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EXCITED STATE RELAXATION OF 9-(4-DIETHYLAMINOSTYRYL)-ACRIDINE

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Excited state dynamics of neutral and protonated 9-(4-diethylaminostyryl)-acridine was investigated by means of picosecond fluorescence and pump-and-probe absorption techniques. Both forms show complex transient absorption spectra evolution on a ps time scale and multicomponental transient absorption relaxation, strongly dependent on the probe wavelength. The protonated form shows very weak fluorescence and its relaxation kinetics slows down in more viscous solvents indicating that conformational changes of molecules take place during relaxation of the excited state and form a significant energy deactivation channel. During the relaxation of the neutral molecules two fluorescent intermediates with lifetimes of 10 and 90 ps are formed. Relaxation dynamics of the neutral molecules is independent of the solvent viscosity excluding role of conformational changes.

Keywords: aminostyrylacridines, acidochromic dyes, photoisomerization, picoseconds, transient absorption

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1. Introduction

Aminostyrylacridines are a sort of acidochromic dyes originally designed as pH sensitive sensitizers that absorb light in the visible and near infrared spectral regions [1]. They are promising indicators to be used in pH measurements in aggressive and/or microscopic media, where direct mechanical contact is deleterious and/or impossible, therefore, noninvasive technologies should be used. This is particularly important to pH measurements in cells and tissues, for example, in cancerous tissue, which was argued to have slightly reduced pH in comparison with normal tissue [2, 3]. An advantage in solving this problem might consist of making use of fluorescent pH probes. Aminostyrylacridines are promising to this application since their fluorescence is sensitive to the solvent acidity. Alterations in fluorescence are relatively easy detectable, however, it is also well known that several mechanisms such as electrochromism, dye reorientation, isomerization, and formation of twisted intramolecular charge transfer states can influence fluorescence properties of dyes [4–8]. Therefore, the processes taking place after molecule excitation should be known in detail in order to take into account the influence of specific environment conditions on fluorescence properties of the probe molecules.

Aminostyrylacridines are also interesting as photosensitizers in the photodynamic therapy of cancer. Due to their specific absorption dependences on pH in the red spectral region, those dyes may be used for more selective treatment of cancerous tissue under red light illumination and/or for the visualization of cancerous tissue.

The acidochromic dyes 9-(4-diethylaminostyryl)-acridines (DSA) depending on the solvent pH may turn into neutral and in protonated forms, structures of which are shown in the insert of Fig. 1. Their pK is fine-tunable by a variation of the electron donor capability of the dialkylaminophenyl moiety via a proper choice of the substituents R_1, R_2, and, to a lesser extent, R_3 [1].

Recently we investigated the excited state relaxation of protonated DSA molecules [9]. It was shown that large range conformational changes controlled by solvent viscosity are involved in the excited state deactivation. In this paper we present time-resolved absorption spectroscopy investigation of the primary processes of excitation energy relaxation in neutral DSA molecules and compare the relaxation mechanisms of both forms.

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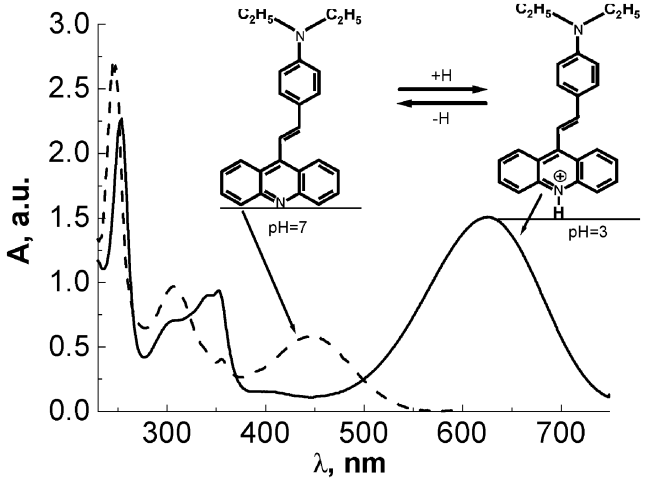

Fig. 1. Steady-state absorption spectra of the neutral and protonated DSA dye forms, measured in 1 cm cuvette, concentration $4.5{\cdot}10^{-5}$M. Insert shows the molecule structure in neutral and protonated forms.

