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Photochemical Activity of Merocyanine Dyes

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Abstract Time-resolved photothermal responses of merocyanine dyes were used to estimate such parameters as the quenching rate constant of dye triplet states and dye capability of singlet oxygen generation, which are helpful in explanation of the dye photochemical activity. The results have shown that the high photostability of the salt form of merocyanine dyes and their resistance against reactive oxygen species as well as dye–oxygen interaction by triplet–triplet energy transfer make merocyanines efficient singlet oxygen generators in simple and complex systems.

Keywords Merocyanine · Photothermal spectroscopy · Singlet oxygen

1 Introduction

Merocyanine (Mero) dyes have been shown to have phototoxic effect on leukemia, lymphoma, and neoplastic cells and viruses [1–3]. The role of oxygen and involvement of singlet molecular oxygen in Mero photodynamic action on artificial and natural cell membranes have been also reported [4,5]. However, the molecular mechanism responsible for the phototherapeutic role of Mero-sensitizer has not been clarified yet. The spectral properties and some photophysical parameters of seven stilbazolium Mero dyes in model systems have been reported [6,7]. The dyes investigated (Fig. 1) differ in the length of the CH_2 chain and its terminal group (R_1) at the heterocyclic ring as well as in the character of side groups attached to the benzene ring (R_2, R_3). It has been shown [6,7] that the molecular structure of Mero dyes influence their intra- and inter-actions, and as a result, the spectrally discernible species of the dye are formed,

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e.g., protonated and/or free-base, showing different photophysical and photochemical properties.

Particularly interesting are the slow non-radiative deactivation processes of the dyes followed by absorption of light energy and related to population of triplet state and energy transfer to molecular oxygen resulting in $^1O_2$ formation. These processes are responsible for photosensitized oxidations involved in phototherapy, in photocarcionogenesis, and in photodegradation of dyes or polymers [8]. In this article, using the data obtained for Mero dyes in organic solvents and in mononuclear blood cells [6,7], the yield of singlet oxygen generation was determined. In addition, the mechanism and quenching rate constant of dye triplet states were estimated. All these parameters as well as the dye photostability are of great importance for successful application of the dye-sensitizer leading to generation of singlet oxygen for increasing photochemical sensitivity.

2 Measurements

The molecular structure and notations (Fig. 1) of the investigated Mero dyes (synthesized by Dr. I. Gruda from Université du Québec, Trois-Rivières, Canada) and the methods of sample preparations and of dye introduction into lymphocytes were described earlier [6,7,9]. The Mero dyes and diazobicyclooctane (DABCO from Sigma) were dissolved in methanol (MeOH) or ethanol (EtOH) (from POCH, Lublin, Poland).

Applying the method proposed by Small et al. [10], the deconvolution of time-resolved photothermal signals for Mero dyes in model systems [6,7], obtained by means of the laser-induced optoacoustic spectroscopy (LIOAS) [11] technique, was performed. The error of k_i and τ_i obtained by the deconvolution method using the program Sound Analysis (Version 1.5) was close to 5 %.

For photodegradation kinetic studies, aliquots of Mero alcohol solutions were irradiated using a xenon lamp and a glass filter, for 60 min for samples without any additions and in the presence of a quencher [12]. The dark control samples were Mero dyes in EtOH (or MeOH). Absorption spectra of the samples investigated were recorded on a Cary 4000 (Varian) spectrophotometer.

