On Novel Fluorescence in the Q_x Region of the Bacteriochlorophyll *a* and Chlorophyll *a* Dimers in Solution†

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Abstract: Pulsed laser induced fluorescence spectra of dimeric and aggregated bacteriochlorophyll *a* (BChl *a*) in solution are presented. Two fluorescence bands, at 783 and 583 nm for BChl *a* in hexane, are assigned to the BChl *a* dimer, These two bands correspond to the two fluorescence bands of the chlorophyll *a* (Chl *a*) dimer in solution at 688 and 625 nm.[1] Comparison of absorption and fluorescence data for the two chlorophyll dimers allows assignment of the shorter wavelength fluorescence transitions at 625 and 583 nm to the dimer Q_x transitions of the Chl *a* and BChl *a* dimers, respectively. The stronger fluorescence bands at 688 and 783 nm are the corresponding dimer Q_y transitions. Q_x fluorescence transitions are not observed for the monomer of either Chl *a* or BChl *a* in solution. The activity of the Q_x fluorescence transitions in the dimers and the observation that the fluorescence lifetime of the Q_y transition is longer than that of the Q_x transition for each of the chlorophyll dimers are discussed in relation to nonradiative channels for deexcitation of the dimers and monomers.

The fluorescence spectrum of the chlorophyll *a* (Chl *a*) dimer in solution has eluded observation until recently, when a pulsed laser induced fluorescence study[1] of Chl *a* in solution showed that the fluorescence spectrum of the dimer is characterized by two bands: in hexane solutions the main band at 688 nm has a fluorescence lifetime $\tau < 5$ ns and the weaker band at 625 nm has a longer lifetime, $\tau \simeq 5$ ns. The fluorescence intensities from dimeric Chl *a* in solution are significantly lower than those from solutions of monomeric Chl *a*, for which a single strong emission band at 680 nm is typically observed.

Several attempts have been made to analyze the visible absorption spectrum of the Chl *a* dimer in terms of an exciton formalism.[2-5] However, the unambiguous assignment of the absorption and fluorescence spectra[1] of the dimer in solution could not be made on the basis of the theoretical models in the literature. The two-electron model of Shipman and co-workers[2] predicts one allowed transition and one forbidden (weakly allowed) transition for the dimer Q_y absorption transitions. This model would be consistent with the assignment of either (i) the stronger fluorescence at 688 nm to the allowed dimer Q_y transition and the weaker fluorescence band at 625 nm to an allowed dimer Q_x transition or (ii) the two observed fluorescence bands to the two split dimer Q_y transitions. The latter assignment would imply a large Q_y exciton splitting of ~ 1400 cm^{-1}.[1]

A more recent four-orbital model for the Chl a dimer[6] predicts two dimer Q_y absorption bands of approximately equal intensity. Assignment of the broad fluorescence band at 688 nm to the *two* components of the dimer Q_y transition, split by ~ 100 cm^{-1}, would be in agreement with this model.[7] The fluorescence band at 625 nm may then be assigned to a weak dimer Q_x transition.[1]

Q_x band fluorescence has not been observed for the Chl *a* monomer. The activity of a Q_x fluorescence transition in the dimer would indicate that the symmetry considerations for the transition moments for the dimer are different from those for the monomer, as a consequence of the relative orientation of the two Chl *a* molecules in the dimer and that nonradiative pathways for deexcitation of the Q_x levels in the monomer are restricted in the dimer. For these reasons it is important to determine whether the 625-nm fluorescence band of the Chl a dimer originates from a dimer Q_x transition. In addition, such a result would exclude the large exciton splitting of ~ 1400 cm^{-1} in favor of a splitting in the order of ~ 100 cm^{-1}.

In Chl *a*, the Q_x absorption transitions lie underneath vibronic sidebands of the more intense Q_y absorption bands.[5,8,9] This hinders attempts to resolve the origin of the 625-nm fluorescence transition by selective excitation of fluorescence at wavelengths in the region which would correspond to the predicted dimer Q_x absorption bands.[1] In contrast, in bacteriochlorophyll *a* (BChl *a*), the Q_y absorption region at ~ 780 nm is separated by more than 4000 cm^{-1} from the Q_x absorption region at ~ 580 nm.[5,8] Since a Q_y exciton splitting in the BChl *a* dimer of sufficient magnitude to shift Q_y components into the 580-nm region is improbable, any emission in this spectral region may be assigned to Q_x transitions for BChl *a* with more confidence than was the case for Chl *a*.[1] The fluorescence study of dimeric and oligomeric BChl a in solution which is reported in this paper is based on the more extensive spectroscopic investigation of the fluorescence of monomeric and dimeric Chl *a* in solution.[1] Since the Chl *a* dimer in solution always exists in equilibrium with small amounts of strongly fluorescent monomeric Chl *a*, and possibly with oligomeric Chl *a*, use was made of the full potential of tunable pulsed laser spectroscopy for wavelength selective excitation of fluorescence, time resolved measurements, and laser intensity-dependent photoquenching experiments to separate the fluorescence spectra of the different species of Chl *a* in solution.