The main aim of this investigation was to clarify the excited state dynamics of the two molecular forms in order to understand the mechanism how acidity and other parameters of environment may change their fluorescence properties.

2. Materials and methods

Details of the synthesis of DSA and related compounds are described elsewhere [1]. The measurements were performed in alcohols of different viscosity. A pH value of solutions was adjusted by adding HCl and NaOH to the solution. Steady-state absorption and fluorescence spectra were recorded using an Ocean Optics spectrophotometer PC1000 and a Perkin–Elmer fluorimeter LS-50B.

The picosecond transient absorption study was performed by a pump-and-probe spectrometer based on a home-made low repetition rate Nd:glass laser, delivering pulses of 2 ps duration. The second (527 nm) and third (351 nm) harmonics of the fundamental radiation were used for excitation, and a white light continuum generated in a water cell was used to probe samples. The continuum was split into two parts passing the sample at different positions, one overlapping with the excitation pulse and another few millimetres beside it, serving as the reference. The probe and reference pulses were focused onto the entrance slit of a monochromator, and their intensities were measured by two photodiodes located at the exit slit. Transient absorption behaviour at selected wavelengths was measured by changing the delay time between the excitation and probe pulses. Transient differential absorption spectra were recorded by scanning the monochromator wavelength at fixed delay times. Simultaneously the delay line was moved in order to compensate for the group velocity dispersion effect.

Fluorescence kinetics were recorded by using Time Correlated Single Photon Counting (TCSPC) technique. For these measurements, a home-made Ti:Sa laser generating pulses of 150 fs duration at 80 MHz repetition rate was used. Samples were excited by the second harmonic (400 nm) of fundamental laser radiation (800 nm). Sample fluorescence dynamics at different wavelengths was detected by a Hamamatsu multichannel plate photomultiplayer R3809U-50.

3. Results and discussion

The absorption spectrum of DSA strongly depends on the medium pH [1], the pK value being about 5.1. Figure 1 shows the steady-state absorption spectra of the neutral (pH $=$ 7) and protonated (pH $=$ 3) forms. A broad, unstructured long-wavelength band in the visible spectral region with the maximum at 444 nm and well-structured bands in the UV region characterize the absorption spectrum of the neutral form. The lowest energy absorption band of the protonated form is strongly shifted to the red side, the maximum being at about 620 nm. This form exhibits a considerably higher molar extinction coefficient than the unprotonated counterpart. The validity of the Beer's law, which has been proved at room temperature, shows that in the concentration range of $5{\cdot}10^{-7}$ to $5{\cdot}10^{-4}$M the dye exists in a monomeric form [10]. Neutral molecules show fluorescence with a very large Stokes shift. The fluorescence of protonated molecules, despite stronger absorption, was too weak to be measured properly.

Figure 2 shows the differential absorption spectrum of neutral DSA molecules at different delays. Because of the formation of the long-lived intermediate, the spectra could not be measured directly; therefore, they were constructed from transient absorption kinetics at 20 different wavelengths. The ΔA spectrum at zero delay time shows the absorption band bleaching and an induced absorption at long wavelengths stretching up to 850 nm. At 4 ps delay time, the spectrum changes substantially: the induced absorption around 600 nm slightly increases, while that around 700 nm is replaced by the negative signal. Since the molecules have no steady-state absorption in this spectral region, the negative signal should be attributed to the stimulated emission. The spectrum at 14 ps has a qualita-

