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Optically detected zero field magnetic resonance studies of the photoexcited triplet states of chlorophyll a and b*

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The zero-field EPR transitions for the triplet states of chlorophyll a and chlorophyll b in *n*-octane solutions at 2°K have been observed by optical detection methods. Both triplet absorption detection of magnetic resonance and fluorescence-microwave double resonance techniques were used. Sharp-line (5–20 MHz) zero-field EPR spectra were recorded for both molecules. The rates of depopulation for the individual triplet state spin sublevels were determined by microwave-modulated fluorescence intensity measurements. These experiments show that the middle spin sublevel is the most active in triplet state intersystem crossing for both chlorophyll a and chlorophyll b.

I. INTRODUCTION

The lowest excited triplet state of the chlorophylls has been of interest to optical and magnetic resonance spectroscopists for several years.[1,2] Triplet–triplet absorption of chlorophyll was first reported by Livingston[3] using flash-excitation techniques and extended by Linschitz and Sarkanen.[4] Chlorophyll phosphorescence is extremely weak, owing to efficient nonradiative deactivation of its low-lying triplet state, but has been observed in the infrared region by Amster[5] and by Krasnovskii *et al.*[6] The triplet state ($\Delta M = 2$) EPR spectrum of photoexcited chlorophyll has been reported by Gribova *et al.*[7] and by Lhoste, who reported the observation of $\Delta M = \pm 1$ photoexcited triplet state EPR transitions for a frozen glass of chlorophyll b at 77°K and measured the triplet state zero field splitting parameters.[8]

Recently, optical detection techniques for the observation of excited state magnetic resonance have been employed for the investigation of the zero field splitting and intersystem crossing dynamics of a wide variety of organic triplet states.[9–12] Of particular interest for weakly phosphorescing systems are triplet absorption detection[10] and fluorescence detection of magnetic resonance.[11,12] We have applied these techniques to an investigation of the lowest triplet states of chlorophyll a and b. The chlorophylls are of interest for several reasons, aside from their obvious biological importance. Recently, the triplet states of porphyrins have been the subject of much work aimed at a characterization of their excited state geometry, intersystem crossing rates, and mechanisms of spin–orbit coupling.[1,11,13] A comparison of these properties in the similar π-electron chlorophyll systems, as well as differences in triplet state properties *between* the chlorophylls, would yield information about the role of the metal center and side chain perturbations on the spin–orbit coupling activity in these systems. Further, it is known that at very low temperatures chlorophyll molecules may be incorporated into straight-chain alkane crystal lattices to provide sharp-line (Shpolskii) absorption and emission spectra.[14] The problem of how a bulky molecule might be incorporated into a simple host matrix may find some solution in the line shape and zero-field splitting parameters measured in the low temperature triplet state magnetic resonance spectra.

In this paper we present optically detected zero-field magnetic resonance spectra of the triplet states of chlorophyll a and b in *n*-octane solutions at 2°K. A discussion of the dynamics of microwave-modulated fluorescence spectroscopy is developed and applied to the measurement of the depopulating rates for the individual triplet spin levels of both molecules. These results are used to discuss possible effects on intersystem crossing in the chlorophylls.

II. EXPERIMENTAL

Chlorophyll a and b (Sigma Chemical Company) were each substantially free of other chlorophylls as confirmed by TLC and absorption spectroscopy. The solvent, *n*-octane, was free of emission in the regions of interest. To facilitate the dissolution of chlorophyll, dry nitrogen was bubbled through the solutions. This also served to deoxygenate the solutions. All samples were freshly prepared prior to each run, and the concentrations were kept low ($\leq 10^{-5} M$) to minimize aggregation.