The formation of dimers and oligomers of BChl *a* in nonpolar solvents has not been studied as intensively as the aggregation of Chl *a* in solution.[5] Although BChl *a* shows a slightly greater tendency to aggregate, the behavior of the two chlorophylls is in many ways analogous. In wet carbon tetrachloride (CCl_4), visible absorption studies have shown that BChl *a* exists as dimer in equilibrium with monomer,[4] as does Chl *a* in CCl_4 and in benzene;[4,10] a recent proton NMR and visible absorption study has also shown that BChl *a* is predominantly dimeric in wet benzene.[11] These results contrast with those from vapor-phase osmometry measurements which suggest that in several *dry* hydrocarbons BChl *a* has an aggregation number of ~ 3 at low concentrations, whereas the aggregation number of Chl *a* in the same solvents

† Research supported by the Natural Sciences and Engineering Research Council of Canada.

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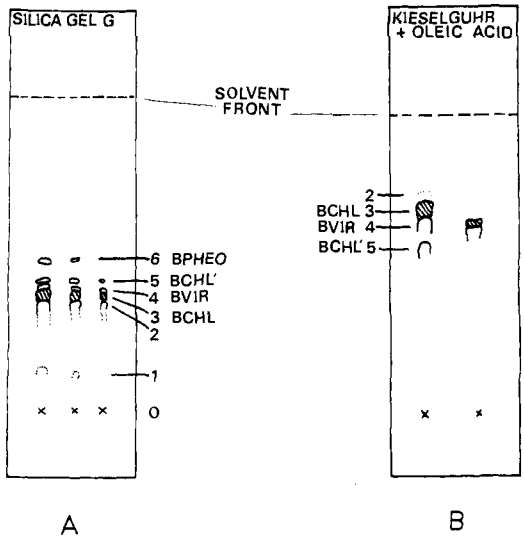

Figure 1. Separation of impurities from BChl *a* samples by (A) thin-layer chromatography (TLC) on silica gel G and (B) reverse-phase TLC on Kieselguhr impregnated with oleic acid. Zones were identified from chromatographic sequence and absorption and fluorescence spectra: 1, unknown (yellow green); 2, unknown (blue grey); 3, BChl *a* (blue green); 4, BVir (yellow green); 5, BChl *a'* (blue green); 6, bacteriopheophytin (pink). Since zones 1, 2, and 6 are absent or barely detectable on the reverse-phase TLC, and the relative amounts of these impurities increase with exposure time to the silica gel G plate, these impurities are believed to be caused by reaction with the siliceous adsorbent.

is 2 or 4, characteristic of multiples of dimeric units.[11]

Studies of aggregates of Chl *a* involving water or other bifunctional ligands (such as alcohols, dioxane, or pyrazine) reveal that the visible absorption bands are generally red shifted with respect to those of the monomer.[12] Polymeric hydrates exhibit large red shifts similar to those characteristic of bacterial antenna complexes in vivo, In addition to NMR and infrared absorption studies, resonance Raman spectroscopy has been used to aid the characterization of BChl *a* interactions in the in vitro aggregates.[13]

A detailed study of solvent effects on the fluorescence lifetimes of monomeric BChl *a* in a large number of solvents has been published.[14] There are also numberous studies of the fluorescence of BChl *a* in vivo,[15] but it appears that there is a paucity of fluorescence studies to complement the absorption data for aggregated BChl *a* in vitro.[15] We therefore present below an analysis of the fluorescence spectra of BChl *a* in benzene, CCl_4, hexane, and pyridine solutions.

Experimental Section

Sample Preparation. BChl *a* was obtained from Porphyrin Products Inc. Chl *a* was extracted from spinach and purified on a column of Sepharose CL-6B according to the method of Omata and Murata.[16] The purity of the chlorophylls was checked by thin-layer chromatography as detailed below. The chlorophyll samples were dried by codistillation with dry CCl_4.[5] CCl_4 (Baker Photrex) and hexane (Caledon Labs. Inc. spectral grade) were dried by passing through columns of neutral activated alumina (Woelm, activity 1).[17] The sample solutions were prepared in a dry nitrogen purged glove box and were sealed in quartz fluorescence cells (10 × 10 mm) fitted with vacuum-tight stopcocks to exclude oxygen and moisture. Titrations of the dry solutions with pyridine (BDH Aristar grade) were carried out with a Hamilton microliter syringe.

