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Photophysical properties of 3,3′-dialkylthiacarbocyanine dyes in organized media: unilamellar liposomes and thin polymer films

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All symmetrical dialkylthiacarbocyanine dyes, with the exception of the diethyl derivatives, are incorporated into liposomes. Absorption and fluorescence data indicate a solubilization site close to the bilayer surface with the alkyl chains penetrating into the lipid bilayer. Incorporation into organized assemblies affects the photophysical parameters of these dyes. Photoisomerization occurring from the first excited state becomes more difficult as the restrictive effect of the solubilization site increases. As a consequence, competing deactivation processes, such as fluorescence and triplet formation, become more efficient with the result that fluorescence quantum yields, triplet yields and singlet oxygen quantum yields are larger in liposomes than in homogeneous solution. Dihexylthiacarbocyanine iodide has a fluorescence quantum yield of 0.27 and 0.10 (25°C) in dimyristoylphosphatidylcholine liposomes and ethanol, respectively, and the singlet oxygen yield increases by a factor three to 0.006 on going from ethanol to liposomes. The effect of a highly organized environment is even more pronounced in thin polymer films. In these systems, photoisomerization is completely inhibited and only triplet formation is observed in the transient absorption spectrum.

Introduction

Photodynamic therapy is a promising treatment involving the use of exogenous chromophores and visible light to effect the selective destruction of neoplastic tissue. During the past several 15 years, several different types and classes of photosensitizers have been the subject of intensive study. Due to extensive work performed with merocyanine 540 (MC540) [1–8], cyanine dyes have attracted interest as a new class of photosensitizing dyes. MC540 and some of its analogues exhibit a high and selective affinity for leukemia and lymphoma cells and have been found to be useful for the light-induced inactivation of such neoplastic cells [1–4,9]. MC540 has now reached the stage of phase-I clinical trials for the extracorporeal purging of autologous bone marrow grafts [10,11].

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Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPBF, 1,3-diphenylisobenzofuran; MC540, merocyanine 540; PMMA, poly(methyl methacrylate); $^1O_2$, singlet molecular oxygen ($^1\Delta_g$); Φ_f, fluorescence quantum yield; Φ_{isc}, quantum yield of intersystem crossing; Φ_Δ, singlet oxygen quantum yield.

Although several other cyanine dyes besides MC540 are currently being studied [12–17], little effort has been made to systematically investigate the relationship(s) in existence between chemical structure, photophysical parameters, photochemistry and photodynamic action. In this work, we present a photophysical investigation of 10 different symmetric 3,3′-dialkylthiacarbocyanine dyes in organized media. The basic structure of these dyes was selected mainly due to the ease of synthetic variation which permits a wide range of future modifications and optimizations. These thiacarbocyanines were prepared with *n*-alkyl chains of different length (C_2 to C_{18}) in order to modify their lipophilic properties. They were also synthesized as both chloride and iodide salts to examine the possibility of a heavy atom effect. Since primary photodamage is believed to occur in cellular membranes [18], the investigation was carried out in two types of model membrane systems having different restrictive properties, i.e., liposomes and thin polymer films. The aim of the study was to determine the effect of compartmentalization on the photophysical properties and to investigate the binding/association behavior of these dyes. The results of this work will contribute to our overall aim of optimizing the photosensitizing abilities of cyanine dyes by appropriate chemical modifications.