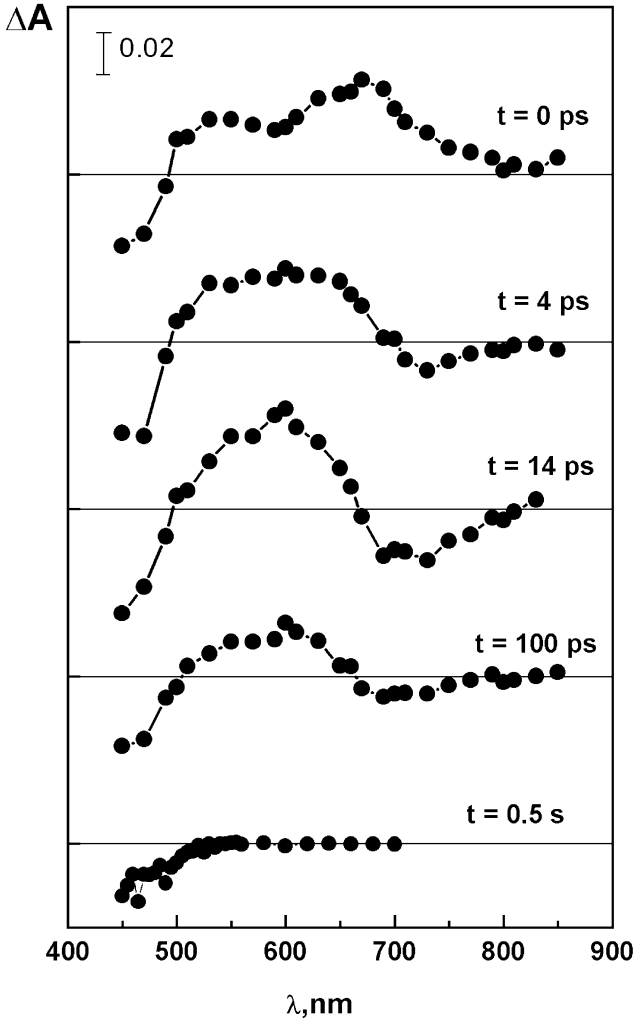

Fig. 2. Transient differential absorption spectra of neutral DSA in ethanol (pH = 7) recorded at various delay times after excitation.

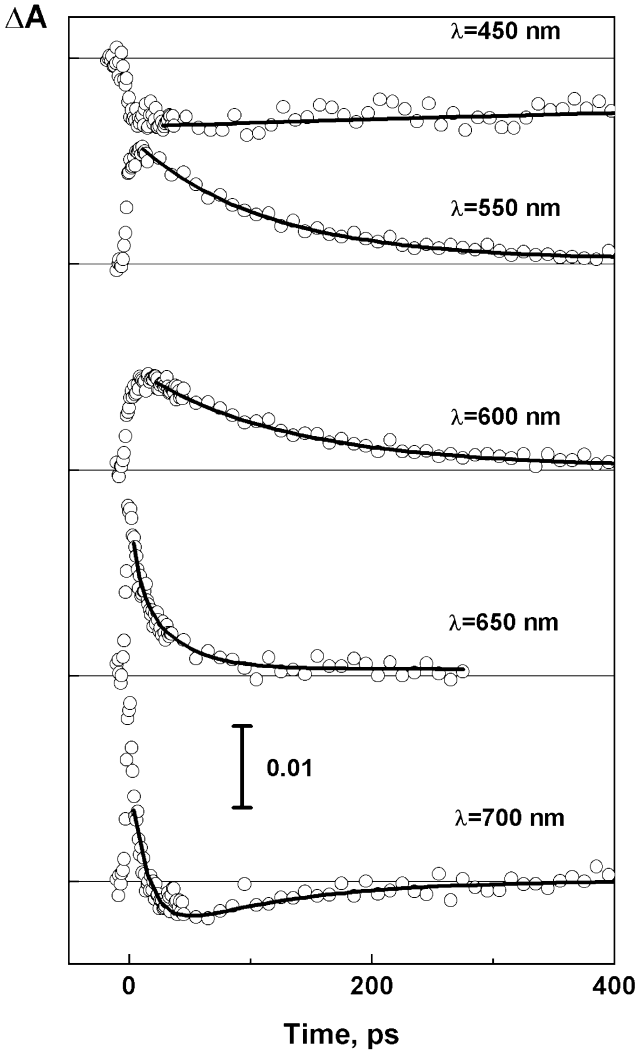

Fig. 3. Transient differential absorption kinetics of neutral DSA in ethanol measured at different probe wavelengths.

tively similar shape, but positive and negative signals at 600 and 700 nm, respectively, are stronger. Hence, an isobestic point at 630 nm is formed. When increasing the delay time further (100 ps) only relaxation of differential absorption spectra can be observed. Figure 5 also shows the differential absorption spectrum measured at 0.5 s after excitation (the delay time in this case was set electronically). At this delay time, only the bleaching of the main absorption band is observed, indicating the existence of long-lived species, which do not absorb in the visible spectral region.