Spectroscopic Methods. Absorption spectra were recorded with a Varian DMS 90 visible/ultraviolet absorption spectrophotometer. Details

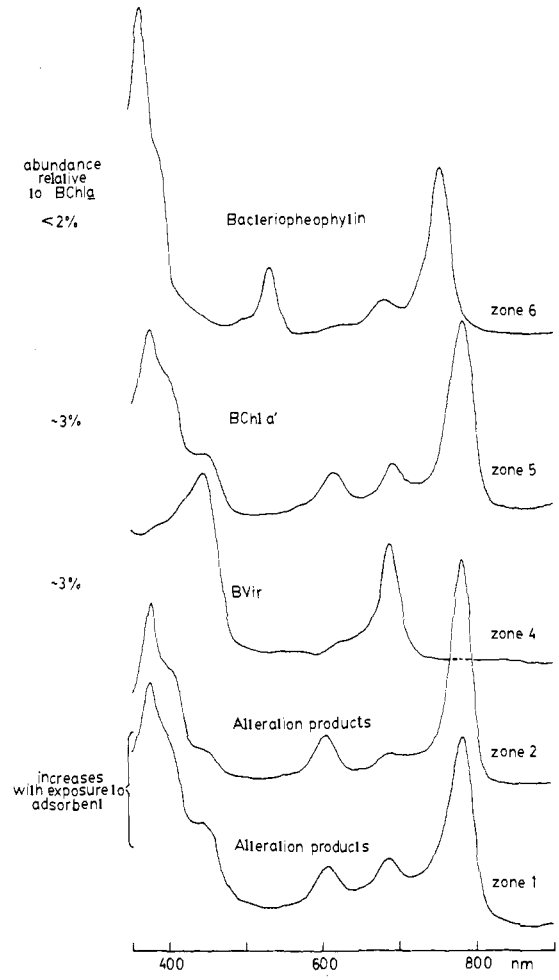

Figure 2. Absorption spectra of the impurity zones eluted from the silica gel G TLC plate. The identity given for each zone is that of the major component. It is noted that complete separation of each component is not achieved: a component absorbing at ~680 nm and ~430 nm, thought to be BVir or a similar pigment, is apparent in each spectrum.

of the pulsed laser and the detection system used for the fluorescence studies have been published elsewhere.[1,18,19] The tunable dye laser (Lambda Physik FL 2000) pumped by a pulsed nitrogen laser (Lambda Physik M 2000) produces 3-ns pulses at a repetition rate of 25 Hz. The average laser power used for the fluorescence experiments was ~1 mW at ~400 nm and ~1.5 mW at ~580 nm. A PTR F100 grating filter was used to exclude superradiance from the dye laser.

The unfocused laser beam (diameter ~2 mm) entered one face of the sample cell at normal incidence, and fluorescence was collected at 90° to the laser beam. The emission was dispersed with a Jarrell Ash 1-m monochromator. In order to eliminate the second-order diffracted laser line in the region of the fluorescence when exciting with laser wavelengths at ~400 nm an Ealing GG-495 cutoff filter was included with the focusing optics in front of the entrance slit of the monochromator. The detection system included a cooled RCA 7102 photomultiplier tube, fast pulse preamplifiers with ×100 gain (Le Croy 101A) and a PAR boxcar integrator, Model 162 mainframe, equipped with Model 165 gated integrator which provides 2-ns time resolution.

Sample Purity. Sample purity was assessed by thin-layer chromatography followed by spectroscopic analysis of any impurities detected. Chl *a* was repeatedly chromatrographed on the Sepharose CL-6B column until thin-layer chromatography on silica gel G (Merck, used without activation) revealed only two spots: Chl *a* accompanied by a minor component of the isomeric Chl *a'* (solvent system hexane–acetone–*tert*-butyl alcohol, 90:5:5).[20]

Chomatography of the BChl a sample on silica gel G (solvent system hexane–acetone–*tert*-butyl alcohol, 9:1:1) showed five minor components

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Table I. Absorption and Fluorescence Data for Bacteriochlorophyll in Solution[a]

	absorption data						fluorescence data					
			absorption maxima						fluorescence maxima			
			Q_y		Q_x				Q_y		Q_x	
	concentration		λ_{max}, ± 1	fwhm, ±10	λ_{max}, ±1	fwhm, ±20	concentration		λ_{laser},	λ_{max}, ±2	fwhm, ±20	λ_{max}, ±2	fwhm, ±20
solvent	BChl *a*, M	pyridine, M	nm	cm^{-1}	nm	cm^{-1}	BChl *a*, M	pyridine, M	nm	nm	cm^{-1}	nm	cm^{-1}
benzene	10^{-4}		781	610	583	~1150	10^{-5}		400	796	670	583	vw
(0.03% H_2O)	10^{-6}		781	690	581	~1040	10^{-5}		580	798	500		
benzene	10^{-6}	2×10^{-2}	781	500	~580 sh		10^{-5}	2×10^{-2}	580	795	460		
					~606								
(0.03% H_2O) +	10^{-6}	4×10^{-2}	781	480	583								
pyridine	10^{-4}	2×10^{-2}	781	480	~586								
					~606								
CCl_4 (dry)	10^{-5}		778	740	584	~1150	10^{-5}		400	795	600	587	
CCl_4 (dry) +	10^{-5}	2×10^{-2}	780	480	~583								
pyridine					~606 sh								
hexane (dry)	10^{-5}		769	830	582	~1080	10^{-5}		385	778	800	585	vw
	10^{-4}		770	940	582	~1195	10^{-4}		400	783	590	583	300
									400	785	700	583	300
									540	784/764	850	585	300
									565	782	450		
									580	783	590	585	vw
									600	785	690		
									620	785	690		
									400	783	430		
							10^{-5}	2×10^{-2}	580	785	430		
hexane (dry) +	10^{-5}	2×10^{-2}	775	430	~578				600	785	450		
					~600								
pyridine	10^{-4}	2×10^{-2}	769	450	~580				620	785	450		
					~604								
pyridine	10^{-5}		780	430	609		10^{-5}		400	795	450		

