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Photophysical properties of thiazine dyes in aqueous solution and in micelles

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Abstract

Depending on the pH of the solution, the excitation of azure A, azure B and azure C leads to the formation of unprotonated (λ_{max} = 420 nm) and protonated (λ_{max} = 370 nm) triplet states and radical cations (λ_{max} = 520 nm). The triplet states are quenched by oxygen with a diffusion-controlled rate constant of $\sim 2\times10^9$ 1 mol^{-1} s^{-1} and with the formation of singlet oxygen. The acid-base equilibrium in the triplet state ($pK_a = 7.2 \pm 0.1$ for azure B) is different from that in the ground state ($pK_a = 0.0 \pm 0.2$). The lifetimes of the triplet states are prolonged in the micellar medium of sodium dodecyl sulfate. The critical micellar concentration of the tenside and its dependence on the ionic strength of the solution can be found from measurement of the concentration of the triplet states of the dye in dependence on the surfactant concentration. The results obtained for sodium dodecyl sulfate are comparable with the previous methods.

Keywords: Thiazine dyes; Photosensitizers; Sodium dodecylsulfate; Micelles; UV/VIS spectroscopy

1. Introduction

Because of its photophysical properties, methylene blue (MB^+, Fig. 1a) is frequently used as a photosensitizer in light-induced reactions. These studies are important particularly from the standpoint of the possibility of their utilization in photodynamic therapy (PDT) [1], photodegradation of environmental contaminants [2–4], molecular recognition and photosensitized modification of DNA [5]. It has recently been found that the thiazine dyes designated as azures (Fig. 1b–d) are promising photosensitizers for PDT [6], and are more stable in aqueous solutions as they do not undergo demethylation [7], in contrast to MB^+.

The course of the photosensitized reactions can be significantly affected when they occur in organized systems, i.e. micro-emulsions, vessicles, thin films and micelles [8,9]. These systems can simulate the environment in biological systems and are thus frequently used as relatively simple models [10]. Surfactants affect the properties of a number of organic compounds, especially the solubility [11], acid-base [12,13] and complexing [14] equilibria, electron-transfer reactions [15] and reaction rates [13,16]. At concentrations higher than the critical micelle concentration (cmc) they form a micellar pseudo-phase, in which the water-insoluble reactants

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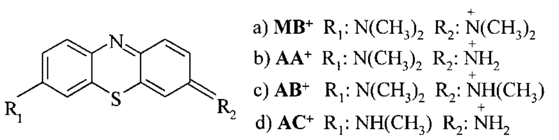

Fig. 1. Structure of thiazine dyes: (a) methylene blue, (b) azure A, (c) azure B and (d) azure C.

and sensitizers can be solubilized as a consequence of hydrophobic interactions The choice of a suitable micellar system is very important, as the electrostatic interactions between the studied substances and the surface charge of the micelles can have a favourable or unfavourable effect on the properties of interest.

Thiazine dyes are suitable for the study of photosensitized reactions in micellar media, because of their low ionization potential caused by the presence of two hetero-atoms. The effect of surfactants on the absorption spectra and equilibrium constants of azure B in the ground singlet state has been described in our previous publications [17,18]. This present investigation is concerned with measurement of the photophysical properties of thiazine dyes (especially azure B) in aqueous solution and in micelles.

2. Experimental

The UV/VIS spectra were recorded on Pye Unicam-Philips (UK) SP8-100 and Perkin–Elmer Lambda 10 spectrophotometers. The time-resolved absorption spectra were measured on the laser kinetic spectrometer (Applied Photophysics, UK) described elsewhere [19,20]. Transient species were generated by a Lambda Physik LPX 205 XeCl laser (excitation wavelength 308 nm, pulse length 20 ns, energy 10–20 mJ/pulse). Changes in the solution absorbance with time at the selected wavelength were detected using an R928 photomultiplier (Hamamatsu). A 250 W xenon lamp (Optical Radiation Corporation) was used as a source of light for absorption spectroscopy and for continuous irradiation of some samples. Singlet oxygen was monitored by measuring the characteristic phosphorescence at 1270 nm. This radiation was separated from the fluorescence by a 1270 nm band-pass filter (Laser Components, Olching, FRG) and detected with a Ge diode (Judson J16-8SP-R05M-HS). The signal was amplified using a laboratory-constructed amplifier, averaged 64 times and recorded on an oscilloscope. The experiments were carried out in D_2O to prolong the lifetime of singlet oxygen [21].

