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Laser Flash Photolysis of Molecules of Medical Relevance.

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LASER FLASH PHOTOLYSIS OF MOLECULES OF MEDICAL RELEVANCE

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In recent years the technique of laser flash photolysis (lfp) has been much used to elucidate the early processes following light absorption by molecules of biological and medical interest. Lfp allows us to measure the variation of optical density of a transient species with time, the equipment most used having a time resolution of a few nanoseconds*(ns). We will concern ourselves with the use of such data to deduce the physical properties of the excited states of some molecules of medical interest including the psoralens, the porphyrins and bilirubin.

The excited states of these molecules studied to date concern mainly the first triplet state (T_1) and lfp has been used to determine the triplet-triplet extinction coefficient (ε_T), triplet-triplet spectra, the efficiency of intersystem crossing from the first excited singlet state (S_1) to T_1 (Φ_{ISC}), and the rate of reaction of T_1 with other molecules, such 'quenching' reactions being important to several processes in biology and medicine. The relationship of the ground state (S_0), S_1, T_1 and the meaning of standard terms such as Φ_{ISC} are well documented as, for example, in reference 1.

Light irradiation of biological systems can lead to processes other than, or subsequent to, the excited states described above and lfp has proven a very useful technique in following such changes, an example being the conformation changes following light absorption by the visual pigments[2].

* Picosecond (10^{-12}s) lfp has also made useful contributions to our understanding of biological processes, particularly photosynthesis and vision

Determination of ε_T

The optical density obtained from a flash photolysis experiment is the difference between the triplet (or transient) optical density and the ground state optical density. This difference optical density (ΔOD) is related to ε_T as $\Delta OD = C(\varepsilon_T - \varepsilon_S)$ for a 1 cm path length where C is the concentration of ground state (extinction coefficient ε_S) converted to the triplet state. In general we measure from lfp ΔOD (as a function of time) and ε_S is already known but both C and ε_T are unknown. Two methods for obtaining ε_T are now described.

1. Complete Conversion

This is the simplest method of determining ε_T and the method has been used in both conventional and lfp. Typically the sample is irradiated by a laser flash with filters being used to vary the laser intensity (I) reaching the sample. The data shown in figure 1 shows a typical plot of ΔOD with I for protoporphyrin IX*(pp) in benzene. Worked Example 1:

The maximum in this plot corresponds to the complete conversion of S_0 to T_1 so that C is simply the starting concentration. For the data given C was 5.44×10^{-6} dm^3mol and path length 0.67 cm so that $\varepsilon_T - \varepsilon_S$ is $\sim$ 31,000 $dm^3mol^{-1}cm^{-1}$ and since ε_S is 4,900 $dm^3mol^{-1}cm^{-1}$ at the wavelength corresponding to the data (450 nm), the value of ε_T was found to be $\sim$ 36,000 $dm^3mol^{-1}cm^{-1}$ at 450 nm. Once ε_T has been obtained at one wavelength it is trivial to convert the triplet-singlet difference spectrum obtained from lfp at different monitoring wavelengths to a triplet-triplet absorption spectrum, the corresponding data for pp being given in figure 2.

* The molecule responsible for the skin photosensitivity diseases of erythropoetic protoporphyria - see later.

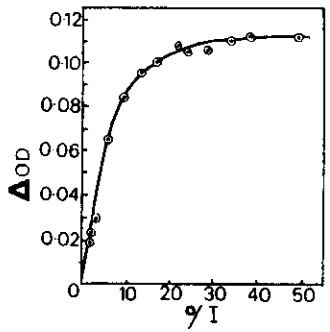

Figure 1. For explanation see text.

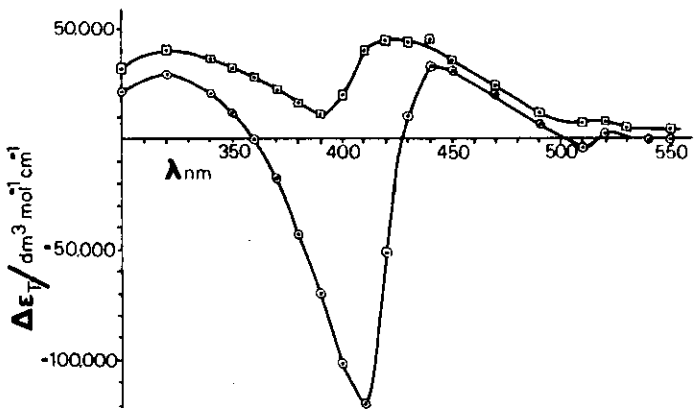

Figure 2. For explanation see text.