The optical and microwave arrangements were as described previously.[15,16] The excitation of chlorophyll a was effected by light filtered through 10 cm of $CuSO_4$ solution and a yellow optical glass filter (PBL L-41) from a 1000 W mercury–xenon lamp. Chlorophyll b was irradiated for the fluorescence detection experiments with the 4579 Å line of an argon-ion laser (Spectra Physics, Model 164) operated in the single line mode and for the triplet absorption detection experiments with the 4880 Å line. In the dynamic experiments, 7000–40 000 sweeps were accumulated on a digital signal averager (Northern Scientific, NS-570), and the resulting curves were computer analyzed for the rate data reported.

III. OPTICAL AND MAGNETIC RESONANCE SPECTRA

Litvin *et al.* have shown that chlorophyll a in *n*-octane solutions produces a quasi-line fluorescence spectrum at temperatures below 77°K, and that at 4°K the narrow lines are superimposed on a relatively intense diffuse background.[14] Figure 1 shows that under argon laser excitation at 2°K chlorophyll b in *n*-octane also produces a quasi-line fluorescence spectrum built on a broad background. The intensities among the lines vary from

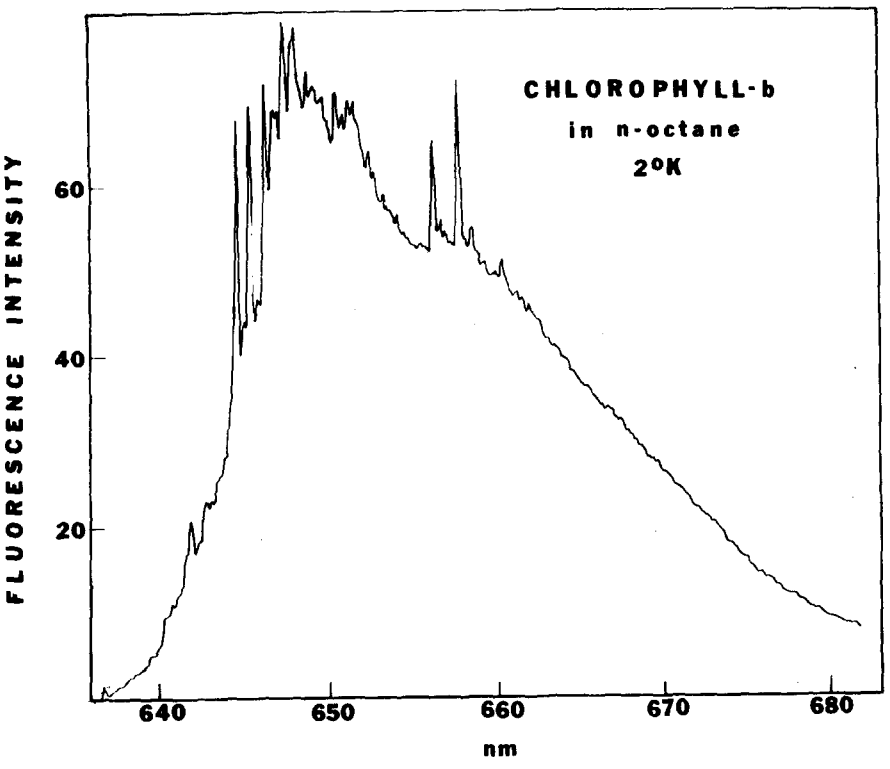

FIG. 1. The fluorescence of chlorophyll b in *n*-octane solution at 2 °K, excited by the 4579 Å line of an argon ion laser. Spectrum is uncorrected for the EMI 9558 phototube response.

sample to sample, depending principally on rate of cooling, but the same peaks are consistently observed. The sharp-line fluorescence peak positions for chlorophyll b in *n*-octane are listed in Table I. The triplet–triplet absorption spectrum could also be observed for both chlorophyll a and b in *n*-octane under CW laser excitation, extending over the region 400–600 nm and duplicated the known triplet–triplet absorption spectra observed by flash experiments in other media.[3,4] No low-temperature phosphorescence could be detected from our samples.

