Journal of Fluorescence, Vol. 4, No. 1, 1994

Delayed Fluorescence of Porphyrins in Different Media

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Received October 18, 1993

Lifetimes of $TPPS_4$ (meso-tetraphenylporphine tetrasulfonate) triplet states were measured for liquid solutions of different acidity and viscosity and as a function of acceptor concentration for different acceptors (bovine serum albumin, tryptophan and furfuryl alcohol). Triplet lifetimes were estimated by monitoring the decay of $TPPS_4$ delayed fluorescence of E-type. The lifetime of delayed fluorescence depends on the concentration of O_2, since the latter is an effective quencher of the triplet state. The lifetime is shown to be influenced mainly by degree of aggregation state of $TPPS_4$ and, therefore, by the pH of the solution, decreasing with pH and for each pH remaining constant over a wide range of acceptor concentrations. The monomeric species is found to have the longest triplet lifetime in aqueous phosphate-buffered saline solution at neutral pH, especially when bound to albumin, despite of the low viscosity and protonated nature of the medium.

KEY WORDS: Porphyrins; delayed fluorescence; photodynamic therapy.

INTRODUCTION

Photodynamic therapy (PDT) is a novel experimental modality for the treatment of cancer. It is based on administering a photosensitizing drug, usually of a porphyrin type, to the patient, with subsequent exposure to laser light of an appropriate dose and wavelength, preferably in the red spectral region. The resulting photochemical process involves energy transfer from the triplet sensitizer to ambient molecular oxygen (triplet), forming singlet oxygen ($^1O_2$), which acts as a cytotoxic agent by oxidizing target biomolecules (acceptors). Therefore, the lifetime of the sensitizer triplet, and the influence of the microenvironment on it, is of particular importance for the PDT efficiency.

$TPPS_4$ (meso-tetraphenylporphine tetrasulfonate) is known to be an effective synthetic sensitizer for PDT, especially in the presence of liposomes or serum proteins [1]. $TPPS_4$ strongly binds to human serum albumin (HSA), with the formation of a complex [2]. It is monomeric in this complex, whereas it is aggregated to a varying extent in aqueous solutions without HSA [3]. The pH of the solution and its ionic strength determine the degree of $TPPS_4$ aggregation, which increases with acidity [4]. Thus, it seems interesting to check the influence of pH and, as a result of the aggregation state, on the triplet lifetime of $TPPS_4$, which can be determined by its delayed fluorescence. Some porphyrins are known to emit long-lived E-type fluorescence, with a lifetime longer than 1 ms, in the presence of albumin under anaerobic conditions and at room temperature [5]. It is expected that $TPPS_4$ shows similar behavior.

EXPERIMENTAL

$TPPS_4$ was obtained from Porphyrin Products (Logan, UT) and used as received. The acceptors for $^1O_2$ were bovine serum albumin (BSA), L-tryptophan (TRP), furfuryl alcohol (FUR), and egg phosphatidylcholine (EPC) liposomes. BSA and TRP were purchased from Sigma Chemical Co. and used as received. FUR (Riedel-de Haën) was freshly doubly distilled before use. Li-

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1053-0509/94/0600-0049$07.00/0

posomes (Sigma) were prepared by sonication, as described in Ref. 4. Triton X-100 (Eastman Kodak) was used as a surfactant.

Aqueous solutions were prepared in phosphate-buffered saline (PBS; 0.9% NaCl buffered with 0.01 *M* NaH_2PO_4–Na_2HPO_4) of appropriate pH. To obtain solutions of different viscosity, mixtures were prepared with an equal volume of methanol or propylene glycol.

Fluorescence measurements were carried out at room temperature in a tightly closed standard cuvette using a fully corrected Perkin–Elmer luminescence spectrometer, LS-50, equipped with a gated photomultiplier (R-928) of improved near-IR detection capability and a xenon flash lamp with a pulse duration of 10 μs at FWHM. Delayed fluorescence (DF) decays were recorded in a point-by-point fashion by averaging signals from at least 3000 lamp flashes at each time point at a fixed wavelength (at maximum emission λ_{em}, when excited at maximum excitation λ_{ex}). Decay kinetics was approximated as a sum of two exponentials (the fast one being the Xe lamp decay and the slow one being the luminescence decay of $TPPS_4$) by a computer least-squares iterative procedure.

