

The effect of solvent deuteration on the photophysics of sulphonated aluminium phthalocyanine

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Abstract

The photophysics of disulphonated aluminium phthalocyanine in protiated and deuterated aqueous phosphate-buffered saline solution are reported. In the deuterated solvent the rate coefficients of fluorescence and intersystem crossing are unaffected by the heavier isotope; however, the rate of internal conversion from S_1 to S_0 has been shown to be considerably reduced. This results in increased quantum yields of fluorescence and triplet-state formation and also increased triplet and singlet lifetimes in deuterium oxide ($\Phi_t = 0.40 \pm 0.04$, $\tau_t = 5.1 \pm 0.1$ ns and $\tau_s = 600 \pm 30$ μ s in H_2O and $\Phi_t = 0.52 \pm 0.06$, $\tau_t = 6.8 \pm 0.1$ ns and $\tau_s = 1590 \pm 100$ μ s in D_2O). The implications of this with respect to the determination of the quantum yields of singlet oxygen formation in D_2O are discussed.

Keywords: Phthalocyanines, solvent deuteration, photosensitization, singlet oxygen, triplet lifetime.

1. Introduction

We wish to report surprising photochemical behaviour from disulphonated aluminium phthalocyanine in deuterated solvents. This compound is of considerable interest as a photosensitizer for the photodynamic therapy (PDT) treatment of tumours, and its phototoxicity is believed to be mediated by the triplet state of the dye which can undergo a type I electron transfer with a substrate or a type II energy transfer reaction to form $O_2(^1\Delta_g)$ (singlet oxygen). Because of this it is important to understand the photophysics of the sensitizer, *e.g.* to quantify the quantum yield Φ_t of triplet formation, and the quantum yield Φ_Δ of singlet oxygen formation. This latter value is often determined in a deuterated solvent because singlet oxygen is deactivated more rapidly in protiated solvents, in particular H_2O , making the measurements more difficult. Surprisingly, there are few reported comparisons of the photophysics of PDT sensitizers in protiated and deuterated solvents despite there being several reports of deuteration affecting the excited state dynamics of porphyrins [1–5], in which exchange of the inner N–H protons for deuterium occurs in deuterated solvents such as D_2O and

CH₃OD. This has been shown to reduce the contribution of non-radiative processes and to increase τ_f , Φ_f and Φ_t ; thus it is to be expected that in D₂O the quantum yield of singlet oxygen formation should also be greater. Deuteration of the ligand bound to magnesium porphyrin has also been shown to affect the non-radiative relaxation of the compound's triplet state [6]. Davila and Harriman [7] observed increases in the quantum yields of fluorescence and triplet formation and also in the fluorescence lifetime of tetrakis-(sulphonatophenyl) porphyrin in deuterium oxide, although they reported no significant change in the photophysics of trisulphonated aluminium phthalocyanine on going from H₂O to D₂O.

2. Experimental details

Disulphonated aluminium phthalocyanine was prepared according to the method of Ambroz *et al.* [8]. The sample used was purified by repeated preparative reverse phase chromatography using aqueous methanol as the eluent; elemental analysis indicated that it is disulphonated aluminium phthalocyanine and does not contain chlorine. Its analytical high performance liquid chromatography (HPLC), shown in Fig. 1, shows that it consists of more than 70% of a single peak. Water was purified by distillation followed by processing in an Elga UHQ; deuterium oxide (99.9 at.% D) was purchased from Aldrich and used as received. Phosphate-buffered saline was prepared from dry powder (Sigma) and contained 120 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer; in water this has a pH of 7.4. Unless stated otherwise, all solutions were prepared in the buffer solution and allowed to stand for at least 4 h prior to use to ensure disaggregation, as indicated by absorption spectroscopy. UV-visible spectra were recorded on a computer-controlled Perkin-Elmer Lambda-2 spectrometer in 1 cm quartz cuvettes. Fluorescence spectra were recorded on a Perkin-Elmer LS-5B fluorometer, and the spectra stored and processed on a computer. Emission spectra

