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The influence of the structure of ketocyanine dyes on the generation of singlet oxygen

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A comparative study of the photophysical properties of a linear ketocyanine dye and its structurally rigid analogue containing a cyclohexanone fragment in the polymethine chain is carried out in this paper. It was found that the introduction of a fixing fragment limits the conformational mobility of the molecule and has a decisive influence on the redistribution of the pathways for deactivation of electronic excitation energy. Restricting the flexibility of the polymethine chain leads to a decrease of the fluorescence quantum yield, that is consistent with a more efficient population of the triplet state. It is shown that the delayed fluorescence lifetimes of both dyes in evacuated solution are practically identical, that indicates comparable kinetic characteristics of the decay of long-lived excited states. The quantum yield Φ_Δ of singlet oxygen O_2 ($^1\Delta_g$) generation is 0.30 ± 0.02 for the linear dye and 0.38 ± 0.03 for the structurally rigid dye. Thus, modifying the chromophore chain by introduction of a cyclohexanone fragment allows for the redirection of excitation energy from the fluorescent channel to the triplet channel. It makes the structurally rigid dye a more effective and promising photosensitizer for use in photodynamic therapy and photocatalysis.

Keywords: ketocyanine dyes; intersystem crossing; phosphorescence; singlet oxygen; photodynamic therapy

Introduction

Singlet oxygen O_2 ($^1\Delta_g$) is one of the most reactive forms of active oxygen and plays an important role in photochemical and photobiological processes [1–5]. O_2 ($^1\Delta_g$) generation is widely used in photodynamic therapy (PDT),

photocatalysis, oxidation processes [1, 2], and environmental photochemistry [6, 7]. Typically, $O_2(^1\Delta_g)$ is formed through energy transfer from the triplet excited state of the photosensitizer to molecular oxygen located in its triplet ground state. The efficiency of this process depends on the photophysical characteristics of the sensitizer, including the probability of intersystem crossing, the lifetime of the triplet state, and the energy transfer efficiency [8].

Organic compounds of various classes: porphyrins, phthalocyanines, xanthene and polymethine dyes are widely used as $O_2(^1\Delta_g)$ photosensitizers [9–11]. Cyanines, whose structure is formed by two heterocyclic fragments linked by a system of conjugated double bonds (a polymethine chain), are of particular interest. Varying the length of the conjugated chain and introducing substituents of different electronic natures make it possible to effectively modulate their spectral and luminescent properties, as well as control the efficiency of intersystem crossing (ISC), which determines the yield of singlet oxygen generation. As noted in a recent fundamental review [1], polymethine dyes (PDs) possess a unique set of properties, including high extinction coefficients and the ability to precisely control their photophysical characteristics through targeted modification of the chemical structure. The authors demonstrated that a key strategy for increasing the photodynamic efficiency of PDs is minimizing the channels for nonradiative deactivation of excitation energy. Specifically, Ref. [1] demonstrated that the introduction of rigid structural fragments into the polymethine chain effectively limits its conformational mobility. It contributes to the stabilization of excited states and optimization of pathways for the generation of reactive oxygen species. This makes PDs promising agents for multifunctional photodynamic therapy (PDT).

Early studies showed that the efficiency of quenching and generation of $O_2(^1\Delta_g)$ by cyanine dyes depends on both structural factors (the length of the polymethine chain, the type of heterocycles) and environmental conditions. In particular, the authors of Ref. [12] measured the quenching constants for 19 cyanines and showed that the quenching mechanism is associated primarily with charge transfer. More recent studies, Ref. [13], show that cyanines with two chromophore fragments provide long-lived triplet states that are consistent with more efficient $O_2(^1\Delta_g)$ generation. The triplet state is an energy donor for molecular oxygen, that is critical for the photosensitizing properties of dyes.