Differential absorption kinetics at 700 nm (see Fig. 3) clearly shows two processes: decay of the induced absorption with 8 ± 2 ps time constant and decay of the delayed stimulated emission with 100 ± 10 ps time constant. Relaxation kinetics at other probe wavelengths is different, however, it may be approximated by using the same relaxation time constants, but different amplitudes. Only the recovery of the absorption bleaching at 450 nm is much slower. We do not see its relaxation during 1 ns. As it is evident from the differential absorption spectra (Fig. 2), weak absorption bleaching is still present at 0.5 s.

Neutral molecules show measurable fluorescence, which evolves in time (see Fig. 4). The maximum of the fluorescence band shifts to the longer wavelength side. The fluorescence spectrum may be decomposed into the short-wavelength and long-wavelength components with time dependent contributions (Fig. 4(b)). The short-wavelength component was obtained by subtracting 220 ps spectrum from 20 ps spectrum after their normalization in the long-wavelength region. The spectra recorded at other delay times may be quite satisfactory modelled as the sum of two components with different contributions. The short-wavelength compo-

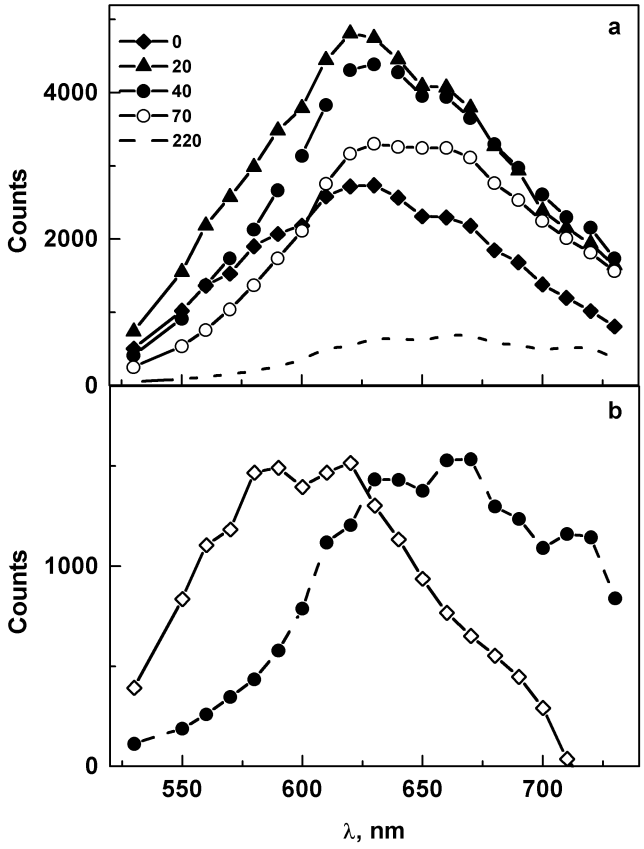

Fig. 4. (a) Time-resolved fluorescence spectra of neutral DSA recorded at different delay times and (b) decomposition of the fluorescence spectrum into two components having 10 ps decay time (open diamonds) and 90 ps decay time (solid circles).