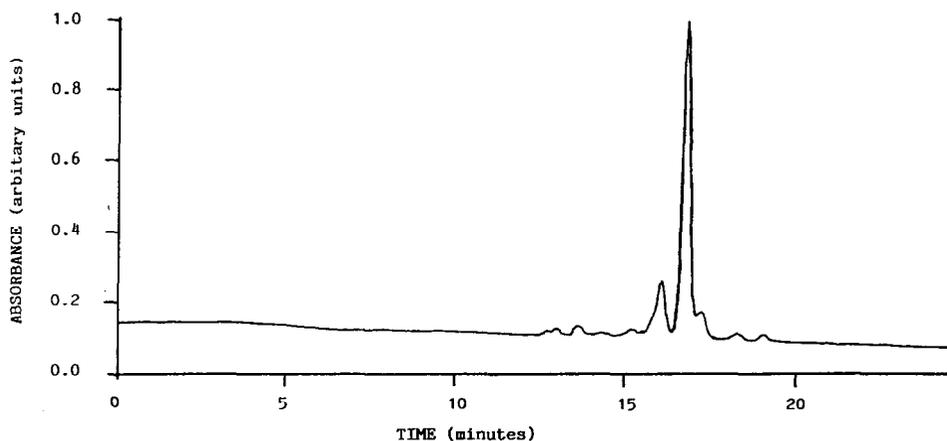


Fig. 1. HPLC trace of the disulphonated aluminium phthalocyanine used in this work. A 10 μ g sample was injected onto a C₁₈ Nova-Pak column and eluted with a linear gradient, changing from 100% 20 mM aqueous ammonium acetate buffer (pH 7) to 20% buffer-80% methanol over 20 min. The eluent was monitored by absorption at 360 nm. Integration shows that mixture contains more than 70% of the large peak.

were corrected according to the manufacturer's method using a calibration curve generated by a standard tungsten filament lamp. Fluorescence quantum yields were determined by the method of Winfield and coworkers [9] and are reported relative to chlorophyll a in ether ($\Phi_f=0.32$) [10] and cresyl violet in methanol ($\Phi_f=0.54$) [11].

Fluorescence lifetimes were recorded by the technique of time-correlated single-photon counting using apparatus pumped by a picosecond dye laser as the excitation source. This system has been fully described elsewhere [12]. The dye laser used DCM in ethylene glycol as the gain medium and provided a 4 MHz train of pulses of less than 10 ps at 630 nm. The fluorescence was collected at 90° and passed through a polarizing filter set at the magic angle, and the wavelength was selected by a monochromator. A Hamamatsu R928 side-on photomultiplier or Hamamatsu R1564-U01 microchannel plate was used to detect the fluorescence together with standard discriminators, time-to-amplitude converter and multichannel analyser, giving typical instrument response times of 300 and 50 ps full width at half-maximum (FWHM) respectively.

Triplet-state studies were carried out using the laser flash photolysis apparatus of the Laser Support Facility, Science and Engineering Research Council (SERC) [13]. Samples with an optical density of 0.1–1.0 at the excitation wavelength were pumped at 610–680 nm with the output of an excimer-pumped dye laser, with a typical pulse duration of 10 ns and energy of 10–2000 μJ . The transients were probed with the output of a quartz–halogen lamp, and the probe wavelength was selected with a monochromator; the transmitted light intensity was monitored with a home-built photodiode–amplifier combination which has a response time of 200 ns FWHM. The output of this diode was fed into a Tektronix 2432A digital storage oscilloscope and the transients transferred to an IBM-AT compatible for analysis by either a spreadsheet package (Lotus 123) or a commercial data analysis package (Grafit). The flash photolysis apparatus also has a time-gated optical multichannel analyser which enables the rapid acquisition of the transient absorption spectra from one to 256 laser shots. The triplet–triplet absorption spectra and extinction coefficients were generated from the difference spectra by the addition of the ground-state spectrum. The relative triplet quantum yields were determined by plotting the intensity of the triplet–triplet absorption at $t=0$ as a function of the absorbed laser energy. The relative gradients in the linear, lower power region of the graph were then compared.

3. Results

Phosphate-buffered saline solutions of disulphonated aluminium phthalocyanine in H_2O and D_2O both exhibit similar UV–visible absorption spectra, with their Q band peaks at 671 nm and 670 nm respectively. There was no evidence of sample aggregation nor of the presence of dimers based upon the absence of their characteristic absorption bands at greater than 700 and 640 nm. The extinction coefficients are very similar: $\epsilon(\text{H}_2\text{O})=1.77\pm 0.06\times 10^5\text{ mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$ and $\epsilon(\text{D}_2\text{O})=1.83\pm 0.06\times 10^5\text{ mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$. The fluorescence spectra from the deuterated solvent also show a small blue shift, with the maxima in H_2O and D_2O occurring at 680 nm and 678 nm respectively. More significantly the quantum yields of fluorescence show a marked difference: $\Phi_f(\text{H}_2\text{O})=0.40\pm 0.04$; $\Phi_f(\text{D}_2\text{O})=0.52\pm 0.06$. The fluorescence decays of the sensitizer also show a pronounced difference; both gave excellent fits to single-exponential decays, $\tau_f=5.1\pm 0.1\text{ ns}$ and $\tau_f=6.8\pm 0.1\text{ ns}$ respectively; typical decays are shown in Fig. 2. In 100 mM phosphate-buffered water the fluorescence lifetime of