This simple method for the determination of ε_T depends on being able to obtain a maximum in the plot of ΔOD against I. For many molecules (particularly if Φ_{ISC} is rather low) this cannot be readily achieved. In such cases an alternative method based on energy transfer is often used.

2. Energy Transfer

This method involves comparing an unknown triplet absorption with another of known ε_T. The method was first applied[3] to pulse radiolysis* data and used a comparison of the extinction coefficient of the benzophenone ketyl radical ($\Phi_2\dot{C}OH$) with that of several aromatic compounds whose triplet levels are below that of benzophenone, a useful review being given by Bensasson and Land (1978)[4]. More recently a number of molecules of known ε_T (including retinol) have been used to determine ε_T of molecules of biological interest. The method is most simply applied when it is arranged that all of the donor triplets (of known ε_T) are quenched with total energy

* This technique often utilises a nano-second pulse of high energy radiation (several MeV) to ionise the solvent molecules. Various ion recombination processes lead to the production of solvent excited singlet and triplet states.

$$\text{Solvent}^+ + e^- \rightarrow S_1, T_1$$
$$\text{Solvent}^+ + \text{Solvent}^- \rightarrow S_1, T_1$$

If a solute (M) is present a variety of charge transfer processes can lead to S_1 and T_1 of the solute, e.g. $S^+ + M \rightarrow M^+$: $M + e^- \rightarrow M^-$ and $M^+ + M^- \rightarrow M$ as S_1 and/or T_1. Detection techniques following high energy pulsed irradiation can be the same as for lfp.

transfer by the acceptor (of unknown ε_T). The ratio of ε_T(donor) / ε_T(acceptor) is then simply equal to the ratio of the donor triplet optical density in the absence of acceptor to acceptor triplet optical density.

Unfortunately the situation is often more complex than this because the donor triplets can decay by means other than energy transfer and the acceptor triplet can decay during its formation. In the general case three reactions must be taken into account:

Donor (T_1) → Donor (S_0); Rate Constant k_1

Donor (T_1) + Acceptor (S_0) → Donor (S_0) + Acceptor (T_1); Rate Constant k_Q

Acceptor (T_1) → Acceptor (S_0); Rate Constant k_3

In order to obtain the 'true' optical density of the acceptor (OD_A^T) it is necessary to determine the rate constants for the decay of the donor in the absence of acceptor (k_1) and in the presence of acceptor $k_2 = k_1 + k_Q[A]$ and the rate constant for the decay of the acceptor (k_3). In addition the maximum optical density of the acceptor is measured (OD_A^M). It can then be shown that[5]:

$$\varepsilon_T^A = \frac{\varepsilon_T^D}{OD_T^D} \times OD_A^M \times \frac{k_2}{(k_2-k_1)} \times \exp - \frac{\ell n\, k_2/k_3}{(k_2/k_3)-1}$$

Worked Example 2

Determination of ε_T for Uroporphyrin* (UP) using Energy Transfer from Biphenyl (BP).

Lfp of biphenyl alone (in benzene) allows the rate constant for the BP triplet to be obtained: $k_1 \sim 8\times10^3 s^{-1}$. Also, ΔOD_T^{BP} at 361 nm = 0.058 and ε_T^{BP} (361 nm) is 27,100 $dm^3mol^{-1}cm^{-1}$. (Note at 361 nm, $\varepsilon_S^{BP} = 0$ so that $\Delta OD_T^{BP} = OD_T^{BP}$).

On adding $3\times10^{-5} dm^3mol^{-1}$ UP, the increased rate of decay of BP or the rate of growth of UP triplet gives $k_2 \sim 3\times10^5 s^{-1}$. The maximum value of ΔOD_T^{UP} (440 nm) was obtained as 0.052.