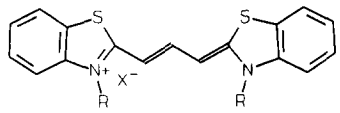
Materials and Methods

Chemicals

3,3′-Diethylthiacarbocyanine iodide C_2I was purchased from Molecular Probes (Eugene, OR, USA) and all other symmetric 3,3′-dialkylthiacarbocyanine dyes (see Fig. 1) were synthesized as previously described [19]. L-α-dimyristoylphosphatidylcholine (DMPC), K_2HPO_4 and KH_2PO_4 were from Sigma (St. Louis, MO, USA) and of 99% + purity. Medium molecular weight poly(methyl methacrylate) (PMMA) was obtained from Aldrich (Milwaukee, WI, USA). MC540 (Sigma), cresyl violet perchlorate (Exciton, Dayton, OH, USA) and rhodamine 101 (Kodak, Rochester, NY, USA) were used as supplied. Rose Bengal from Sigma was purified as described in the literature [20]. 1,3-Diphenylisobenzofuran (DPBF) supplied by Aldrich was used without further purification, since its molar absorption coefficient corresponded to the pure dye [21]. Actinochrome 475/610 was purchased from AMKO (Tornesch, Germany) and Sepharose CL-2B was obtained through Pharmacia (Piscataway, NJ, USA). Water was purified with a Nanopure II-4-module system equipped with an ultrafiltration manifold (Barnstead, Newton, MA, USA) and had a resistivity of > 17.3 MΩ/cm. Deuterium oxide from Sigma was of 99.9 atom% D. Ethanol (Aldrich or Sigma) was of spectroscopic grade, and chloroform was purchased through Sigma.

Liposomes

Unilamellar liposomes with a diameter of 60–80 nm were prepared as described by Kremer et al. [22]. A 0.03 M potassium phosphate buffer (pH 7.0 at 20°C) was used in order to keep pH variations with temperature negligible small. Typically, 0.225 ml of an ethanolic solution, which was 0.0211 M in DMPC and 0.002–1.2 mM in dye, was injected with a speed of 0.042 ml/min into 3 ml of the aqueous buffer. The phosphate buffer was magnetically stirred and kept at 35°C. The incorporation of dyes into liposomes was checked by gel filtration on Sepharose CL-2B (eluent, 0.03 M potassium phosphate, 0.2 M KCl (pH 7.0); column, 1.5 × 36 cm; sample size, 1 ml). The absorbance of gel-filtration fractions (220 nm for liposomes, 560 nm for dye) was determined using a Perkin-Elmer λ4C spectrophotometer (Perkin-Elmer, Norwalk, CT, USA).



I: X=I C$_2$: R=C_2H_5 C$_{12}$: R=$C_{12}H_{25}$

Cl: X=Cl C$_6$: R=C_6H_{13} C$_{18}$: R=$C_{18}H_{37}$

C$_8$: R=C_8H_{17}

Fig. 1. 3,3′-Dialkylthiacarbocyanines: chemical structure and nomenclature.

Polymer films

Preparations of the dyes supported on polymer films were achieved by addition of the desired amount of solid dye to a solution of PMMA in chloroform (25% (w/w)). The viscous solution was added to one end of a 3 × 10 cm quartz plate and drawn out to form a film using a wet film, wire-wound Meier Rod (Paul N. Gardner, Pompano Beach, FL, USA). Solvent was then removed by heating the plates in an oven at 90°C for 30 min. Dry film thickness was approx. 100 μm.

Absorption spectra

Ground-state absorption spectra were measured on a Perkin-Elmer λ4C spectrophotometer equipped to handle turbid samples (Perkin-Elmer), or a Hewlett-Packard 8451A UV-visible diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA).

Fluorescence

Corrected steady-state emission and excitation spectra were obtained using a 4800C SLM spectrofluorimeter (SLM Instruments, Urbana, IL, USA). Samples were temperature controlled within ±1°C. Fluorescence quantum yields (Φ_f) were determined at λ_{exc} = 520 nm with MC540 as reference. Beforehand, Φ_f for MC540 was determined relative to cresyl violet perchlorate ($\lambda_{exc} = 560$ nm, $\Phi_f = 0.54 \pm 0.3$ in methanol [23]) and to rhodamine 101 ($\lambda_{exc} = 520$ nm, $\Phi_f = 1.0 \pm 0.2$ in ethanol [24]), giving a $\Phi_f = 0.14 \pm 0.01$ at 25 ± 1°C for MC540 in methanol. Typically, reference and sample were matched to have absorbances between 0.01 and 0.04. Fluorescence quantum yields were calculated using the following relationship:

$$\Phi_u = \frac{(1-10^{-A_s})F_u n_u^2}{(1-10^{-A_u})F_s n_s^2}\Phi_s \quad (1)$$

where the u subscript refers to the unknown and s to the standard, A represents the absorbance at the excitation wavelength, F the integrated emission area across the band and n the index of refraction at the sodium D line at the temperature of the specific measurement. The refractive index for DMPC liposomes (1 mg lipid/ml of liposome solution) was measured with a temperature-controlled refractometer (Bausch and Lomb, Rochester, NY, USA) giving a value n_D of 1.372 at 20°C. In order to correct for possible scattered light, the fluorescence of liposomes without dye was subtracted from the fluorescence of liposomes with dye.