In the ref. [14], a new series of thio-pentamethine cyanine (TCy5) photosensitizers with reduced ΔE_{ST} was developed, that ensured accelerated intersystem crossing, high quantum efficiency of $O_2(^1\Delta_g)$ generation, and effective destruction of tumor cells in vitro and in vivo. These results demonstrate the potential of TCy5 as modern photosensitizers for photodynamic cancer therapy. Additionally, it was shown that a number of heptamethine cyanines absorbing in the near-infrared range are also capable of efficiently generating $O_2(^1\Delta_g)$ upon photoirradiation. Such compounds are particularly promising for biomedical applications because their absorption spectra lie within the "biological transparency window", where tissues absorb minimally.

Squarylium cyanines, which are structurally closely related to ketocyanine compounds, represent one of the most intriguing classes of cyanine dye deriva-

tives. A several studies have been devoted to the investigation of their photosensitizing properties. In particular, it was shown in Ref. [15] that the quantum yields of $O_2(^1\Delta_g)$ generation for some derivatives reach values of 0.4–0.5. These results were confirmed both by methods of recording the infrared phosphorescence of $O_2(^1\Delta_g)$ and by the use of chemical traps. The authors also noted that the substitution of heavy atoms and variation of heterocyclic fragments enhance intersystem crossing, that indicates the high photosensitizing capacity of squarylium dyes.

Of particular interest are ketocyanine dyes containing a carbonyl group in the chromophore. According to Ref. [16], the presence of a carbonyl fragment changes the distribution of electron density and promotes donor–acceptor interactions, which affects the energy position of excited states and the relaxation dynamics. However, the photochemical properties of ketocyanines was studied significantly less. Questions related to the mechanisms of triplet state formation, the quantum yields of $O_2(^1\Delta_g)$ generation, and the influence of structure on the efficiency of intersystem crossing remain open.

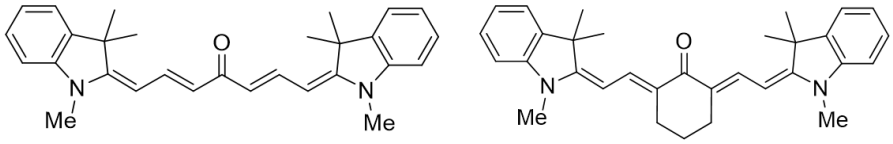
Thus, studying the spectral and photochemical properties of ketocyanine dyes, as well as determining the quantum yields of $O_2(^1\Delta_g)$ generation, is an important task in modern photochemistry and can facilitate the development of new, effective photosensitizers for various applications.

This work presents the results of a comparative study of the photophysical properties of a linear ketocyanine dye (KC1) and its structurally rigid analogue (KC2) containing a cyclohexanone fragment in the polymethine chain. The aim of the study is to investigate the influence of the conformational rigidity of the dye structure on the evaluation of the $O_2(^1\Delta_g)$ generation efficiency and on the redistribution of excited state deactivation pathways associated with the population of triplet levels. The established mechanism of excitation energy redistribution between radiative and nonradiative pathways for the deactivation of excited electronic states of the sensitizer opens the possibility for the targeted optimization of the photosensitizing activity of this class of dyes. The obtained results serve as an important basis for the rational design of promising agents for photodynamic therapy.

Experimental

Ketocyanine (KC) dyes belong to a class of neutral symmetric and asymmetric polymethine compounds (merocyanines) containing an electron-withdrawing carbonyl group in the chromophore system. The studied dyes belong to the same structural type and differ in the conformational flexibility of the chromophore. KC1 has an open polymethine chain, while KC2 contains a rigid cyclohexanone fragment, which restricts intramolecular rotation. These compounds were chosen as model objects to elucidate the relationship between the rigidity of the molecular framework and the photophysical properties of the chromophore. The dyes were synthesized at the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine. The structural formulas of the ketocyanines studied are

shown in Figure 1.



КС1 **КС2**

Figure 1. Structural formulas of ketocyanine dyes.

