotenoporphyrin dyad 6 have shown[7] that the carotenoid quenches the porphyrin triplet state with a rate constant >1 × 10^8 s^{-1}. The rate-determining step for 1 is thus the *endergonic* interporphyrin triplet energy transfer (step 5). This suggests that triplet transfer should cease at low temperatures. Indeed, when a butyronitrile solution of 1 was cooled to 77 K and the resulting glass excited with a laser pulse at 660 nm, no carotenoid triplet was detected.

The photochemistries of zinc-containing dyad 4 and triad 2 are quite different from those of their free-base analogs, as can be shown with reference to Scheme II. Excitation of the zinc

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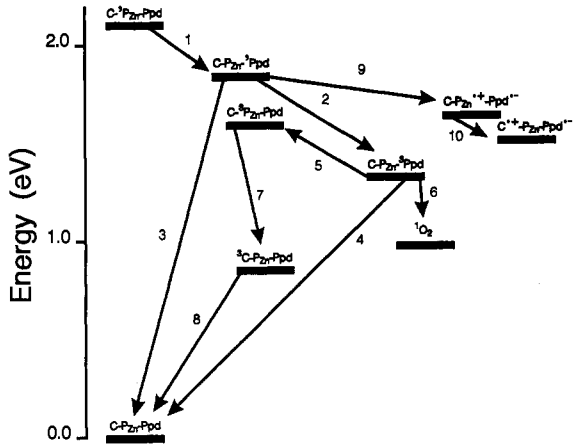
Scheme II



porphyrin moiety of **4** is followed by essentially complete singlet energy transfer to the attached pyropheophorbide (step 1), which can also, of course, be excited directly with 670-nm light. As was the case for the free-base compounds, the pyropheophorbide singlet state decays by fluorescence, intersystem crossing, and internal conversion with rate constants essentially identical to those for **1**, **3**, and **5**. The time-resolved fluorescence results reveal that an additional decay pathway exists. We suggest that the excited singlet state may accept an electron from the attached zinc porphyrin to create a charge-separated state (step 9). The reasonably high quantum yield for this quenching, as judged by the reduction in fluorescence quantum yield for the pyropheophorbide moiety upon introduction of zinc into the adjoining porphyrin, is consistent with the stabilization of the positive charge on the porphyrin by zinc, which contributes to an estimated overall driving force for the electron transfer of about 0.2 eV.[26] In the case of the free-base dyad and triad, the lack of this stabilization and resulting small free energy change preclude significant electron-transfer quenching. Once formed, the charge-separated state of **4** could decay to the ground state or, in principle, to the pyropheophorbide triplet state.

Similar processes were observed for triad **2**. In this case the proposed C–$P_{Zn}^{\bullet+}$–$Ppd^{\bullet-}$ charge-separated state could, in principle, decay by electron transfer from the attached carotenoid to yield a $C^{\bullet+}$–P_{Zn}–$Ppd^{\bullet-}$ charge-separated state (step 10). Formation of this state was not detected in **2**, possibly due to rapid and efficient charge recombination of C–$P_{Zn}^{\bullet+}$–$Ppd^{\bullet-}$ to yield the ground state. However, related photoinitiated electron-transfer processes have been reported in a series of carotene–diporphyrin triad molecules.[16]

The pyropheophorbide triplet state of **2** effectively sensitizes the production of singlet oxygen by step 6 in the presence of air. In nitrogen-purged solutions, it decays slowly by the usual photophysical processes (step 4) and by an energy-transfer relay to the carotenoid similar to that observed for **1** (steps 5 and 7). The rate constant for the rate-determining step 5 in the relay is $3.7 \times 10^4\ s^{-1}$, as determined from the data in Figure 5. Although this quenching by the carotenoid is reasonably efficient in the absence of oxygen (the calculated energy-transfer efficiency is 0.92), very little carotenoid triplet absorption was observed at 540 nm (Figure 5). This is the case because the rate of decay of the carotenoid triplet is substantially faster than its rate of formation. We ascribe the slow triplet–triplet transfer to the fact that the energy of the porphyrin triplet state has been raised from 1.44 to 1.59 eV by insertion of zinc,[23-25,28] rendering step 5 more endergonic. Because the triplet–triplet transfer rate in **2** is so slow, triplet transfer cannot compete with sensitization of singlet oxygen in aerated solutions, and the carotenoid provides no photoprotection via quenching of the pyropheophorbide triplet state.

