tributable to an excited zwitterion is observed. In each case this appears to arise from excitation of a ground-state conformer in which there is intramolecular H bonding between the phenolic proton and the carbonyl oxygen. It is a consistent feature of the three derivatives that the zwitterion ground-state precursor absorbs at longer wavelengths than other ground-state conformers. In the case of salicylamide and salicylanilide, the zwitterion emission is strongly overlapped by emission attributable to excited phenolate. This fact, together with the weakness of the uncharged molecule emission, means that at most pH values the emission appears as a single band and shows marked excitation wavelength dependence. That this single band contains two contributions is, in the case of salicylamide, elegantly demonstrated by kinetic measurements. The very low quantum yield makes this impossible in the case of salicylanilide. Streak camera measurements with a time resolution of 5 ps have shown that proton loss to form the phenolate and proton transfer to form the zwitterion are both very rapid processes. A noteworthy feature of all three molecules is the shortness of the lifetime of the zwitterionic species. Although the zwitterion lifetimes are to some degree solvent dependent, their shortness in comparison to those of the phenolate species in all solvents indicates that intramolecular proton transfer opens up a particularly efficient nonradiative channel in these molecules. The nature of this nonradiative process remains to be elucidated. Temperature dependence measurements could prove informative.

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Electrochemical Production of Chlorophyll a and Pheophytin a Excited States

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Abstract: The reaction of chlorophyll a$^+$ (Chl a$^+$) with either Chl a$^-$ or pheophytin a$^-$ (Pheo a$^-$) in addition to the reaction of Pheo a$^+$ with Pheo a$^-$ was studied in butyronitrile (BCN), BCN–1% THF, THF, and DMF. The electrochemically produced radical ion pairs Chl a$^+$–Chl a$^-$ and Pheo a$^+$–Pheo a$^-$ react in each solvent to produce a 10^{-7}–10^{-6} yield of luminescent states on the basis of the initial number of radical pairs. The Chl a$^+$–Pheo a$^-$ reaction produces no observable luminescence in any of the solvents examined. The luminescence maximum for the Pheo a$^+$–Pheo a$^-$ reaction occurs at 730 nm in each solvent and is strongly red-shifted relative to the fluorescence maxima for optically excited Pheo a in these solvents. A similar result is obtained for the Chl a$^+$–Chl a$^-$ reaction in BCN. However, emission from the Chl a$^+$–Chl a$^-$ reaction in the other three solvents occurs at 680 nm and corresponds more closely to normal fluorescence from optically excited Chl a. The red-shifted spectra are consistent with the formation of excimers. AC voltammetry of Chl a in BCN provides evidence that Chl a is aggregated in the ground state in this solvent. Chl a reduction shows four waves in BCN and two waves in the other three solvents. Thus, the Chl a$^+$–Chl a$^-$ reaction in BCN does not form a true excimer, whereas the Pheo a$^+$–Pheo a$^-$ reactions in each solvent do. The luminescence efficiencies of these charge-transfer neutralization reactions are discussed in terms of the geometric constraints on electron-transfer reactions in photosynthetic reaction centers.

Introduction

The primary processes of both green plant and bacterial photosynthesis involve photoinduced electron transfer from a chlorophyll species to an electron acceptor.[1,2] Evidence based on ESR and ENDOR data suggests that the primary donors in reaction center proteins of photosystem I (PS I) in green plants and of the *Rhodopseudomonas sphaeroides* bacterium consist of a special pair of chlorophyll a (Chl a) and of bacteriochlorophyll a (Bchl a) molecules, respectively.[3,4] In photosystem II (PS II) of green plants and in some strains of photosynthetic bacteria the monomeric or dimeric nature of the primary donor is not yet established. In addition, both optical and ESR evidence indicate that the first electron acceptor in *R. sphaeroides* reaction centers is bacteriopheophytin a.[5,6] However, the nature of the first acceptor in green plant photosystems remains an open question. The two most likely candidates are pheophytin a (Pheo a) and Chl a.[7-12]

For the past several years our research has addressed questions of reaction center structure and function by examining in vitro models possessing two or more chlorophyll-type chromophores for which the geometry of the chromophores relative to one another is constrained by linking the chromophores through several covalent bonding schemes.[13-15] Recently, we have examined photoinduced electron transfer from a dimeric pyrochlorophyll a system to one of two pheophorbide a macrocycles attached to the dimer by coordination bonds.[16] In this model the forward reaction

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proceeds in a few picoseconds, whereas the lifetime of the proposed charge-transfer state is more than 10 ns. An important feature of this system is the fact that the charge-transfer state decays directly to the ground state. No long-lived triplet states of the dimer or delayed light emission have been observed. On the other hand, it is a well-known fact that spin-polarized triplet states and delayed light emission result from the back-electron-transfer reactions in vivo for reaction centers in which secondary electron transport is blocked.[17,18]