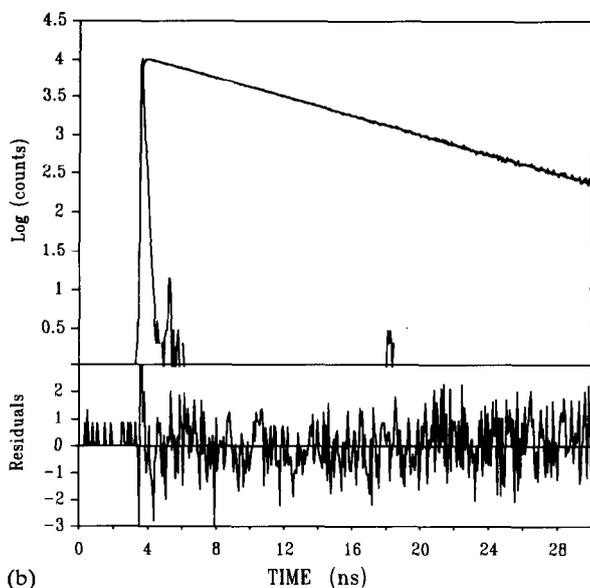
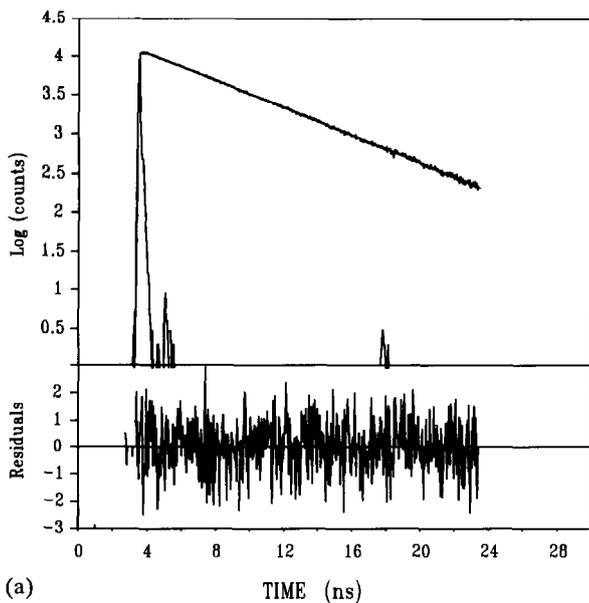


Fig. 2. Fluorescence decays of the phthalocyanine in phosphate-buffered saline solution in (a) H_2O and (b) D_2O . Fitting by iterative reconvolution and least-squares analysis gives the following lifetimes and reduced χ^2 values: (a) $\tau_f = 5.1 \pm 0.1$ ns, $\chi^2 = 1.02$; (b) $\tau_f = 6.8 \pm 0.1$ ns, $\chi^2 = 1.07$.

the dye was constant at 5.1 ± 0.1 ns in the pH range 5.5–9.0 and at pH 10 it was reduced to 4.9 ± 0.1 ns. A similar effect was observed in D_2O .

The triplet state of the phthalocyanine was studied by laser flash photolysis. The triplet–triplet absorption spectra in the two solvents were identical as were the triplet