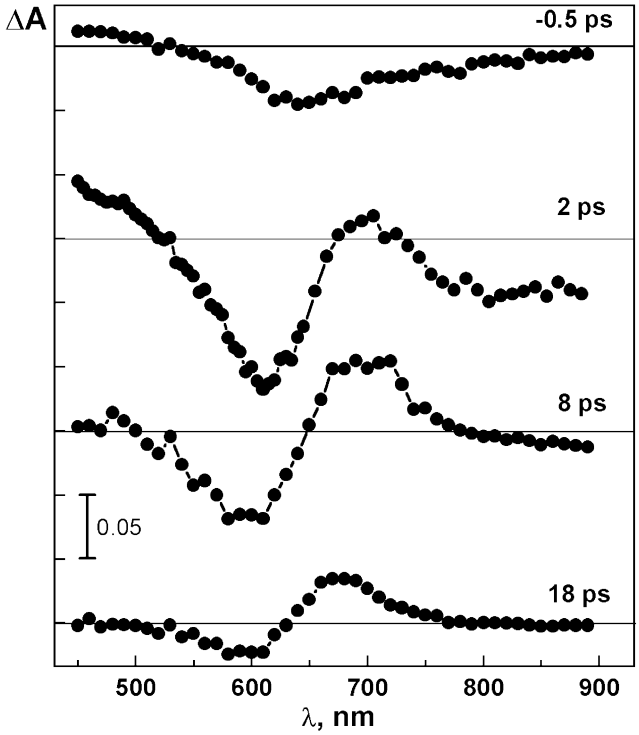

Fig. 5. Transient differential absorption spectra of protonated DSA in ethanol (pH = 3) recorded at various delay times after excitation.

nent is replaced by the long-wavelength component with about 10 ps time constant. The latter decays with about 100 ps time constant. This interplay between two fluorescence bands coincides with evolution of differential absorption spectra, where growth of transient absorption at 600 nm and stimulated emission at 700 nm in the time scale of 8 ps are observed.

For comparison, Figs. 5 and 6 show the main experimental results on the excited state relaxation of the protonated DSA molecules. The time-resolved differential absorption spectra of the protonated DSA in ethanol are presented in Fig. 5. Three characteristic shapes of the differential absorption spectrum observed at various delay times may be distinguished. At 0.5 ps, the wide negative differential absorption band around 650 nm should be attributed to the ground state absorption bleaching and stimulated emission, whereas the transient absorption around 420 nm is caused by the excited state absorption. At 2 ps delay time, the bleaching in the centre of the absorption band and the stimulated emission around 800 nm become stronger, however, bleaching around 700 nm turns into a weak induced absorption. At 18 ps delay, the short-wavelength induced absorption and the stimulated emission vanish indicating that the excited state is already depopulated, and only bleaching of the short-wavelength side of the absorption band and the induced absorption on the long-wavelength side are observed. This transient spectrum is caused by the shift of the ground state absorption band to the long-wavelength side. Figure 6 shows dynamics of the transient absorption. The monotonic differential absorption decay is observed only at 600 and 800 nm. At other wavelengths the decays have both positive and negative components, or long rise time, like at 550 nm. Time constants are different at different wavelengths what is typical of conformational changes. The transient absorption dynamics in isobuthanol, which is about four times more viscous than ethanol, is qualitatively similar but relaxation times are about 4–6 times longer.

The DSA molecule structure allows variety of conformational arrangements, and different arrangements may be energetically profitable in the ground and excited states. The transient absorption dynamics and its dependence on the solvent viscosity are typical of the excited state twisting. The relaxation process evidently involves three excited states: the Franck–