Since sharp-line emission (fluorescence or phosphorescence) is frequently accompanied by sharp-line zero-field triplet state magnetic resonance spectra,[11,13,17] *n*-octane was chosen as the host matrix for the investigation of the zero-field EPR of the chlorophylls. We have observed zero field magnetic resonance transitions of the lowest triplet state of chlorophyll a and b in *n*-octane solutions at 2 °K by triplet absorption detection of magnetic resonance (TADMR) and by fluorescence-microwave double resonance (FMDR). For chlorophyll a, only two of the triplet state zero-field magnetic resonance transitions were observed at 724 and 953 MHz. Both transitions are sharp, with linewidth of ~ 15 MHz, and both correspond to an increase in fluorescence intensity. The frequencies of these transitions given zero field splitting parameters of $|D| = 0.0280$ cm^{-1}, $|E| = 0.0038$ cm^{-1}. In the case of chlorophyll b transitions could be observed at ~ 840, 1000, and ~ 1080 MHz. The 1000 MHz transition could be observed only under highest microwave power (incident power ~ 1 W) in high concentration solutions and may correspond to aggregated chlorophyll centers. Under less severe power conditions (10–20 mW) using concentrations of $\leq 10^{-5} M$, only the 840 and 1080 MHz transitions were observed.

In the TADMR spectra of chlorophyll b, the peaks at ~ 840 and ~ 1080 MHz appeared to be made up of several sharp peaks (linewidths on the order of 5 MHz). All signals corresponded to an increase in triplet–triplet absorption intensity.[12] In the fluorescence-detected spectra, the exact frequency of the signals observed in the region of 840 and 1080 MHz varied with the detecting wavelength over the range 825–850 and 1069–1103 MHz. The intensity of the FMDR signals, all of which corresponded to a decrease in fluorescence, also varied with wavelength; the strongest magnetic resonance peaks were observed at 6436 Å, corresponding to ~ 1% decrease in the fluorescence intensity.[12] Undoubtedly, the chlorophyll molecules exist in several magnetically distinct sites in frozen *n*-octane solutions at 2 °K, which is reflected in the frequency variation of the zero-field transitions observed in FMDR, as one detects on different fluorescence bands.[18] In the case of TADMR, however, the zero-field transitions from *all* sites are viewed simultaneously (a composite of the individual zero-field peaks observed in FMDR), since the broad triplet–triplet absorption spectrum from each site overlaps at any observing wavelength and does not allow the wavelength discrimination among sites which is found in FMDR.[16,17] To minimize possible effects from the overlapping spectra of different sites, the dynamics experiments described in the following section were performed using microwave-modulated fluorescence. It should be noted that there is no unambiguous way to show that the chlorophyll spin levels are perfectly isolated in our experiments; however, a very short triplet lifetime and low temperature are usually sufficient conditions for isolation in organic systems.[11,13,17] Further, there is no observable intensity change on the zero-field transitions over the temperature range 2–4.2 °K in our experiments.

TABLE I. Wavelength of bands observed in laser excited fluorescence spectrum of chlorophyll b in *n*-octane at 2 °K. Wavelengths accurate to ± 2 Å.

λ(Å)	Intensity
6360	w
6408	mw
6418	w
6421	w
6422	w
6433	mw
6436	ms
6439	mw
6443	ms
6446	w
6451	ms
6455	m
6458	m
6462	vs
6464	m
6468	ms
6475	m
6480	w
6492	mw
6487	mw
6590	w
6592	mw
6597	w
6500	w
6502	mw
6512	mw
6516	mw
6520	w
6527	m
6530	w
6535	m
6550	ms
6555	m
6565	ms