An alternative explanation for the lack of spectroscopic observation of a significant amount of carotenoid triplet state in **2** would be that the conformation of the molecule precludes the orbital overlap necessary for rapid transfer. The observation of effective transfer in **1**, which has a closely similar structure, rules out this interpretation.

Conclusions

These results demonstrate that carotenoid polyenes can provide effective photoprotection from singlet oxygen by rapid quenching of tetrapyrrole sensitizers via a triplet relay mechanism. However, the structural and energetic constraints on the stepwise transfer are relatively severe. In the type of system investigated here, for example, the electronic coupling between the pyropheophorbide donor and the porphyrin intermediate and between that intermediate and the carotenoid must be relatively strong, and in addition the thermodynamic barrier to triplet energy migration must not be too high. In the case of triad **1**, for example, the combination of electronic coupling (presumably via the amide linkages)[7] and a weakly endothermic initial triplet–triplet transfer step (step 5) leads to a reduction in the yield of singlet oxygen by a factor of approximately 2 relative to dyad **3** or pyropheophorbide **5**. However, the carotenoid moiety of triad **2**, which features similar electronic coupling, is completely ineffective in this photoprotective role because of the enhanced endergonicity of step 5.

As mentioned above, both carotenoids and cyclic tetrapyrroles can quench singlet oxygen after it has been formed. Carotenoid polyenes are more efficient than porphyrins or pyropheophorbides in this connection. However, the results of this study demonstrate clearly that photoprotection from singlet oxygen damage by rapid quenching of the sensitizer is much more effective than scavenging already-produced material. In this connection, it is interesting to note that the ϕ_Δ values for triad **2** and dyad **4** are very similar. This demonstrates that effects due to the propinquity of the carotenoid to the site of singlet oxygen generation in these molecules are insignificant. Efficient quenching of singlet oxygen by carotenoid while $^1O_2$ is trapped within a solvent cage or similar structure is not observed.

The triplet relay in triad **1** is similar in many ways to that proposed for the *Rb. sphaeroides* reaction center.[12-14] In the reaction center, the bacteriochlorophyll special pair has a triplet energy of about 0.94 eV, whereas the bridging bacteriochlorophyll has its triplet state at about 1.02 eV.[12] Thus, activated triplet transfer might be expected in this case as well. Indeed, the rate of triplet transfer slows significantly below about 50 K, and at 10 K triplet transfer to the carotenoid is not observed.[13,14,29,30]

It was suggested above that in triad **2**, excitation of the pyropheophorbide led to photoinitiated electron transfer to yield C–$P_{Zn}^{\bullet+}$–$Ppd^{\bullet-}$. Although electron donation from the carotenoid to produce a $C^{\bullet+}$–P_{Zn}–$Ppd^{\bullet-}$ charge-separated state was not observed, many photosynthetic model systems featuring carotenoid polyenes as electron donors have been reported.[31,32] It has also been noted that in photosystem II reaction centers, carotenoids can act as electron donors to $P680^+$ under certain conditions.[33,34]

(26) This estimate is based on an oxidation potential of 0.77 V vs SCE for the zinc porphyrin moiety,[16] a reduction potential for the pyropheophorbide moiety of 0.87 V (as reported for ethyl pheophorbide-*a*),[27] and the pyropheophorbide first excited singlet-state energy of 1.84 eV.

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These observations suggest a reason for a triplet relay architecture in some reaction centers. A carotenoid polyene in van der Waals contact with a special pair could indeed rapidly quench the triplet state of the special pair, but it would also be an efficient electron donor to the special pair radical cation for systems having suitable energetics. Thus, the carotenoid would interfere with normal electron transfer. By inserting a chlorophyll molecule between the carotenoid and the special pair, rapid electron transfer can be prevented on thermodynamic grounds while triplet state quenching is retained via the relay mechanism. Thus, these triplet relays act as conductors of triplet energy and simultaneously as insulators for electron transfer. Synthetic photoprotective triplet relays could, in principle, be incorporated into artificial molecular photosynthetic devices in order to protect them from singlet oxygen degradation.