[a] Abbreviations: sh = shoulder; λ_{laser} = laser excitation wavelength; vw = too weak to measure bandwidth reliably.

in addition to the major spot of BChl *a* (figure 1A). To rule out the presence of fluorescent impurities which could be responsible for the new fluorescence bands reported in this article, the zones from a number of chromatograms were scraped off the plates and eluted with pyridine, and absorption and fluorescence spectra of each impurity band were recorded. The absorption spectra are shown in Figure 2. These samples were exposed to air and light, and therefore it is expected that the proportion of degradation products to BChl *a* is higher than that found in the samples prepared in dim light in the dry nitrogen purged glovebox, from which the fluorescence spectra shown in Figures 4 and 5 were recorded.

Siliceous adsorbants have been reported to cause alteration of the chlorophylls and may contribute to the degradation products observed. A separation on a less reactive reverse-phase thin-layer system was therefore also employed. Kieselguhr (Merck) plates were impregnated by dipping in a 1% solution of oleic acid in petroleum ether (bp 30–60 °C) and allowed to dry for 30 min at 60 °C. The plates were developed by the sandwich plate method using methanol–acetone–water (20:10:5). This method revealed, even under heavy loading, only three impurities in the BChl *a* sample (Figure 1B). Since the two additional impurity spots observed by TLC on the silica gel G were absent, and it was observed that the relative amount of these additional spots increased on prolonged exposure to the silica plate; zones 1 and 2 are believed to be degradation products caused mainly by reaction with the siliceous adsorbent. It is noted that the results of the reverse-phase TLC are in agreement with the analysis of the degradation products of BChl *a* by Kim[21] using a similar reverse-phase thin-layer technique.

The impurities were identified if possible from known absorption spectra, and the identities and the relative abundances of the known impurities are given with the absorption spectra in Figure 2. Emission spectra of the samples eluted from each zone showed fluorescence corresponding only the the main red absorption band of each impurity, that is, at ~780 nm for zones 1, 2, and 5, at ~760 nm for zone 6, and at ~690 nm for zone 4. Zone 4 was identified as bacterioviridin (BVir) known also as 2-acetyl-2-devinylchlorophyll *a* or Δ3,4 BChl *a*.[9,11,22,23] The fluorescence quantum yield of this component was higher than that of BChl *a* or the other impurities, for laser excitation at 400 nm. From the relative abundances and quantum yields of the impurities, it is estimated that the fluorescence intensity from impurities 1,2,5, and 6 (that is, two unidentified alteration products, a compound with an absorption spectrum similar to BChl *a* thought to be BChl *a'* and bacteriophenophytin (BPheo), respectively), in the unpurified samples of BChl *a* prepared in a dry nitrogen atmosphere, is 10^{-2} times that of BChl *a*, while that of impurity 4 (BVir) should be 10^{-1} times that of BChl *a*. Thus, BVir is expected to be the only impurity with detectable fluorescence in the unseparated BChl *a* sample. It has been noted by several authors[21,11,14] that separation of this impurity from BChl *a* is difficult and that even highly purified samples of BChl *a* are not free from fluorescence at 690 nm. We observed that this oxidation product accumulated after prolonged laser irradiation of the BChl *a* samples, and subsequently, irradiated samples degraded completely if left overnight in the dark to yield BVir as the major product.

Experimental Results

A. Absorption Spectra. The absorption spectrum of a 10^{-5} M solution of dried BChl a in dry hexane is shown in Figure 3A. The spectrum shows a broad Q_y absorption band at 770 nm, a weaker Q_x absorption band at 582 nm, and strong absorptions in the Soret region with maxima at 360 and 390 nm.[12,13] Titration of the dry solution with pyridine disaggregates dimeric and oligomeric BChl *a* present in the dry solution to form the pyridinated monomer.[5] The titrated solution yields the absorption shown in Figure 3B. The absorption bands of the monomeric BChl *a* are considerably narrower than those of the aggregated solution. It should be noted that spectrum 3B shows a double Q_x absorption band indicating that, after addition of pyridine to the dry solution, both five- and six-coordinate BChl *a* monomers are present in the solution.[24,25] BChl *a* appears to have a strong tendency toward five coordination in the dry solvent, even in the presence of a large