Mero	R_1	R_2	R_3
B	$(CH_2)_{11}-OH$	H	H
B*	$(CH_2)_{11}-OH$	H	H
T*	$(CH_2)_{11}-OH$	OCH_3	H
W*	$(CH_2)_{15}-CH_3$	OCH_3	H
H	$(CH_2)_6-OH$	H	NO_2
I	$(CH_2)_6-OH$	NO_2	NO_2
U*	$(CH_2)_{10}-CH_3$	NO_2	NO_2

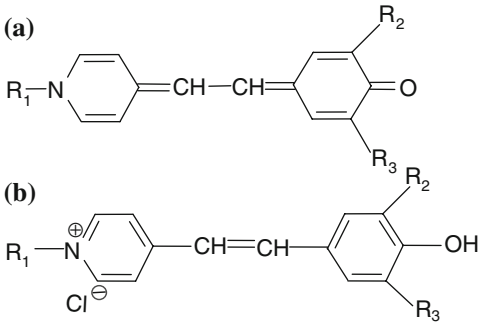

Fig. 1 Molecular structure of (**a**) merocyanine (Mero) dye and (**b**) its salt (Mero with *) form

3 Results

On the molecular level, changes in the dye photoactive behavior can be monitored by various parameters (e.g., absorption and fluorescence properties, thermal deactivation, yields of triplet population (Φ_T), and singlet oxygen formation (Φ_Δ), etc.) estimated by means of optical (absorption and fluorescence spectroscopy) and photothermal (LIOAS [11,13]) techniques. Some of these parameters obtained for Mero dyes in alcohol and polyvinyl alcohol (PVA) solutions, and in PVA film as well as in healthy and pathologically changed lymphocyte cells, have been already presented [6,7]. But the most interesting Φ_Δ parameter describing the photochemical activity of Mero dyes has not been estimated yet. The ability of a dye to generate singlet oxygen plays a key role in phototoxicity of a dye-sensitizer. For the time-resolved photothermal signals recorded for Mero dyes in simple (alcohol solutions) [6] and complex (biological materials) [7] systems, the Small et al. [10] method was applied, first of all, to calculate (from the following equation) the Φ_Δ parameter:

$$\Phi_\Delta = \frac{(1-k_1)E_{h\nu} - \Phi_F E_S - E_{h\nu}k_2}{E_\Delta}, \tag{1}$$

where the values of k_i are related to the energy thermally deactivated in different time-dependent processes and can be evaluated by the deconvolution method of photothermal signals, $E_{h\nu}$ is the molar energy of the incident photons at excitation wavelengths used in LIOAS experiments, Φ_F, E_S describe the yield of fluorescence and the energy of the Mero dye singlet state estimated earlier [6], and E_Δ represents the energy of the lowest singlet molecular oxygen state, known from the literature [14].

Before applying the deconvolution method [10], the assumption was made that the LIOAS signal recorded in an air atmosphere is a sum of three exponential components ($k_i \exp(-t/\tau_i)$), each of them characterizing a certain radiationless process. The first component (k_1 for $\tau_1 < 0.5\,\mu s$) is related to the thermal deactivation of Mero dye singlet states (i.e., $S_n \rightarrow S_1$ and $S_1 \rightarrow S_0$ as well as $S_1 \rightarrow T_1$ transitions). The second component (k_2, τ_2) can be correlated with the two possible pathways of the triplet state deactivation involving triplet–triplet energy transfer from this state of Mero to the oxygen and/or non-radiative decay to the ground state, the analysis implies that to a good approximation the parameters k_2 and τ_2 can describe the dye triplet state quenching by molecular oxygen causing $^1O_2$ generation. And finally, the third component (k_3, τ_3) represents the singlet oxygen radiationless decay. The results of LIOAS signal analyses for Mero dyes in alcohol solutions are compiled in Table 1. The analyses have been performed assuming that the depopulation of the excited singlet state of an oxygen molecule is a simple exponential decay process. The time τ_3 describing the decay time of singlet oxygen (τ_Δ) in certain solvents (taken from the literature [15]) was used for the LIOAS signal deconvolution. The τ_Δ values in air-saturated alcoholic solvents are 7.8 μs to 11.0 μs or 6.8 μs to 15.0 μs for EtOH or MeOH, respectively.