In order to better understand charge-transfer neutralization reactions involving the cations and anions of the photosynthetic pigments, we have examined the electronic states produced by the reaction of electrogenerated Chl a cations with both Chl a and Pheo a anions. The production of emissive states from the reaction of cation radicals of large conjugated organic molecules with their corresponding anion radicals is a well-known phenomenon. Extensive work in the area of electrogenerated chemiluminescence (ECL) has been carried out principally in the laboratories of Bard and Faulkner. These workers have recently reviewed this field.[19]

The first report of ECL from Chl a is due to Krasnovskii and Litvin.[20] However, Saji and Bard were able to show later that the Krasnovskii and Litvin result was probably due to the presence of oxygen and possibly impurities in their DMF–LiCl electrolyte system.[21] Saji and Bard showed that both Chl a cations and anions are stable in rigorously purified DMF–TBAP electrolyte on the cyclic voltammetry time scale and that no ECL could be observed in degassed solutions at their level of light detection. Moreover, they observed substantial luminescence due to the reaction of oxygen with Chl a anions. We will show in this paper that Chl a and Pheo a both yield low level luminescence when their respective cation anion pairs react. Moreover, we will show that the nature of this emission is strongly solvent dependent as is the voltammetry of these species. We will discuss these results in terms of the back-electron-transfer processes of the photosynthetic pigments in reaction centers.

Experimental Section

Materials Purification. Butyronitrile (BCN) (Aldrich) was refluxed over a mixture of $KMnO_4$ and Na_2CO_3 (10 g of each/L of solvent) for 4 h followed by distillation. The distilled solvent was redistilled from P_2O_5 and stored over Linde 4A molecular sieves in a N_2-filled drybox. After 24 h, the solvent was transferred onto fresh sieves. Tetrahydrofuran (THF) (Burdick and Jackson spectrograde) was refluxed for 4 h over $LiAlH_4$ and distilled under N_2 into a flask containing Linde 4A molecular sieves. The flask was stored in a N_2 drybox for 24 h. The solvent was then poured onto fresh sieves and kept in the N_2 drybox.

Dimethylformamide (DMF) (Burdick and Jackson spectrograde) was bubbled with N_2 and stored over Linde 4A molecular sieves for 24 h in the N_2 drybox. The solvent was then poured onto fresh sieves and stored in the N_2 drybox. Further purification was postponed until the electrolyte solution was prepared. Tetra-*n*-butylammonium perchlorate (TBAP) (Eastman, electrochemical grade) was recrystallized once from ethyl acetate–hexane and dried in vacuo at 100 °C for 48 h. The resulting electrolyte was stored in the N_2 drybox.

Pheophytin a was obtained from large scale extraction of the blue-green alga *Spirulina maxima*.[22] Chlorophyll a was prepared from pheophytin a by using the literature procedure.[23] Both Chl a and Pheo a were purified prior to use by high-pressure LC. Chl a was passed through a Waters 30 cm × 3.9 mm i.d. μ-Bondapak-C_{18} column, elution with CH_3CN–5% THF. Pheo a was passed through a Waters 30 cm × 3.9 mm i.d. μ-Porasil column, elution with toluene–2.5% acetone.

Apparatus. The ECL cell design is an adaptation of the one used by Bezman and Faulkner.[24] The Pt disk working electrode is made from a $^1/_4$-in. long piece of $^1/_8$-in. diameter Pt rod. The Pt disk is polished and has an electrochemical area of 0.08 cm^2. The Pt counterelectrode loop is well removed from the working electrode in order to prevent detection of spurious luminescence from this electrode. A Pt wire quasireference electrode (QRE) extends the length of the cell to the vicinity of the Pt disk. The Pt disk working electrode is positioned about 1 mm from each wall and the optical window of the cell. This feature and the use of low pigment concentrations (10^{-4} M) serve to minimize shifts in the luminescence spectra due to self-absorption effects.[25]

The cell is mounted in an integrating sphere constructed from a 3-L round bottom flask containing a 24/40 joint and modified to include a 5-cm diameter side port for coupling to the photomultiplier tube housing. The inside of the sphere is coated with Eastman white reflectance paint. The output port of the sphere is coupled to a custom made filter holder for 2 in. × 2 in. filters which in turn butts up against the wall of an aluminum light tight box. On the outside of the box are a shutter and a Pacific Photometric Model 3378 low-temperature housing containing a RCA C31034A photomultiplier tube. In all our experiments the tube was cooled to −78 °C. The cell is potentiostated with a PAR Model 173 unit controlled by a PAR Model 175 waveform programmer. The reference buffer amplifier of the Model 173 is placed inside the light tight box to facilitate good potential control. The output of the PMT was detected by a PAR Model 1105 photon counter. The pulse output from the amplifier-discriminator of the 1105 was counted as a function of time by the single channel scaler of a Tracor-Northern TN-1203 signal processor. A PDP-11/04 computer controlled the TN-1203 and allowed scan averaging of the transient light emission. The data was displayed on a Tracor-Northern TN-1300 display unit. An LA-36 Decwriter, DEC RX01 dual floppy disks, and an HP-7221A digital plotter complete the data acquisition system. The overall timing of the experiment is controlled by clock pulses from an ORTEC 4710 pulse generator that pulses every t_D and simultaneously triggers the potential step program and the start of the data acquisition. Normally, 1024 data points were acquired with a dwell time of 200 μs per channel.

