Triplet-state photophysics of aluminium phthalocyanine sensitizer in murine cancer cells

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Abstract

Diffuse-reflectance laser flash photolysis has been used to record transient spectra and decay kinetics of the photodynamic therapy sensitizer disulfonated aluminium phthalocyanine in two murine cancer cell lines, P815 derived from white mouse mast cells, and EL4, a lymphoblast derived from black mouse lymphocytes. In contrast to results with bacterial cells and yeasts, no transient other than the triplet state of the sensitizer was detected, suggesting that unlike the case in microbes, Type I electron-transfer processes play no role in the photodestruction of the murine cells studied.   ©2000 Elsevier Science S.A. All rights reserved.

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1. Introduction

The diffuse-reflectance laser flash photolysis (DRLFP) technique, which is described fully elsewhere [1,2], was used in earlier studies to identify the triplet state (and chemical species derived from it) of sensitizers for photodynamic therapy (PDT) in microbes [1,2]. From the results it was clear that in the case of Gram-negative bacteria, and the yeast *Candida albicans*, a Type I electron-transfer mechanism was probably of importance in the deactivation of the microbe in addition to the Type II singlet-oxygen mechanism. The existence of the Type I process was clearly indicated by the observation of the transient spectrum of the radical anion of the sensitizer.

In the present paper, we have extended such measurements to two murine cancer cell lines. P815 is a mastocytoma cell line delivered from white mice; EL4 is a chemical lymphoma lymphoblast derived from black mice.

2. Experimental

Mouse cell lines were the gift of Athena Koulourianos, Department of Immunology, Kings College, London. P815 DBA/2 mouse mastocytoma and EL4 chemically induced C57BL/6N mouse lymphoma cell lines (European Collection of Cell Cultures, Salisbury, UK) were obtained as 2–3 day cultures in 15 ml RPMI-1640 medium with 10% foetal calf serum (FCS) containing $(1–5) \times 10^7$ cells. Some early experiments used the P1.HTR line derived from P815 by selection for enhanced gene transfection characteristics. Cells were harvested by centrifugation at $600g$ for 5 min, and sensitized with disulfonated aluminium phthalocyanine $(AlPcS_2)$ with a 60 min incubation. Rigorous quantitative determinations of cell viability were not conducted. A variety of indirect qualitative methods were employed to assess cell viability and integrity following photophysical measurements based upon trypan blue exclusion; 50% of murine cells remained viable after laser flash photolysis.

$AlPcS_2$ was prepared and used as described elsewhere [3]. It consisted of a mixture of isomers, the major component (85%) being that shown below.
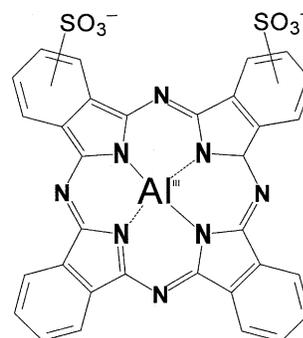
DRLFP measurements were made using the apparatus described in full elsewhere [1–3].

3. Results and discussion

Fig. 1 shows the transient absorption spectra obtained by DRLFP of the two murine cell lines sensitized with AlPcS$_2$. As with the bacterium *Streptococcus mutans*, spectra were less well resolved than those from cells with extracellular sensitizer. The contribution to the observed signal of the sensitizer in the supernatant liquid was estimated to be less than 15% by collecting the supernatant liquid by centrifugation and filtering, and adding the appropriate concentration

of unsensitized cells to mimic the scattering properties of the original suspensions. No transient was observed when the sample was bubbled with oxygen.

The absorption maxima at 500 and 490 nm, respectively, agree well with the solution-phase triplet spectrum of AlPcS$_2$ [3]. Transient absorption in the region 700–750 nm is particularly intense in these samples. In P815 particularly, the transient absorption peaking at 700 nm is clearly a discrete band displaying considerable overlap with the ground-state Q-band.