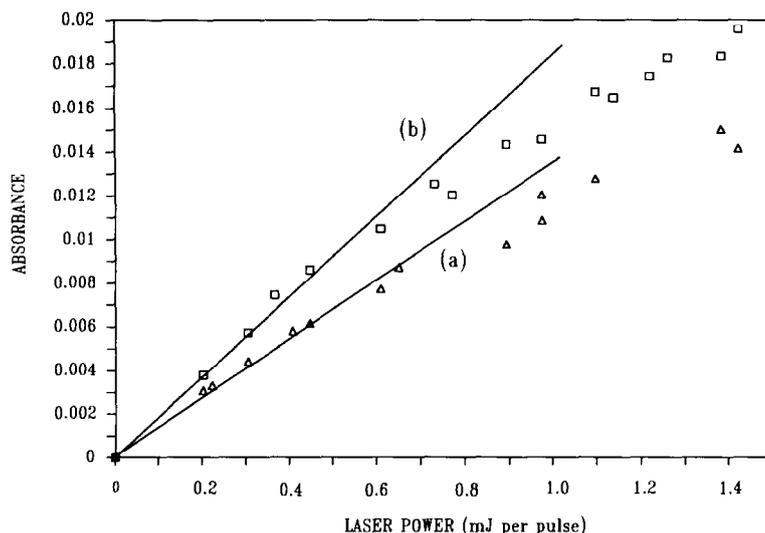


Fig. 3. Relative triplet quantum yield determinations. The triplet-triplet absorption at 490 nm immediately after the excitation pulse is plotted as a function of laser energy for disulphonated aluminium phthalocyanine in phosphate-buffered saline solution in H₂O (line (a)) and D₂O (line (b)).

extinction coefficients at 490 nm: $\Delta\epsilon_t = 3.7 \pm 0.2 \times 10^4 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$. The ratio of the triplet quantum yields determined from the power dependence of the triplet state absorption at 490 nm was measured as $\Phi_t(\text{D}_2\text{O})/\Phi_t(\text{H}_2\text{O}) = 1.3 \pm 0.1$ (Fig. 3). In the range of pump energies used, the triplet lifetimes in degassed solution were independent of light dose but did vary with the sensitizer concentration. All the triplet-state decays fitted well to a single-exponential decay, as illustrated in Fig. 4, and the observed lifetime τ_t fitted an equation of the form

$$\tau_t = \frac{1}{k_r} + k_q[\text{Pc}]$$

where k_r is the rate coefficient of the triplet decay at zero concentration, k_q is the rate coefficient of quenching by the ground state and $[\text{Pc}]$ is the concentration of the sensitizer. In H₂O, $k_r = 1670 \pm 120 \text{ s}^{-1}$ and $k_q = 1.1 \pm 0.1 \times 10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ and, in D₂O, $k_r = 630 \pm 30 \text{ s}^{-1}$ and $k_q = 1.0 \pm 0.2 \times 10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$; a plot of $1/\tau_t$ vs. concentration is shown in Fig. 5. In both solvents the triplet state is quenched by oxygen with a rate constant of $2.0 \pm 0.1 \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$. The same values were obtained in 100 mM phosphate buffer in the pH range 5.5–9.0.

4. Discussion

The fluorescence quantum yield and lifetime in H₂O are very similar to those reported by Davila and Harriman [7, 14] for the trisulphonated aluminium phthalocyanine ($\tau_f = 5.3 \text{ ns}$; $\Phi_f = 0.44$). However, in marked contrast with their work we have observed a significant increase in the fluorescence lifetime and quantum yield when deuterium oxide is used as solvent. Strictly speaking it is not possible to compare the pH of the

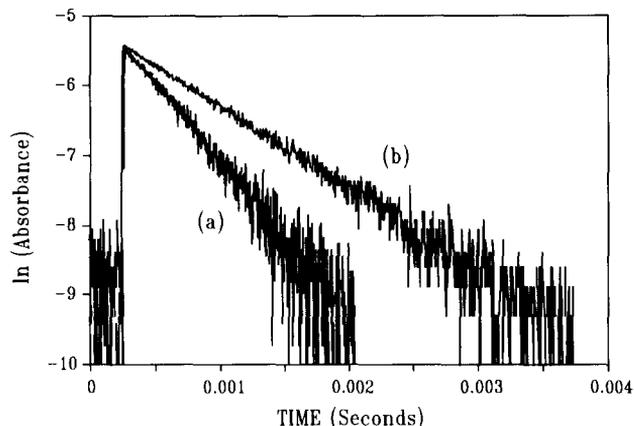


Fig. 4. Decay of triplet-triplet absorption at 490 nm for 5 μM solutions of disulphonated aluminium phthalocyanine in phosphate-buffered saline solution in H_2O (trace (a)) and D_2O (trace (b)). The observed triplet lifetimes are 450 μs and 770 μs respectively.