Laser flash photolysis

The excitation source for the laser flash photolysis was a Quantel YG660 Nd/YAG laser (Continuum,

Santa Clara, CA, USA) emitting 8-ns pulses at 355 or 532 nm. The laser energies were attenuated to < 10 mJ/pulse by neutral density filters. The analyzing light was from a 75 W xenon arc lamp (Photon Technology International, South Brunswick, NJ, USA) aligned to overlap the laser beam at 90° within a 10 × 10 mm path length quartz cuvette containing the sample. The analyzing light transmitted through the sample was focused into the input slit of a 1681 monochromator (Spex Industries, Edison, NJ, USA) for wavelength selection before impinging on a R928 photomultiplier (Hamamatsu, Bridgewater, NJ, USA), powered from a 415B high-voltage power supply (Fluke, Everett, WA, USA). Cut-off filters were placed in the analyzing beam path before and after the sample to reduce exposure and emission from the sample reaching the photomultiplier. The signal from the photomultiplier then passed through a back-off unit (Kinetic Instruments, Austin, TX, USA) in order to measure the pre-pulse signal level (I_0) and then on to a 9400 digital oscilloscope (LeCroy, Chestnut Ridge, NY, USA). Data acquisition was controlled by a Macintosh II computer using programs written using the LabView 2 software package in conjunction with NB-GPIB and LAB-NB boards (National Instruments, Austin, TX, USA). Signal averaging was routinely performed to increase the signal-to-noise ratio. Uniblitz fast shutters (Vicent Associates, Rochester, NY, USA) were placed in the path of both laser and lamp beams in order to minimize sample exposure to both light sources. The shutters were controlled programmatically allowing background corrections, i.e., for fluorescence or scattered light, to be made when necessary. The synchronization of events in the experiment was achieved through a combination of sequence generator and laser controller units (Kinetic Instruments, TX, USA) and delay generator units (Berkeley Nucleonics, Richmond, CA, USA) which controlled the triggering of the laser, shutters, back-off, energy meter and digitizer. A beam splitter was placed in the path of the laser beam and a fraction of the beam directed to a calibrated RJP-735 pyroelectric probe connected to an RJ-7610 energy meter (Laser Precision, Utica, NY, USA) in order to measure laser energies incident on the sample. Experimental control was carried out also using LabView programs and GPIB interfaces on digitizer, energy meter, and Kinetic Instruments devices in addition to RS232 interface of a CD2A monochromator driver (Spex Industries, Edison, NJ, USA) which controls the wavelength selection of the monochromator. Kinetic analysis was carried out using Levenbeg-Marquardt non-linear fitting programs on a Macintosh IIci computer.

Time-dependent transient absorption spectra were recorded using a point-by-point approach where an average of approx. 5 shots were taken at successive wavelength increments across a chosen range and the resulting spectrum constructed by extraction of absorption values at chosen time windows from the individual kinetic traces recorded at each wavelength.

Singlet oxygen quantum yields

Singlet oxygen quantum yields (Φ_Δ) were measured directly by the infrared luminescence method and by using DPBF as a chemical singlet oxygen ($^1O_2$) scavenger. In both methods, rose bengal was used as a reference ($\Phi_\Delta = 0.75$ in liposomes [25,26]). For the direct detection of $^1O_2$ by the time-resolved infrared luminescence technique, a germanium-diode-based system was used, as previously described [27]. Singlet oxygen quantum yields in liposomes were estimated by comparison of the slopes of the energy-dependence plots for the luminescence for both dye and reference compound. Samples were prepared with deuterated buffer and were bubbled with oxygen prior to irradiation. With DPBF, steady-state irradiation experiments were typically performed with 2 ml of air saturated solution. Samples were placed in a 1-cm path length quartz cuvette and irradiated through a GG475 cut-off filter, a 560 ± 10 nm interference filter (both filters from Ealing Electro-Optics, South Natick, MA, USA) and various calibrated neutral density filters (Oriel, Stratford, CT, USA) with a 150 W xenon arc lamp (Photon Technology, Princeton, NJ, USA). Actinometry was performed with Actinochrome 475/610 as described [28].