The decay kinetics were different from those of other cells studied to date, namely, bacteria and the yeast *Candida albicans*. The shapes of the transient spectra for both cell lines were independent of delay time, indicating that the spectra
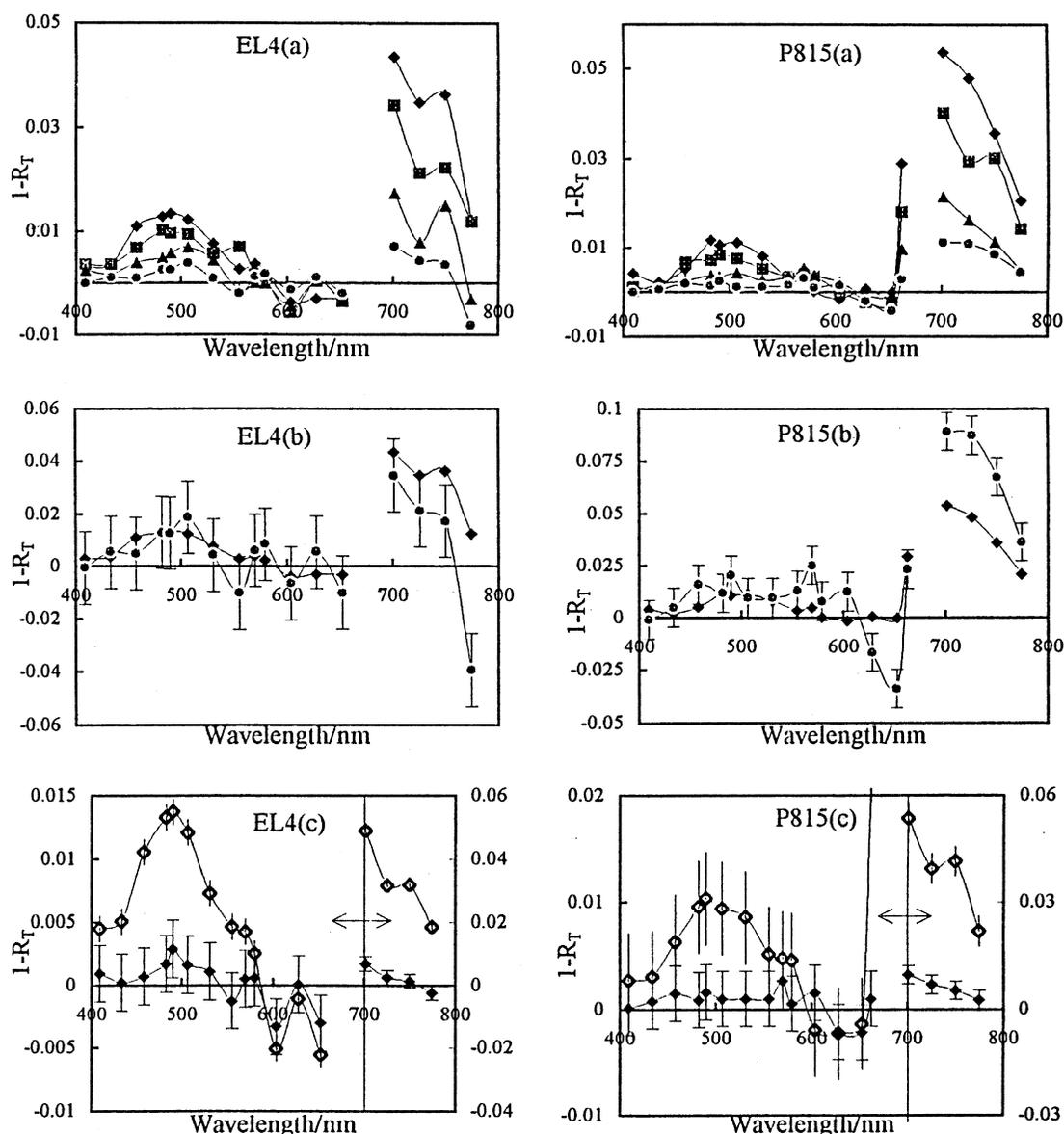