Experimental Section

Spectroscopic Measurements. The ^{1}H NMR spectra were obtained at 300 or 400 MHz from ≤1% solutions in chloroform-*d* with tetramethylsilane as an internal reference. The carotenoid and porphyrin resonances are reported using the numbering system previously reported.[7] Mass spectra (FAB) were obtained on a Kratos three-sector instrument, EBE design, operating as a double-focus mass spectrometer and using argon atom bombardment and CsI/glycerol matrices. The UV–vis spectra were recorded on a Hewlett-Packard 8450A diode array spectrometer. Corrected fluorescence and fluorescence excitation spectra were measured using a SPEX Fluorolog-2. Excitation was produced by a 450-W xenon lamp and single grating monochromator. Fluorescence was detected at a 90° angle to the excitation beam via a single grating monochromator and an R928 photomultiplier tube having S-20 spectral response operating in the photon counting mode. Fluorescence decay measurements were made on ≤1 × 10^{-5} M solutions using the time-correlated single photon counting method. The excitation source was a frequency-doubled, mode-locked Nd-YAG laser coupled to a synchronously pumped, cavity-dumped dye laser. Detection was via a microchannel plate photomultiplier (Hamamatsu R2809U-11), and the instrument response time was ca. 35 ps.[35]

The singlet oxygen luminescence was studied in air-saturated pigment solutions at room temperature. Excitation was produced by a Lambda Physik FL2000 dye laser pumped by a Lambda Physik EMG-50 xenon chloride excimer laser. The excitation wavelengths were tunable over the 630–680-nm range. The maximum single flash intensity of the dye laser was 0.1 mJ, the flash duration was about 10 ns, and the frequency of flash repetition was about 10 Hz. The luminescence was detected by a cryogenic germanium diode detector (North Coast Instruments model EO-817PP) through an interference filter with the transmission maximum at 1270 nm. The analog signal from the diode was amplified and then digitized with a LeCroy Transient Recorder and analyzed using a computer. Decay kinetics of singlet oxygen luminescence having lifetimes exceeding 1 μs could be detected. Time-resolved absorption measurements were made with a spectrometer based on the same laser system, which has been described elsewhere.[36]

