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PHOTOPHYSICAL AND PHOTOCHEMICAL PROPERTIES OF HITC INDOTRICARBOCYANINE DYE MOLECULES IN SOLUTIONS

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Results of studies on the photonics of HITC indotricarbocyanine dye molecules with I^-, Br^-, and ClO_4^- counterions were presented. Introduction of an additional salt (tetrabutylammonium bromide) (10^{-2} M) to slightly polar solutions of the tricarbocyanine dye was shown to cause significant changes in the dye photophysical and photochemical characteristics. It was concluded based on a comparison of HITC dye photophysical properties with I^-, Br^-, and ClO_4^- counterions in slightly polar dichlorobenzene and highly polar EtOH; an analysis of trends in HITC photobleaching in deoxygenated and air-saturated solutions; and data from femtosecond spectroscopy that the features of the photophysical and photochemical processes in slightly polar dichlorobenzene were due to superfast charge transfer in tight ion pairs followed by formation of free radicals.

Keywords: *indotricarbocyanine dyes, tight ion pairs, spectral properties, femtosecond transient absorption spectroscopy, photobleaching, singlet oxygen.*

Introduction. Cationic polymethine dyes are widely used as various biological labels [1]. It seemed promising to use them as photosensitizers for cancer photochemotherapy [2, 3]. The phototoxicity mechanism of this class of compounds goes beyond a scheme in which tumor cells are damaged exclusively by the formation of singlet oxygen [3]. Indotricarbocyanine dyes were found to be localized in cancer cells in regions of low dielectric permeability and were present primarily as tight ion pairs [4, 5]. This circumstance and data that indotricarbocyanine dyes retain their photocytotoxicity under hypoxic conditions [2] suggested that photo-induced formation of free radicals as a result of charge transfer in the tight ion pairs was the starting mechanism for cancer cell damage. The occurrence of charge transfer in tight ion pairs with the formation of free radicals was confirmed by studies of transient absorption spectra of HITC dye with iodide (I^-) and perchlorate (ClO_4^-) counterions in the slightly polar solvent *o*-dichlorobenzene (DCB) and polar EtOH [6]. The transient spectrum of the dye in DCB was interpreted as a superposition of the absorption spectrum from the excited electronic state of the neutral molecules and the absorption spectrum of free radicals that formed practically instantaneously [6]. The high formation rate of the radicals ($k \geq 0.6 \cdot 10^{13}$ s^{-1}) and the rather long lifetime of the S_1-state of the studied compound (1.8 ns) were explained by assuming that rapid phototransfer of an electron from the anion to the HITC dye cation and formation of An$^\bullet$ and HITC$^\bullet$ free radicals occurred only for a small part of the (tight) ion pairs.

The spectral properties of identical ion pairs can vary slightly depending on the solvent and can have different average distances between the ions [7]. Tight ion pairs with various distances between the ions are in dynamic equilibrium in an actual solution. Such pairs have similar spectral properties but can differ radically with respect to the possibility of electron transfer. It must be noted that the concept of penetrating ion pairs in which the volume of the ion pair is much less than the sum of the volumes of its component ions was introduced for polymethine dyes with specific bulky counterions in order to interpret results from phototransfer of charge in tight ion pairs [8].

Herein we present results from studies of the spectral-kinetic and photochemical properties of HITC dye under conditions where the ionic equilibrium was shifted toward an increased fraction of tight ion pairs by adding an additional salt to the solution.

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Experimental. The dye 1,3,1;1′,3′,3′-hexamethyl-2,2′-indotricarbocyanine (HITC) in three forms with different counterions (ClO_4^-, I^-, and Br^-) that were designated HITC(ClO_4), HITC(I), and HITC(Br) was studied. The purity of the dyes was checked by high-performance liquid chromatography (HPLC) and was also confirmed by the fact that their fluorescence spectra in EtOH were independent of excitation wavelength and their fluorescence excitation spectra were independent of recording wavelength.