Comparing the result of LIOAS analysis for the dyes with an H or OCH_3 side group attached (B, B*, T*, and W*) (Fig. 1), it can be observed that the k_1 parameter describing fast radiationless depopulation processes is slightly higher in air (Table 1) than in a N_2 [6] atmosphere. This effect is well correlated with a shortening of decay time τ_2

Table 1 Results of LIOAS signals analysis of the Mero dyes in air-saturated alcohol solution

Mero	λ_{exc}[a] (nm)	k_1[b]	k_2[b]	k_3[b]	$\sum k_i$ (±0.05)	τ_2[c] (μs) (±0.18)	Φ_Δ[d] (±0.06)	S_Δ[e]	$k_T \times 10^{-9}$[f] (Ms^{-1}) (±0.05)
B	384	0.57	0.14	0.28	0.99	3.62	0.77	0.96	0.13
B*	384	0.61	0.14	0.26	1.01	3.74	0.68	0.92	0.13
T*	415	0.50[g]	0.07	0.38	0.95	1.23	0.82	1.22	0.39
W*	415	0.64	0.09	0.11	0.84	$\ll$0.5	0.19	0.63	$\ll$0.97
H	420	0.78[g]	0.17	0.03	0.98	0.94	0.18	0.40	0.50
I	420	0.14	0.25	0.61	1.00	1.27	0.95	0.95	0.37
U*	420	0.99	0.01	–	1.00	2.18	0.06	1.00	0.22

[a] λ_{exc}—excitation wavelength used in LIOAS measurements
[b] k_i—pre-exponential factors
[c] τ_2—decay times
[d] Φ_Δ—yield of singlet oxygen formation (Eq. 1)
[e] S_Δ—fraction of dye triplet states quenched by oxygen
[f] k_T—the rate constant of triplet state quenching by molecular oxygen
[g] Results adopted from [6]

for the samples in an air-saturated solvent, but still for Mero dyes with hydrogen in the R_2 and R_3 positions, the decay time τ_2 around 3.7 μs was obtained, which is longer than that observed for the other Mero dyes. For these dyes, about 36 % to 50 % of the energy absorbed is deactivated into heat at times ranging from 0.5 μs to 5 μs. The estimated parameters suggest that the presence of oxygen increases the depopulation process rate of photoexcited states of these Mero dyes. Such behavior is not observed for Mero H, I, and U* with acceptor (NO_2) side groups attached (Table 1).

Comparing the results obtained for the whole set of Mero dyes, it seems that the terminal CH_3 group (R_1) at the heterocyclic ring (Mero W* and U*, both in salt form) have great influence on fast thermal deactivation processes. For Mero W*, about 64 % of the energy is converted into heat as a result of deactivation of the singlet states of the dye, and this dye weakly interacts with oxygen as confirmed by a very short τ_2 time, while for Mero U* almost all the energy absorbed is deactivated in a time shorter than the time resolution of the apparatus used (close to zero value of k_2). Also, a significant part of energy is lost in the radiationless processes related to deactivation of the singlet state of Mero H. For Mero I, whose molecular structure differs from that of Mero H only by the side group in the R_2 position, the course of these processes is different and it deactivates 86 % of the energy absorbed in the processes taking place at times longer than 0.5 μs. It turn out that two NO_2 groups and a short CH_2 chain (R_1) in the molecular structure of Mero I can affect the dye sensitizing potential determining the dye ability to induce photodynamic reactions fundamental for their phototherapeutic activity revealed after irradiation.

The LIOAS analysis results in an air atmosphere show that when applying three exponential components, the sum of k_i (in most cases) is almost unity with the optimum accuracy of calculation. It indicates that the analysis has been well performed and describes the real processes of heat release taking place in the samples studied. The parameter S_Δ (Table 1), calculated as a quotient of Φ_Δ and Φ_T where Φ_T is the yield of population of the triplet state, has been determined earlier [6]. The high value of the S_Δ parameter indicates that a huge fraction of the dye triplet states is quenched by oxygen, which shows the dye ability to produce $^1O_2$.