Triad 1. A 16.8-mg (0.303 mmol) portion of 2-desvinyl-2-carboxymethylpyropheophorbide-*a*[37] was dissolved in 2 mL of dry toluene and 0.5 mL of dry pyridine, and the solution was stirred under nitrogen for 5 min. One drop of thionyl chloride was added, and after 5 min a small aliquot of the mixture was mixed with 1 drop of methanol. Thin-layer chromatography (silica gel) of this mixture indicated the quantitative formation of the methyl ester. The volatiles were distilled from the original mixture at reduced pressure, and 20 mL of dry toluene was added and distilled under reduced pressure. The residue was kept at high vacuum for 15 min and redissolved in 2 mL of dry toluene and 0.5 mL of dry pyridine. The requisite carotenoporphyrin amine **8**[36] (17.5 mg, 0.0147 mmol) was dissolved in 2 mL of toluene and 1 mL of pyridine and added to the acid chloride solution. After 5 min, thin-layer chromatography indicated the complete consumption of **8** and the appearance of a brightly fluorescent new spot which was more polar than **8**. The workup involved extraction of the crude mixture in chloroform twice with a saturated aqueous solution of sodium bicarbonate and once with water. The solvent was evaporated, and the crude product was purified by flash chromatography on silica gel with chloroform to afford 24.9 mg (98%) of pure triad **1**. ^{1}H NMR in $CDCl_3$ (400 MHz): δ −2.70 (2H, s, porphyrin pyrrole-NH), −2.18 (1H, s, pyropheophorbide pyrrole-NH), −0.40 (1H, s, pyropheophorbide pyrrole-NH), 1.04 (6H, s, 16C-CH_3, 17C-CH_3), 1.46–1.49 (2H, m, 2C-CH_2), 1.58–1.64 (2H, m, 3C-CH_2), 1.72 (3H, s, 18C-CH_3), 1.74 (3H, t, *J* = 7.9 Hz, 4b-CH_3), 1.84 (3H, d, *J* = 7.3 Hz, 8a-CH_3), 1.99 (3H, s, 19C-CH_3), 2.00 (3H, s, 20C-CH_3), 2.01 (3H, s, 20′C-CH_3), 2.03 (2H, b t, 4C-CH_2), 2.07 (3H, s, 19′C-CH_3), 2.27–2.40 (2H, m, 7a′,b′-CH_2CH_2), 2.59–2.73 (2H, m, 7a,b-CH_2-CH_2), 2.73 (6H, s, 10,20Ar-CH_3), 3.39 (3H, s, 3a-CH_3), 3.61 (3H, s, -OCH_3), 3.67 (3H, s, 5a-CH_3), 3.76 (2H, q, *J* = 7.7 Hz, 4a,a′-CH_2), 3.81 (3H, s, 1a-CH_3), 4.27 (1H, b d, *J* = 7.1 Hz, 7-H), 4.55 (1H, q, *J* = 7.3 Hz, 8-H), 5.04 (1H, d, *J* = 20.6 Hz, 10a′-CH), 5.14 (1H, d, *J* = 20.6 Hz, 10a-CH), 6.14 (1H, d, *J* = 16 Hz, 8C-H), 6.19 (1H, d, *J* = 7.5 Hz, 10C-H), 6.21 (1H, d, *J* = 16 Hz, 7C-H), 6.27 (1H, d, *J* = 9.4 Hz, 14C-H), 6.33 (1H, d, *J* = 11.2 Hz, 14′C-H), 6.36 (1H, d, *J* = 15 Hz, 12C-H), 6.41 (1H, d, *J* = 11.7 Hz, 10′C-H), 6.47 (1H, d, *J* = 14.5 Hz, 12′C-H), 6.61 (1H, d, *J* = 15.8 Hz, 7′C-H), 6.64–6.71 (4H, m, 11C-H,11′C-H, 15C-H,15′C-H), 7.00 (1H, d, *J* = 15.8 Hz, 8′C-H), 7.57 (2H, d, *J* = 8.2 Hz, 1′C-H, 5′C-H), 7.57 (4H, d, *J* = 8.4 Hz, 10,20Ar3-H, Ar5-H), 7.94 (2H, d, *J* = 8.2 Hz, 2′C-H, 4′C-H), 8.06 (2H, d, *J* = 8.3 Hz, 15Ar3-H, Ar5-H), 8.11 (4H, d, *J* = 8.4 Hz, 10, 20Ar2-H, Ar6-H), 8.18 (1H, s, Ar-NHCO), 8.24 (2H, d, *J* = 8.3 Hz, 15Ar2-H, Ar6-H), 8.42 (2H, d, *J* = 6.0 Hz, 5Ar3-H, Ar5-H), 8.46 (2H, d, *J* = 6.0 Hz, 5Ar2-H, Ar6-H), 8.78 (1H, s, δ-H), 8.89 (2H, d, *J* = 4.6 Hz, 17,13 pyrrole-H or 18,12 pyrrole-H), 8.91 (2H, d, *J* = 4.6 Hz, 17,13 pyrrole-H or 18,12 pyrrole-H), 8.94 (2H, d, *J* = 8.9 Hz, 3,7 pyrrole-H or 2,8 pyrrole-H), 9.01 (1H, br s, Ar-NHCO), 9.04 (2H, d, *J* = 3.9 Hz, 3,7 pyrrole-H or 2,8 pyrrole-H), 9.50 (1H, s, α-H), and 9.98 (1H, s, β-H).