The half-wave potentials for cation and anion formation were measured by using AC voltammetry. Two cell arrangements were employed. First, voltammograms were obtained on the samples contained in the ECL cell prior to the luminescence experiments. Potentials were measured relative to the Pt QRE. The value of $\Delta E_{1/2}$ between the potentials for cation and anion formation and the wave shape of the voltammograms remained constant for a given sample.

The voltammetric measurements were repeated in a standard 3-electrode cell using a 0.08 cm^2 Pt disk working electrode, Pt counterelectrode, and an SCE reference electrode. The voltammograms obtained in this fashion were identical with those obtained in the ECL cell and yielded half-wave potentials relative to a stable reference. The IR compensator of the PAR 173 was employed in all voltammetric measurements and the ferrocene–ferrocinium redox couple was used to estimate junction potential changes upon changing solvents.[26]

A 10-mV p–p 100-Hz sin wave from an HP-4204A signal generator was superimposed on the DC ramp from the PAR 175. The AC currents both in-phase with and quadrature to the applied potential were measured with an Ithaco Model 391A lock-in amplifier. The resulting AC voltammograms exhibited excellent signal to noise. The coulometric parameters were measured with the PAR 179 coulometer plug-in for the PAR 173.

Sample Preparation and Measurement Techniques. The electrolyte solutions were prepared by dissolving the TBAP in the appropriate solvent in the N_2 drybox and pouring the solvent onto alumina (Woelm, activated at 450 °C for 24 h, about 2 g of alumina/10 mL of solution) and stirring the mixture in the N_2 drybox at least 48 h prior to use. The alumina serves to remove nucleophilic impurities from the solvent.[27] The electrolyte was filtered through a dry 0.2-μm Teflon Millipore filter into the flask containing the pigment sample. The pigment sample had been azeotropically dried by three evaporations of solutions of the pigment in dry toluene. The concentrations of Chl a and Pheo a were each 10^{-4} M, measured spectrophotometrically. Fluorescence spectra of each solution in a 0.1-mm cell were measured by front surface excitation both before and after the chemiluminescence experiments.[28] About 25 mL of the sample solution was transferred to the degassing bulb of the ECL cell, and the sample was subjected to four freeze–pump–thaw cycles to 10^{-4} torr and allowed to return to room temperature. The sample was then poured into the main body of the cell. After placement of the cell in the light tight box the potentiostat was turned on and the QRE allowed to

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Table I. Redox Potentials of Chl a and Pheo a (V vs. SCE)[a]

solvent	$E_{1/2}^{+}$	$E_{1/2}^{+2}$	$E_{1/2}^{-}$	$E_{1/2}^{-2}$	$\Delta E_{1/2} = E_{1/2}^{+} - E_{1/2}^{-}$
			Chl a		
BCN	0.62	0.86	−1.20, −1.37	−1.64, −1.81	1.82, 1.99
BCN–1% THF	0.63	0.87	−1.17, −1.36	−1.62, −1.80	1.80, 1.99
THF	0.81	1.07	−1.20	−1.59	2.01
DMF	0.60	0.84	−1.12	−1.56	1.72
			Pheo a		
BCN	1.04	1.36	−0.94	−1.23	1.98
BCN–1% THF	1.03	1.35	−0.95	−1.23	1.98
THF	1.13	1.46	−0.97	−1.25	2.10
DMF	0.98	1.30	−0.87	−1.20	1.85

[a] Half-wave potentials listed are uncorrected for liquid junction potential changes as a function of solvent. The oxidation potential of ferrocene in each solvent is BCN = 0.51 V, BCN-1% THF = 0.51 V, THF = 0.57 V, and DMF = 0.52 V.

equilibrate for 30 min. AC voltammograms of the sample were then recorded. Pulsed light emission as a function of time was then averaged over 100 cation–anion generation cycles. Emission spectra were obtained by averaging the light emitted during 250 pulse sequences with an interference filter (Corion) having 10-nm fwhm bandwidth at a given wavelength in the filter holder between the integrating sphere and the PMT.

The absolute photometric calibration of the emission detection system was performed with an expanded laser beam (He–Ne, 632.8 nm) of known photon flux impinging on the bottom window of the empty ECL cell placed in the integrating sphere. Neutral density filters were used to vary the light intensity over 6 orders of magnitude. The number of photons counted by the photon counter over a 10-s integration time was determined for 20 different light intensities all the way down to the 5–10 counts s^{-1} dark count of the PMT. A plot of counts vs. photon flux was linear over the entire range measured and yielded a calibration factor of 7.98×10^3 photons/count registered.