The solvents EtOH and DCB were purified by standard methods [9]. An important factor in choosing the solvents was their practically identical viscosities at room temperature because the rate constants of intramolecular dissipation of electronic excitation energy in polymethine dyes depend on the medium viscosity and temperature [10, 11]. The photophysical properties of these dyes should be compared with identical solution viscosity and temperature-controlled samples.

Fluorescence and fluorescence excitation spectra and the degree of fluorescence polarization were recorded using a Fluorolog spectrofluorimeter (Spex); electronic absorption spectra, using a PV 1251A (Solar) or Specord M40 spectrophotometer. Fluorescence decay kinetics of the dyes in the nanosecond range were analyzed using a pulsed spectrofluorometer operating in the Spectroscopy Laboratory of IAPP [12]. The photodestruction quantum yield (Φ) of the dyes was determined by the literature method [13]. Solutions were degassed by multiple freeze–thaw cycles using a vacuum pump (down to 10^{-3} Torr).

Luminescence of singlet oxygen near 1270 nm was recorded using an apparatus constructed in the Laboratory of Molecular Photonics, IP, NAS, Belarus [14]. Radiation from a semiconducting laser with $\lambda = 667$ nm was used to excite the dyes. The experimental method was described before in detail [15]. The quantum yield of singlet-oxygen formation γ_Δ was determined by a relative method. The standards were 5,10,15,20-tetrakis(4-N-methylpyridyl)porphyrin in EtOH ($\gamma_\Delta = 0.77 \pm 0.04$) [16] and tetraphenylporphine (TPP) in DCB ($\gamma_\Delta = 0.74 \pm 0.05$). It is noteworthy that the emission lifetime of singlet oxygen was determined during the measurements with photosensitization by the standards and experimental compounds. The singlet-oxygen lifetime in DCB was 60 ± 2 μs; in EtOH, 12 ± 1 μs.

Transient absorption spectra were recorded using a femtosecond spectrometer [17] based on a Ti:sapphire original generator of femtosecond pulses [18] that was pumped synchronously by a pulsed Nd:YAG laser in passive synchronization mode and negative feedback. This system allowed pulses of length ~150 fs and energies up to 1 mJ to be tuned in the range 760–820 nm at repetition rate 10 Hz. Pulses of the main frequency that were produced at the amplifier output were divided into two parts with intensity ratio 1:4. The more intense beam passed through a controlled delay line and was used as the pump pulse. Changes of the optical density of the samples were recorded using a supercontinuum generated in a 1-cm cuvette with distilled H_2O by focusing on it the smaller part of the main frequency pulse. The resulting continuum allowed a probe signal in the range 350-1000 nm to be formed taking into account the spectral sensitivity of the photomultiplier. Radiation of the continuum was divided by a semi-transparent mirror into two pulses of identical intensity (reference and signal) that were focused in the sample using an optical mirror. The reference pulse passed through the cuvette with the test compound before passage of the exciting pulse. The signal pulse passed through the excited volume with a certain delay relative to the exciting pulse. Spectra of both pulses for each laser flash were recorded by a system based on a polychromator and CCD-array with subsequent data processing.

The half-width of the cross-correlation function was ~350 fs for all probe wavelengths. The minimal recorded change of optical density for each delay-line position with signal averaging over 100 pulses was $\sim 0.5 \cdot 10^{-3}$. The dye concentrations in samples for studies using the femtosecond spectrometer were $4 \cdot 10^{-6}$–10^{-5} M. Measurements were made in 2-mm cuvettes. The optical densities of the samples were adjusted to be identical at the excitation wavelength in order to ensure similar measurement conditions. The energy of the exciting pulses was ~200 μJ with pump beam diameter in the overlap region of the exciting and probe pulses of 6 mm. Band intensities and shapes of transient absorption spectra before the start and after the finish of a series of measurements did not differ, which indicated that the compounds were photostable.

Results and Discussion. The studies were carried out in EtOH and DCB for HITC(ClO_4), HITC(I), and HITC(Br), and also for HITC(Br) with tetrabutylammonium bromide (TBAB) added to the solution. This was done in order to determine if the ClO_4^- and I^- counterions were displaced from the dye molecules by Br^- upon adding the additional salt.