Substitution of this data into the above equation gives $\Delta\varepsilon_T^{UP} \sim 25{,}000\ dm^3mol^{-1}cm^{-1}$ (at 440 nm) and since ε_T^{UP} is known to be $\sim 6{,}000\ dm^3mol^{-1}cm^{-1}$ (at 440 nm) $\varepsilon_T^{UP} \sim 31{,}000\ dm^3mol^{-1}cm^{-1}$ at 440 nm.

We have thus considered two methods of using the data obtained from a lfp experiment to determine transient extinction coefficients and hence transient or triplet absorption spectra; we next consider methods of determining the efficiency of intersystem crossing from $S_1 \rightarrow T_1$ (Φ_{ISC}).

* Molecule responsible for severe skin photosensitivity in several hepatic porphyrias.

Comparative Technique for Determination of Intersystem Crossing Efficiency

This method is based on comparing the concentration of triplets (or other transient species, such as the electron following a photo-ionisation process) with the concentration of triplets formed by the same number of incident quanta on a solution containing some molecule of known Φ_{ISC}. The OD of the ground state of both solutions at the wavelength of laser excitation are made equal so that for an unknown X and a standard S

$$\varepsilon^S (S) = \varepsilon^X (X)$$

where ε^S and ε^X, (S), and (X) are, respectively, the molar extinction coefficients and molar concentrations of molecules S and X in their singlet ground state (S_0). Using these conditions both solutions will absorb the same number of photons, so that:

$$\Phi^X_{ISC} = \Phi^S_{ISC} \cdot \frac{(T)^X_{max}}{(T)^S_{max}}$$

where $(T)^X_{max}$ and $(T)^S_{max}$ are the maximum triplet concentrations observed after the laser pulse (the laser pulse duration is much shorter than the triplet lifetime). Thus

$$\Phi^X_{ISC} = \Phi^S_{ISC} \frac{OD_T{}^X/\varepsilon_T{}^X}{OD_T{}^S/\varepsilon_T{}^S}$$

where $OD_T{}^X$ and $OD_T{}^S$ are the maximum optical densities of the transient absorptions due to the triplet-triplet transitions, and $\varepsilon_T{}^S$ and $\varepsilon_T{}^X$ are the known molar extinction coefficients of these triplet transitions at the wavelength of the triplet optical density absorption.

It is vital in this type of laser experiment to ensure that significant amounts of laser light is not absorbed by molecules in their excited singlet or triplet states or as products and also that the depletion of the molecules S and X in their ground states is < 10%. If this latter condition is not maintained it can lead to intensity-dependent quantum yields[6].

Excitation Wavelengths and Reference Φ_{ISC} Values

265 nm The actinometer usually chosen for this wavelength (obtained from frequency quadrupling a neodymium pulse of 1060 nm) is naphthalene. The value of Φ_{ISC} for this molecule is established at 0.75±0.03 based on the independent technique of fluorescence quenching by xenon[7].

353 nm and 347 nm The actinometer usually used for these wavelengths (frequency tripled neodymium and frequency doubled ruby laser) is anthracene with $\Phi_{ISC} = 0.71$.

530 nm Actinometers for this wavelength (frequency doubled neodymium laser) are much less well established. One useful molecule for this wavelength is $Ru(bipyr)_2C\ell$ for which Φ_{ISC} has been reported as 1.0[8].

694 nm No widely accepted actinometer is available for the primary ruby line. A useful molecule being metal-free phthalocyanine for which $\Phi_{ISC} = 0.2$[9].

Worked Example 3

Determination of Φ_{ISC} for Protoporphyrin from lfp Data

Using a frequency doubled ruby laser with output ∿ 400 mJ at 347 nm and the laser intensity reduced to ∿ 1-2% and anthracene as actinometer the following data was obtained -

Actinometer (Anthracene) in cyclohexane

ε_T (422 nm) = 64,700

$\Delta OD_T{}^A$ (422 nm) = 0.023 (≡ $OD_T{}^A$ because $\varepsilon_S{}^A = 0$ at 422 nm)

Unknown (pp) in benzene.

ε_T (450 nm) = 36,000 ($\Delta\varepsilon = \varepsilon_T - \varepsilon_S$ = 31,000) - See page 4.