As a secondary standard the oxidation of five known concentrations of luminol in Me_2SO contained in the bottom 0.5 cm of the ECL cell was performed as suggested by Lee and Seliger[29] and Michael and Faulkner.[30] This work yielded a calibration factor of 7.24×10^3 photons/count. The agreement between the laser and luminol calibrations substantially confirms the RCA specification that the response of the C31034A is flat within about 10% over the range of about 400–800 nm. The largest contributor to the magnitude of our calibration factor is the geometry of the detection system. The photocathode of the C31034A is only 1.0 cm × 0.3 cm and is set back 10 cm from the integrating sphere because of the low-temperature housing.

Results and Discussion

Voltammetric Measurements. There have been a variety of sometimes conflicting reports of redox potentials for the photosynthetic pigments in aprotic solvents.[12,21,31–46] It is only recently that the sensitivity of the electrochemistry of these molecules to solvent, electrolyte, and impurities in the electrolytic media has been more fully characterized. Most notable in this regard is the work of Bard on Chl a in DMF,[21] the experiments of Cotton and Van Duyne on Bchl a in various solvents,[46] and the studies of Fajer concerning the effect of solvent and electrolyte on Chl a oxidation potentials.[12]

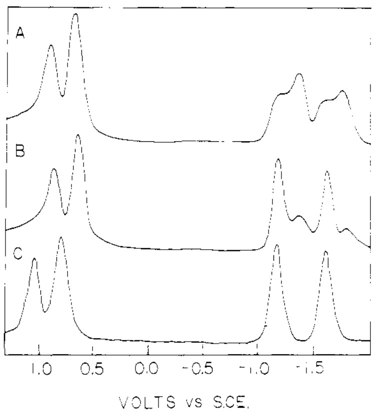

Figure 1. AC voltammograms of 10^{-4} M Chl a in (A) BCN, (B) BCN–1% THF, and (C) THF. The AC signal was 10-mV p–p at 100 Hz. The DC scan rate was 50 mV s^{-1}.

Half-wave potentials for Chl a and Pheo a redox processes in a variety of highly purified solvents appropriate for ECL measurements are presented in Table I. Butyronitrile (BCN) was chosen both for its wide potential limits and the ready solubility of the pigments in it. The AC voltammogram of Chl a in BCN, shown in Figure 1A, exhibits two reversible one-electron oxidations. The measured oxidation potentials agree with those reported previously in this solvent.[45] However, the reduction of Chl a in BCN yields a total of four AC voltammetric waves, each of which is 90 ± 5 mV wide (fwhm) and possesses a 43–45° phase angle relative to the applied AC potential (corrected for residual iR drop) at its current maximum. These parameters are completely consistent with and diagnostic for the reversibility of each reduction. This observation contrasts with that of Bard for Chl a in DMF.[21] In DMF Bard reported two reversible one-electron waves by using cyclic voltammetry. However, our results in BCN are similar to the observations of Cotton and Van Duyne regarding Bchl a reduction in CH_3CN.[46] These workers observed a distinct shoulder at a more positive potential on both the first and second reduction waves of Bchl a by using cyclic differential pulse voltammetry. Further, they were able to show that these shoulders were not due to Bchl a adsorption processes on the Pt electrode but were probably due to reduction of an aggregate of Bchl a. They added 1% THF to the CH_3CN and the shoulders disappeared, yielding two clean one-electron reversible reduction waves. Since the phase angle of the faradaic AC current at each Chl a reduction wave maximum in BCN was 43–45°, no significant adsorption processes were involved. The phase angle in the presence of pronounced adsorption tends to be much greater than 45°.[47]

Changing the solvent to BCN–1% THF results in the AC voltammogram shown in Figure 1B. Note that the current peak at −1.20 V has grown at the expense of that at −1.37 V. The same is true for the reduction wave at −1.65 V which increased as that at −1.82 V decreased. While this observation is similar to the type of behavior seen by Cotton and Van Duyne, the hypothetical

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"aggregated species" of Chl a is reduced at potentials more negative than those of the pentacoordinate Chl a monomer. This is just the opposite of that observed for Bchl a.[46] On the other hand, the oxidation potentials for both Chl a and Chl a cation change very little in the solvent with 1% THF.

In pure THF the oxidation potentials of Chl a are somewhat higher than those determined in BCN. This is consistent with the recent observation of Fajer.[45] The reduction of Chl a in THF, Figure 1C, exhibits two reversible one-electron waves at potentials very close to those of the first and third reduction waves of Chl a in pure BCN.

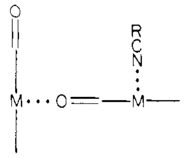
In DMF both the oxidation and reduction characteristics of Chl a indicate a single species undergoing two one-electron oxidations and two one-electron reductions. The redox potentials and voltammetric behavior observed for Chl a in DMF are consistent with those reported by Saji and Bard.[21]

Pheo a was examined in the same solvents, and the results are presented in Table I. There are only two one-electron reversible reduction waves for Pheo a in BCN. In general, the redox potentials for Pheo a exhibit relatively small solvent dependencies.