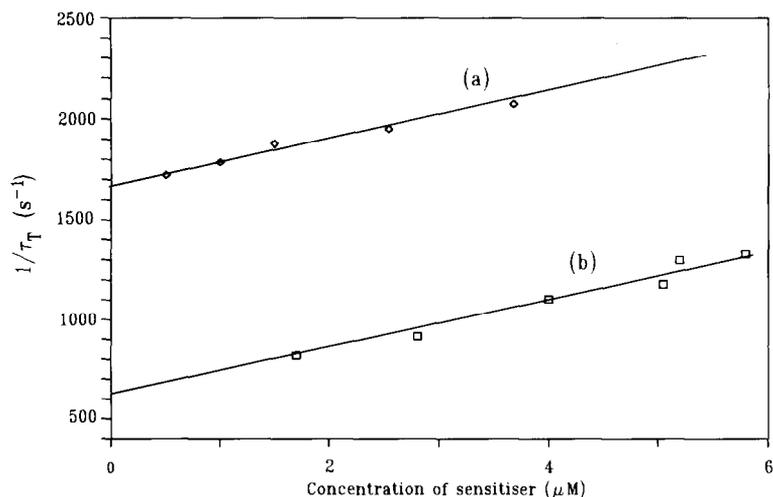


Fig. 5. Plot of reciprocal triplet lifetime *vs.* sensitizer concentration showing the calculation of self-quenching rate coefficients and triplet lifetimes at zero concentration in H_2O (line (a)) and D_2O (line (b)).

H_2O solution with the pD of the D_2O solution; however, it is necessary to show that the differences in the fluorescence are not caused by differing degrees of phthalocyanine ionization [15]. In 100 mM phosphate-buffered solution we have demonstrated that the fluorescence lifetime is constant in the pH range 5.5–9.0, and that at higher pH values the lifetime decreases slightly. Thus we conclude that in the phosphate-buffered saline solutions at pH 7.4 and pD 7.8 the differences in the fluorescence quantum yields and lifetimes do not originate from the ionization of the sulphonated phthalocyanine. From the values of τ_f and Φ_f the radiative rate constant k_f may be calculated:

$$k_f = \frac{\Phi_f}{\tau_f}$$

The values of k_f in the two solvents are the same within experimental error; $k_f = 7.8 \pm 0.7$ and $8.1 \pm 0.8 \times 10^7 \text{ s}^{-1}$. This is supported by the molar extinction coefficient not being significantly affected by the deuterated solvent. The relative ratio of intersystem crossing rate coefficients, k_{isc} , in H_2O and D_2O may be calculated

$$k_{isc} = \frac{\Phi_t}{\tau_f} \quad \frac{k_{isc}(\text{D}_2\text{O})}{k_{isc}(\text{H}_2\text{O})} = \frac{\Phi_t(\text{D}_2\text{O})\tau_f(\text{H}_2\text{O})}{\Phi_t(\text{H}_2\text{O})\tau_f(\text{D}_2\text{O})}$$

Again, the observed ratio of the rate coefficient is unaffected by the deuteration since $k_{isc}(\text{D}_2\text{O})/k_{isc}(\text{H}_2\text{O}) = 0.97 \pm 0.05$.

From this we conclude that the differences in the singlet-state photophysics are caused by a reduction in the rate of internal conversion from S_1 to S_0 in the deuterated solution. If in the deuterated solvent we assume that ' $\Phi_f + \Phi_{isc} = 1$ ', i.e. the quantum efficiency, Φ_{ic} , of internal conversion is zero, then we can calculate that the maximum value for the triplet quantum yield is 0.48 ± 0.06 , and therefore it follows that the maximum triplet yield for the protiated solvent is 0.37 ± 0.06 . This is only a little lower than the triplet quantum yields reported by Davila and Harriman [14] (0.45 ± 0.05) and Keir *et al.* [16] (0.42), suggesting that our original assumption of $\Phi_{ic} = 0$ in D_2O is good. Furthermore, it shows that there is a very significant change in Φ_{ic} , which in the protiated solvent must have a value of at least 0.23 ± 0.10 and a very much smaller value in deuterium oxide.