The dye concentration in the ethanol was 10^{-5} M, that ensured an optical density $D < 0.1$ at the excitation wavelength. Absorption spectra were recorded on a Cary-300 spectrophotometer, fluorescence – on a Cary Eclipse (Agilent Technologies). The fluorescence quantum yield (φ_{fl}) was determined by the relative method (standard is rhodamine 110 in ethanol, $\varphi_{\text{fl}} = 0.92$) [17]. Fluorescence kinetics were recorded by the time-correlated single photon counting method (TCSPC, Becker&Hickl). Excitation was performed with a picosecond laser ($\lambda_{\text{exc}} = 488$ nm, $\tau = 120$ ps). Lifetimes were calculated by SPCImage software using the deconvolution method. The kinetics of delayed fluorescence of dyes and $O_2(^1\Delta_g)$ phosphorescence were recorded on an FLS1000 spectrometer (Edinburgh Instruments) equipped with a Hamamatsu IR detector. The phosphorescence of $O_2(^1\Delta_g)$ was recorded at atmospheric pressure. For long-lived luminescence measurements, the cuvette with the solution was preliminarily evacuated to a pressure of $P \approx 133.32 \cdot 10^{-5}$ Pa (10^{-5} mm Hg) over the frozen solution.

The quantum yield of $O_2(^1\Delta_g)$ generation (Φ_Δ) was determined by the relative method using the following equation [18]:

$$\Phi_\Delta = \Phi_\Delta^{st} \cdot \frac{I}{I_{st}} \cdot \frac{D_{st}}{D} \tag{1}$$

where Φ_Δ^{st} – is the quantum yield of $O_2(^1\Delta_g)$ sensitized by the standard, I and I_{st} – integrated phosphorescence intensities of $O_2(^1\Delta_g)$ sensitized by the studied photosensitizer and the standard, D and D_{st} – are the optical densities of the photosensitizer solution and the standard at the excitation wavelength, respectively.

To measure the quantum yield of $O_2(^1\Delta_g)$ using the relative method, solutions of the sensitizer under study and the standard were prepared in ethanol with the same optical density at the excitation wavelength ($\lambda_{\text{exc}} = 532$ nm). Rose Bengal (RB) was used as the standard ($\Phi_\Delta = 0.86$) [19, 20].

The $O_2(^1\Delta_g)$ phosphorescence decay kinetics ($\lambda_{\text{reg}} = 1270$ nm) were measured at room temperature in an open cuvette (1 cm) to allow oxygen access. The total intensity was obtained by numerically integrating the experimental $O_2(^1\Delta_g)$ phosphorescence decay kinetics using the trapezoidal rule implemented in the OriginPro 8.0 program.

Results and Discussion

The absorption maxima of the studied ketocyanines (KC1, KC2) in ethanol are located at 520 nm and 522 nm, respectively. The fluorescence spectral maxima of KC1 and KC2 are located at 620 nm and 610 nm, respectively (Figure 2(a)). Unlike classical symmetric polymethine systems, these ketocyanine dyes exhibit broadened absorption and fluorescence bands.