Results

Absorption and fluorescence

Thiacarbocyanine dyes with *n*-alkyl chains of $n \geq$ hexyl (C_6I–$C_{18}I$, and C_6Cl–$C_{18}Cl$, see Fig. 1) are incorporated into DMPC liposomes. The absorption maxima in DMPC liposomes are 562–563 nm and have molar absorption coefficients of $(1.3 \pm 0.1) \cdot 10^5$ $M^{-1}cm^{-1}$. The formation of dimers or aggregates, common for cyanine dyes and generally reported to appear as an enhanced shoulder blue-shifted to the monomeric peak [29,30], was not observed even after reducing the lipid/dye ratio to 160:1. Since these cyanines were found to exhibit a hypsochromic solvatochromism [19], the observed absorption maxima suggest a solubilization site with a micropolarity between that of ethanol and n-butanol. Fig. 2 shows a representative absorption spectrum in DMPC liposomes. Gel-filtration experiments on Sepharose CL-2B confirm the results obtained by absorption spectroscopy: these cyanine dyes were always eluted in fractions together with liposomes, and unbound dye which would be found in later fractions was never present. In contrast, both C_2I and C_2Cl resist incorporation into the liposomal bilayer, as evidenced by absorption spectroscopy and gel filtration. The absorption maxima of both dyes undergo

a blue shift to 557 nm, indicating a much more polar solubilization site than the longer chain analogues. This is in agreement with results obtained from gel filtration: these dyes were not eluted together with liposomes, but appeared in much later fractions which correspond to unbound dye.

Corrected fluorescence emission spectra in DMPC liposomes were recorded above and below the phase transition temperature of 23°C [31] (Fig. 2). Emission spectra at 5°C and 35°C are identical after normalization, suggesting that dimerization is not promoted in the gel-like phase as has previously been observed for MC540 [30]. In addition, varying lipid/dye ratios from 13000:1 to 160:1 has no influence on Φ_f values. Fluorescence quantum yields, however, are strongly temperature dependent and are also somewhat influenced by the length of the *n*-alkyl chain: Φ_f values decrease with increasing temperature, and slightly increase as the *n*-alkyl chain becomes longer (Fig. 3A) up to a maximum *n* at $C_{12}I$ and $C_{12}Cl$. On the other hand, the size of the counter ion has no influence on the fluorescence of these dyes (Table I), which is in agreement with previous findings in solution [19]. Generally, fluorescence quantum yields in DMPC liposomes follow the same trend as in homogeneous media. However, most likely due to the more restrictive and rigid environment of the bilayer, Φ_f values are larger, e.g., Φ_f values increase approx. 3-fold on going from ethanol to DMPC liposomes (Table I). In an additional experiment, the temperature dependence of the fluorescence for dyes C_6I and $C_{12}I$ in DMPC liposomes was investigated in greater detail. As Fig. 3B shows, a biphasic behavior is observed in liposomes which is absent in homogeneous solution. The change in biphasic behavior occurs at 23°C, the characteristic phase-transition temperature of DMPC liposomes.