Triad 2. The selective metalation of the porphyrin moiety of **1** was accomplished by titrating a dilute solution of zinc acetate in methanol into a stirred solution of the triad in dichloromethane. The progress of the reaction was monitored by thin-layer chromatography. Immediately after the first addition of zinc acetate, a more polar, brownish-orange product could be detected. It became more prominent as more zinc acetate was added. When a third, more polar spot started to develop, the reaction was stopped. Triad **1** has an R_f of 0.40, triad **2** has an R_f of 0.28, and the dimetalated triad is the most polar of the three compounds with an R_f of *ca.* 0.1 on silica gel plates (Analtech GHL) with 2.5% methanol in dichloromethane as the eluant. A preparative-scale thin-layer chromatography with the same type of plates and solvent was developed, and the second band was separated and mixed with a few milliliters of dichloromethane. The solids were removed by filtration, and the solvent was evaporated. The ^{1}H NMR spectrum in $CDCl_3$ (400 MHz) of the material extracted from the second band was consistent with the structure shown for triad **2**, with resonances at δ −1.97 (1H, s, pyropheophorbide pyrrole-NH), −0.03 (1H, s, pyropheophorbide pyrrole-NH), 1.04 (6H, s, 16C-CH_3, 17C-CH_3), 1.44–1.48 (2H, m, 2C-CH_2), 1.58–1.65 (2H, m, 3C-CH_2), 1.72 (3H, s, 18C-CH_3), 1.75 (3H, t, *J* = 7.7 Hz, 4b-CH_3), 1.87 (3H, d, *J* = 7.2 Hz, 8a-CH_3), 1.98 (3H, s, 19C-CH_3), 1.99 (3H, s, 20C-CH_3), 2.00 (3H, s, 20′C-CH_3), 2.02 (2H, b t, 4C-CH_2), 2.09 (3H, s, 19′C-CH_3), 2.30-2.40 (2H, m, 7a′,b′-CH_2CH_2–), 2.60-2.80 (2H, m, 7a,b-CH_2-CH_2), 2.74 (6H, s, 10,20Ar-CH_3), 3.37 (3H, s, 3a-CH_3), 3.64 (3H, s, -OCH_3), 3.71 (3H, s, 5a-CH_3), 3.75 (3H, s, 1a-CH_3), 3.77 (2H, q, *J* = 7.7 Hz, 4a,a′-CH_2), 4.37 (1H, br d, *J* = 8.3 Hz, 7-H), 4.59 (1H, q, *J* = 7.3 Hz, 8-H), 5.16 (1H, d, *J* = 19.7 Hz, 10a′-CH), 5.30 (1H, d, *J* = 19.7 Hz, 10a-CH), 6.14 (1H, d, *J* = 16 Hz, 8C-H), 6.16 (1H, d, *J* = 7.2 Hz, 10C-H), 6.21 (1H, d, *J* = 16 Hz, 7C-H), 6.27 (1H, d, J = 9.8 Hz, 14C-H), 6.33 (1H, d, *J* = 10.6 Hz, 14′C-H), 6.36 (1H, d, *J* = 15 Hz, 12C-H), 6.44 (1H, d, *J* = 12.0 Hz, 10′C-H), 6.48 (1H, d, *J* = 14.5 Hz, 12′C-H), 6.61 (1H, d, *J* = 15.8 Hz, 7′C-H), 6.64-6.71 (4H, m, 11C-H,11′C-H, 15C-H,15′C-H), 7.05 (1H, d, *J* = 15.8 Hz, 8′C-H), 7.59 (4H, d, *J* = 8.0 Hz, 10, 20Ar3-H, Ar5-H), 7.61 (2H, d, *J* = 7.8 Hz, 1′C-H, 5′C-H), 7.96 (2H, d, *J* = 8.0 Hz, 15Ar3-H, Ar5-H), 7.98 (2H, d, *J* = 7.8 Hz, 2′C-H, 4′C-H), 8.14 (4H, d, J = 8.0 Hz, 10, 20Ar2-H, Ar6-H), 8.17 (2H, d, *J* = 8.0 Hz, 15Ar2-H, Ar6-H), 8.28 (2H, d, *J* = 7.1 Hz, 5Ar3-H, Ar5-H), 8.38 (2H, d, J = 7.1 Hz, 5Ar2-H, Ar6-H), 8.66 (1H, s, Ar-NHCO), 8.80 (1H, s, δ-H), 8.97 (2H, d, *J* = 4.7 Hz, 17,13 pyrrole-H or 18,12 pyrrole-H), 9.00 (2H, d, *J* = 4.7 Hz, 17,13 pyrrole-H or 18,12 pyrrole-H), 9.06 (2H, d, *J* = 4.3 Hz, 3,7 pyrrole-H or 2,8 pyrrole-

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H), 9.12 (2H, d, J = 4.3 Hz, 3,7 pyrrole-H or 2,8 pyrrole-H), 9.63 (1H, s, α-H), and 9.92 (1H, s, β-H).