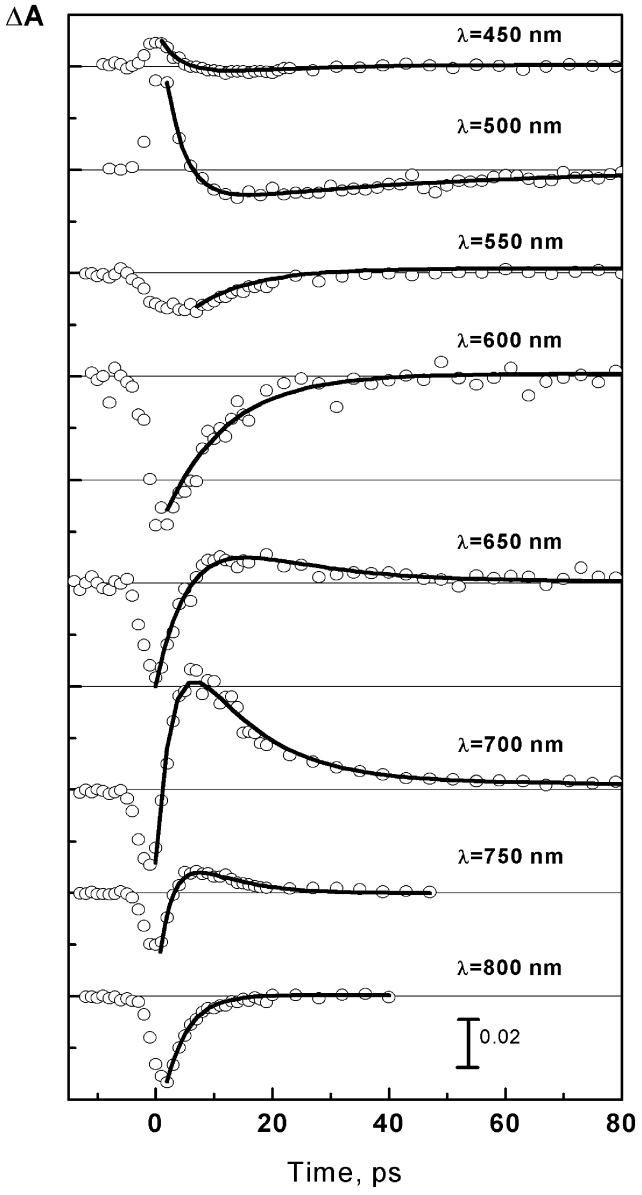

Fig. 6. Transient absorption kinetics of protonated DSA in ethanol (pH = 3) at various probe wavelengths.

Condon excited state, relaxed excited state, and the nonrelaxed ground state. Immediately after excitation, the molecule appears in the Franck–Condon excited state, which is nonequilibrium. The main optical properties of this state in ethanol are reflected by the 0.5 ps type ΔA spectrum. During about 2 ps, excited state twisting takes place. The ΔA spectrum at 2 ps reflects optical features of the molecules in the twisted excited state. The stimulated emission spectrum in this state is shifted to the long-wavelength side, and therefore, a gap in the negative signal appears around 700 nm. Decay of the stimulated emission at 800 nm and decay of the excited state absorption in the short-wavelength region with about 4 ps time constant reflect relaxation of the twisted excited state to the electronic ground state, which is also non-equilibrated. This state is characterized by the absorption shifted to the long-wavelength side. Relaxation of the shift-caused spectral changes within about 10–20 ps represents ground state stabilization.

The neutral molecules, as well as protonated, pass a sequence of at least three transient states, however, their properties are different from those participating in the excited state relaxation of the protonated molecules. The existence of isobestic points in differential absorption spectra and quite similar transient absorption and fluorescence lifetimes obtained at different wavelengths indicate that in this case the relaxation process may be described by abrupt transitions between different states, rather than by their gradual evolution. It is an evidence that the spectral evolution is not caused by conformational changes of molecules. This conclusion is also supported by investigations of the neutral DSA in a more viscous solvent, isobuthanol. Despite different viscosity, relaxation kinetics in both solvents within the experimental accuracy is identical. It indicates that the solvent controlled large amplitude molecular motions do not play any important role in the relaxation process.

Another important difference is revealed by the relaxation kinetics at 450 nm. The kinetics shows only slow absorption recovery, much slower than kinetics observed at other wavelengths. The absorption bleaching observed at 0.5 s confirms that intermediates nonabsorbing in the visible spectral region and with the lifetime of the order of subseconds are formed in course of the picosecond relaxation process.

Let us shortly discuss presumptive processes, which may be responsible for the excited state dynamics of neutral DSA. Triplet state formation [11–13], excited state reduction [14, 15], and relaxation between excited $\pi\pi^*$ and $n\pi^*$ states [11, 16–18] were reported for different acridine derivatives. The triplet state formation in some of acridines takes place very efficiently, however, it has already been reported [10] that there is no evidence of triplet state population of DSA dyes. Moreover, triplet state formation as an origin of the about 8 ps dynamics may be eliminated because of strong fluorescence and stimulated emission of molecular species formed during this process. These features are not typical of triplet states.