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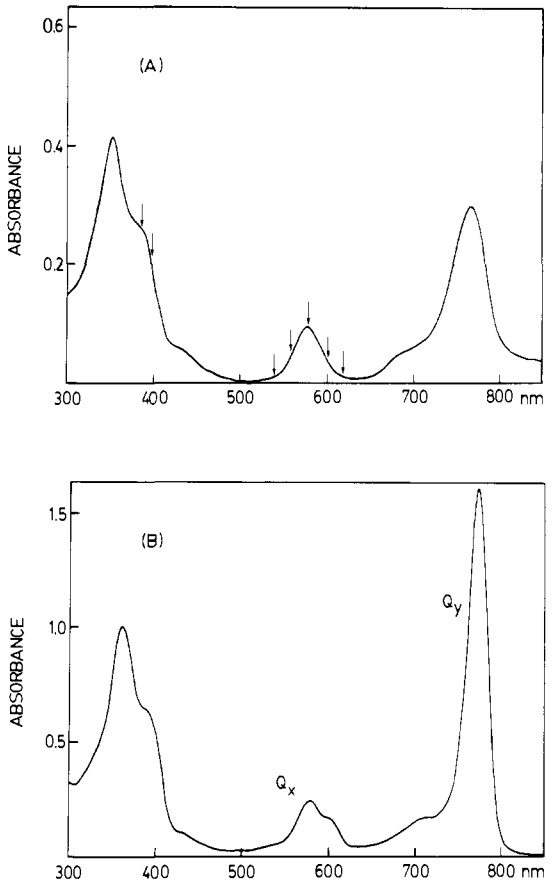

Figure 3. (A) Absorption spectrum of $\sim 10^{-5}$ M BChl *a* in dry hexane. The arrows indicate the laser wavelengths employed for excitation of fluorescence of the solution (10-mm path length cell). (B) Absorption spectrum of the same solution as in Figure 1A after addition of pyridine (2×10^{-2} M) to disaggregate the dimeric and oligomeric BChl *a*. The Q_x and Q_y absorption bands are indicated (10-mm path length cell). The double peak in the Q_x absorption region indicates the presence of pentacoordinated ($\lambda_{abs} \simeq 580$ nm) and hexacoordinated ($\lambda_{abs} \simeq 600$ nm) monomeric BChl *a*.

molar excess of a nucleophilic ligand.[25]

The absorption maxima and the full width at half-maxima (fwhm) of the Q bands of BChl *a* in several solvents are listed in Table I. For solutions in wet benzene, in which NMR studies have shown that BChl *a* is mainly dimeric,[11] the fwhm of the Q_y absorption band is broadened with respect to the monomeric absorption band in the same solvent but not to as great an extent as observed for the dry solution of BChl *a* in hexane. This suggests that the hexane solution contains significant amounts of oligomers larger than the dimer. Although the oligomeric solutions show considerable broadening of the absorption bands, there is no red shift as observed for oligomeric Chl *a* in solution.[5] The monomeric bandwidths are slightly narrower in hexane (~ 440 cm^{-1}) than in CCl_4 and in benzene (~ 480 cm^{-1}).

B. Fluorescence Spectra. The maxima and fwhm of the observed fluorescence bands of solutions of BChl *a* in benzene, CCl_4, and hexane are summarized in Table I together with complementary absorption data. Fluorescence spectra for the solutions of BChl *a* in hexane are shown in Figure 4 for excitation at wavelengths in the region of the blue (Soret) absorption band at 401.3 nm and in Figure 5 for excitation at five wavelengths in the region of the yellow Q_x absorption band. The maxima and fwhm of the observed fluorescence bands of solutions of BChl *a* in benzene, CCl_4, and hexane are summarized in Table I together with the complementary absorption data.

In Figure 4, the fluorescence spectrum of 10^{-5} M BChl *a* in dry hexane (Figure 4A) is compared with the fluorescence spectrum from the same solution after titration with pyridine to disaggregate dimeric or oligomeric BChl *a* (Figure 4B). The intensity of the fluorescence from the dry solution is ~ 5 times

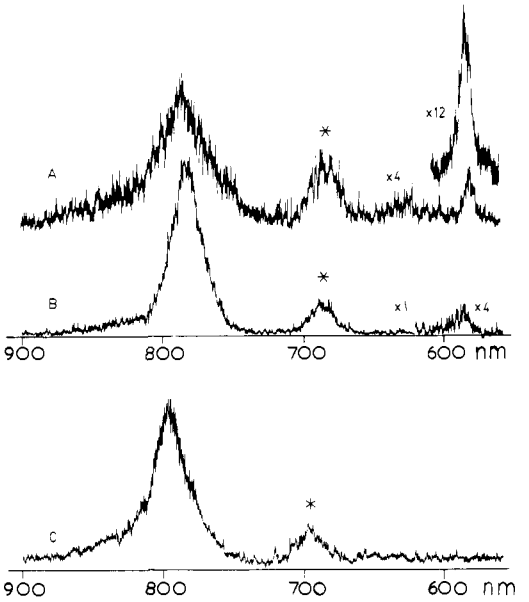

Figure 4. Fluorescence spectra excited at 401.3 nm from $\sim 10^{-5}$ M BChl *a* in solution in (A) dry hexane, (B) dry hexane titrated with pyridine to disaggregate the dimeric and oligomeric species of BChl *a*; and (C) pyridine. The relative amplification factors of the spectra are given. The bands marked by * are impurity fluorescence from the BChl *a* oxidation product BVir.