The dependence of Chl a reduction electrochemistry on the amount of coordinating solvent present in the electrolyte, and the apparent independence of Pheo a to solvent changes suggest that the coordinatively unsaturated magnesium atom in Chl a is responsible for this behavior. It is well-known that Chl a undergoes a wide variety of coordination phenomena both with added ligands and with the nucleophilic 9-keto carbonyl oxygen atom of other Chl a molecules.[48] Briefly, in the absence of added nucleophiles in noncoordinating solvents the magnesium atom of Chl a achieves pentacoordination through self-aggregation, whereas the addition of a nucleophilic ligand (L) can displace the coordination equilibrium to yield monomeric Chl a-L. In the limit as the donor strength of L increases hexacoordinate Chl a-L_2 prevails. The equilibria for many of these processes have been measured.[49]

Since the nitrogen atom of the nitrile group is known to be only weakly nucleophilic,[50] it is possible that Chl a in BCN may be partially self-associated. The most economic model one may propose is that the 9-keto carbonyl oxygen atom of one Chl a and the BCN nitrogen atom compete for the fifth coordination site of a second Chl a. It is certainly possible that the two electroactive species in solutions Chl a-L and Chl a_2 could have different reduction potentials and similar oxidation potentials. This type of behavior may also account for the similar Bchl a results in CH_3CN.[46] A comparison between the peak current of the first oxidation wave with the sum of the peak currents of the first two reduction waves for Chl a in BCN indicates that the same number of species are being oxidized at 0.62 V as are being reduced at both −1.20 and −1.37 V together. This suggests that there are at least two species undergoing independent one-electron redox processes.

The optical spectrum of Chl a is diagnostic for the coordination state of its magnesium atom and ligation by the oxygen atom of its 9-keto carbonyl group.[48] In BCN, BCN–1% THF, and DMF the $Q_y(0,0)$ transition occurs at 663 nm while the $Q_x(0,0)$ transition appears at 575 nm. These spectral parameters are consistent with pentacoordination for each Chl a molecule in solution. On the basis of this fact perhaps a better candidate for the Chl a species reduced at −1.37 V in BCN might be Chl a_2-L:



In this species each Chl a is pentacoordinate, yet the two Chl a molecules are nonequivalent due to the use of one carbonyl group as a ligand whereas the other remains free. Since the electronic spectra give no indication as to the nature of the species that disappears upon addition of THF, it is difficult to assign a specific structure that could account for the observed solvent dependence of the Chl a reduction electrochemistry.

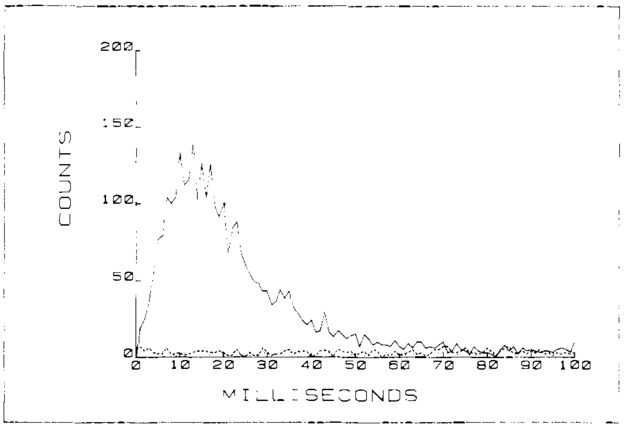


Figure 2. ECL of 10^{-4} M Chl a in BCN–1% THF. The solid line is the photon counter response when Chl a^- is generated from 0–100 ms following a 100-ms Chl a^+ generation pulse. The dashed line is the photon counter response for Chl a^+ generation alone from 0–100 ms while the dotted line is the photon counter response for Chl a^- generation alone from 0–100 ms. The traces are each the average of 100 pulse sequences.

The addition of THF to BCN shifts the equilibrium with the result that most of the Chl a in BCN–1% THF presumably adopts conventional Chl a-THF pentacoordinate ligation. In pure THF it is well established that Chl a is predominantly hexacoordinate.[48,49] Yet, the reduction potentials of Chl a do not change significantly. This is quite different from the large changes observed in Chl a oxidation potentials upon increasing the magnesium atom coordination number from five to six.[45] Apparently, there is only one doublet state of Chl a^- populated for both five and six coordination unlike the two low-lying doublet states of Chl $a^{+\cdot}$ whose relative population is controlled by the coordination state of the magnesium atom.[45]

The difference between the half-wave potential for formation of the cation radical and that of the anion radical is the quantity of prime concern in determining whether ECL is energetically possible for Chl a and Pheo a. The $\Delta E_{1/2}$ values are given in Table I. Since these values are proportional to ΔG for the charge-transfer neutralization, an estimate of $T\Delta S$ is necessary to compare the resulting ΔH for the charge transfer with the energies of the singlet and triplet states of these molecules. The value of $T\Delta S$ is usually estimated to be 0.1 ± 0.1 eV so that the values of $\Delta E_{1/2}$ listed in Table I may need to be reduced by as much as 0.2 eV to estimate ΔH.[51] One immediately recognizes that since the Chl a first excited singlet state lies 1.86 eV above the ground state, the Chl^+a–Chl^-a reaction of monomeric Chl a may not yield sufficient energy to directly populate the singlet state. Even the reaction between the supposedly aggregated Chl a species in pure BCN may be only marginally energy sufficient. The Pheo a^+–Pheo a^- reaction may be marginally energy sufficient for lowest excited singlet state formation in all the solvents examined. Nevertheless, all the pigments liberate more than enough energy in their cation–anion reactions to populate the lowest excited triplet state of each pigment, since this state for Chl a is 1.30 eV above the ground state while that of Pheo a is 1.34 eV.[52]