Fluorescence quantum yields in Table I and Fig. 3A also demonstrate the anomalous behavior of C_2I and C_2Cl in liposomes. These dyes have much smaller yields than their analogues with longer alkyl chains, and their Φ_f values have values close to yields measured in ethanol. These fluorescence results are in agreement with data from absorption spectroscopy and gel filtration which suggest that both diethyl derivatives

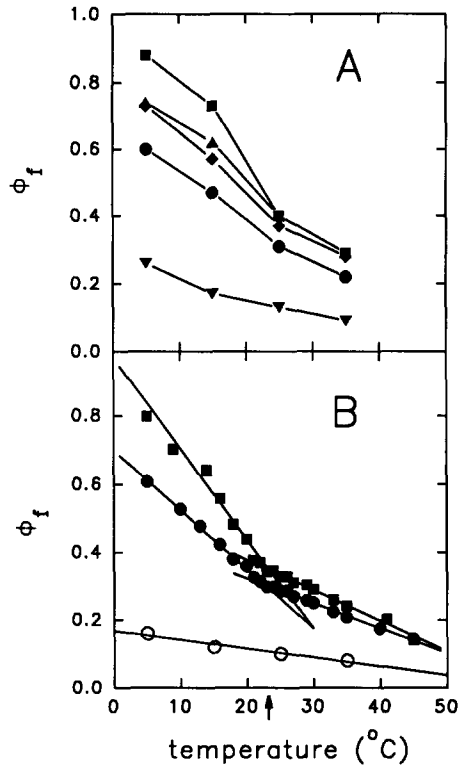


Fig. 3. (A) Temperature dependence of fluorescence quantum yields (Φf) in DMPC liposomes. (▼) C_2I; (●) C_6I; (♦) C_8I; (■) $C_{12}I$; (▲) $C_{18}I$. (B) Detailed temperature dependence of Φ_f for selected dyes in DMPC liposomes and in ethanol. (●) C_6I in liposomes; (■) $C_{12}I$ in liposomes; (○) C_6I in ethanol. Arrow represents the phase-transition temperature of DMPC liposomes (23°C).

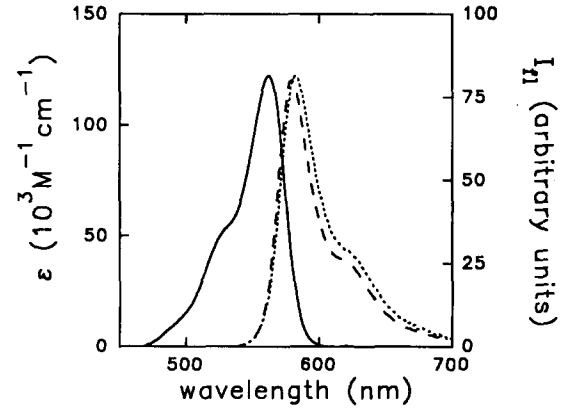


Fig. 2. Absorption and normalized corrected emission spectra of $C_{18}I$ in DMPC liposomes. (———) absorption; (— · —) fluorescence emission at 5°C; (· · · · · ·) fluorescence emission at 35°C.

TABLE I

Fluorescence quantum yields at 25°C in DMPC liposomes and in ethanol

Fluorescence quantum yields (Φ_f) were determined with MC540 as reference and at λ_{exc} = 520 nm. The temperature was kept constant at 25±1°C. In DMPC liposomes, Φ_f values were independent of lipid/dye ratios between 13000:1 and 160:1. In order to correct for possible scattered light by liposomes, the fluorescence of liposomes without dye was subtracted from the fluorescence of liposomes containing dye. Values are means±S.D.

Dye	DMPC liposomes	Ethanol
C_2I	0.13±0.02	0.12±0.01
C_6I	0.27±0.04	0.10±0.01
$C_{18}I$	0.37±0.05	0.15±0.02
C_2Cl	0.14±0.02	0.07±0.01
C_6Cl	0.28±0.04	0.10±0.01
$C_{18}Cl$	0.37±0.05	0.12±0.01