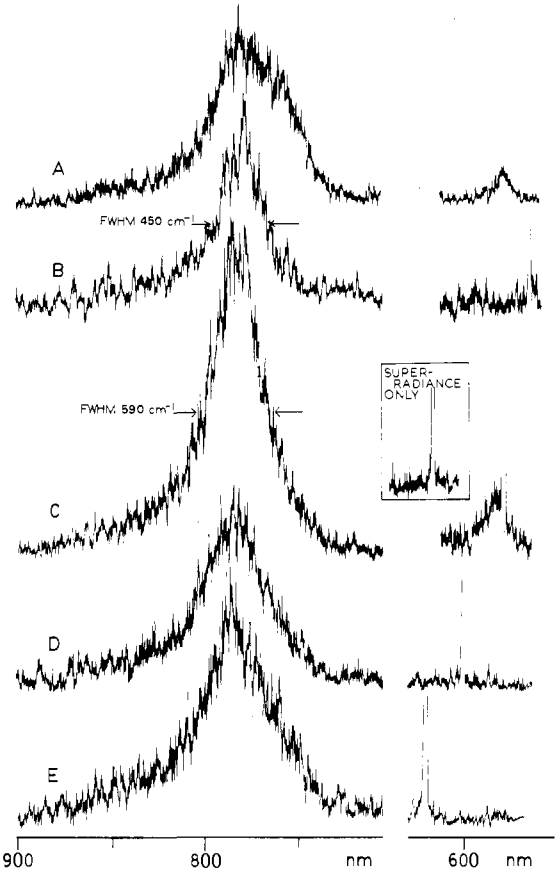

Figure 5. Fluorescence spectra from $\sim 10^{-5}$ M BChl *a* in dry hexane excited in the yellow (Q_x) absorption band at (A) 540.0 nm, (B) 565 nm, (C) 579.5 nm, (D) 599.8 nm; and (E) 619.8 nm.

weaker than that from the monomeric solution in hexane. While the main band at 783 nm is not shifted significantly the bandwidth is considerably broader (590 cm^{-1}) in the aggregated solution than that in the monomeric solution (430 cm^{-1}). In addition, in the dry solution, a weaker band at 583 nm is apparent (fwhm 300 cm^{-1}), which diminishes in intensity on addition of pyridine to the

solution Figure 4B. After titration of the dry solution with sufficient pyridine to completely form monomeric BChl *a*·pyridine$_2$, the spectrum resembles that of the six-coordinated monomeric BChl *a* in pure pyridine shown in Figure 4C.

The bands at 695 nm in all spectra in Figure 4 are due to fluorescence[14] from BVir which is an impurity in the solutions. The intensity of this band increases on prolonged exposure of the BChl *a* solutions to the laser radiation if the sample is not thoroughly deoxygenated.

In all solutions which have been titrated with pyridine to form the monomeric pyridinates of BChl *a*, the fluorescence is characterized by a relatively narrow band (430–460 cm^{-1}) with a maximum between 783 and 795 nm depending on the solvent; the bandwidths agree well with those found for the Q_y absorption bands of monomeric BChl *a* in the corresponding solvents (Table I).

Analysis of the absorption and fluorescence data for BChl *a* in the different solvents listed in Table I shows that the fluorescence spectrum in Figure 4A originates from both dimeric and oligomeric BChl a species in solution in dry hexane. This conclusion may be reached on the basis of the observed fluorescence spectra of monomeric BChl *a* and the knowledge that the wet benzene solution contains predominantly the dimer with minor amounts of the monomer and larger oligomers.[11] Excitation of fluorescence at different wavelengths in the region of the Q_x absorption bands results in selectivity of several different fluorescence bands from BChl *a* in dry hexane. These spectra are shown in Figure 5. Excitation at ~565 nm results in a spectum which is characteristic of the monomeric emission (Figure 5B) while excitation near the peak of the Q_x absorption band of the solution at 580 nm results in a spectrum consisting of a symmetrical band at 783 nm, accompanied by a weaker band at 585 nm (Figure 5C). The bandwidth of the main band at 783 nm is 590 cm^{-1}, intermediate between that of the monomeric emssion and the broad fluorescence band observed by excitation of the solution at 401,3 nm (Figure 4A). Excitation at laser wavelengths further to the red, at 600 and 620 nm, resulted in broad fluorescence bands (fwhm ~690 cm^{-1}) at 783 nm, but it appeared that at these wavelengths emission at 583 nm could not be excited. The similarity of spectrum 5C with that obtained from the solutions of BChl *a* in wet benzene (Table I) suggests that the spectrum excited at 580 nm (Figure 5C) is that of the BChl *a* dimer in hexane.