Fig. 1. Transient absorption spectra of EL4 and P815 murine cell lines sensitized with AlPcS$_2$. (a) Time-resolved spectra at 20 μs (diamonds), 100 μs (squares), 400 μs (triangles) and 850 μs (circles) after the laser pulse. (b) Comparison of 20 μs (diamonds) and 850 μs (circles) spectral shapes (normalized at 480 = 490 nm). (c) Fitted triplet amplitudes (open symbols) and offsets (filled symbols) obtained from global analysis of decays. Note the change of scale at 700 nm. Excitation at 673 nm, 2.8 mJ pulse^{-1}, 4×10^7 cells ml^{-1}, 40 μg ml^{-1} AlPcS$_2$. Samples purged with Ar. Points typically the average of 30 channels.

are due entirely to the sensitizer triplet state (Fig. 1(b)). A suggestion of a longer-lived component in the region of 575 nm in the P815 spectrum was attributed to noise, as it did not appear in the results of the global analysis. These are shown for an exponential with offset model described fully elsewhere [1–3] in Fig. 1(c) (see below). The residuals showed clearly that the decays were not first order, particularly for EL4. The results of earlier models developed for analysis, the single exponential plus offset and biexponential models [1–3], are summarized in Table 1 [1,2]. The former gave the best results, based upon residuals; however, neither model was as accurate in this system as the former model was in microbial suspensions [1–3]. A triplet–triplet annihilation model has not been tested, since second-order kinetics dependent upon the concentration of transient absorbers cannot be described analytically in DRLFP. Part of the complexity observed here may be attributable to the failure of these samples to meet the assumption of infinite optical thickness upon which the analysis relies. The effect of this has not been quantified, but would result in a non-exponential depth dependence of the transient concentration that may then affect the relation between the time dependence of the transient decay and the transient concentration. Quantitative results for these cell lines must be considered with this limitation in mind.

An inspection of Fig. 1(c) shows that for the EL4 cell line the spectrum of the offset has exactly the same shape as the triplet spectrum (if normalized, they overlap very well). This suggests that the entire decay is due to the decay of the $AlPcS_2$ triplet state, which experiences a range of environments within the cell and hence displays a distribution of lifetimes. The biexponential model was applied to explore the distribution further, but as Table 1 shows, it returned the same parameters (within error) as the single exponential with offset model, with the second lifetime approaching an offset. This was found to be a feature of the biexponential model when applied to noisy data and so does not imply that there are no shorter components. Analysing the blue and the red absorption bands separately for EL4 revealed that both displayed the same kinetics. The results for the P815 cell line

were less clear but are amenable to the same interpretation. An alternative explanation, which cannot be discarded at this stage, is that the longer component is an artefact of the sample preparation. The transmission of these suspensions was higher than any other studied, and it is possible that the departures from the theoretical assumptions underpinning the analysis have resulted in the longer components appearing as an artefact. A small amount of sensitizer leaking into the buffer could have accounted for the longer component.

However, there is a precedent to suggest that this result is not an artefact. Firey et al. observed a biexponential decay under aerated conditions for zinc phthalocyanine in mouse myeloma cells [4]. From a biological standpoint, such an observation is perhaps also not surprising. Eucaryotic cells are compartmentalized and the local environment is likely to vary from compartment to compartment. $AlPcS_2$ is known to localize in a variety of different organelles and is transported into the cells via an endocytotic mechanism in vacuoles [5]. It would not be surprising for $AlPcS_2$ to experience an inhomogeneous distribution of local environments, giving rise to a distribution of lifetimes. In particular, the triplet lifetime of $AlPcS_2$ is considerably extended by interaction with proteins, and this may also introduce biexponential quenching behaviour [6].