For all Mero dyes, the rate constant of triplet state quenching by molecular oxygen (k_T) (Eq. 2) was estimated by

$$k_T[O_2] = \frac{1}{\tau_2(\text{air})} \tag{2}$$

taking into account the oxygen concentration ($[O_2] = 2.07 \times 10^{-3}$M or 2.12×10^{-3}M) in air-saturated EtOH or MeOH, respectively, at room temperature [13,15].

As follows from Table 1, the k_T parameter has a value lower than $1/9k_{diff}$ equal $2.7 \times 10^9 Ms^{-1}$ or $3.4 \times 10^9 Ms^{-1}$ for EtOH or MeOH, respectively. Hence, we can conclude that the mechanism of $^1O_2$ formation is rather related to physical quenching by the triplet–triplet energy transfer between the dye and oxygen molecules than to formation of charge transfer complexes.

From an analysis of results reported in Table 1, we can conclude that no simple correlation is observed between the lengths of the CH_2 chain (R_1), the character of R_2 and R_3 side groups attached, as well as the salt form of the dye and the distribution

of energy in radiationless processes as well as the efficiency of oxygen generation. It is seen that for the Mero dyes with the same CH_2 chain length (10 to 11 carbon atoms), the incorporation of the electron donor OCH_3 group in the R_2 position of the benzene ring results in an increase of the rate constant k_T and the Φ_Δ value, whereas the electron-attracting NO_2 group reduces the singlet oxygen generation quantum yield (Table 1). The analogous situation has been observed earlier [16] for porphyrin molecules. The estimated times τ_2 and high value of k_3 for Mero B, B*, T*, and I as well as the Φ_Δ value confirm that these Mero dyes have a high ability to interact with oxygen molecules and generate singlet oxygen. This feature could be used not only in photomedicine, but also for photo- or/and bio-degradation processes. It has been reported [17] that Mero dyes, such as Mero B, B*, and T*, with the chains containing 11 to 12 carbons terminated with a hydroxyl group, have a high affinity to penetrate into a hydrophobic cell membrane.

For the Mero dyes, their photostability in alcohol solutions towards singlet oxygen generated by excitation to triplet state dye was checked. The Mero samples without any additions and in the presence of DABCO, which is known from the literature [12] as a $^1O_2$ quencher, were studied. As an example, the absorption changes of Mero B and B* at maximum absorption under illumination without and with any further addition are shown in Fig. 2. Yang et al. [18] suggested that the dyes with a positive charge on the heterocyclic moiety have great influence on the photostability of Mero dyes. The decay of the absorption at 400 nm did not vary significantly under and without illumination for Mero B* and B, respectively (Fig. 2, curves 4 and 3). As shown in Fig. 2 (curve 1), the strong photobleaching of Mero B in EtOH is observed for the first 20 min. The photodegradation products did not have absorptions in the visible region. The slope of the plot of $\ln(A/A_t)$ (where A, A_t are the initial and the changed in time absorbance, respectively) versus irradiation time gives a straight line in this time range (not shown) which means that the photodegradation of Mero B follows quasi-first-order kinetics. But when DABCO is added to a Mero B alcohol solution, it does not give any obvious change in dye absorption, indicating that singlet oxygen is mainly responsible for photodegradation and $^1O_2$ quenching protected the dye against photobleaching (Fig. 2, curve 2).

The dyes (Mero B, T*, and I) used for lymphocytes coloration have been shown to have poor ability to form mixed complexes with a polymer and weak interaction with water molecules [6,7], which suggests that these dyes can easily accumulate and/or penetrate the hydrophobic inner structure of the cell organelle and membranes. For these dyes the LIOAS signal was analyzed into the exponential components by deconvolution and the value of Φ_Δ was estimated (Table 2). The τ_3 parameter applied was the decay time of singlet oxygen in water solutions [15]. Taking into regard the sum of the k_i parameters, we can conclude that the convolution into the three exponential components gives a full description of all possible radiationless transitions leading to conversion of the excitation energy into heat in the biological samples studied. The results obtained suggest considerable changes in the thermal properties of Mero I, which shows the highest k_1 value and a significantly lower Φ_Δ value in comparison with the values in an organic solvent (Tables 1, 2). Smaller changes were observed for Mero B and T*. Both dyes in lymphocytes have still a significant ability to produce $^1O_2$. The values of τ_2 for the second exponential component describing the triplet–triplet