Methylene blue (MB^+, CI 52015, Fig. 1a, Lachema, Czech Republic), azure A (AA^+, CI 52005, Fig. 1b, Aldrich), azure B (AB^+, CI 52010, Fig. 1c, Loba-Chemie, Austria) azure C (AC^+, CI 52002, Fig. 1c, Aldrich) and sodium dodecyl sulfate (SDS, Serva Heidelberg, FRG) were used without further purification. The pH was adjusted in some cases by addition of HCl and NaOH and the ionic strength (I) was adjusted by addition of $NaNO_3$. The pK_a value was measured in 0.07 mol l^{-1} phosphate buffer. The experiments were carried out in 1×1 cm quartz cuvettes. The solutions were saturated with air. In some cases oxygen was removed by purging with argon for 30 min. The initial dye concentration was 1×10^{-4} mol l^{-1}.

3. Results and discussion

3.1. Aqueous medium

Depending on pH, methylene blue can be present in aqueous solution in two forms: MB^+ ($\lambda_{max} = 664$ nm) and MBH^{2+} ($\lambda_{max} = 760$ nm), protonated on the nitrogen in the heterocycle (pK_a = 0) [22]. Similar acid-base equilibria (pK_a = 0.0 ± 0.2, Fig. 2a) were found for AA^+ ($\lambda_{max} = 630$ nm) and AAH^{2+} ($\lambda_{max} = 710$ nm), AB^+ ($\lambda_{max} = 644$ nm) and ABH^{2+} ($\lambda_{max} = 730$ nm), and AC^+ ($\lambda_{max} = 620$ nm) and ACH^{2+} ($\lambda_{max} = 695$ nm). In addition, a proton is dissociated

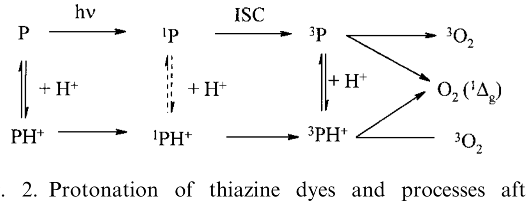

Fig. 2. Protonation of thiazine dyes and processes after absorption of a photon.

from the amino or methylamino groups of the azures in alkaline media (pH > 11), with the formation of the deprotonated AA ($\lambda_{max} = 535$ nm), AB ($\lambda_{max} = 530$ nm) and AC ($\lambda_{max} = 630$ nm) forms. A weak maximum is present in the 550–600 nm region of the absorption curve for all the studied unprotonated forms, corresponding to dye aggregates (dimers).

The processes that occur during the interaction of radiation with MB^+ and MBH^{2+} have been described [2–4] and are depicted in Fig. 2 ($P = MB^+$). In addition to the formation of ${}^3MB^+$ and ${}^3MBH^{2+}$, minor products include some radicals ($MB^{2+\bullet}$ etc.). Our results indicate that, during the excitation of azure A, azure B and azure C ($P = AA^+$, AB^+, AC^+), which contain the same phenothiazine chromophore, in all cases similar protonated (${}^3PH^+$, $\lambda_{max} = 370$ nm, Fig. 3a) and unprotonated (${}^3P$, $\lambda_{max} = 420$ nm, Fig. 3a) triplet states are formed, along with the radical cations $AA^{2+\bullet}$ and $AB^{2+\bullet}$ ($\lambda_{max} = 520$ nm, Fig. 3a). The formation of $AC^{2+\bullet}$ was not observed. ${}^3AA^+$, ${}^3AB^+$ and ${}^3AC^+$ are quenched by oxygen with the formation of O_2 (${}^1\Delta_g$) with a diffusion-controlled rate constant $k_{O2} \sim 2 \times 10^9$ l mol^{-1} s^{-1}. Substituents bonded directly to the chromophore do not have a marked effect on the position of the absorption bands or of the acid-base equilibrium of the triplet states, but affect other photophysical parameters and the quantum yield of the singlet oxygen (Table 1). At pH > 1 in the ground state, azure B is present in the unprotonated form AB^+ [18]. Following excitation, the excited singlet and triplet states are protonated (Fig. 4a). In the excited singlet state, equilibrium is apparently not completely established between AB^+ and ABH^{2+}, because of their short lifetimes (< 10 ns). Equilibrium is established between the protonated and unprotonated triplet states in air-saturated phosphate buffer at times of less than 1 μs after the excitation pulse (Fig. 4).