IV. DYNAMICS OF THE TRIPLET STATE OF CHLOROPHYLL

A. Description of microwave-induced fluorescence intensity changes

In order to discuss the experimental results that determine the population dynamics of the chlorophyll triplet state, we will examine the time dependence of the population changes among the triplet spin sublevels with and without a microwave field present. Microwave-induced fluorescence changes depend on the *over-all* triplet state population being altered at the expense of the ground state (and, therefore, the fluorescing singlet state), and microwave modulation of the fluorescence intensity may be used to measure the rates at which each of the triplet spin sublevels undergo intersystem crossing into the ground state. A saturating microwave field connecting two of the triplet spin sublevels will produce a characteristic buildup to a new steady state population in the triplet state and a subsequent change in the ground state at a rate that mirrors the time dependence of the triplet population. The basic problem of the time dependent behavior of the over-all triplet state population has been analyzed previously in work on microwave-induced changes in triplet–triplet absorption in organic molecules,[15,16] and in the present work we will recast and extend that treatment for microwave-induced fluorescence.

The fluorescing singlet level S_1 can be described by a simple first order kinetic equation:

$$\frac{dS_1}{dt} = k_0 S_0 - (k_1 + k_2) S_1 , \tag{1}$$

where S_0 is the ground state population and the rate constants k_0, k_1, k_2 describe all the processes by which S_1 is populated (k_0), depopulated by internal conversion and fluorescence (k_1), and depopulated by intersystem crossing (k_2). In our experiments on the triplet state we will be concerned with population changes occurring among the triplet spin levels which take place on a time scale much slower than the population and decay of S_1. Thus, it is reasonable to assume that S_1 is *always* present at a steady state concentration, i.e., equilibrium with respect to S_1 occurs much more rapidly than equilibrium in T_1. Therefore, the population in S_1 is given by its steady state value

$$S_1 = \frac{k_0}{k_1 + k_2} S_0 , \tag{2}$$

and since the total number of molecules $N = S_0 + S_1 + T_1$,

$$S_1 = \frac{k_0}{k_0 + k_1 + k_2} (N - T_1) \equiv K(N - T_1) . \tag{3a}$$

The fluorescence intensity observed is proportional to the concentration of the lowest excited singlet state S_1. S_1 may, in fact, change with time as T_1 changes, but Eq. (2) assumes that S_1 exists at a steady state level with respect to its own population and decay channels throughout such a change in T_1.[19] We may also write an expression comparable to Eq. (1) for each of the triplet spin sublevels of T_1 (T_1^l, T_1^m, T_1^n) in the absence of any interactions among the spin sublevels as

$$\frac{dT_1^i}{dt} = k_2^i S_1 - k_3^i T_1^i \quad (i = l, m, n) , \tag{3b}$$

where k_2^i is the populating rate and k_3^i is the total depopulating rate for spin sublevels i.

Consider the effect of microwave saturation of the transition between two of the spin sublevels, e.g., $T_1^l \rightleftharpoons T_1^m$, in a system which had been allowed to come to steady state equilibrium in both S_1 and T_1 ($T_1 = T_1^l + T_1^m + T_1^n$) under continuous illumination. The population in the triplet sublevels following the application of microwaves will be

$$\frac{d(T_1^l + T_1^m)}{dt} = (k_2^l + k_2^m) S_1 - \tfrac{1}{2}(k_3^l + k_3^m)(T_1^l + T_1^m) . \tag{4}$$

There will, of couse, be no change in T_1^n from its steady state value, if the levels are effectively isolated, i.e., $dT_1^n/dt = 0$. Substituting into Eq. (4) the steady state values for S_1 from Eq. (3) and defining $N' = N - T_1^n$ gives the time dependence of the population change in T_1 as

$$\frac{d(T_1^l + T_1^m)}{dt} + [K(k_2^l + k_2^m) + \tfrac{1}{2}(k_3^l + k_3^m)](T_1^l + T_1^m) = K(k_2^l + k_2^m) N' , \tag{5}$$

which has the solution

$$T_1 = C\exp\{-[K(k_2^l + k_2^m) + \tfrac{1}{2}(k_3^l + k_3^m)]t\} + \frac{K(k_2^l + k_2^m)N'}{K(k_2^l + k_2^m) + \tfrac{1}{2}(k_3^l + k_3^m)}\ , \quad (6)$$

where C is the constant determined by the initial steady state populations and $T_1 = T_1^l + T_1^m$. Thus, upon microwave saturation the triplet state population changes exponentially from its initial value to a new steady state value; it is important to note that the rate constant describing the exponential population change depends on the populating rate k_0 and, therefore, on the light intensity of the excitation source. In the limit of low light levels ($k_0 \to 0$), Eq. (6) reduces to the simplified form obtained previously,[16] viz.