$\Delta OD_T{}^{pp}$ (450 nm) = 0.012

Substituting into the above equation

$$\Phi^{pp}_{ISC} = 0.71 \times \frac{0.012}{31{,}000} \times \frac{64{,}700}{0.023} \simeq 0.8$$

An alternative method for determining Φ_{ISC} (mentioned above for naphthalene) is based on relative measurements of the influence of heavy atoms on the fluorescence yield and triplet-triplet absorption. We will not consider this technique in detail because it is well documented[10] and because it has not been applied to systems of medical interest. Nevertheless (using conventional flash photolysis) the technique has been used to obtain Φ_{ISC} for some porphyrins and metalloporphyrins[11].

Application of Comparative Methods to Molecules of Medical Interest

Lfp techniques (only some of which have been described above) have been used to study numerous molecules of chemical, biological and medical interest . We will choose the following systems of medical interest to exemplify the technique:
1. Psoralens (furocoumarins), 2. Porphyrins, 3. Bilirubin.
The emphasis will be on the psoralens, the other systems being considered in much less detail.

The Psoralens (Furocoumarins)

Psoralens are important drugs used in the phototherapy of psoriasis and vitiligo. Systemic or topical application of 8-

methoxypsoralen (8-MOP) followed by long wavelength UV irradiation is an effective treatment of psoriasis, a debilitating skin disease in man. The treatment, sometimes called PUVA* treatment, should, however, be regarded as potentially hazardous since prolonged UV irradiation of animals treated with 8-MOP has been shown to induce squamous cell carcinomas, and because the phototherapy does not cure psoriasis and must be repeated periodically.

In psoriasis DNA synthesis and cell division (in the basal cells of the epidermis) is accelerated by up to ten times compared to normal skin. The mode of action of the psoralen drugs is by intercalating between the base pairs in duplex DNA; light absorption by the psoralen then results in covalent linkages being formed between the psoralen and the pyrimidine bases of DNA. Some typical structures are shown in figure 3.

The crosslinks are caused by the formation of cyclobutane adducts resulting from the psoralen 3,4 pyran and 4'5' furan double bonds photoreacting with two pyrimidine base 5,6 double bonds. Both types of intermediate mono-adduct have been isolated. The 4'5' mono-adduct absorbs up to 380 nm[12] whereas the 3,4 mono-adduct only absorbs appreciably below 320 nm[13]. Thus after the first quantum of UVA light has been absorbed by the psoralen itself to form a mono-adduct, the second quantum has to be absorbed by a 4'5' mono-adduct, rather than a 3,4 mono-adduct, to form a crosslink.

There is evidence that at least one of the steps of this two-step process involves the psoralen triplet state[14], and typical lfp data[15,16,17] of psoralen triplet states will now be discussed.

* Psoralen plus UVA light

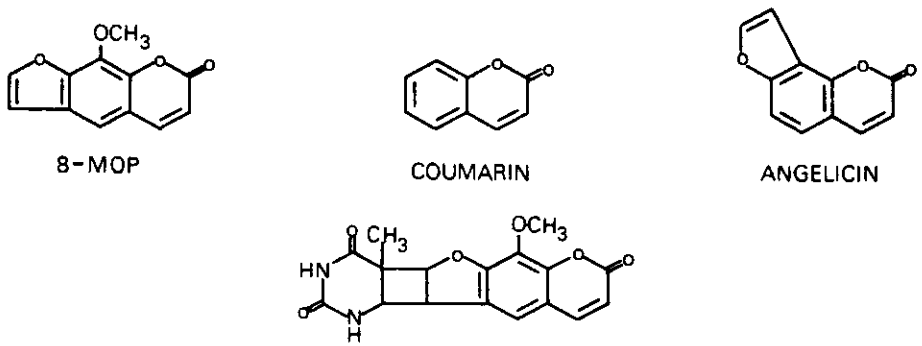

Figure 3. For explanation see text.