Acid-base equilibrium between the protonated and unprotonated triplet states of azure B can be described by Eq. (1):

$$ {}^3ABH^{2+} \overset{pK_{a1}}{\rightleftharpoons} {}^3AB^+ \overset{pK_{a2}}{\rightleftharpoons} {}^3AB \qquad (1)$$

The values of ΔpK_{a1} (difference in pK_{a1} between the triplet and ground states) can be calculated using Eq. (2) [24],

$$\Delta pK_{a1} = 0.00209(\tilde{\nu}_{AB^+} - \tilde{\nu}_{ABH^{2+}}) \qquad (2)$$

where $\tilde{\nu}_{AB^+}$ and $\tilde{\nu}_{ABH^{2+}}$ are the corresponding wavenumbers of ${}^3AB^+$ and ${}^3ABH^{2+}$, respectively.

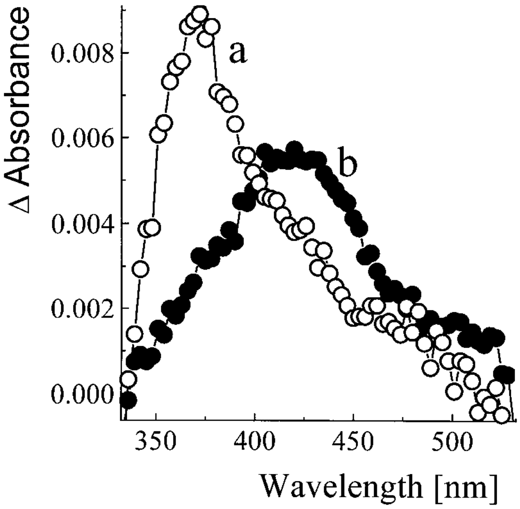

Fig. 3. Difference absorption spectra of azure B, 200 ns after excitation pulse in phosphate buffer, (a) pH 4.9 (${}^3ABH^{2+}$) and (b) pH 9.1 (${}^3AB^+$).

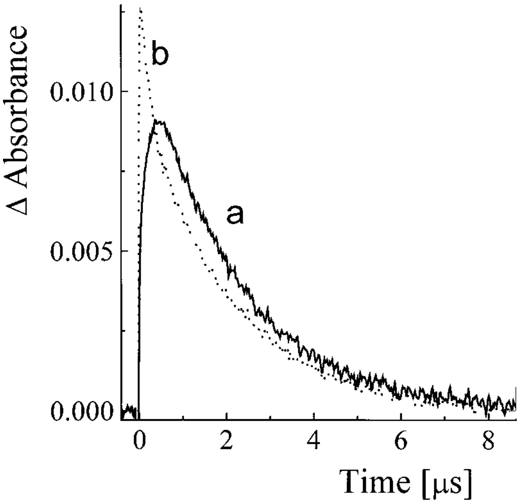

Fig 4. Transient kinetic traces of (a) ${}^3ABH^{2+}$ ($\lambda_{max} = 370$ nm) and (b) ${}^3AB^+$ ($\lambda_{max} = 420$ nm) after excitation of AB^+ in phosphate buffer, pH 7.

Table 1
Photophysical data of thiazine dyes in aqueous solution: absorption maxima (λ_{max}), lifetimes (τ_T) of triplet states under oxygen-free conditions and quantum yield of singlet oxygen in D_2O (Φ_Δ)

Species	λ_{max} [nm]	τ_T [μs]	Φ_Δ
$^3MB^+$	420	37	0.52[a]
$^3MBH^{2+}$	370	4.4	
$^3AA^+$	420	30	0.56[b]
$^3AAH^{2+}$	370	5.5	
$^3AB^+$	420	40	0.47[b]
$^3ABH^{2+}$	370	6.5	
$^3AC^+$	420	21	0.71[b]
$^3ACH^{2+}$	370	4.0	

[a] Literature value [23].
[b] Error less than 20%.

The value of $\Delta pK_{a1} = 6.8$, calculated from Eq. (2), is in good agreement with the value of $pK_{a1} = 7.2 \pm 0.1$, obtained from the experimentally determined values of the concentration — pH dependence of the protonated form in the triplet state (Fig. 3a). Comparison of the dissociation constants of azure B in the ground and excited triplet state indicates that both dissociation equilibria are shifted in the excited state towards the more basic pH region [Eq. (1)]. While in the ground state the values $pK_{a1} = 0 \pm 0.2$ and $pK_{a2} = 12$ [18] were determined, in the triplet state $pK_{a1} = 7.2 \pm 0.1$ and the value of pK_{a2} cannot be determined as it apparently lies in the highly alkaline region.