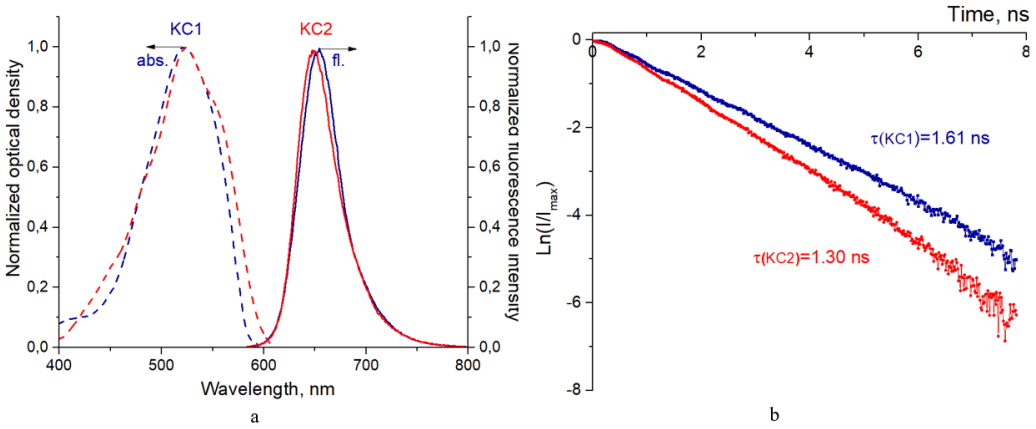


Figure 2. (a) Normalized absorption (dashed curves) and fluorescence (solid curves) spectra of ketocyanine dyes KC1 and KC2 in ethanol ($\lambda_{exc} = 520$, 522 nm, respectively); (b) Decay kinetics of fluorescence of ketocyanine dyes.

The key factor determining the differences between the two studied dyes is the conformational mobility of the polymethine chain. While the flexible analogue of KC1 has high molecular mobility, in the case of KC2 dye, the introduction of a rigid six-membered cyclohexanone fragment effectively restricts the rotational and vibrational degrees of freedom of the molecular framework [21]. Such a restriction of intramolecular mobility in the excited state prevents deep geometric relaxation of the chromophore. It leads to a hypsochromic (blue) shift of the emission maximum of the rigid structure of KC2 molecule (610 nm) relative to KC1 molecule with the open chain (620 nm). Moreover, both compounds retain a significant Stokes shift, that confirms a significant rearrangement of the solvate shell of the dyes in the singlet excited state (S_1) due to a change in their dipole moment upon photoexcitation.

To analyze in detail the deactivation mechanisms of singlet excited states of the studied ketocyanines, the fluorescence decay kinetics (Figure 2(b)) were recorded at a wavelength of $\lambda_{reg} = 618$ nm.

As can be seen from the presented kinetic dependences, the luminescence intensity decay for both compounds is monoexponential. Mathematical deconvolution yielded fluorescence lifetimes of 1.61 ± 0.08 ns for the open-chain dye KC1 (blue curve) and 1.30 ± 0.06 ns for the structurally rigid analog KC2 (red curve).

To obtain a more detailed understanding of the photophysical processes, we also investigated the long-lived luminescence of these dyes. The corresponding parameters of the studied systems are summarized in Table 1 and their spectral characteristics are shown in Figure 3. As can be seen from the presented data, the luminescence maxima of both dyes are localized in the long-wavelength region of 615–625 nm.