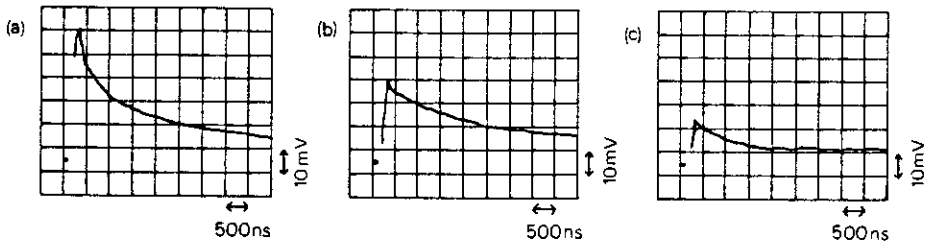

Figure 4. For explanation see text.

Oscillograms following 347 nm (ruby) laser flash photolysis of 0.15 mM aqueous 8-MOP are shown in figure 4. Oscillogram (a) concerns an argon-saturated solution monitored at 580 nm and (b) a N_2O saturated solution also monitored at 580 nm.

Oscillogram (a) shows a complex transient decay which is composed of three portions: (i) a fast decaying species with $\tau \approx 200$ ns, (ii) a longer lived species with $\tau \approx 1.6$ µs and (iii) a very long lived species which does not decay during the time of the monitoring pulse, i.e. with a lifetime much greater than 10 µs. The lifetimes quoted above are obtained from other oscillograms with time scales appropriate to each component.

As can be seen from (b) the short lived transient has been removed following N_2O saturation; this is consistent with it being due to the hydrated electron which is quenched by the process

$$e^-_{aq} + N_2O \xrightarrow{H_2O} N_2 + OH^\cdot + OH^-$$

Oscillogram (c) shows the transient absorption from the same solution following air saturation. Once again the very short lived transient is quenched ($e^-_{aq} + O_2 \rightarrow O_2^-$) but the 1.6 µs transient is also quenched. Assuming an oxygen concentration of 0.265 mM the quenching constant for the 1.6 µs transient was calculated as 3.3×10^9 $dm^3mol^{-1}s^{-1}$. These results support the assignment of the short lived species as e^-_{aq} and identify the 1.6 µs transient as the 8-MOP triplet.

One technique which can be used to understand the origin of the various transients produced (the e^-_{aq}, triplet and long-lived species for 8-MOP) is to determine the effect of laser intensity on these yields. Such data is shown in figure 5.

The slope of the plot for both e^-_{aq} and the long-lived species is 1.8 which implies that both arise from a biphotonic process mainly whilst the triplet is formed by a monophotonic process (slope = 1.0). Consequently oscillograms at low laser intensities show little or no e^-_{aq} or long-lived transient but still show triplet absorption.

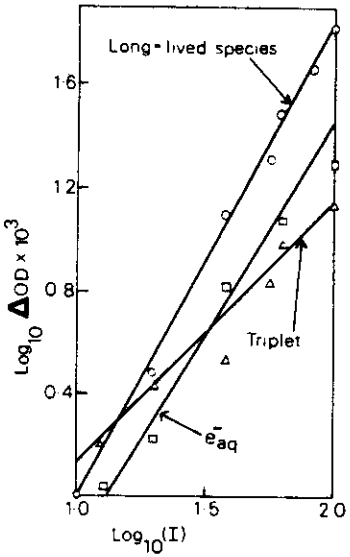

Figure 5. For explanation see text.

Since only low intensities of irradiation are used in psoralen phototherapy the biphotonic transients are unlikely to be of any relevance to the medical treatment. However, this example, does illustrate the care which must be taken in interpreting lfp data since the first published spectrum of 8-MOP obtained from lfp work did not allow for such biphotonic effects at high laser intensities and was consequently in error at wavelengths near to the absorption maximum of e^-_{aq} (700 nm). Figure 6 shows both the 'true' triplet-triplet spectrum obtained from 347 nm laser excitation of 0.226 mM 8-MOP with contributions from the e^-_{aq} and the long-lived species (radicals, as described below) subtracted. The insert shows the previously reported spectrum[15] with the absorption rising at wavelengths > 500 nm due mainly to contributions from the biphotonic photo-ionisation process.

The identity of the long-lived species shown in the oscillogram was obtained from the transient spectra at times after both e^-_{aq} and the triplet had decayed - these were very similar in shape to the known spectra of the radical anion of 8-MOP[18].