It was found that adding TBAB to EtOH solutions of HITC(Br) at concentrations up to 10^{-2} M did not cause changes in absorption and fluorescence spectra of the dye. The half-width and position of the spectral maxima did not change (Fig. 1, Table 1). The fluorescence quantum yield and lifetime in addition to the quantum yield of singlet-oxygen generation and photodestruction also did not change. Therefore, adding TBAB did not affect the photophysical and photochemical parameters of the dye molecules in EtOH solutions where the dye was found as the free ions.

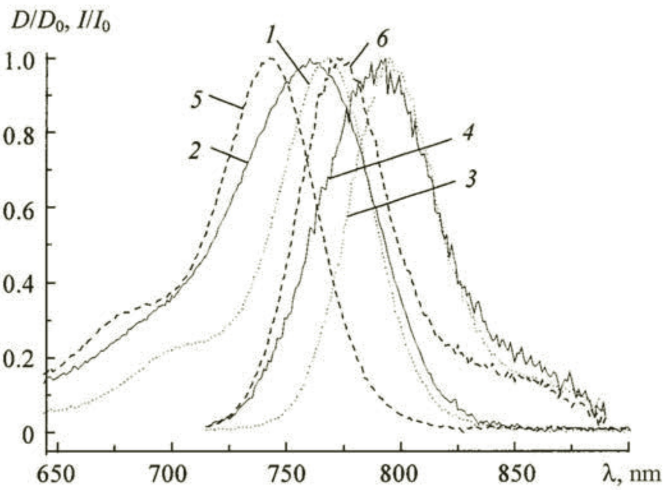

Fig. 1. Absorption (1, 2, 5) and fluorescence (3, 4, 6) spectra of HITC(Br) dye in EtOH (5, 6) and dichlorobenzene (1-4) and with added tetrabutylammonium bromide (10^{-2} M) (2, 4).

TABLE 1. Photophysical Properties of HITC(Br) Dye in Solutions

Solvent	λ^{a}_{max}, nm	$\Delta\lambda^{a}_{1/2}$, nm	λ^{f}_{max}, nm	$\Delta\lambda^{f}_{1/2}$, nm	τ, ns	φ_f	Φ	γ_Δ
Dichlorobenzene	768	54	795	45	2.0	0.42	$1.2 \cdot 10^{-6}$	$0.7 \cdot 10^{-2}$
Dichlorobenzene + 10^{-2} M TBAB	763	67	791	59	1.6	0.34	$4.3 \cdot 10^{-6}$	$1.1 \cdot 10^{-2}$
EtOH	742	53	773	48	1.4	0.28	$4.7 \cdot 10^{-7}$	$0.5 \cdot 10^{-2}$
EtOH + 10^{-2} TBAB	742	53	773	48	1.4	0.28	$4.7 \cdot 10^{-7}$	$0.5 \cdot 10^{-2}$

Note. λ^{f}_{max} is the fluorescence wavelength maximum; $\Delta\lambda^{f}_{1/2}$, the fluorescence spectrum half-width; λ^{a}_{max}, the absorption wavelength maximum; $\lambda^{a}_{1/2}$, the absorption spectrum half-width; τ, the lifetime of the molecules in the first excited singlet state recorded at the fluorescence spectrum maximum; φ_f, the fluorescence quantum yield; Φ, the photodestruction quantum yield; γ_Δ, the singlet oxygen generation quantum yield.