It was suggested in [14, 15] that the excited state relaxation of acridine occurs through its photoreduction, taking place in the $n\pi^*$ state. A similar pro-

cess, excited state electron transfer between acridines and different solvents or DNA, was also widely discussed [19]. Thus, excited state dynamics and formation of the long-lived intermediate could be related to the molecule photoreduction. However, photoreduction would lead to the formation of the protonated form, which has completely different spectral properties than those of the long-lived intermediate; namely, strong absorption in the visible spectral region.

The $n\pi^*$ states, which in acridines are close in energy to the lowest singlet $\pi\pi^*$ state, may also cause the relaxation peculiarities. These states were found to play a crucial role in the excited state relaxation of some acridine derivatives [11, 16–18]. In most cases, the $n\pi^*$ state of acridines is slightly higher than the $\pi\pi^*$ state, however, it was also suggested that positions of $\pi\pi^*$ and $n\pi^*$ states in aprotic solvents may be inverted [12, 20, 21]. But not of the 9-aminoacridines, where the gap between the $\pi\pi^*$ and $n\pi^*$ states is significantly larger [17]. This is evidently because DSA molecules have enlarged π electron system, which leads to the $\pi\pi^*$ state energy reduction, whereas it should not influence the energy of the $n\pi^*$ state. Therefore, $\pi\pi^*$ and $n\pi^*$ state energy inversion is not likely to take place in DSA molecules.

These considerations show that none of the widely discussed relaxational processes of acridines can explain excited state relaxation and formation of the long-lived intermediates. Further investigation is necessary to fully clarify the relaxation mechanism.

4. Conclusions

Spectral properties and excited state relaxation of the 9-(4-diethylaminostyryl)-acridines in neutral and acidic aqueous solutions were investigated. Absorption and fluorescence spectra and fluorescence quantum yield are very sensitive to the solvent acidity; the fluorescence yield drastically decreases in acidic solvents. The differences are related to the protonation of molecules in acidic solvents.

Both neutral and protonated 9-(4-diethylaminostyryl)-acridines show ultrafast excited state dynamics taking place on a time scale of tens of ps followed by slower relaxational processes, however, relaxation mechanisms of the two forms are different. The excited state evolution and relaxation of the protonated form are controlled by the solvent viscosity and are explained in terms of twisting in the excited state, and back twisting in the ground state. The relaxation mechanism of the neutral DSA form is different. The product state with the subsecond lifetime is formed during the excited state relaxation and the relaxation kinetics is not sensitive to the solvent viscosity excluding the role of the large amplitude conformational changes. None of several widely discussed excited state relaxation processes of acridines can fully explain the complex relaxation peculiarities.

Our investigation results explain the strong difference in the fluorescence efficiency of the neutral and protonated forms of DSA molecules. They predict strong dependence of the fluorescence efficiency of the protonated form on temperature and solvent viscosity. It should be taken into account in application of these molecules as pH sensors. Whereas, fluorescence of the neutral form, which is much stronger and independent of the solvent viscosity, is a good indicator of the environment acidity.

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References

[1] H. Lindauer, P. Czerney, and U.W. Grummt, 9-(4-dialkylaminostyryl)-acridines – a new class of acidochromic dyes, J. Prakt. Chem. **336**, 521 (1994).

[2] J.R. Griffiths, Are cancer cells acidic?, Br. J. Cancer **64**, 425 (1991).

[3] I.F. Tannock and D. Rotin, Acid pH in tumors and its potential for therapeutic exploitation, Cancer Res. **49**, 4373 (1989).

[4] A. Mishra, G.B. Behera, M.M.G. Krishna, and N. Periasamy, Time-resolved fluorescence studies of aminostyryl pyridium dyes in organic solvents and surfactant solutions, J. Lumin. **92**, 175 (2001).