Clearly the processes

$$\text{8-MOP} \xrightarrow{2h\nu} \text{8-MOP}^{\cdot+} + e^-_{aq}$$

and

$$\text{8-MOP} + e^-_{aq} \rightarrow \text{8-MOP}^{\cdot-}$$

occur; furthermore lfp can be used to determine the rate of e^-_{aq}

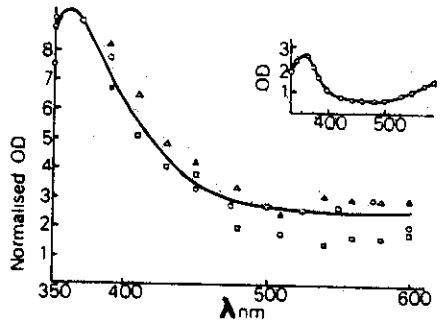

Figure 6. Triplet Spectrum of 8-MOP

○ e^-_{aq} contribution removed with N_2O and radical removed with t-BuOH
△ low laser intensities
□ transients measured after the decay of e^-_{aq} (1 μs)

addition to 8-MOP by monitoring the rate of decay of the hydrated electron (at 700 nm) as a function of 8-MOP concentration - the second-order rate constant so obtained being $\sim 3 \times 10^{10}$ $dm^3mol^{-1}s^{-1}$.

Having obtained a 'true' triplet spectrum of 8-MOP as described above, it is now possible to apply the technique of energy transfer described earlier to obtain ε_T and then the comparative method to obtain Φ_{ISC}. Furthermore, the rate of the triplet psoralen reaction with other species such as pyrimidine bases and amino acids can now be established by monitoring the increased rate of decay of the psoralen triplet on adding such species. Also, as will be shown below, it is possible (at least in some cases) to use lfp to deduce the type of reaction occurring between a psoralen triplet and another species such as an amino acid.

Triplet-Triplet Extinction Coefficient

Values of ε_T for several psoralens and related molecules obtained using the energy transfer technique are given in Table 1. Generally it has proven easier to determine $\varepsilon^T_{psoralen}$ in benzene using the nano-second pulse radiolysis technique, while in more polar solvents (alcohols) lfp has been used with retinol as standard ($\varepsilon^T_{Retinol}$ = 80,000 $dm^3mol^{-1}cm^{-1}$ at 405 nm). Typical results[15,16] are shown in Table 1.

Φ_{ISC}

Table 1 also gives the reported values of Φ_{ISC} for several psoralens and coumarins measured using 353 neodymium or 347 nm ruby laser excitation. The number of triplets produced in a psoralen or coumarin solution by a given number of laser quanta at 347 nm or 353 nm was compared with the number of triplets

Table 1. Values of ε_T and Φ_{ISC} for Psoralens and Coumarins

Compound	ε_{T-T} ($dm^3mol^{-1}cm^{-1}$) in Benzene (λ), nm	Φ_{ISC} Benzene	Φ_{ISC} Water
Psoralen	8,100 (450)	0.034	0.45
8-MOP	10,000 (480)	0.011	0.14†
5-MOP (Bergapten)	10,200 (450)	0.067	0.01
Angelicin (Isopsoralen)	4,700 (450)	0.009	0.33
Coumarin	11,100 (400)	-	0.054*
4',5' dihydropsoralen	15,700 (500)	-	0.068*

* Laser excitation wavelength 347 nm, all other laser excitation wavelengths are 353 nm.
† Value in Methanol 0.03.

number of laser photons absorbed by anthracene as standard.

Clearly there is an increase in Φ_{ISC} when comparing the non-polar solvent benzene to the polar solvent water. This implies that the actual environment of psoralen in the skin is very important with respect to photoreactions occurring via the triplet state. In fact the values of Φ_{ISC} in both solvents are higher than the quantum yields for the photoreaction of psoralens with native DNA, for example[19] the quantum yield for 8-MOP is 4.6×10^{-3}.

Such photoprocesses could therefore involve the corresponding triplet states. Also given in Table 1 is Φ_{ISC} for the model of a psoralen-pyrimidine 4'5' mono-adduct (4'5' dihydropsoralen), and this data is consistent with DNA psoralen photoexcitation occurring via the triplet state of the 4'5' mono-adduct. If both cross-link formation and mono-adduct formation were to occur via the corresponding triplet state the yield of cross-link formation would be related to the product of Φ_{ISC} for both processes.