Dyad 3. A suspension comprised of 57 mg (0.10 mmol) of 2-desvinyl-2-carboxymethylpyropheophorbide-*a*,[37] 20 mL of benzene, and 0.12 mL (1.50 mmol) of pyridine was treated with 37 μL (0.50 mmol) of thionyl chloride and stirred under a nitrogen atmosphere for 40 min. The solvent was evaporated under vacuum, as was a 20-mL portion of benzene which was subsequently added to the reaction mixture. To the residue were added a mixture of 20 mL of dichloromethane containing 0.16 mL of pyridine followed by 67.2 mg (0.10 mmol) of 5-(4-aminophenyl)-10,15,20-tris(4-methylphenyl)porphyrin. The solution was stirred at room temperature under nitrogen for 30 min. The reaction mixture was diluted with dichloromethane (60 mL) and washed with aqueous sodium bicarbonate. The solvent was removed by evaporation under vacuum, and the residual pyridine was azeotropically distilled with a portion of toluene. The residue was chromatographed on silica gel (dichloromethane/1–2% acetone) to afford 98 mg (80%) of the desired dyad. ^{1}H NMR in $CDCl_3$ (300 MHz): δ –2.67 (3H, s, porphyrin pyrrole-NH, pyropheophorbide pyrrole-NH), –1.28 (1H, s, pyropheophorbide pyrrole-NH), 1.67 (3H, t, J = 7.5 Hz, 4b-CH_3), 1.68 (3H, d, J = 6.1 Hz, 8a-CH_3), 1.82–2.57 (4H, m, 7a′,7b′,7a,7b-CH_2CH_2-), 2.71 (3H, s, 15Ar-CH_3), 2.73 (6H, s, 10,20Ar-CH_3), 3.20 (3H, s, 3a-CH_3), 3.38 (3H, s, -OCH_3), 3.68 (2H, q, J = 7.7 Hz, 4a,a′-CH_2), 3.74 (3H, s, 5a-CH_3), 3.80 (3H, s, 1a-$CH_3$3.87 (1H, br d, J = 10.0 Hz, 7-H), 4.33 (1H, q, J = 6.1 Hz, 8-H), 4.47 (1H, d, J = 20.0 Hz, 10a′-CH), 4.60 (1H, d, J = 20.0 Hz, 10a-CH), 7.57 (2H, d, J = 6.5 Hz, 15Ar3-H, Ar5-H), 7.59 (4H, d, J = 7.8 Hz, 10, 20Ar3-H, Ar5-H), 8.13 (2H, d, J = 6.5 Hz, 15Ar2-H, Ar6-H), 8.16 (4H, d, J = 7.8 Hz, 10, 20Ar2-H, Ar6-H), 8.45 (2H, d, J = 8.1 Hz, 5Ar3-H, Ar5-H), 8.60 (2H, d, J = 8.1 Hz, 5Ar2-H, Ar6-H), 8.64 (1H, s, δ-H), 8.90 (4H, br s, 12,13,17,18 pyrrole-H), 8.97 (1H, b s, Ar-NHCO), 8.98 (2H, d, J = 4.7 Hz, 3,7 pyrrole-H or 2,8 pyrrole-H), 9.11 (2H, d, J = 4.7 Hz, 3,7 pyrrole-H or 2,8 pyrrole-H), 9.58 (1H, s, α-H), and 9.88 (1H, s, β-H). FAB/MS: m/z 1220 (M + H).