$$T_1 \propto \exp[-\tfrac{1}{2}(k_3^l + k_3^m)t]\ . \quad (7a)$$

When the microwave field is turned off, the triplet spin sublevels T_1^l and T_1^m return to their original steady state values, according to Eq. (3b). Substituting the S_1 steady state value into Eq. (3b) for both triplet spin sublevels leads to the coupled kinetic equations

$$\frac{dT_1^l}{dt} = Kk_2^l(N' - T_1^m) - (Kk_2^l + k_3^l)T_1^l \quad (7b)$$

and

$$\frac{dT_1^m}{dt} = Kk_2^m(N' - T_1^l) - (Kk_2^m + k_3^m)T_1^m\ . \quad (7c)$$

The solution of these coupled equations leads to an overall change in the triplet state population of

$$T_1 = T_1^l + T_1^m = C_1 e^{-\alpha_+ t} + C_2 e^{-\alpha_- t}\ , \quad (8)$$

where

$$\alpha_\pm = \tfrac{1}{2}\{(k_3^l + k_3^m) + K(k_2^l + k_2^m) \pm [(k_3^l + k_3^m + Kk_2^l + Kk_2^m)^2 - 4(Kk_2^l k_3^m + Kk_3^l k_2^m + k_3^l k_3^m)]^{1/2}\}$$

and the constants C_1 and C_2 are obtainable from the steady state population conditions. Again, in the limit of low exciting light intensity, Eq. (8) reduces to a simple form,[16]

$$T_1 = C_1^l e^{-k_3^l t} + C_2^l e^{-k_3^m t}\ . \quad (9)$$

Thus, a microwave-modulated fluorescence experiment will follow the time dependent triplet state population changes described in Eqs. (6) and (8). However, the fluorescence intensity changes will bear a simply interpreted relationship to the depopulating rates of the individual spin levels only in the limit of low exciting light intensity; increasing light intensities will have the effect of *increasing* the observed rate constants.[20]

B. Intersystem crossing rates

The time-dependent response of the fluorescence to a modulated saturating microwave field has been measured for all the observed zero field transitions of chlorophyll a and b. A typical response curve is shown in Fig. 2. The results quoted in this section are the result of several reproducible decay curve runs. The experiments were routinely run at the lowest power levels practical to minimize distortions of the microwave-modulated curves presented here. Experiments were also run with varied incident exciting light power (either by varying the laser power or by defocusing the laser pump light), but we could detect no power dependence for any of the microwave-modulated curves reported here. Further, the fluorescence detection wavelength was varied among several of the quasi-line peaks in the emission spectra, but, again within our experimental limitation, the microwave-modulated fluorescence curves showed no differences in rate with differences in detection wavelength.

Therefore, since we were satisfied that we were performing our experiments in the "low-power" limit, we