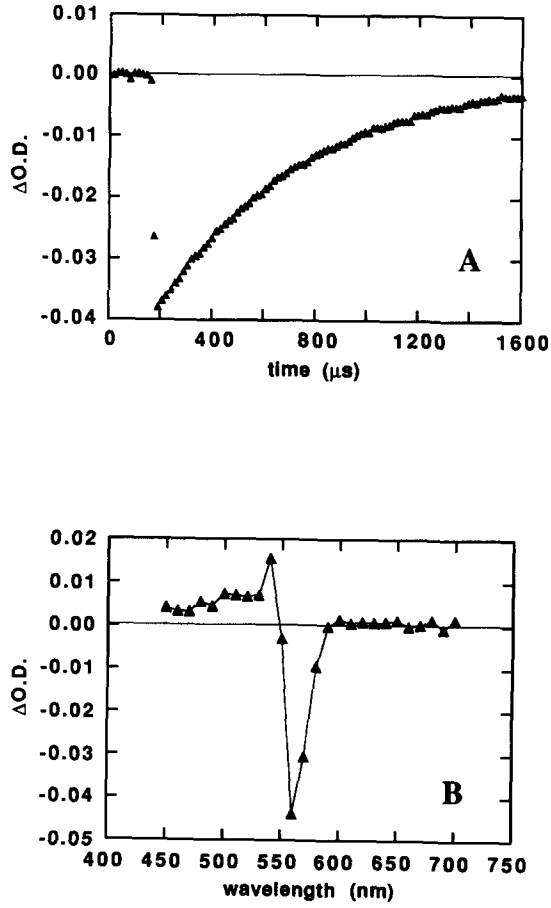


Fig. 4. (A) Decay of photoisomer observed at 560 nm on 532 nm irradiation of $C_{18}I$ in DMPC liposomes. (B) Transient difference absorption spectrum corresponding to the photoisomer of $C_{18}I$ in DMPC liposomes.

are neither incorporated nor associated with DMPC liposomes.

Laser flash photolysis

Laser flash photolysis experiments allow the observation of the time-resolved changes in absorption which occur following absorption of light from the laser pulse. On 532 nm excitation of all dyes in DMPC liposomes, a two-component kinetic decay was observed similar to that observed in homogeneous solution [19]. The transient phenomena were very similar for all dyes irrespective of the length of the alkyl chain or the counter ion. A weak absorption due to a species decaying with a lifetime of 55 μs in a nitrogen-saturated solution was ascribed to the triplet state through oxygen quenching experiments and its spectral similarity ($\lambda_{max} = 620$ nm) to the triplet state in ethanol [19]. A much longer-lived species with a lifetime of 550 μs was also observed with its decay being unaffected by the presence of oxygen (Fig. 4A). This species is the photoisomer of the dye. There is negligible difference between the absorption spectra of both species in liposomes (Fig. 4B) compared to homogeneous solution. Similarly, triplet state lifetimes are comparable but a major difference in the lifetime of the photoisomer is observed. The lifetime of > 20 ms in ethanol is reduced to 550 μs in liposome solutions (Fig. 4A), which is most likely due to the absence of solvent reorganization in organized assemblies.

Comparison of the intensity of triplet absorption produced on excitation of optically matched solutions of dye in ethanol and liposome solutions shows a 2-fold increase in triplet absorption in the latter. This observation is in agreement with the photoisomerization process being hindered by the more viscous nature of the lipid bilayer compared to ethanol. Using the previously measured value of 0.004 for the quantum yield of intersystem crossing (Φ_{isc}) of C_2I in ethanol [19], a Φ_{isc} of approx. 0.008 in DMPC liposomes is determined.

Polymer films

In order to further investigate the effect of a more rigid environment, $C_{18}I$ was incorporated in a thin PMMA film such that its absorbance was comparable to those used in solution studies. Given the shorter pathlength of approx. 100 μm, the concentration of dye is 2–3 orders of magnitude higher in the film than in solution. However, using the 532 nm laser emission to excite into the monomer band of the dye allows comparison of the results to solution studies. A non-exponential absorption decay is observed at all wavelengths (Fig. 5A). However, the transient absorption spectrum is invariant with time. Thus, we observe only one transient species with the spectrum shown in Fig. 5B. This spectrum with $\lambda_{max} > 600$ nm matches that of the triplet state. The rigid medium of the polymer film shuts down the isomerization pathway with the result that the triplet state is the only observable intermediate. Non-exponential triplet decays are not uncommon in films [32] as is the relative longevity of the triplet state in films under air atmosphere due to slow diffusion of oxygen within the film compared to the liquid solution.