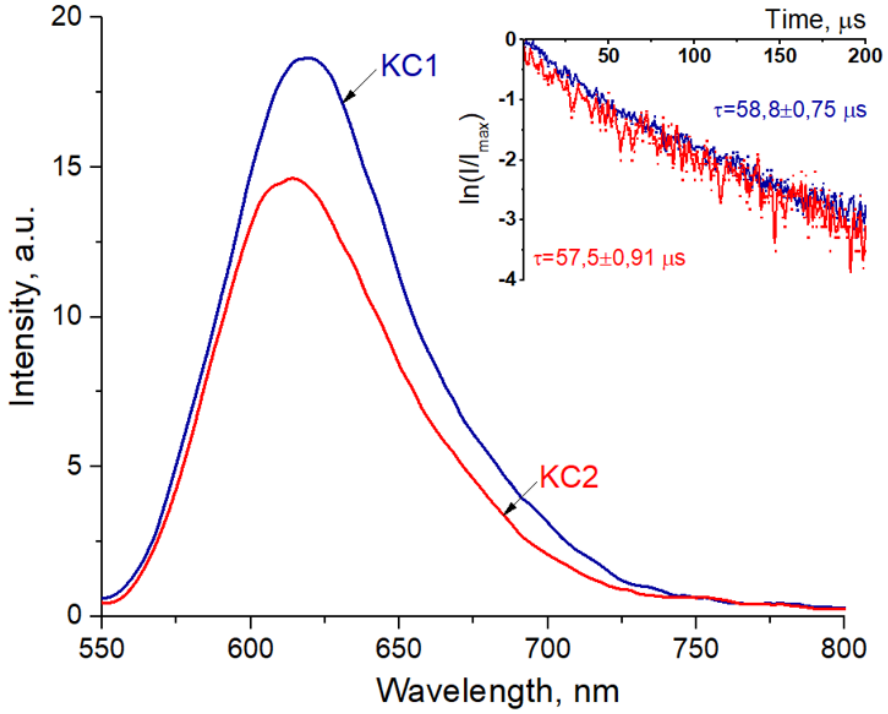


Figure 3. DF spectra of the ketocyanine dyes KC1 and KC2 in ethanol ($C = 10^{-5}$ M). The inset shows DF decay kinetics.

The coincidence of the band shape and the position of the maxima of the recorded long-lived fluorescence with the fast fluorescence spectra clearly indicates that the observed fluorescence is DF. Moreover, the intensity of the delayed fluorescence (I_{DF}) of the KC1 dye is 26% higher than that of the KC2 dye. This is due to the fact that the quantum yields of fluorescence of the studied compounds are 0.28 ± 0.02 for KC1 and 0.17 ± 0.01 for KC2.

The delayed fluorescence lifetimes calculated via linear approximation are 58.8 ± 0.75 μs for KC1 and 57.5 ± 0.91 μs for KC2. Temperature-dependent measurements demonstrated that the delayed fluorescence of the dyes is associated with thermal activation from the T_1 state to the S_1 state [22].

As can be seen from the obtained data, the KC1 is characterized by a higher efficiency of radiative decay from the singlet state S_1 ($\varphi_{\mathrm{fl}} = 0.28 \pm 0.02$) compared to the structurally rigid analogue KC2 ($\varphi_{\mathrm{fl}} = 0.17 \pm 0.01$).

An important step in studying of the photophysical properties of the presented compounds was investigating their ability to generate $\mathrm{O}_2\,(^1\Delta_g)$, since the efficiency of this process is directly determined by the population of the long-lived triplet state of the photosensitizer.

Figure 4 shows the kinetic curves for the decay of $\mathrm{O}_2\,(^1\Delta_g)$ phosphorescence of KC1 and KC2 dyes in ethanol, and the insets show the dependence of signal intensities on the concentration of the photosensitizers.

Furthermore, at high concentrations (10^{-4} mol/L), the singlet oxygen lifetime is reduced to 12.7 ± 0.15 μs for KC1 and 10.5 ± 0.06 μs for KC2, that indicates quenching of $\mathrm{O}_2\,(^1\Delta_g)$ by dye molecules in the ground state. τ_{Ph} values remain stable in the concentration range of $5 \cdot 10^{-5} - 10^{-5}$ mol/L, while further dilution

of the solution to 10^{-6} mol/L leads to a decrease in lifetime (down to 7.4 ± 1.07 μs for KC2).