Adding TBAB (10^{-2} M) to slightly polar DCB shifted the HITC(Br) absorption spectrum by 5 nm to shorter wavelength and increased its half-width by 7 nm. The fluorescence spectrum of HITC(Br) shifted upon adding TBAB by 4 nm to shorter wavelength. The half-width increased by 14 nm. The fluorescence quantum yield of HITC(Br) decreased from 0.42 to 0.34; the fluorescence lifetime, from 2.0 to 1.6 ns. The photodestruction quantum yield increased from $1.2 \cdot 10^{-6}$ to $4.3 \cdot 10^{-6}$ whereas the quantum yield of singlet-oxygen formation increased from $0.7 \cdot 10^{-2}$ to $1.1 \cdot 10^{-2}$. The mechanism of singlet-oxygen generation involved transfer of excitation energy from dye molecules in the triplet state [19]. Thus, the increase by 1.5 times in the yield of $^1O_2$ formation in DCB upon adding the additional salt was consistent with a corresponding increase in the yield of HITC molecules in the triplet state. It should be considered that HITC molecules in DCB, in contrast with EtOH, were found as tight ion pairs [6]. Because the effect of an outer heavy atom on the probability of intersystem crossing depends on the distance [20–22], the increased formation of HITC(Br) molecules in the triplet state in DCB upon adding TBAB and the lack of change of this parameter in EtOH provided evidence that the distance between the dye cation and Br anion in the ion pair in DCB had decreased.

Thus, the recorded substantial changes of photophysical and photochemical properties of HITC in slightly polar DCB and their lack of change in highly polar EtOH indicated that adding TBAB shifted the dynamic equilibrium to the side of an increased fraction of ion pairs with closer ions (tight ion pairs).

Adding TBAB (10^{-2} M) to EtOH also did not cause any changes in HITC transient absorption spectra. Adding TBAB to slightly polar DCB increased the contribution of the band with $\lambda_{max} = 736$ nm that was due to absorption of free radicals

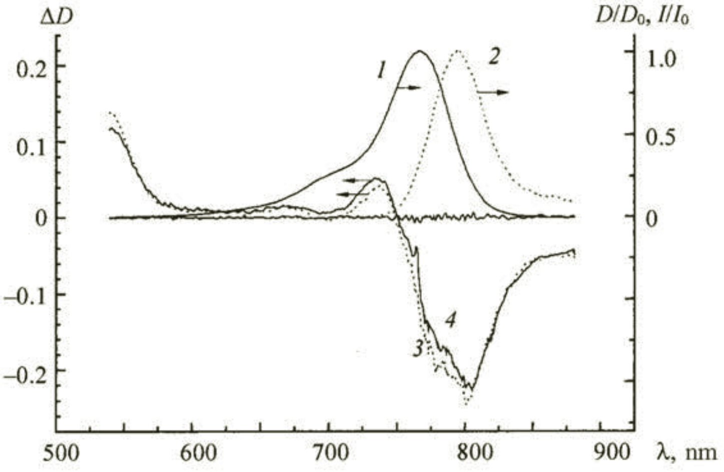

Fig. 2. Absorption (1), fluorescence (2), and transient absorption spectra of HITC(Br) dye in dichlorobenzene (3) and with added tetrabutylammonium bromide (10^{-2} M) (4).

TABLE 2. Photodestruction Quantum Yield of HITC (ClO_4^-, Br^-, I^-) in Dichlorobenzene and EtOH in Air-Saturated and Deoxygenated Solutions with Photodestruction by 740-nm Radiation

Counterion	ClO_4^-		I^-		Br^-			
Solvent	DCB	EtOH	DCB	EtOH	DCB	DCB + 10^{-2} M TBAB	EtOH	EtOH + 10^{-2} M TBAB
$^3O_2$	$1.6 \cdot 10^{-6}$	$1.3 \cdot 10^{-6}$	$3.2 \cdot 10^{-6}$	$1.4 \cdot 10^{-6}$	$1.2 \cdot 10^{-6}$	$4.3 \cdot 10^{-6}$	$1.2 \cdot 10^{-6}$	$1.2 \cdot 10^{-6}$
w/o $^3O_2$	$7.9 \cdot 10^{-8}$	$7.2 \cdot 10^{-8}$	$8.9 \cdot 10^{-8}$	$7.4 \cdot 10^{-8}$	$8.0 \cdot 10^{-8}$	$2.8 \cdot 10^{-7}$	$7.0 \cdot 10^{-8}$	$6.0 \cdot 10^{-8}$

to the total HITC(Br) transient absorption spectrum that was calculated for an identical number of excited molecules (Fig. 2). As noted above, adding TBAB to the slightly polar solvent also caused the quantum yield and the dye fluorescence lifetime to decrease. It should be noted that the proportion of the changes agreed approximately with the intensity increase of the new band. The results indicated that adding the additional salt to dye in the slightly polar solution led to an increased contribution from charge transfer in the ion pairs that formed free radicals to deactivation of the HITC(Br) excited state.