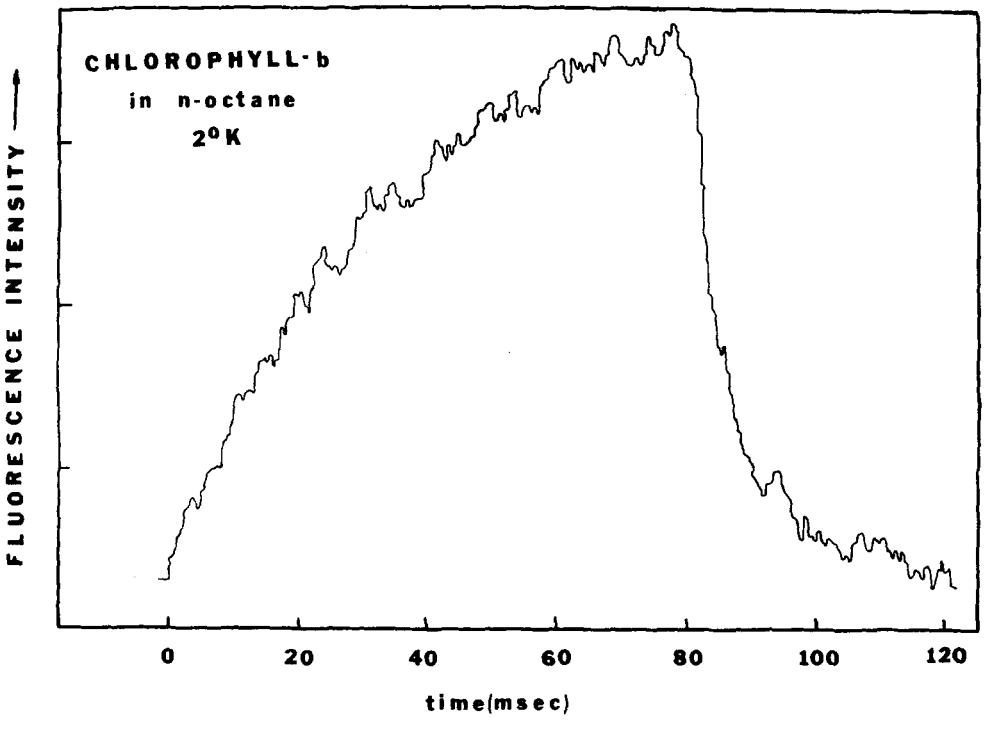

FIG. 2. Microwave induced intensity changes in the fluorescence band at 6436 Å of chlorophyll b at 2 °K. At $t = 0$ the 1085 MHz microwave field is turned off, and at $t = 80$ msec the microwaves are switched on. Rise time of the microwave field is < 1 μsec. The picture is an average of 3100 sweeps.

applied the simplified intensity expressions given in Eqs. (7) and (9) of the previous sections to extract the decay rates of the individual triplet spin sublevels from the microwave-modulated intensity change curves depicted in Fig. 2. Even without the observation of the third ($2E$) zero-field transition, the curves for the two remaining zero-field transitions provide four measurements (microwaves on–microwaves off for each transition) of the three decay rates in the low-power limit. *All* the microwave-modulated intensity curves were exponential within the accuracy of our experiments, as seen in the semilog plots of intensity change vs time (Figs. 3, 4) for the various transitions, even on the microwave-off cycle. From Eq. (9), this behavior implies that one of the zero-field levels dominates the population expression for the return to equilibrium after the microwave field is switched off. The results of an analysis of the data in Figs. 3 and 4 in terms of Eqs. (7) and (9) for chlorophyll a and b give the depopulation rates listed in Table II. The lifetime obtained by averaging the decay rates in the table for chlorophyll a gives 1.4 msec, which compares very well with the phosphorescence lifetime for chlorophyll a in ethanol of 1.6 msec.[6] For chlorophyll b, the lifetime calculated from our numbers is 3.4 msec, compared with 2.2 msec from the phosphorescence lifetime measurements[6] and 3 msec from high field EPR.[8]