Selective excitation in the region of the Soret band was limited by the inability of the laser system to pump ultraviolet laser dyes efficiently. It was not therefore possible to excite the solutions of BChl *a* at the peak of the Soret absorption band near 360 nm. However, the spectrum excited at 385 nm from the solutions of BChl *a* in dry hexane is significantly different from that shown in Figure 4A which was excited at 410,3 nm and is similar to that shown in Figure 5A which was excited at 540.0 nm. The spectrum of Figure 5A shows a broadening of the main band to the blue, apparently due to an additional band at ~760 nm. The total Q_y fluorescence bandwidth in this spectrum is ~800 cm^{-1}, in agreement with that of the absorption spectrum of the same aggregated solution (Figure 3A). The broad band in the fluorescence spectrum is therefore thought to result from excitation of multiple species of oligomeric BChl *a* in the dry solution, which are characterized by blue shifted absorption and fluorescence bands.

In summary, spectrum 5C is characterized by a main band at 783 nm (fwhm ~590 cm^{-1}) and a weaker band at 583 nm (fwhm ~300 cm^{-1}). Time-resolved studies show that the fluorescence lifetime of the 583-nm band is longer than that of the 783-nm band, which has approximately the same radiative lifetime as the monomeric fluorescence ($\tau \simeq 3$ ns).[14] This spectrum is assigned to the BChl *a* dimer for the following reasons:

(1) This spectrum is characteristic only of solutions which are known to contain a significant proportion of BChl *a* as the dimer.

(2) Titration of the dry aggregated solution with pyridine to disaggregate the dimer causes disappearance of the 583-nm fluorescence band and narrowing of the main band at 783 nm, yielding the fluorescence spectrum characteristic of monomeric BChl *a* in solution.

(3) There are no detectable impurities having a main absorption band at ~580 nm present in sufficient concentration to account for the 583-nm fluorescence intensity.

(4) An impurity fluorescence would be expected to remain when the solution was titrated with pyridine, and the intensity of fluorescence from any degradation product of BChl *a* produced by laser irradiation should increase on prolonged irradiation. These effects were not observed for the 583-nm band and the broadening of the 783-nm band.

Discussion

In our previous study of the fluorescence spectrum of Chl *a* in solution[1] the fluorescence spectra of dimeric Chl *a* in solution were obtained by using selective excitation at wavelengths resonant with the Soret absorption band. For a 10^{-5} M solution of Chl *a* in hexane (containing ~80% Chl *a* as dimer) with excitation at 430.5 nm, near the peak of the monomeric absorption band, the fluorescence spectrum was essentially that of the monomer, showing a main band at ~680 nm and vibronic structure toward 720 nm. Excitation at wavelengths in the region 440–450 nm, wherein lies the peak of the dimeric absorption band, resulted in two new bands, one at 688 nm and a weaker band at 625 nm, which have been assigned to the Chl *a* dimer in hexane.

The fluorescence bands observed at 783 and 583 nm for the BChl *a* dimer in solution in hexane (Figure 5C) are analogous to the bands at 688 and 625 nm, respectively, for the Chl *a* dimer in solution, including the observation that the shorter wavelength fluorescence band in each case has the longer lifetime. However, whereas it was previously not possible to make a definite assignment of the 625-nm band of the Chl *a* dimer, it is clear that the 583-nm fluorescence band of the BChl *a* dimer lies in the region of the Q_x absorption band. This band also lies too far to the blue to originate in a Q_y transition of the BChl *a* dimer. It is therefore concluded that the 783-nm fluorescence band corresponds to the Q_y absorption band of the BChl *a* dimer in solution at 770 nm, and the 583-nm fluorescence band corresponds to the dimer Q_x absorption band at 580 nm. Because of the correspondence of the Q_x and Q_y bands of the fluorescence and absorption spectra of the BChl *a* dimer, and the similarity of the corresponding spectra of the Chl *a* dimer, it is concluded that the 688- and 625-nm fluorescence bands of Chl *a* correspond to the Q_y and Q_x fluorescence transitions, respectively.

Observation of split components in the absorption bands in the circular dichroism spectra of several dimeric chlorophylls in solution[7] has been interpreted as showing exciton splittings in the Q_y and Soret absorption bands. Assignment of the two fluorescence bands of the Chl *a* and BChl *a* dimers to Q_x and Q_y transitions as above excludes the possibility of a a Q_y exciton splitting of 1400 cm^{-1} but otherwise does not provide new evidence for the exciton splittings of either the Q_y or Q_x transitions. The enigma of whether each of the dimeric Q_y and Q_x absorption bands, and their corresponding fluorescence bands, arises from one to two exciton components remains unresolved. Recently, a nonlinear process observed in the Raman excitation profiles[28] of vibrational modes of dimeric Chl *a* in solution in hexane has been used to reveal the presence of two closely spaced electronic origins in the region of the Soret absorption band of the Chl *a* dimer. It is therefore anticipated that similar nonlinear spectroscopic studies may provide direct evidence for the exciton splittings of the lower lying singlet levels of the Chl *a* and BChl *a* dimers in solution.