The mechanism of formation of the radical cation ($P^{+\bullet}$) following laser excitation has been studied for proflavin [25], phenothiazine [26] and methylene blue [27]. Several mechanisms have been suggested for its formation, including loss of an electron from the triplet state in neutral or acidic medium [Eq. (3)] and interaction of O_2 in the ground state with the triplet state of the dye, with formation of a superoxide anion [Eq. (4)].

$$^3P \rightarrow P^{+\bullet} + e^-(aq) \tag{3}$$

$$^3P + {}^3O_2 \rightarrow P^{+\bullet} + O_2^- \tag{4}$$

The band corresponding to $AB^{2+\bullet}$ appears immediately following excitation and is characterized by single exponential deactivation kinetics. $AB^{2+\bullet}$ tends to be formed by direct photoionization (3) rather than by a bimolecular process (4), where $AB^{2+\bullet}$ would be gradually formed as a consequence of the lifetime of $^3AB^+$ (1.5 μs). The lifetime of $AB^{2+\bullet}$ in air-saturated solutions, pH 7, was measured as 52 ± 4 μs. The absorption band of $AB^{2+\bullet}$ was not found in oxygen-free solutions as it is apparently overlapped by the absorption bands of $^3AB^+$.

In neutral medium (pH 7), the dyes AA^+, AB^+ and AC^+ generate singlet oxygen with high quantum yield Φ_Δ (Table 1), comparable with MB^+ within experimental error; these substances can thus be used as sensitizers for photochemical and photobiological applications. Measurements of Φ_Δ in acid media were not carried out; however, lower Φ_Δ values can be expected because of the shorter lifetime of ABH^{2+}, similar to experiments with MBH^{2+} [28].

3.2. Micellar medium

In the micellar medium of an anionic surfactant (SDS), i.e. at SDS concentrations higher than the critical micelle concentration (cmc), changes occur in the UV/VIS spectra of the ground state [17,18]. The individual dye molecules are stabilized here by electrostatic forces and the tendency to form aggregates decreases (in contrast to solutions with free surfactant molecules or in the formation of premicellar aggregates of surfactant at surfactant concentrations lower than cmc, in which dye aggregation increases). Because of electrostatic interactions, an increase in the ionic strength (addition of inorganic salts) also affects dye aggregation and its absorption spectrum.

The positions of the absorption maxima of $^3ABH^{2+}$ (370 nm) and $^3AB^+$ (420 nm) in the triplet state and AB^{2+} (520 nm) are not changed in micellar SDS medium; however, the absorbance values are decreased. The triplet states are apparently stabilized and the lifetimes are increased. Where the lifetimes of the triplet states in air-saturated aqueous media are equal to 1.6 μs for AB^+ and 0.3 μs for ABH^{2+}, these values equal 16.1 and 5.9 μs, respectively, in micellar media. Micellar medium also increases the quantum yield of singlet oxygen for AB^+ (the value of $\Phi_\Delta = 0.71 \pm 0.15$ in SDS micellar medium). Similar results were also published for MB^+ [12].

The presence of micelles of the anionic tenside SDS greatly affects the acid-base equilibrium between $^3AB^+$ and $^3ABH^{2+}$. The processes cannot be described in terms of Eq. (2), as the dye molecules are present both in the micelle and the aqueous phase. In the pH 5–9 range only the AB^+ form is present in the micelles [19]; following excitation, this yields $^3AB^+$. The results indicate that the concentration of $^3AB^+$ does not greatly change with pH, and $^3ABH^{2+}$ (formed by protonation of $^3AB^+$) is present only in low concentration (Fig. 5b). The elevated stability of $^3AB^+$ can be explained on the basis of the theory of the pseudo-phase ion-exchange model [12]. Dye molecules are bonded by hydrophobic interactions and, when oppositely charged, also by electrostatic interactions in the micelle pseudo-phase. The degree of penetration into the interior of the micelle depends on the hydrophobicity of the dye; a polar dye can be bound only to the surface (Stern) micelle layer. In the presence of micelles of an anionogenic surfactant (SDS), highly polar AB^+ will be attracted electrostatically to the Stern layer, where it will be bonded to the oppositely charged SDS molecules, increasing its stability (lifetime) and decreasing its affinity for H^+ ions.