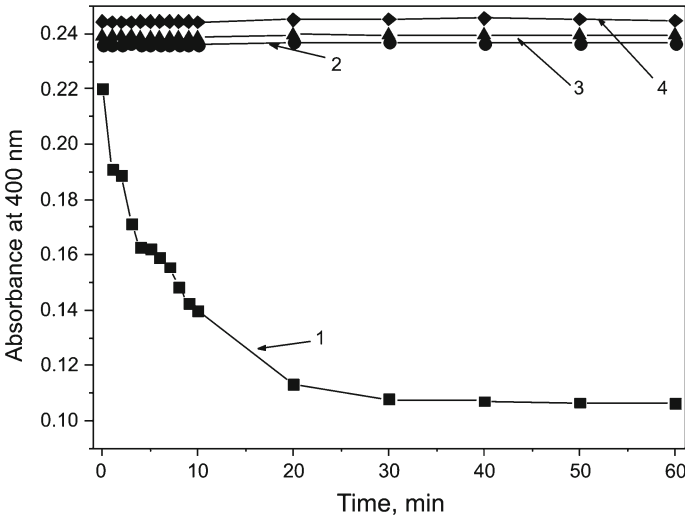

Fig. 2 Absorption changes at 400 nm for Mero B (1–3) and B* (4) in ethanol solution under illumination (1, 2, 4), in dark (3), and in the presence of DABCO (2)

Table 2 Results of LIOAS signals analysis of Mero dyes incorporated into pathologically changed lymphocytes

Mero	λ_{exc} (nm)	k_1^a	k_2	k_3	$\sum k_i$ (± 0.05)	τ_2 (μs) (± 0.04)	Φ_Δ (± 0.06)
B	384	0.53	0.14	0.31	0.98	0.63	0.75
T*	415	0.76	0.02	0.21	0.98	0.69	0.64
I	420	0.93	0.02	0.04	0.99	0.49	0.19

Description as in Table 1

[a] Results adopted from [7]

energy transfer between the dye and molecular oxygen were considerably shortened. In such circumstances, the concentration of molecular oxygen in the cells and the localization of the dye in the biological materials are important as only the molecules of the dye and oxygen, being in a close neighborhood, are able to transfer energy to each other in such a short time (ca. 0.60 μs) leading to oxygen excitation. On the basis of the images obtained under a fluorescence confocal microscope, it was suggested that [17] because of its lipophilic properties Mero dyes are mainly localized in the cell membrane of the mitochondria, which permits their effective interaction with oxygen.

4 Conclusions

In search for a new, inexpensive, and easy-to-synthesize dye, which would exhibit excellent photochemical properties and photostability useful in different applications, a group of Mero dyes were studied to compare the effect of substituents on their photosensitizing activity. The Mero dyes whose heterocyclic ring hold a charge had a

donor group (such as OCH_3) or/and H atom and had 11 carbon atoms in the CH_2 chain terminated with the OH group were found to show higher photostability and higher ability to generate singlet oxygen than the other Mero dyes of different molecular structure.

The results obtained for Mero dyes confirm that Mero T* and B* dyes are the best candidates for therapeutic applications. The photostability of the salt form of this dye is important to trigger the photochemical reaction induced by prolonged illumination used for dye therapeutic excitation. However, such dyes as Mero B, in view of its spectral properties [6] and course of kinetics of photodegradation reaction in alcohol (Fig. 2) and a biological environment [7], can be used for staining cells for diagnostic purposes.

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