The standard procedure of deriving DF lifetimes (τ_{df}) was as follows: A solution of $TPPS_4$ ($5 \cdot 10^{-6}$ *M*, unless stated otherwise) in PBS, together with an appropriate concentration of acceptor, was placed in the cuvette. Fluorescence spectra of emission and excitation were recorded to obtain λ_{em} and λ_{ex} and to determine the initial intensity of emission, which was used afterward for normalization of intensity levels at each point of the DF decay curve. An Intralux 6000 (Volpi AG, Switzerland) lamp with a bandpass glass filter (340–460 nm) was used as an irradiation source for PDT modeling photoreactions leading to oxygen consumption, which were carried out in the same cuvette. DF decays were recorded after each irradiation period until no more changes in τ_{df} were observed.

RESULTS AND DISCUSSION

The response characteristics of our instrument allows measuring only τ_{df} longer than 0.25 ms, thus we cannot determine τ_{df} for aerated solutions. During PDT modeling photoreactions, ambient oxygen was consumed, and depletion of this quencher caused τ_{df} to increase. Figure 1 illustrates this dependence, using BSA as an acceptor. After appropriate irradiation τ_{df} reaches a maximum, which is estimated to be equal to the triplet lifetime of $TPPS_4$ under anaerobic conditions. The resulting τ_{df} values are summarized in Table I.

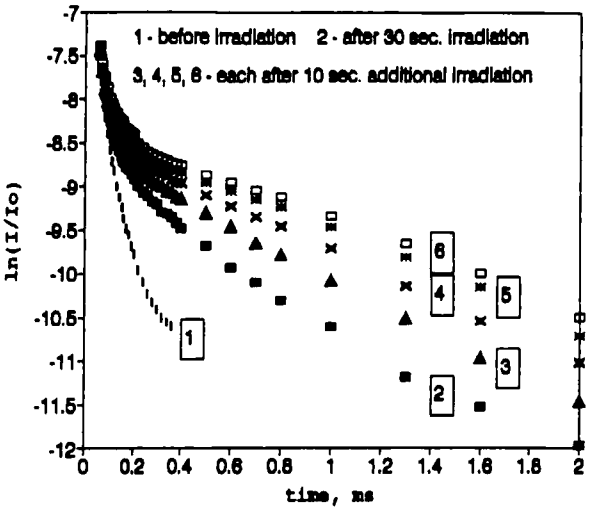

Fig. 1. Delayed fluorescence dependence on oxygen consumption.

τ_{df} was shown to be independent of $TPPS_4$ concentration (10^{-6}–10^{-5} *M*). Therefore, the DF of $TPPS_4$ was concluded to be of the E type, i.e., the first singlet state of the sensitizer (^1S) is formed from the long-lived triplet state (^1T) as a result of collisions with "hot" (high-vibrational state) molecules of the solvent. Thus, for a hot solution (under irradiation), the long fluorescence lifetime is equivalent to that of the sensitizer triplet state.

It can be seen from Table I that τ_{df} is identical for solutions of TRP, FUR, and EPC in PBS and is in good agreement with the triplet lifetime of $TPPS_4$ in buffered solution under anaerobic conditions, obtained by flash photolysis measurements [3,6]. The concentration of acceptors had no effect on the measured τ_{df}, because these acceptors, reacting with $^1O_2$, do not react with $TPPS_4$ or influence its degree of aggregation. In contrast, the addition of a surfactant (Triton x-100) increases τ_{df} dramatically, since it monomerizes $TPPS_4$ completely.

