Preliminary Note

Deuterium isotope effect on intersystem crossing for pheophytins

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1. Introduction

Recently a number of investigators have been interested in the properties of the lowest photoexcited triplet state T_1 of chlorophyll systems, both *in vitro* and *in vivo* (for a recent review see ref. 1). The discovery by Dutton and coworkers [2 - 5] that triplet state electron paramagnetic resonance signals associated with reaction center chlorophyll are observed from reduced preparations of photosynthetic bacteria has prompted studies aimed at utilizing triplet state properties to probe the structure of the reaction center [6]. Further, chlorophylls are large complex molecules of low symmetry that present an interesting challenge to theorists in the interpretation of $T_1 \rightarrow S_0$ intersystem crossing (ISC) processes [7].

It has been pointed out [8] that an investigation of position-dependent deuterium isotope effects on the T_1 lifetime of partially deuterated molecules and measurement of the individual triplet spin sublevel decay rates can be an effective approach to studying the "mechanisms" thought to control ISC for planar aromatic molecules. We take such an approach here to gain insight concerning $T_1 \rightarrow S_0$ radiationless decay for the photosynthetic pigments pheophytin a and pheophytin b, the free-base forms of the corresponding chlorophylls.

2. Experimental

The fluorescence onset method [9, 10] was used to determine triplet state lifetimes. This technique involves measuring the rate at which the initial intensity of fluorescence decreases to a steady state level immediately after the exciting light has been turned on. The exponential loss of intensity is the result of a build-up of steady state population in the metastable triplet state at the expense of the ground state population. The rate of decay is measured as a function of exciting light intensity and is extrapolated to its value at zero light intensity to obtain the true triplet state decay constant [10]. For each system studied the decay rates were measured at the wavelength of maximum fluorescence. Exchange of the central protons was accomplished by dissolving the pheophytin molecule in ethanol-d_1.

3. Results and discussion

Previous work from our laboratory [11] has shown that deuteration of the N—H positions of simple free-base chlorins greatly increases their triplet state lifetimes. Presented in Table 1 is an extension of this study to the more complicated pheophytins. Although it has been predicted [12] that deuteration of the N—H positions of pheophytins would not have a large effect on their triplet lifetimes, the contrary is observed. Also presented in Table 1 is the triplet decay rate of chlorophyll b in ethanol and in ethanol-d_1. It is seen that the chlorophyll b rate does not change, within experimental error, on going from the undeuterated to the deuterated solvent. It therefore appears that the deuterium effect observed for the pheophytins is caused primarily by exchange of the central protons and not by any secondary effect associated with hydrogen bonding of the deuterated solvent to the carbonyl side groups, since the latter effect would be expected to influence the chlorophyll b rate as well.

TABLE 1

$T_1 \rightarrow S_0$ ISC decay constants

Compound	k_T^{H} [a] (s^{-1})	k_T^{D} [b] (s^{-1})	λ_{fl} [c] (nm)
Pheophytin a	760 ± 80	500 ± 25	669
Pheophytin b	624 ± 58	325 ± 28	655
Chlorophyll b	355 ± 26	366 ± 44	667

[a]Decay constant in ethanol solvent at 77 K.
[b]Decay constant in ethanol-d_1 solvent at 77 K.
[c]Fluorescence wavelength monitored.

It has been determined by previous workers that total deuteration of chlorophyll a [13] and deuteration of the C—H ring positions of simple free-base porphyrins [14, 15] have relatively small effects on their triplet decay rates. Although the effects of deuteration at positions other than N—H have not been studied for pheophytins, it appears safe to assume through analogy with chlorophyll a and porphyrins that they will be relatively small. Thus it is demonstrated that N—H vibrations are important modes in ISC for pheophytins, just as they have been found to be for simple porphyrins [15] and chlorins [11]. A question then arises. To what extent are N—H vibrations functioning as promoting modes and to what extent as accepting modes?

Bowman [13] has postulated that N—H out-of-plane bending is the most important promoting mode in $T_1 \rightarrow S_0$ ISC for pheophytins. This assignment is based in part on the observation reported by Ponte Goncalves and Burgner [16] of a spin-dependent deuterium isotope effect on the T_1 decay rates of free-base meso-tetra(m-fluorophenyl)porphin (TFPP). However, previous optically detected magnetic resonance studies from our laboratory [11] have shown that deuteration of the N—H positions of free-base tetraphenylchlorin (TPC) results in a large but spin-independent effect.