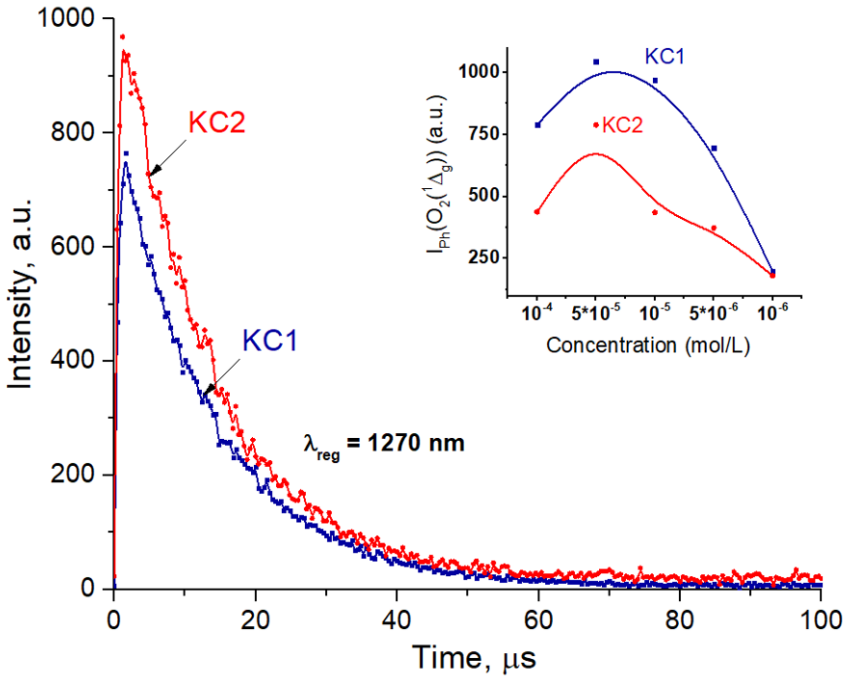


Figure 4. Decay kinetics of O_2 ($^1\Delta_g$) phosphorescence sensitized by KC1 and KC2 dyes in ethanol. The inset shows the dependences of singlet oxygen phosphorescence intensity (I_{Ph} O_2 ($^1\Delta_g$)) on dye concentration.

Table 1.
Lifetimes of O_2 ($^1\Delta_g$) sensitized with ketocyanine dyes KC1 and KC2 in ethanol at different concentrations.

N	Concentration of KC1 (mol/L)	$\tau_{Ph}(O_2(^1\Delta_g))$ (μs)	N	Concentration of KC2 (mol/L)	$\tau_{Ph}(O_2(^1\Delta_g))$ (μs)
1	10^{-4}	12.7 ± 0.15	1	10^{-4}	10.5 ± 0.06
2	$5 \cdot 10^{-5}$	13.4 ± 0.11	2	$5 \cdot 10^{-5}$	12.9 ± 0.10
3	10^{-5}	13.5 ± 0.11	3	10^{-5}	13.3 ± 0.14
4	$5 \cdot 10^{-6}$	13.1 ± 0.15	4	$5 \cdot 10^{-6}$	13.2 ± 0.17
5	10^{-6}	12.9 ± 0.35	5	10^{-6}	7.4 ± 1.07

That is associated with a sharp weakening of the useful signal and an increase in measurement error in highly diluted solutions. The obtained values of the lifetime of singlet oxygen phosphorescence are consistent with the obtained data in the work [23].

Conclusion

A comparative study of the photophysical properties of ketocyanine dyes – the linear KC1 and the structurally rigid KC2 containing a cyclohexanone fragment in the polymethine chain is carried out in this paper. It was experimentally demonstrated that the conformational rigidity of the KC2 molecule has a significant effect on the redistribution of the deactivation pathways for electron excitation

energy. The introduction of a stabilising fragment leads to a decrease of the quantum yield of fluorescence from 0.28 ± 0.02 to 0.17 ± 0.01, that is consistent with more efficient population of the triplet state at the initial photoexcitation stage.

The DF lifetimes for both dyes in evacuated solution are virtually identical (58.8 ± 0.75 μs for KC1 and 57.5 ± 0.91 μs for KC2), that indicates comparable parameters of the decay kinetics of long-lived excited states under the studied conditions. A consequence of more efficient population of the triplet state enhanced of the rigid structure of KC2 is a significant increase of the efficiency of $O_2(^1\Delta_g)$ photosensitisation. The quantum yield of $O_2(^1\Delta_g)$ generation is 0.30 ± 0.02 for KC1 and 0.38 ± 0.03 for KC2. At the same time, the phosphorescence decay traces recorded at 1270 nm exhibited similar $O_2(^1\Delta_g)$ lifetimes, consistent with those typically observed in ethanol.

Thus, modification of the chromophore chain by incorporating a cyclohexanone fragment allows for a targeted weakening of the fluorescent channel in favor of the triplet channel, making KC2 a more effective photosensitizer of $O_2(^1\Delta_g)$, that opens up prospects for its use in photodynamic therapy and photocatalysis.

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