A higher viscosity of the solution increases the DF lifetime (Fig. 2), due to the reduced probability of physical quenching. For each particular medium, τ_{df} has a specific value, e.g., for a solution containing 50% propylene glycol or for one containing 50% methanol, but τ_{df} is independent of the nature of the acceptor or its concentration. Thus, it can be concluded that $TPPS_4$ dissolves much better in these solvents, characterized by moderate polarity, than in water, being in a less aggregated form in alcohols.

Albumin forms a complex with $TPPS_4$ monomers [2], therefore it is expected that τ_{df} in this complex is longest, because of the monomeric nature of $TPPS_4$ and the high viscosity of the BSA solution. Since BSA

Table I. Delayed Fluorescence Lifetimes of $TPPS_4$ (ms)

Acceptor	pH	[C]	100% PBS	50% methanol	50% propylene glycol
BSA	5.0		1.3–1.6		0.9–1.2
	7.0	0.1–3.0%	1.7–1.9	0.7–0.8	1.0–1.3
	9.0		1.7–1.9	0.7–0.8	1.1–1.3
FUR		0.005–0.05 *M*			
TRP	7.0	0.005–0.05 *M*	0.25–0.35	0.5–0.8	1.0–1.3
EPC		0.1%			
FUR		0.005–0.05 *M*			
TRP	7.0 + Triton	0.005–0.05 *M*	1.3–1.6	0.6–0.8	1.0–1.3
EPC		0.1%			

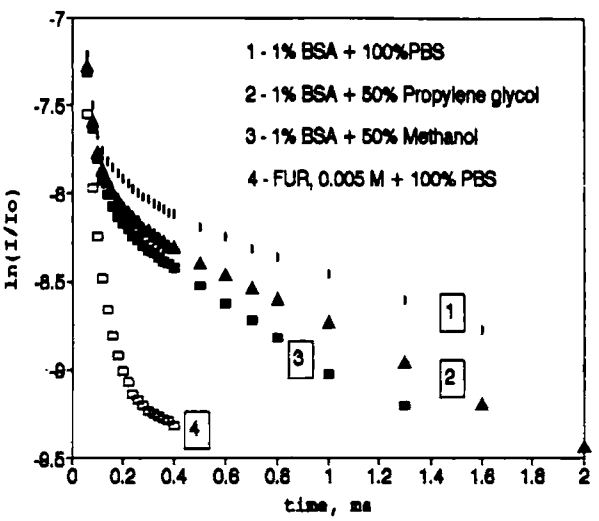

Fig. 2. Dependence of delayed fluorescence on solution viscosity.

undergoes denaturation in the presence of methanol, there is no doubt that its addition destroys the $TPPS_4$–BSA complex, resulting in the τ_{df} of a $TPPS_4$–BSA solution in methanol being characteristic for $TPPS_4$ in methanol. Similarly, the τ_{df} measured in a $TPPS_4$–BSA solution in propylene glycol is equal to that measured in propylene glycol for other acceptors. This fact may be interpreted by destruction of the $TPPS_4$–BSA complex in propylene glycol.

The most interesting results have been obtained for $TPPS_4$–BSA buffered solutions of different acidity. As shown previously [4], the pH of the solution is important for the extent of aggregation of $TPPS_4$, which increases with the acidity of the solution. In this work we tested $TPPS_4$–BSA buffered solutions for pH values 5.0, 7.0, and 9.0 with and without the addition of methanol or propylene glycol. For alcohol-containing solutions τ_{df} did not depend on the acidity of the solution, which confirmed our conclusions on the disaggregation of $TPPS_4$ in these alcohols. However, regarding buffered solutions there was a clear difference in τ_{df} between a solution of pH 5.0 and one of pH 7.0, where the aggregation state is changed [4], but it remained the same between pH 7.0 and pH 9.0, which is in good agreement with our previous results [4].

ACKNOWLEDGMENTS

This research was supported by The German-Israeli Foundation for Scientific Research and Development, GIF. I.L. was supported by The Posnansky Trust (British Technion Society) and by The Bat-Sheva De Rothschild Fund.

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