Since simple chlorins are models that more closely resemble pheophytins than porphyrins do, it is quite possible that the pheophytins do not show a spin dependence in the deuterium isotope effect.

At this point a look at radiationless transition theory is helpful for a further discussion of ISC in pheophytins. According to the approach of Henry and Siebrand [8], the rate of $T_1 \rightarrow S_0$ radiationless decay for a planar aromatic molecule is proportional to the expression

$$|H_{10}^{(3)}|^2 = F(E) \left[\sum_k \left(\hbar^3 \frac{\omega_k^{(0)}}{2u_k} \right)^{1/2} \sum_l (E_l - E_0)^{-1} \times \right.$$

$$\times \left\{ \langle S_0|H_{SO}|T_l\rangle \left\langle T_l \left| \frac{\partial}{\partial Q_k} \right| T_1 \right\rangle + \right.$$

$$\left. \left. + \left\langle S_0 \left| \frac{\partial}{\partial Q_k} \right| S_l \right\rangle \langle S_l|H_{SO}|T_1\rangle \right\} \right]^2 \tag{1}$$

where $F(E)$ represents the Franck—Condon factor and $\omega_k^{(0)}$ and u_k are the frequency and the reduced mass respectively of the vibrational promoting mode. T_1 is of $\pi\pi^*$ character, whereas the intermediate states T_l and S_l are of $\sigma\pi^*$, $\pi\sigma^*$ and $n\pi^*$ character. Symmetry requires that the promoting modes correspond to out-of-plane bending modes. Expansion of the integrals in eqn. (1) reveals the local (one-center) nature of vibronic spin—orbit coupling for planar aromatic molecules, i.e. the theory of Henry and Siebrand predicts that $T_1 \rightarrow S_0$ radiationless decay is promoted most effectively when atomic centers carrying high π-electron density in the highest occupied molecular orbital (HOMO) and in the lowest unoccupied molecular orbital (LUMO) are coupled with large out-of-plane bending amplitudes. It is believed that C—H out-of-plane bending modes are the most important promoting modes for planar aromatic hydrocarbons. From this it follows that triplet radiationless decay is expected to be most effectively inhibited when deuterium substitution occurs at positions carrying the highest π-electron density in the HOMO and LUMO. Recent experimental results [17, 18] support this view. A further prediction of the approach of Henry and Siebrand is that, since the vibronic spin—orbit coupling mechanisms associated with the decay of the individual spin sublevels of T_1 are in general different, a spin-dependent deuterium isotope effect is expected.

The absence of a spin-dependent deuterium isotope effect for TPC has been interpreted by us [11] in terms of Gouterman's [19] four-orbital model of the electronic structure of molecules derived from the porphyrin ring. According to this picture the HOMO and LUMO have symmetry nodes located on the N—H nitrogens. It therefore follows from a simple application of the theory of Henry and Siebrand that N—H out-of-plane bending vibrations are not expected to be important promoting modes. The observation of a spin-dependent effect for the free-base porphyrin mentioned earlier is consistent with the four-orbital model since T_1 of TFPP is believed to

correspond to either $^3(b_{1u} \rightarrow b_{2g})$ or $^3(b_{1u} \rightarrow b_{3g})$ under D_{2h} symmetry [16]. According to the four-orbital model, the b_{1u} orbital has high π-electron density on the N—H nitrogens, allowing for effective coupling with out-of-plane N—H bending modes to promote $T_1 \rightarrow S_0$ ISC. Although the four-orbital model can be successfully applied to simple porphyrins and chlorins, we must be concerned with how well the model holds for pheophytins, which are more complex in structure and have low symmetry. To answer these concerns we turn to the recent *ab initio* configuration interaction calculations on ethyl pheophorbide a by Petke *et al.* [20]. Their results show that the general features of the four-orbital model do hold. The low-lying excited singlet and triplet states are composed of configurations involving the two highest filled and the two lowest unfilled molecular orbitals. The lowest triplet state arises from a pure configuration (94%) corresponding to $^3[(HOMO) \rightarrow (LUMO)]$. Further, their electron density contour maps do indeed show nodes on the N—H nitrogens for the HOMO and LUMO. Thus it appears that TPC can be used as a reasonable model for pheophytins in discussing $T_1 \rightarrow S_0$ ISC and that N—H vibrations may not be effective promoting modes for pheophytins. This implies that the observed decrease in the rate of triplet state decay can be ascribed primarily to the change in Franck—Condon factors that accompanies deuteration. If this is the case, further work is needed to understand better why N—H vibrations act so much more effectively as accepting modes than C—H vibrations do.

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