Radical formation should influence the efficiency of irreversible dye photobleaching. The principal photodestruction mechanism of cationic polymethine dyes in air-saturated solutions is self-sensitized oxidation by singlet oxygen [19]. Degassing solutions decreases by about two orders of magnitude the quantum yield of dye photodestruction [19], i.e., dye photodestruction by the self-sensitized oxidation mechanism is substantially decreased in de-oxygenated solutions. The manifestation of irreversible photobleaching as a result of the interaction of dye molecules with radicals or with the products of their reaction with the solvent should become more probable because of this. Therefore, the photodestruction quantum yields of HITC with I^-, Br^-, and ClO_4^- counterions in air-saturated and de-oxygenated solutions and with added additional salt that increased the fraction of tight ion pairs in solution were compared.

Table 2 presents the resulting quantum yields of HITC (ClO_4, Br, I) photodestruction in DCB and EtOH in air-saturated and de-oxygenated solutions for photolysis of samples by radiation with $\lambda = 740$ nm. Solutions with identical molar concentrations of the compounds were studied because of the dependence of the photodestruction quantum yield on the dye concentration.

Photodestruction of dyes with different counterions occurred with identical quantum yields in air-saturated EtOH solutions. Adding TBAB salt to a solution of HITC(Br) also did not change its value. The quantum yield of photodestruction of all dyes in de-oxygenated EtOH solutions decreased by more than an order of magnitude. The photodestruction quantum yields of HITC with different counterions were identical and did not change (within experimental uncertainty) upon adding TBAB to HITC(Br) solution.

The photodestruction quantum yields differed considerably in DCB for air-saturated solutions of dyes with different counterions, in contrast with highly polar EtOH. The photodestruction quantum yield for HITC(I) was double that of HITC(ClO_4) or HITC(Br). Taking into account the low solution concentrations (~10^{-5} M), the results indicated that the efficiency of intersystem crossing was substantially higher in the slightly polar solvent for the dye containing iodide anion than for dye with perchlorate or bromide. Such a correlation was indicative of the manifestation of the heavy-atom effect, which was possible only for a rather close approach of the anion and the polymethine chain of the dye cation [20–22].

Like in EtOH, degassing dye solutions in slightly polar DCB decreased the dye photodestruction quantum yield by more than an order of magnitude. However, this parameter was identical for dyes with different counterions, in contrast with the air-saturated solutions (Table 2). Also, the quantum yield of singlet-oxygen generation in slightly polar DCB was 9% for HITC(I); 0.5%, HITC(ClO_4); and 0.7%, HITC(Br), i.e., the values differed by more than an order of magnitude. Because sensitization of singlet-oxygen formation occurred as a result of transfer of electron excitation energy from HITC molecules in the triplet state [19], the fact that the photodestruction quantum yield in de-oxygenated DCB was independent of the type of counterion indicated that the HITC photodestruction mechanism under such conditions was not related to the population of the molecular triplet state and that self-sensitized oxidation was not a credible process for the de-oxygenated solutions. In addition, adding TBAB to de-oxygenated solutions of HITC(Br) dye in DCB, in contrast with polar EtOH, increased significantly (by 3.5 times) its photodestruction quantum yield. As shown above, adding TBAB was accompanied by an increase of induced absorption in the band due to radicals. This indicated that free radicals that formed by superfast electron transfer in tight ion pairs played the definitive role in HITC photodestruction in the de-oxygenated slightly polar solution.

Conclusion. A comparison of the photophysical properties of HITC dye with I^-, Br^-, and ClO_4^- counterions in slightly polar DCB and highly polar EtOH, an analysis of the trends of irreversible photobleaching of HITC in de-oxygenated and air-saturated solutions, and data from femtosecond spectroscopy led to the conclusion that free radicals formed as a result of superfast electron transfer upon photoexcitation of tight ion pairs.

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