Measurement of changes in the concentrations of the triplet states of organic dyes, which can be incorporated into the micelles, in dependence on the surfactant concentration, can be a suitable method for determining the critical micellar concentration of the surfactant (cmc). Although there are many ways of measuring cmc [29], this method has not yet been utilized. Spectrophotometric methods are based mainly on measuring the dependence of the absorbance [30] or steady-state fluorescence [31] of the solution on the surfactant concentration. The dependence of changes in the absorbance of $^3AB^+$ on the SDS concentration in media with various ionic strengths is depicted in Fig. 5. The initial decrease in the concentration of triplet states can be explained by the formation of dye-rich premicellar aggregates of the surfactant, leading to the formation of dye aggregates, similarly to absorption [18] or fluorescence [31] measurements. The monomer-dimer association constant will be higher than in water solutions (~4000 M^{-1} for MB^+ [32]). Excitation of aggregates of organic dyes does not lead to the formation of triplet states [33]. In addition, triplet states formed from the monomer form of AB^+ can be quenched by the ground state of AB^+. The sharp increase in the concentration of triplet states of the dye when a certain SDS concentration is attained can be

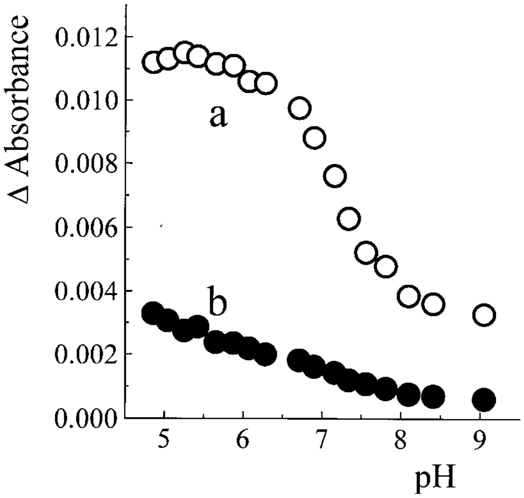

Fig. 5. Relative concentration of $^3ABH^{2+}$ vs pH monitored at 370 nm, 200 ns after excitation pulse in aqueous medium (a) and in micelles (b).

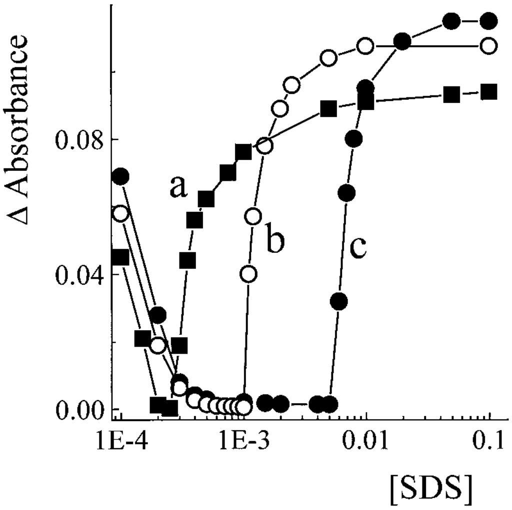

Fig. 6. Relative concentration of $^3AB^+$ monitored at 420 nm vs concentration of SDS in the presence of 0.01 M (a), 0.1 M (b) and 0.5 M (c) $NaNO_3$.

explained analogously to the measurement in the ground state through the formation of micelles, and thus corresponds to cmc. In micellar medium, electrostatic forces lead to stabilization of the triplet states of the dye as the individual molecules are separated and their interaction is limited.

The measured cmc values decrease with increasing ionic strength (I), i.e. with the concentration of hydrated sodium ions $[\mathrm{Na}^+]_{\mathrm{aq}}$. As the concentration of surfactant counter-ions increases, the electrostatic repulsion decreases between the ionic groups of surfactant molecules and the number of detergent monomers N_A forming the micelle (aggregation number) increases [Eq. (5)] [29],

$$N_A = \kappa([\mathrm{Na}^+]_{\mathrm{aq}})^{\gamma} \qquad (5)$$

where κ and λ are constants. The values of cmc = 1.5×10^{-3} ($I = 0.01$, Fig. 6a), cmc = 7×10^{-4} ($I = 0.1$, Fig. 6b) and cmc = 4×10^{-4} ($I = 0.5$, Fig. 6c) mol l^{-1} are comparable with the values obtained by measuring the surface tension of SDS [32] and measuring the absorbance in the ground state [18].

Acknowledgements

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