In principle, with the depopulating rates known, the *relative* populating rates of the individual triplet state spin levels can be obtained from a measurement of the ratio of fluorescence intensity with and without microwaves for each of the zero-field transitions, as was done for triplet absorption detection in previous work.[15,16] The microwave-induced population changes are determined by both the populating and depopulating rates for the triplet spin levels, and it is a straightforward matter to express the fluorescence (or triplet absorption intensity) ratios in terms of these rates.[15,16] However, the microwave-induced changes in these experiments are so small that all the ratios are very close to unity, and the value of such calculations is questionable. The ordering of the relative populating rates is predictable from the depopulating rates and the qualitative features of the observed spectra, i.e., relative intensities among the zero-field transitions. To be consistent with the spectral features of either fluorescence or triplet absorption requires a steady state population in each level found by assuming the middle level to be the most efficiently populating (P_y), and $P_y > P_x \gg P_z$ (x, y, and z are the top, middle, and bottom levels, respectively) for

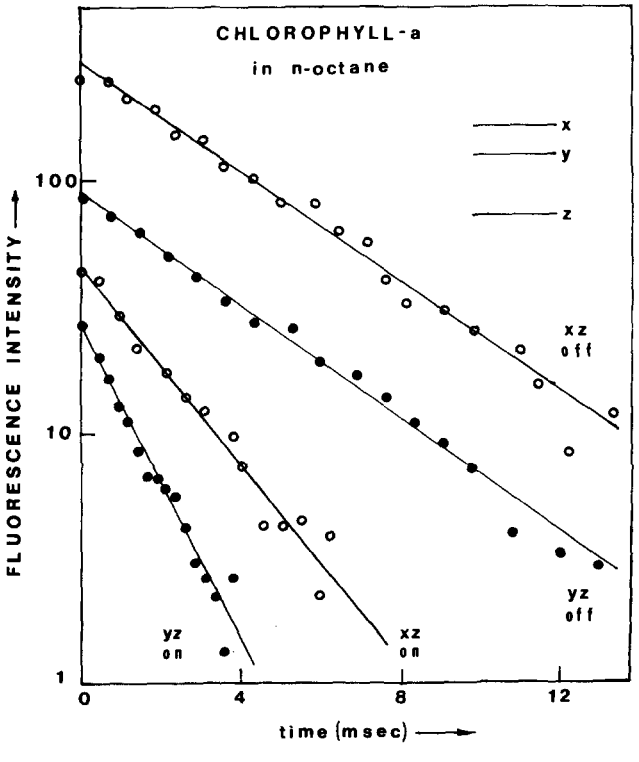

FIG. 3. Semilog plot of the changes in the fluorescence intensity vs time (msec) for chlorophyll a in n-octane at 2 °K upon application and extinction of a saturating microwave field. The xz microwave frequency is 952 MHz and the yz frequency is 724 MHz.