ECL Measurements. A triple potential step program was used to generate the Chl a and Pheo a radical ions in the ECL experiments.[24] The potential was stepped to a value at which one of the radical ions is produced at a diffusion-controlled rate. The duration of this step was typically 100 ms. The potential was then rapidly stepped to a value at which the oppositely charged radical ion is produced at the diffusion-controlled rate. The duration of

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Table II. ECL Data

solvent	photons emitted[a]	charge passed (coulombs)[b]	ϕ_c
	Chl a		
BCN	2.52×10^5	4.93×10^{-7}	8.2×10^{-8}
BCN-1% THF	1.18×10^6	4.85×10^{-7}	3.9×10^{-7}
THF	1.30×10^6	5.09×10^{-7}	4.1×10^{-7}
DMF	6.46×10^5	1.61×10^{-6}	6.4×10^{-8}
	Pheo a		
BCN	9.76×10^5	8.92×10^{-7}	1.8×10^{-7}
BCN-1% THF	3.05×10^6	9.04×10^{-7}	5.4×10^{-7}
THF	2.84×10^6	8.80×10^{-7}	5.2×10^{-7}
DMF	3.41×10^6	9.90×10^{-7}	5.5×10^{-7}

[a] $t_f = t_r = 0.1$ s. [b] During t_f.

the second step was equal to that of the first step. The final step was to 0 V vs. the QRE and served to remove all radical species from the solution. This pulse sequence was repeated at 10-s intervals. In each experiment the photomultiplier tube response was time averaged by the computer over at least 100 pulse sequences. The overall shape and intensity of the individual transients did not vary significantly from scan to scan. The solid line in Figure 2 shows a typical emission intensity profile as a function of time for the Chl a$^+$–Chl a$^-$ reaction in BCN–1% THF. In this instance the cation was generated first, but the same response is obtained if the anion is generated first. In order to check for luminescence arising from side-reactions of Chl a$^+$ and Chl a$^-$ alone, we used a double potential step of 100-ms duration to generate each ion separately. In Figure 2 the photomultiplier response during Chl a$^-$ formation is depicted by the dotted line, while the response during Chl a$^+$ generation is shown by the dashed line. Luminescence occurs only during the second potential step of the triplet step sequence when both Chl a$^+$ and Chl a$^-$ are present.

Similar luminescence decay curves were obtained for Chl a$^+$–Chl a$^-$ and Pheo a$^+$–Pheo a$^-$ reactions in each solvent studied. The shapes of the curves are very similar with the emission intensity falling to the level of the background well before completion of the second radical-formation pulse. The integrated intensity of the emission varies substantially in different solvents but is rather low in all solvents employed in these experiments. The integral of each curve corrected for background, normalized to a single scan, and scaled using the absolute photometric calibration factor for our apparatus is listed in Table II. In order to determine the efficiency of Chl a and Pheo a ECL, we determined the so-called coulometric efficiency for each reaction according to the method of Faulkner[53] (Table II).

The coulometric efficiency ϕ_c is the ratio of the number of photons emitted to the number of electrons passed in the initial potential step.

A mixture of 10^{-4} M Chl a and 10^{-4} M Pheo a was prepared in each of the four solvents employed in this study. The potential of the working electrode in the ECL cell was stepped from that at which only Chl a$^+$ is produced to that at which only Pheo a$^-$ is produced. Care was taken to monitor potential overshoot to avoid formation of Pheo a$^+$ or Chl a$^-$. Under no conditions did the Chl a$^+$ or Pheo a$^-$ ion pair reaction yield measurable luminescence. Changing the order of radical ion formation or collecting 500 transients did not yield detectable luminescence. Stepping the cathodic potential out to the value at which Chl a is reduced in addition to Pheo a resulted in a return of luminescence albeit at somewhat lower intensity than that of Chl a alone in each solvent. Similarly, stepping the anodic potential out to the value at which Pheo a$^+$ is formed results in a return of luminescence.

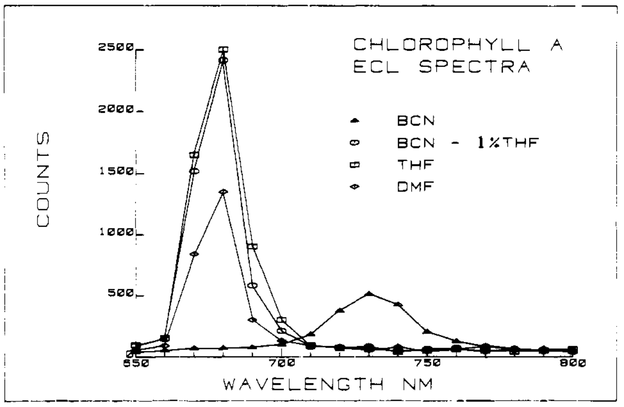

Figure 3. ECL spectra of Chl a in the solvents indicated. The intensity at each point is the integrated luminescence output of 250 pulse sequences.