Rates of Reaction of Psoralen Triplets with Nucleic Acid Bases and Amino Acids

These rates are obtained relatively easily from lfp data by measuring the increased rate of decay of triplet upon addition of the amino acid or the base. Such reactions of triplet states can occur by several mechanisms including energy transfer and partial or complete charge transfer, i.e.

$$P(T_1) + Q(S_0) \rightarrow P(S_0) + Q(T_1)$$
$$P(T_1) + Q(S_0) \rightarrow P^{\cdot+} + Q^{\cdot-}$$

a useful general review of quenching (Q is quencher) processes has been given by Wilkinson[20].

Table 2 gives the second order rate constants obtained by quenching some psoralen and coumarin triplets with thymine and tryptophan.

Table 2. Quenching Rate Constants.

Compound	Second Order Rate Constant ($dm^3mol^{-1}s^{-1}$) Triplet + Thymine	Triplet + Tryptophan
Psoralen	7.5×10^8	3.1×10^9
8-MOP*	$< 6 \times 10^6$	3.5×10^8
Angelicin	1.1×10^9	2.1×10^9
Coumarin	2.4×10^8	3.1×10^9
4',5' dihydrocoumarin	2.5×10^7	1.7×10^9

* Solvent was Methanol - in all other cases solvent was water.

It can be seen, for example, that 8-MOP is quenched at more than two orders of magnitude slower than psoralen. This variation implies that it is unlikely that psoralen triplet states lead to mono-adduct formation. Possibly the 4'5' mono-adduct is formed via the excited singlet state (S_1) and the cross-link formation then occurs via the triplet state. Certainly, as Table 2 shows the model 4'5' dihydropsoralen triplet was quenched by thymine.

Table 2 also gives the rates of reaction of tryptophan with psoralen and coumarin triplet states*. For coumarin and 4'5' dihydropsoralen long-lived transients remain after the triplets have been quenched. By comparison of the spectra of such long-lived species with the spectra of the radical anion of coumarins (intense band at 360 nm) and with the deprotonated tryptophan radical cation evidence has been presented[16] that the quenching process occurs by charge transfer, i.e.

$$\text{Coumarin}\ (T_1) + \text{Tryptophan}\ (S_0) \rightarrow \text{Coumarin}^{\cdot-} + \text{Tryptophan}^{\cdot+}$$

Since nucleic acid bases such as thymine also have electron donor properties it is possible that such molecules also quench psoralen triplet by a (partial) charge transfer mechanism.

To summarise, lfp has shown that significant yields of triplet states are formed on light excitation of psoralens and that in water these triplets often react efficiently with several amino acid and nucleic acid bases; lfp data has also indicated that the mechanism of such reactions may be via a charge transfer process.

Porphyrins

The porphyrins which arise in the hereditary diseases known as the porphyrias are generated by a disfunction of haem biosynthesis and vary in structure depending on the particular step in the biosynthetic pathway at which the enzymic disfunction occurs. For example in erythropoetic protoporphyria (epp) high levels of proto-

* Triplet energy transfer is precluded by the energy levels involved.

porphyrin (pp) arise, while in cutaneous hepatic porphyria (porphyria cutanea tarda) it is the uroporphyrin (up) which is present in high levels. All porphyric diseases lead to skin photosensitivity of varying severity.

Porphyrins are known to sensitise the formation of singlet oxygen and it is reasonable to assume that this reactive intermediate is one cause of tissue damage and that the singlet oxygen arises via energy transfer from the porphyrin triplet. Furthermore it has been claimed that carotenoids and particularly β-carotene reduces photosensitivity in epp and it has been speculated that this effect also occurs via energy transfer, i.e. quenching of pp triplet

$$pp\ (T_1) + \beta\text{-carotene}\ (S_0) \rightarrow pp\ (S_0) + \beta\text{-carotene}\ (T_1)$$

and/or $^1O_2 + \beta\text{-carotene}\ (S_0) \rightarrow {}^3O_2(\text{ground state}) + \beta\text{-carotene}\ (T_1)$

It is because of this medical relevance that lfp has recently been used to attempt to characterise and follow the reactions of porphyrin triplet state in various environments.