Singlet oxygen formation

Our time-resolved luminescence apparatus proved not to be sensitive enough for quantitative measurements of $^1O_2$ production in liposome solution (deuterated buffer solution, limit: $\Phi_\Delta > 0.05$) and therefore $^1O_2$ formation was monitored indirectly by using DPBF as a chemical scavenger. Singlet oxygen quantum yields in DMPC liposomes are $(6.3 \pm 1.2) \cdot 10^{-3}$ for all cyanine dyes and they are not affected by varying the lipid/dye ratio from 1500:1 to 100:1. Since C_2I and C_2Cl do not incorporate into liposomes, Φ_Δ determinations were omitted. Singlet oxygen yields

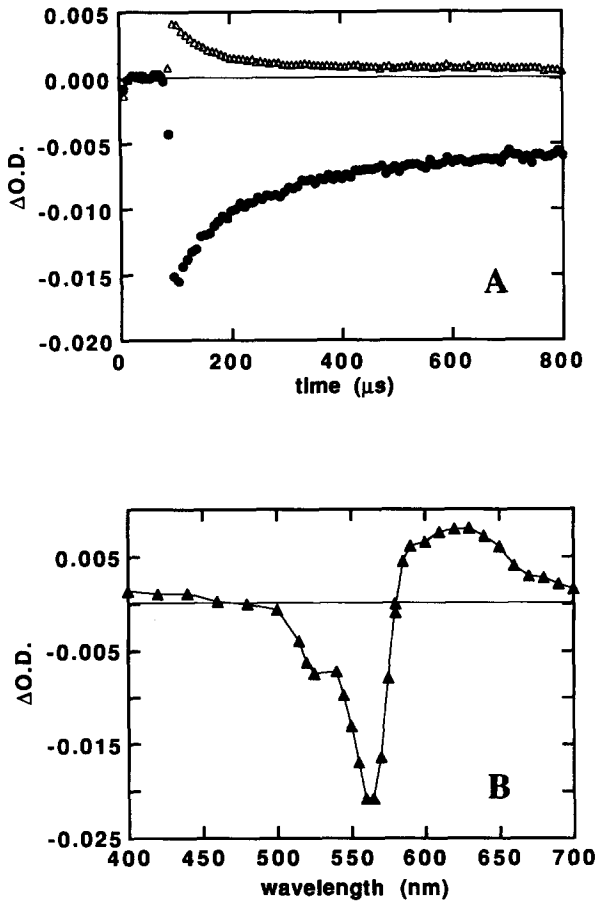


Fig. 5. (A) Transient absorption decays observed on 532 nm excitation of $C_{18}I$ in thin polymer films of PMMA detected at (△) 620 nm and (●) 570 nm. (B) Transient difference absorption spectrum of $C_{18}I$ in thin polymer films of PMMA.

were also independent of the length of the *n*-alkyl chains and the size of the counter ion. These Φ_Δ values are in good agreement with a Φ_{isc} value of $8 \cdot 10^{-3}$ determined by flash photolysis.

Discussion

The incorporation of symmetric *n*-dialkylthiacarbocyanine dyes into liposomes is dependent on the length of the alkyl chain. Absorption, fluorescence and gel-filtration data all indicate that the two diethyl derivatives, C_2I and C_2Cl, are neither solubilized into the lipid bilayer nor associated with liposomes, whereas all other dyes under investigation are incorporated and interact with the hydrocarbon interior of the liposomes. Absorption and fluorescence data further suggest that the positively-charged chromophoric system of incorporated dyes is located in proximity of the bilayer surface with both *n*-alkyl chains reaching into the hydrophobic section of the liposome membrane. This is also supported by the observation that Φ_f values slightly increase with increasing chain length until maximum values at the C_{12} derivatives are reached. Lower Φ_f values for the C_{18} derivatives are probably due to the fact that DMPC has a shorter alkyl-chain length, i.e., C_{14}, than these cyanine dyes, allowing some coiling and therefore more freedom of motion.