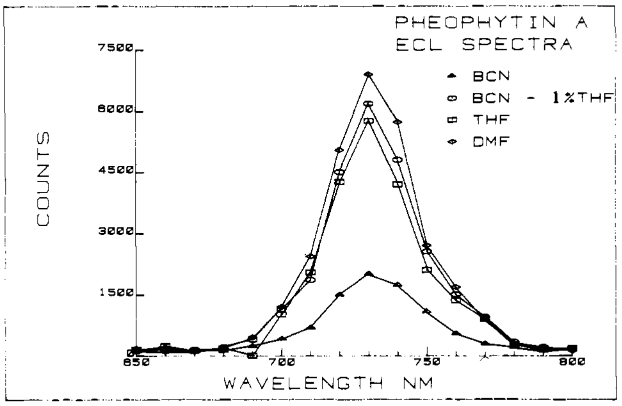

Figure 4. ECL spectra of Pheo a in the solvents indicated. The intensity at each point is the integrated luminescence output of 250 pulse sequences.

Table III. Fluorescence Maxima of Chl a and Pheo a in Electrolytic Solutions (nm)

solvent[a]	Chl a[b]	Pheo a[c]
BCN	668	674
BCN-1% THF	668	674
THF	669	674
DMF	668	674

[a] All solutions are 0.1 M in TBAP. [b] 430-nm excitation. [c] 410-nm excitation.

The ECL spectra of Chl a and Pheo a reflect to some degree the aggregation phenomena occurring in the various solvent systems. Figure 3 shows that the Chl a ECL spectra resemble normal Chl a fluorescence spectra with maxima about 680 nm except in pure BCN in which the emission is strongly red-shifted and broadened. There is no difference in the Chl a ECL spectra in BCN for radical anion formation at either −1.20 or −1.37 V vs. SCE. In each solvent in which the emission occurs at 680 nm the voltammetry indicates that the ground state is predominantly monomeric Chl a-L. The approximately 15-nm red shift of the ECL spectra for Chl a in BCN–1% THF, THF, and DMF relative to the fluorescence maxima is probably due to residual self-absorption effects. There is little change in the emission maximum upon changing from five-coordinate Mg in DMF to six-coordinate Mg in THF. This observation is consistent with the fluorescence maxima for optically excited Chl a listed in Table III. However, Table III also shows that the fluorescence maximum of Chl a in BCN is 668 nm. As we have seen above, the voltammetry indicates that there is probably some aggregation of Chl a in the ground state in BCN. The fact that the ECL spectrum is red-shifted suggests that this aggregation perists through the intermediates which precede formation of the excited singlet state responsible for the ECL.

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(53) Feldberg originally proposed an analysis of ECL decay curves to distinguish between singlet and triplet mechanisms. Feldberg, S. W. *J. Am. Chem. Soc.* **1966**, *88*, 390; *J. Phys. Chem.* **1966**, *70*, 3928. The results of the treatment are often difficult to interpret when triplets are involved and thus it is not applied here, see ref 59.

In contrast to the Chl a results, Figure 4 shows that the Pheo a ECL spectra are red-shifted relative to the normal fluorescence maximum of Pheo a in *every* solvent system examined. In this case, there is no evidence from either optical spectra or the voltammetric data to suggest the presence of ground-state aggregates. This is consistent with the fact that Pheo a aggregates by π stacking at higher concentrations than were employed in these experiments. The 730-nm emission is not Pheo a phosphorescence because the triplet is known to emit at about 930 nm.[52] The cation and anion of Pheo a may react to ultimately yield a singlet excimer which then emits at 730 nm. Excimer and exciplex formation in polar electrolytic solvents by the reaction of electrogenerated radical cations with radical anions is well documented.[19] Since the singlet state energy of Chl a is very close to that of Pheo a, 1.86 and 1.84 eV, respectively, it is not surprising that the emission from the aggregated, excited Chl a species is close to that of the assumed true excimer of Pheo a. Moreover, the 730-nm emission maxima are consistent with the magnitude of the red shifts observed in the fluorescence spectra of covalently linked Chl a dimers in their stacked conformation.[16]

Since emission from the excited aggregates of Chl a and Pheo a occurs at 730 nm, their lowest excited singlet state lies 1.70 eV above the ground state.

An examination of $\Delta E_{1/2}$ for both Chl a and Pheo a in Table I taking into account the 0.1 ± 0.1 eV $T\Delta S$ term shows that direct singlet excimer formation is at least marginally energy sufficient for Chl a and almost certainly energy sufficient for Pheo a.