The fluorescence yield of both BChl *a* and Chl *a* monomers is larger than that of the dimers, which indicates that nonradiative pathways for the deexcitation of the dimers are different from those of the monomers. For example, in Chl *a*, excitation of the monomer at wavelengths in the spectral region from 400 to 680 nm results in strong Q_y fluorescence, while no emission is observed in the Q_x region. Even if the optical pump is tuned to the Q_x region

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fluorescence is observed originating only from Q_y transitions. This suggests that there is extremely efficient coupling between these two states in the monomer, but the observation of Q_x emission in the dimer shows that nonradiative pathways between the Q_x and Q_y levels are less probable in the dimer.

We observe that the radiative lifetime of the Q_x band fluorescence is longer than that of the Q_y emission. This difference is consistent with the suggestion that there is reduction in the number of nonradiative pathways from Q_x in the dimer compared to the monomer.

From a comparison of the corresponding absorption spectra of monomer and dimer of BChl *a* and of Chl *a*, it is noted that the integrated absorptions over the Q_y bands of the monomeric and dimeric species in solution are similar. This is also true for the Q_x absorption bands. If we assume that the direct matrix elements from the ground state to Q_y and to Q_x are of the same order of magnitude,[14] then, from the relative integrated absorption strengths of the two bands, the lifetimes of the two states, $\tau_{abs}^{Q_y}$ and $\tau_{abs}^{Q_x}$, may be shown to be such that $\tau_{abs}^{Q_x} < \tau_{abs}^{Q_y}$. Lifetimes measured for fluorescence are reduced by the existence of nonradiative channels, but the longer lifetime of the Q_x fluorescence band compared with that of the Q_y fluorescence is not inconsistent if nonradiative channels from Q_x are less effective in the dimer. It is not known to what degree triplet states may play a role in these nonradiative processes, but this may be determined from nonlinear absorption studies.[26,27]

The fluorescence yield of monomeric BChl *a* in solution ($\Phi_{fl} \sim 0.2$) is less than that of Chl *a* in solution ($\Phi_{fl} \sim 0.3$).[14] The fluorescence intensities of the dimeric solutions of BChl *a* are observed to be weaker than that of dimeric Chl *a* in solution. This suggests that there are more nonradiative channels for deexcitation of BChl *a* than in Chl *a*; this may be a result of the lowering of the symmetry in BChl *a* relative to Chl *a* by the additional carbonyl group at C_{2a} in BChl *a* which is in conjugation with the π system of the macrocycle.

It is interesting that the dimer and oligomers of Chl *a* exhibit a marked red shift in their absorption and fluorescence maxima with respect to those of the monomer, while the maxima of the fluorescence and absorption spectra of the BChl *a* dimer and oligomers, if shifted at all, show a blue shift. The sensitivity of the shift and bandwidth of dimeric chlorophylls to geometrical structure has been noted for a number of synthetic linked chlorophyll dimers.[28-31] This observation indicates that the detailed structures of the dimers of Chl *a* and BChl *a* may not be identical, although the fluorescence results reported above for the two chlorophyll dimers are analogous in the observation of the activity of the Q_x fluorescence transition.

Registry No. BChl *a* dimer, 18025-10-0; Chl *a* dimer, 18025-08-6; BChl *a*, 17499-98-8; Chl *a*, 479-61-8.

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Discrimination of Rotational Isomeric States in Cycloalkanes by Solid-State CP-MAS ^{13}C NMR Spectroscopy†

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Abstract: The solid-state behavior of three cycloalkanes, cyclododecane, cyclotetraeicosane, and cyclohexatriacontane, was investigated by means of temperature-dependent magic angle cross-polarization ^{13}C NMR experiments. For the two smaller ring molecules a state of high internal mobility like the "rotator phase" in *n*-alkanes was detected. It could be correlated with a phase transition in the solid state visible by means of DSC. In the case of $(CH_2)_{12}$ this is 151 K below the melting point, and in the case of $(CH_2)_{24}$ it is 25 K below the melting transition. The CP-MAS ^{13}C NMR spectra show a transition from the fast exchange to the slow exchange regime of magnetically nonequivalent states. By comparison with X-ray diffraction data the well-resolved resonance signals for the low-temperature phases were assigned to molecular segments distinguished by the rotational isomeric states of the carbon-carbon bonds. Chemical shift differences due to conformational isomerism were as large as 12 ppm; thus, they exceed "packing effects" by far.

Recently cycloalkanes found rising interest as an appropriate model system to investigate specific defect structures in poly(ethylene) crystals. Studies on cycloalkanes were reported using infrared spectroscopy, X-ray diffraction, and the longitudinal acoustic modes, measured by Raman spectroscopy. Some interesting details were reported about the thermal behavior of cycloalkanes. Several of the ring alkanes undergo one or more phase transitions in the solid state. Some of those show a greater heat and also a greater entropy of transition than does the melting transition.[1-7]

^{13}C NMR spectroscopy has been reported as a potentially useful tool for the observation of rotational isomeric states in aliphatic chain molecules.[8-13] However, often the high exchange rates of

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