Strong triplet absorptions (using conventional flash photolysis) are well established for several porphyrins and prior to the application of lfp it was know that Φ_{ISC} for several porphyrins was high. Recently[21] lfp has been applied to several porphyrins known to occur in porphyric diseases. Values of $\Delta\varepsilon_T$ were reported based on the complete conversion method and Φ_{ISC} estimated by the comparative method; in addition the triplet lifetimes and rates of reaction with oxygen and some carotenoids were obtained. Some of these results are summarised in Table 3.

Table 3. Porphyrin Ester Triplet Data obtained from lfp

Porphyrin	ε_T ($dm^3mol^{-1}cm^{-1}$)	Φ_{ISC}	Triplet Decay Rate (s^{-1})	Rate of Triplet Quenching by O_2 ($dm^3mol^{-1}s^{-1}$)
pp DME	35,000 (at 450 nm)	0.80	-	2.7×10^9
Coproporphyrin I TME	32,000 (at 440 nm)	0.88	4.0×10^3	1.8×10^9
Uroporphyrin I OME	29,000 (at 440 nm)	0.72	3.7×10^3	1.5×10^9

(TME ≡ tetramethyl ester and OME ≡ octamethyl ester)

These results show no correlation between triplet parameters and porphyrin structure. Since differences in photosensitising results are implied by photohaemolysis work[22] the lfp data would imply that these cannot be attributed to different triplet state properties.

Protoporphyrin and pp DME have been the subject of detailed study[21,23] by both lfp and pulse radiolysis and the triplet state

properties reported as a function of porphyrin environment. Thus pp DME in benzene, pp H DME (monocation) in acetic acid/methanol, pp H_2 DME (dication) in benzene/trifluoacetic acid and pp DME solubilised in detergent gives rise to strong triplet absorptions with Φ_{ISC} in the range 0.5-0.9. Also, preliminary results on pp in human albumin also showed a high triplet yield. On the other hand in aqueous environments only near zero Φ_{ISC} yields were obtained probably due to pp aggregation. However, since pp is bound to serum protein and is not present in the aqueous phase the lfp results on pp in water may not be relevant to the 'in vivo' system.

Bilirubin

Bilirubin (BR) is a yellow compound produced from the degradation of haemoglobin. The photo-reactions and excited states of BR are of importance because the disease of neonatal hyperbilirubinemia is due to an excess of BR and can be treated by irradiation with visible light. 'In vitro' photoexcitation of BR leads both to a number of oxidation products[24] and also to isomerisation[25]. It has been suggested that the oxidative reactions occur via singlet oxygen ($^1\Delta g$) produced by energy transfer from triplet BR

$$^3BR + O_2\ (^3\Sigma^-g) \rightarrow BR\ (S_0) + O_2\ (^1\Delta g)$$

whereas little or no evidence has been presented on the mechanism of photo-isomerisation. In both reactions the BR is converted into more water soluble products and thus both reactions could be relevant to the phototherapeutic process.

Little direct information is available on the excited states of BR, however, Land[26] has applied both lfp and pulse radiolysis to this molecule (in benzene) and established several properties of the triplet state. Thus ε_T at 500 nm (obtained by the energy transfer method) is 8,800 $dm^3mol^{-1}cm^{-1}$ and Φ_{ISC} was estimated at ≤ 0.1. In addition the energy level of BR triplet was shown to lie between 123 and 176 kJ mol^{-1}, and the triplet lifetime to be 9 μs. Thus while the triplet lifetime is sufficiently long for reaction with oxygen to occur the low value for Φ_{ISC} does not support the self-sensitised oxidation of BR via the triplet. However some cross-over to the triplet could still occur and be partially responsible for the phototherapeutic effect although the currently accepted major process occurring during phototherapy is photo-isomerisation of BR to polar isomers thus explaining the sudden excretion of bile pigment when jaundiced babies are irradiated with visible light. Very recently[27] lfp has been applied to BR-albumin complexes and has shown that photo-isomerisation may be detected by this technique with some evidence that the isomerisation of human serum albumin complexed BR is occurring via a triplet state. However it is difficult to reconcile a value of Φ_{ISC} much below 0.1 with the rapid rate of photo-isomerisation reported in relatively low light intensities.

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