In view of the possible involvement of both singlet and triplet pathways contributing to the observed ECL we will briefly discuss the ECL efficiency in terms of each mechanism.[53] In general, the coulometric efficiency of the ECL process ϕ_c listed in Table II does not equal the ECL efficiency ϕ_{ecl} because a fraction of the radical ions produced in the first potential step are consumed in the second step. Digital simulation of the triplet step sequence has shown that ϕ_{ecl} should be about 8% larger than ϕ_c.[54] For our purposes $\phi_{ecl} \simeq \phi_c$ and the values listed in Table II may be regarded as ϕ_{ecl}.

If a purely singlet mechanism for excimer formation and emission prevails, then

$$R^+ + R^- \rightarrow {}^1R_2^*$$

$$ {}^1R_2^* \rightarrow 2R + h\nu$$

where $\phi_{ecl} = \phi_{ct}\phi_f$, ϕ_{ct} is the efficiency of singlet state production via the radical ion annihilation, and ϕ_f is the fluorescence efficiency of the excimer. If preequilibria involving aggregation prior to radical ion annihilation exist as may well be the case for Chl a in BCN, then ϕ_{ct} becomes the efficiency of excited singlet state production from the reaction of these aggregated radical ions. Since total light output of these reactions is very low, the source of inefficient ECL could be a poor yield of excited states in the charge-transfer reaction, a low fluorescence yield for the singlet excimer, or both. Singlet excimers do not necessarily have intrinsically low fluorescence yields.[55] It is well-known that the quantum yield of fluorescence of Chl a dimers depends greatly on the geometry and ligation of the Chl a.[56,57] For example, self-associated noncovalently linked Chl a dimers have fluorescence yields close to 10^{-2},[56] whereas covalently linked Chl a dimers in which the Chl a molecules occupy roughly parallel planes relative to each other have fluorescence quantum yields of 0.3–0.4.[57] Since the self-associated Chl a dimers are thought to possess a geometry relative to one another that is something other than parallel planar, the large change in quantum yield must reflect the detailed geometry of the excited state. Even when Chl a dimers possess very similar geometries, we have shown that small solvation changes can greatly affect the fluorescence yields of the dimers.[57] If one assumes the fluorescence yields of both the Chl a and Pheo a excited aggregates produced in the ECL experiments are at most about 0.4, then the inefficiency of the ECL process is predominantly due to ϕ_{ct}. Even if $\phi_f \simeq 10^{-2}$ the overall value of ϕ_{ecl} is still dominated by ϕ_{ct}. Thus, this suggests that the charge-transfer neutralization process leads predominantly to vibrationally excited states of the ground electronic state.

If on the other hand a triplet mechanism (eq 1–3) is operative

$$R^+ + R^- \rightarrow {}^3R^* + R \qquad (1)$$

$$ {}^3R^* + {}^3R^* \rightarrow {}^1R_2^* \qquad (2)$$

$$ {}^1R_2^* \rightarrow 2R + h\nu \qquad (3)$$

the efficiency of the triplet–triplet annihilation step ϕ_{tt} must be evaluated in order to gauge the efficiency with which triplets are formed in the radical ion annihilation. Parker has determined that ϕ_{tt} for Chl a in ethanol is about 0.1.[58] This value should not change greatly for Pheo a and should be at least of similar order of magnitude in the solvents we employed. However, the triplets are quenched effectively by the surrounding radical ions. Faulkner has determined that ϕ_{tt} should be roughly competitive with the quenching process.[59] Thus, once again the dominant inefficient step in the ECL process is most likely the formation of triplets in the radical ion annihilation step. These observations are consistent with the dominance of nonradiative mechanisms for the decay of charge-transfer states observed in our previous studies of reaction center models.[16] Our earlier results showed that if substantial charge-transfer character is produced optically in a Chl a type donor–Pheo a type acceptor system for which the geometry of the donor relative to the acceptor is only partially constrained, the collapse of the charge separation does not yield a luminescent state or even substantial quantities of triplets. Radiationless decay to the ground state is the only observed deactivation mechanism. It is interesting that the ECL experiments most analogous to the reaction center model studies, the reaction of Chl a$^{+\cdot}$ with Pheo a^{-}, yielded no observable light.

Reaction centers of photosynthetic bacteria and PS I and PS II in chloroplast preparations are also chemiluminescent when normal electron transport is artificially blocked.[18] In these cases, especially for the bacteria, the participation of the triplet radical pair state of the donor–acceptor and the triplet excited state of the donor in the mechanism determining the quantum yield of chemiluminescence is highly likely. Quantum yields of delayed luminescence in vivo have been estimated to be 10^{-4}–10^{-3},[18] at least 3 orders of magnitude higher than ϕ_c for our ECL experiments. Once again the importance of donor–acceptor geometry is manifest. The reaction center donor and acceptor are fairly rigidly fixed relative to one another by the protein matrix so that factors such as collisional deactivation of the excited states do not influence the luminescence yield greatly. Moreover, the dimeric chlorophyll donors in vivo are presumably also under similar geometric constraints. Thus, the efficiencies of the ECL processes of Chl a and Pheo a observed in vitro are consistent with those measured in vivo.

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