The effect of dye incorporation into the liposomal bilayer is also observed in the temperature dependence of Φ_f shown in detail in Fig. 3B. This temperature dependence exhibits the typical biphasic behavior of liposomes with a break at 23°C where DMPC is well-known to undergo phase transition. Below the phase transition temperature, the slope of the temperature plot is about 6-times larger than in ethanol. Above phase transition, the effect is reduced, but the slope is still 2-times larger than in homogeneous solution. These results can be rationalized in terms of an activated process (i.e., isomerization from the first excited singlet state): by going from ethanol to the liquid-like state and then to gel-like state of the liposomes, the viscosity/rigidity of the environment increases making the isomerization process more difficult and hence rendering competing deactivation processes of the excited singlet state, i.e., fluorescence and intersystem crossing, more efficient. Indeed, after solubilization into liposomes, photoisomerization decreases and triplet as well as $^1O_2$ production increase: photoisomerization is reduced in DMPC liposomes compared to ethanol, as seen from transient spectra, and Φ_{isc} and Φ_Δ increase 2–3-fold to 0.008 and 0.006, respectively, after incorporation into liposomes. In thin polymer films where the incorporated dyes experience an even higher degree of organization from the environment, the effect of the restricted medium is further pronounced: photoisomerization is now completely inhibited and only triplet formation can be observed in the transient absorption spectrum (Fig. 5B).

The effect of incorporation into liposomes can be quantified by estimating rate constants for the deactivation processes of the first excited singlet state. The radiative rate constants can be calculated from absorption and steady-state fluorescence data according to the Strickler-Berg relationship (Eqn. 2) [33]:

$$\frac{1}{\tau_f} = k_{fo}\Phi_f^{-1} = 2.880 \cdot 10^{-9} n^2 \nu_f^3 \Phi_f^{-1} \int \epsilon \, d \ln \nu \qquad (2)$$

where τ_f represents the fluorescence lifetime, k_{fo} the radiative rate constant, n the refractive index of the medium ($n_D = 1.372$ at 20°C for DMPC liposomes with 1 mg lipid/ml of liposome solution, see Materials and Methods), ν_f the mean fluorescence wavenumber, ϵ the molar absorption coefficient and ν the wavenumber of the absorption band. These calculations resulted in a radiative rate constant in DMPC liposomes of

$(3.1 \pm 0.2) \cdot 10^8$ s^{-1} for all dyes with an alkyl chain $\geq C_6$. By using this radiative rate constant and experimentally obtained Φ_f values and Φ_{isc} values, a rate constant for intersystem crossing of $8 \cdot 10^6$ s^{-1} and a combined rate constant for photoisomerization and internal conversion of $6 \cdot 10^8$ s^{-1} are obtained. In comparison to results in ethanol [19], the rate of photoisomerization and internal conversion in liposomes decreases by a factor of 3, while the radiative rate constant and the rate constant for intersystem crossing remain unchanged.

The observation that the photophysics in liposomes and thin polymer films is not affected by the size of the counter ion excludes the presence of a significant internal heavy atom effect which is in agreement with data previously obtained in homogeneous solution. In addition, the finding that the length of the alkyl chains has only a small effect on the photophysical parameters coincides with the work in solution. However, absorption and fluorescence data reveal that the presence of two *n*-alkyl chains of the same length hinders the ability of the chromophoric system to solubilize at different locations inside the bilayer. It becomes evident that asymmetrically substituted cyanine dyes should be prepared in order to increase the range of association/incorporation capabilities. On the other hand, solubilization into the lipid bilayer affects the photophysical parameters and provides information for further structural optimization. Incorporation into liposomes results in an increase in $^1O_2$ production and Φ_Δ values similar to MC540 in this medium are obtained. Nevertheless, these cyanines remain very modest producers of $^1O_2$, with a maximum possible Φ_Δ of approx. 3% (assuming complete inhibition of photoisomerization and internal conversion). This demonstrates that incorporation into membranes is not sufficient, but rather, further chemical modifications are necessary to raise Φ_Δ values. The results from this study together with data from a previous work in solution provide important information for the optimization of these cyanine dyes. At present, an investigation of such chemically modified dyes is in progress and results are encouraging.

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