In a microbial cell such as *S. mutans,* on the other hand, there is no intracellular compartmentalization. The sensitizer may therefore be expected to experience a narrower distribution of environments, and hence a narrower distribution of lifetimes that can be adequately described by a single exponential. Single-exponential kinetics have been observed in eucaryotic cells, specifically for haematoporphyrin in aerated fibroblast cells [7] and in deoxygenated white blood cells [8], for a merocyanine derivative in nitrogen-purged suspension of L1210 leukaemia cells [9] and for benzoporphyrin derivative monacid A (BPDMA) in rat bladder cancer and mouse monocyte macrophage cell lines.

85–90% of triplets in EL4 or P815 cells decay with kinetics characterized by a lifetime of less than 300 μs in the absence of oxygen. This compares with a lifetime of 400 μs for $AlPcS_2$ in PBS, 300–400 μs in PBS with cells that partly quench

Table 1
Fit parameters of earlier analysis models applied to EL4 and P815 cell lines

Fit parameter		EL4	P815
Single exponential + offset model [1,2]	τ_2/μs	270 ± 40	299 ± 4
	R/A [a]	0.13 ± 0.18	0.13 ± 0.17
		$0.97, 1.0$	$0.97, 0.95$
Biexponential model [1,2]	τ_1/μs	241 ± 15	210 ± 130
	τ_2 [b]$/\mu$s	>2000	>2000
	A_2/A_1	0.18 ± 0.5	0.36 ± 0.63
	χ_g^2 [c]	$0.97, 1.0, 1.16, 0.95(5)$	$0.98, 0.95$

[a] R = Offset, A_i = amplitude of component i with lifetime τ_i.

[b] The fit range was 10–895 μs, so any $\tau_2 > 2000$ μs is not sufficiently defined to be evaluated.

[c] The number of values listed indicates the number of separate data sets analysed to generate the remaining data. All fits were to 9–10 separate decays unless indicated otherwise in brackets.

AlPcS$_2$ by Type I processes, and over 500 μs for AlPcS$_2$ in organic solvents and in *S. mutans* where no evidence of radical quenching was found. Previous reports show lifetimes in the absence of oxygen that are both shorter than those found in aqueous solutions [8] and comparable to those found in organic solvents [9,10].

The rate constant for quenching of the AlPcS$_2$ triplet state by oxygen was not measured, but the reduction in the triplet lifetime to less than that resolvable by the instrument when the suspension was purged with oxygen indicates that it is greater than 10^8 M^{-1} s^{-1}. This is consistent with two previous determinations in cell suspensions [4,7].

The decay kinetics of AlPcS$_2$ in these cell lines suggest a wide distribution of localized environments experienced by the sensitizer. This may be due to compartmentalization of the cell or binding of a fraction of the sensitizer to cell proteins. No evidence of Type I reactions was found in AlPcS$_2$-sensitized El4 and P815 murine cell lines. Redmond et al. and Aveline et al. have also failed to find spectroscopic evidence of radical formation in mammalian cells [9,10]. This suggests that ^{1}O$_2$ may be the dominant cytotoxic species in this cell system. Baker and Kanofsky have shown that L1210 leukaemia cells quench 99% of the ^{1}O$_2$ generated in their membranes, and that the membrane lifetime of ^{1}O$_2$ may be an order of magnitude shorter in these cells than it was estimated to be in *Porphyromonas gingavalis* [11,12]. Dahl [13] has shown that a range of mammalian cells (murine hepatocytes, FaDu squamous carcinoma cells, rat basophilic leukaemia and Chinese hamster V79 fibroblasts) can be killed by extracellularly generated ^{1}O$_2$ using an experimental system that does not permit type I processes. He estimated that 10^{12}–10^{13} molecules of ^{1}O$_2$ were required to inactivate a cell, relatively independently of cell type [13]. While this is suggestive of a relatively standard mode of fatal ^{1}O$_2$ damage in mammalian cells, it also suggests that even very low yields of Type I products might compete with ^{1}O$_2$. Further investigations in systems of this type are warranted. The failure to observe Type I processes again highlights the role that cell-wall components must play in microbes.

Acknowledgements

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