Dyad 4 was prepared in a way similar to the preparation of dyad **3**; the crude product was purified by column chromatography on silica gel (toluene/10–20% ethyl acetate) to give 62 mg (48%) of the desired compound. ^{1}H NMR in $CDCl_3$ (300 MHz): δ –2.50 (1H, s, pyropheophorbide pyrrole-NH), –0.09 (1H, s, pyropheophorbide pyrrole-NH), 1.65 (3H, t, J = 7.6 Hz, 4b-CH_3), 1.78 (3H, d, J = 7.2 Hz, 8a-CH_3), 2.11–2.33 (4H, m, 7a′,7b′,7a,7b-CH_2CH_2-), 2.64 (3H, s, 15Ar-CH_3), 2.65 (6H, s, 10,20Ar-CH_3), 3.29 (3H, s, 3a-CH_3), 3.55 (3H, s, -OCH_3), 3.57 (3H, s, 5a-CH_3), 3.67 (2H, q, J = 7.6 Hz, 4a,a′-CH_2), 3.69 (3H, s, 1a-CH_3), 4.22 (1H, br d, J = 8.0 Hz, 7-H), 4.48 (1H, q, J = 5.6 Hz, 8-H), 5.00 (1H, d, J = 20.1 Hz, 10a′-CH), 5.13 (1H, d, J = 20.1 Hz, 10a-CH), 7.47 (2H, d, J = 8.6 Hz, 15Ar3-H, Ar5-H), 7.50 (4H, d, J = 7.8 Hz, 10, 20Ar3-H, Ar5-H), 8.04 (2H, d, J = 8.6 Hz, 15Ar2-H, Ar6-H), 8.07 (4H, d, J = 7.8 Hz, 10, 20Ar2-H, Ar6-H), 8.32 (4H, br s, 5Ar3-H, Ar5-H, Ar2-H, Ar6-H), 8.71 (1H, s, δ-H), 8.86 (4H, br d, 12,13,17,18 pyrrole-H), 8.93 (2H, d, J = 4.6 Hz, 3,7 pyrrole-H or 2,8 pyrrole-H), 9.02 (2H, d, J = 4.6 Hz, 3,7 pyrrole-H or 2,8 pyrrole-H), 9.45 (1H, s, α-H), and 9.83 (1H, s, β-H). FAB/MS: m/z 1282.4627 $(M + H)^+$.

Pyropheophorbide 5. A suspension of 50 mg (0.088 mmol) of 2-desvinyl-2-carboxymethylpyropheophorbide-*a*,[37] 20 mL of dichloromethane, and 180 μL (2.21 mmol) of pyridine was treated with 64 μL (0.88 mmol) of thionyl chloride and stirred at room temperature for 40 min. The solvent was removed under vacuum as was an added portion of benzene (20 mL). The residue was redissolved in dichloromethane (20 mL), and to the solution was added 0.5 mL of aniline. After 15 min, the reaction mixture was diluted with dichloromethane, washed successively with 1M hydrochloric acid and aqueous sodium bicarbonate, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to a solid which was chromatographed on silica gel (dichloromethane/3–5% acetone) to give 51 mg (90%) of the desired chlorin. ^{1}H NMR in $CDCl_3$ (300 MHz): δ –2.82 (1H, s, pyropheophorbide pyrrole-NH), –1.41 (1H, s, pyropheophorbide pyrrole-NH), 1.59 (3H, t, J = 7.6 Hz, 4b-CH_3), 1.61 (3H, d, J = 7.2 Hz, 8a-CH_3), 1.56–2.55 (4H, m, 7a′,7b′,7a,7b-CH_2CH_2-), 3.14 (3H, s, 3a-CH_3), 3.17 (3H, s, -OCH_3), 3.57 (2H, q, J = 7.7 Hz, 4a,a′-CH_2), 3.59 (3H, s, 5a-CH_3), 3.71 (3H, s, 1a-CH_3), 3.80 (1H, b d, J = 10.1 Hz, 7-H), 4.25 (1H, q, J = 7.2 Hz, 8-H), 4.42 (H, d, J = 20.0 Hz, 10a′-CH), 4.53 (1H, d, J = 20.0 Hz, 10a-CH), 7.35 (1H, t, J = 7.5 Ar4-H), 7.60 (2H, t, J = 7.7 Ar3,5-H), 8.15 (2H, d, J = 7.8 Ar2,6-H), 8.50 (1H, s, δ-H), 8.86 (1H, s, Ar-NHCO), 9.58 (1H, s, α-H), and 9.88 (1H, s, β-H). FAB/MS: m/z 643 $(M + H)^+$.

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