A similar effect was reported by Kajii *et al.* [5] who studied the dynamics of the excited singlet state of porphyrin- H_2 and porphyrin- D_2 . They found that the quantum yield of internal conversion in the isotopically substituted porphyrin was 15 times smaller than that in the parent and argued that this was because the inner N-H vibrational modes played an important role in the radiationless decay. Deuteration of the ring has been shown to have a smaller effect on $S_1 \rightarrow S_0$ internal conversion [4]. Others have shown that the non-radiative decay of the triplet state is also affected by deuteration of the inner N-H groups [1, 3]. However, in the present case there is strong evidence from the Q-band in the visible absorption spectrum, which is characteristic of a metallophthalocyanine, and also from the elemental analysis that the material used in this work has an aluminium ion occupying the centre of the ring; hence the exchange of the inner N-H groups cannot be responsible for the observed isotope effect. In aqueous solution the metal is believed to be solvated by two axial water ligands [17], which would be expected to exchange deuterium for hydrogen rapidly in D_2O .

The work presented here suggests that the vibrational modes of these ligands play a significant role in the non-radiative decay of the excited singlet state by $S_1 \rightarrow S_0$ internal conversion. The -OH and -OH₂ ligands represent polar groups on the otherwise hydrophobic face of the macrocycle; indeed it is these ligands which inhibit aggregation of the aluminium phthalocyanines, and it is anticipated that there will be a strong solvent-solute interaction. By analogy with the work of Burgner and Ponte Goncalves [1], who attributed their observations of decreased non-radiative rate coefficients in deuterated porphyrins to reduced Franck-Condon vibrational overlap integrals, we suggest that in D_2O the lower vibrational frequency of the ligand results in a smaller Franck-Condon factor and hence a less efficient energy relaxation pathway. This is not the case for the aromatic C-H bonds present in the system which are expected to have a weak interaction with the solvent. Previously, Gradyushko *et al.* [6] have reported that deuteration of alcohol ligands bound to magnesium porphyrin cause an increase in the triplet lifetime.

The triplet lifetimes of the sensitizer in solution are also affected by the solvent, although the rates of quenching by O_2 and self-quenching by ground-state phthalocyanine are unaffected by the substitution. The rate coefficient for the latter process has been determined to be $1.1 \pm 0.1 \times 10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ in H_2O and $1.0 \pm 0.2 \times 10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ in D_2O , and these values may be compared with the previously reported value of $2.3 \times 10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ for aluminium phthalocyanine chloride in aqueous dimethyl formamide and aqueous dimethyl sulphoxide [18]. Quenching of the triplet state by the ground-state dye has a very significant effect upon the observed triplet lifetimes in degassed solutions. Thus a $2.5 \mu\text{M}$ solution of the dye in H_2O has an observed triplet lifetime of $510 \pm 30 \mu\text{s}$ but extrapolation back to zero concentration for a range of sensitizer concentrations gives a value of $600 \pm 30 \mu\text{s}$. As in the porphyrins the increase in triplet lifetime in D_2O can be attributed to the decrease in non-radiative $T_1 \rightarrow S_0$ relaxation due to the deuteration of the axial ligand. In aerated solutions this change in τ_1 is expected to have little effect upon the efficiency of formation of singlet oxygen by the dye in aqueous solution; however, in an oxygen-deficient environment, such as that found when the sensitizer is bound to a protein, the increased triplet lifetime would allow a more efficient photodynamic effect.

The effect of deuteration upon the singlet and triplet state photophysics reported above appears not to be limited to the disulphonated aluminium phthalocyanine in water. Preliminary results from the disulphonated aluminium phthalocyanine in deuterated methanols and also tri- and tetra-sulphonated aluminium phthalocyanine in water have demonstrated this to be a general phenomenon that must be considered when studying these dyes in deuterated solvents [19].

5. Conclusion

The influence of the solvent upon the relaxation processes of the excited states of disulphonated aluminium phthalocyanine has been demonstrated by the significant reduction in internal conversion from S_1 . Such an effect has been reported previously for many porphyrin-containing species, although to date only one has indicated that this also occurs when the ligand bound to a central metal ion is deuterated. The implication is that the coupling of the ligand's vibrational modes affects the quantum yield of triplet formation by the dye and therefore influences the efficiency of photodynamic action. Binding of the sensitizer to a biological molecule may displace the water ligands and therefore allow more (or less) efficient quenching of the singlet or triplet state. The effects of such binding are currently under examination in our laboratories.

The results also emphasize the importance of comparing the excited-state dynamics of a dye in protiated and deuterated solvents, particularly if a triplet-mediated process such as singlet oxygen formation is being determined. Finally, this effect may be used to our advantage; by preparing selectively deuterated dyes in which internal conversion is suppressed, the generation of cytotoxic singlet oxygen may be increased.

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