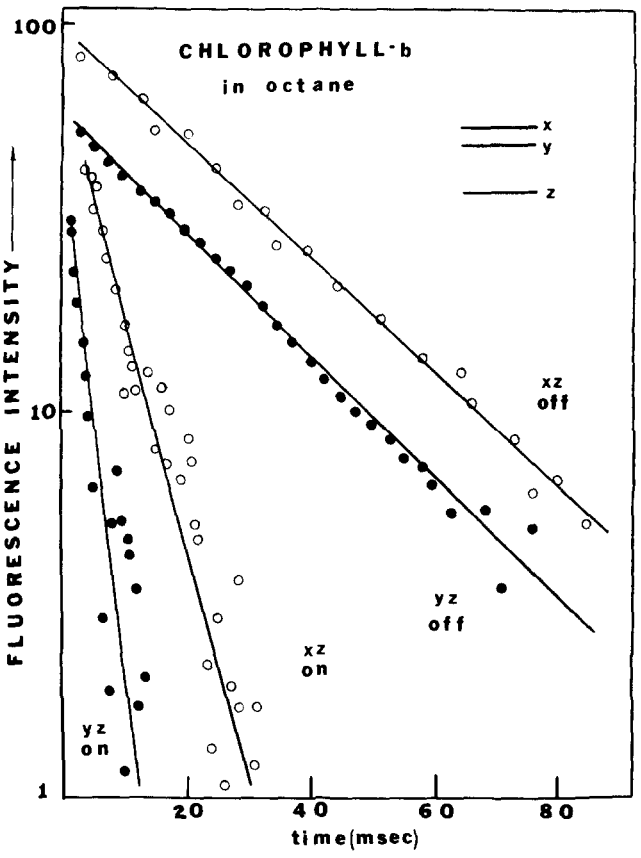

FIG. 4. Semilog plot of the changes in the fluorescence intensity vs time (msec) for chlorophyll b in n-octane at 2 °K upon the application and extinction of a saturating microwave field. The xz microwave frequency is 1085 MHz and the yz frequency is 840 MHz.

TABLE II. Rate constants (in sec^{-1}) for the depopulation of the individual triplet state spin sublevels of chlorophyll a and b in n-octane.

	k_x	k_y	k_z
chlorophyll a	661 ± 89	1255 ± 91	241 ± 15
chlorophyll b	268 ± 34	570 ± 54	34 ± 4

both chlorophyll a and b. Thus, the individual spin levels are populated with a relative efficiency which duplicates the relative individual depopulating rates (Table II), with the middle spin level the most active in both the buildup and decay of the chlorophyll triplet state.

V. CONCLUSIONS

As has been found in the zero-field magnetic resonance studies of porphyrins,[11,13] *n*-octane provides a suitable low-temperature host matrix for high resolution analysis of the fine structure in the triplet state of the chlorophylls. No attempt at a detailed investigation of the nature or number of the guest molecule sites was carried out, but the presence of the chlorophyll phytol chain will undoubtedly lead to complex guest–host interactions. The most significant observation is that, whatever the differences that exist among sites, they are not sufficiently severe as to produce observable differences in triplet spin level depopulating rates from site to site.

An assignment of the principal axes of the spin–spin interaction tensor in the molecular framework cannot be made on the basis of a zero-field EPR experiment. However, it is clear both from a simple dipole model and from previous high-field EPR studies[8,13] that the top two zero-field levels (which we have labelled x and y) are in-plane spin levels and the bottom level (z) corresponds to spins precessing about an out-of-plane direction. No assignment is implied by the in-plane designations, and a high-field EPR experiment on an oriented sample is required to fix precisely the directions of x and y. As has been observed for the $\pi\pi^*$ triplet states of several aromatic molecules, the in-plane triplet spin levels for both chlorophylls are the most active in intersystem crossing.[16,21] The relatively small change in structure upon substituting a methyl for a CHO group in chlorophyll a vs chlorophyll b substantially increases all three depopulating rates, with the largest effect (by almost an order of magnitude) on the depopulating rate for the out-of-plane (z) level. Such a change suggests a distortion away from planarity in chlorophyll a greater than that for chlorophyll b in the triplet state leading to more efficient spin–orbital coupling of z with σ states. Such a distortion may also change the position of the central metal ion relative to the coordinated ring structure, affecting the spin–orbit interactions for all three spin levels and leading to an over-all shortening of the triplet state lifetime in chlorophyll a compared to chlorophyll b. The increased efficiency of intersystem crossing out of the triplet state of chlorophyll a relative to chlorophyll b is consistent with previous observations of lower phosphorescence intensity[6] and weaker triplet state EPR signals[7,8] for chlorophyll a.

It should be noted that a recent measurement of the triplet state intersystem crossing rates for the similar π-electron system, Zn porphin, show that in contrast to chlorophyll, the out-of-plane (lowest energy) spin level dominates the population and decay rates.[13] In this system, however, the relatively heavy central atom undoubtably exerts a stronger influence in the spin–orbit coupling of the individual spin levels than does the Mg atom in the chlorophylls. Studies of chlorophyll derivatives, such as the pheophytins and chlorophyllides, are required to further define the relative importance of the metal center and side chains on the intersystem crossing mechanisms in the chlorophylls. Such experiments are presently underway.

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