[5] D. Braun, W. Rettig, S. Delmond, J.-F. Letard, and R. Lapouyade, Amide derivatives of DMABN: A new class of dual fluorescent compounds, J. Phys. Chem. **101**, 6836 (1997).

[6] R. Lapouyade, K. Czeschka, W. Majenz, W. Rettig, E. Gilbert, and C. Rulliere, Photophysics of donor–acceptor substituted stilbenes. A time-resolved fluorescence study using selectively bridged dimethylamino cyano model compounds, J. Phys. Chem. **96**, 9643 (1992).

[7] W. Rettig, Ladungstrennung in angeregten Zuständen entkoppelter Systeme – TICT – Verbindungen und Implikationen für die Entwicklung neuer Laserfarbstoffe

sowie für den Primärprozess von Sehvorgang und Photosynthese, Angew. Chem. **98**, 969 (1986).

[8] W. Rettig, Photoinduced charge separation via twisted intramolecular charge transfer states, in: *Topics in Current Chemistry, Electron Transfer I*, ed. J. Mattay (Springer Verlag, Berlin, 1994) p. 169, 253.

[9] P. Grigaravičius, V. Gulbinas, U.-W. Grummdt, and R. Rotomskis, Excited state relaxation of protonated 9-(4-diethylaminostyryl)-acridine, Lithuanian J. Phys. **42**, 93 (2002).

[10] U.W. Grummt, E. Birckner, H. Lindauer, B. Beck, and R. Rotomskis, Deactivation of excited 4′-dialkylamino-9-styrylacridines, J. Photochem. Photobiol. A: Chem. **104**, 69 (1997).

[11] O. Rubio-Pons, L. Serrano-Andres, and M. Merchan, A theoretical insight into the photophysics af acridine, J. Phys. Chem. A **105**, 9664 (2001).

[12] L.A. Diverdi and M.R. Topp, Subnanosecond time-resolved fluorescence of acridine in solution, J. Phys. Chem. **88**, 3447 (1984).

[13] S.L. Shapiro and K.R. Winn, Picosecond kinetics of acridine in solution, J. Chem. Phys. **73**, 5958 (1980).

[14] K. Kikuchi, K. Kasama, A. Kanemoto, K. Uji-ie, and H. Kokubun, Reaction and deactivation of excited acridine in ethanol, J. Phys. Chem. **89**, 868 (1985).

[15] A. Kira, S. Kato, and M. Koizumi, Studies of the photoreduction of acridine in ethanol by the flash technique, Bull. Chem. Soc. Jpn. **39**, 1221 (1966).

[16] K. Kasama, K. Kikuchi, S. Yamamoto, K. Uji-ie, Y. Nishida, and H. Kokubun, Relaxation mechanism of excited acridine in nonreactive solvents, J. Phys. Chem. **85**, 1291 (1981).

[17] K. Kasama, K. Kikuchi, Y. Nishida, and H. Kokubun, Deactivation mechanism of excited acridine and 9-substituted acridines in water, J. Phys. Chem. **85**, 4148 (1981).

[18] V. Zanker and G. Prell, Blitzlichtphotolyse an Acridin und Acridan im Temperaturbereich von $+25\,^{\circ}$C bis $-180\,^{\circ}$C, Ber. Bunsenges. Phys. Chem. **73**, 791 (1969).

[19] K. Fukui, K. Tanaka, M. Fujitsuka, A. Watanabe, and O. Ito, Distance dependence of electron transfer in acridine-intercalated DNA, J. Photochem. Photobiol. B: Biol. **50**, 18 (1999).

[20] V. Sundstrom, P.M. Rentzepis, and E.C. Lim, Electronic relaxation of acridine as studied by picosecond spectroscopy, J. Chem. Phys. **66**, 4287 (1977).

[21] O. Rubio-Pons, L. Serrano-Andrés, and M. Merchán, A theoretical insight into the photophysics of acridine, J. Phys. Chem. A **105**, 9664 (2001).

9-(4-DIETILAMINOSTIRIL)-AKRIDINO SUŽADINTOSIOS BŪSENOS RELAKSACIJA

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Santrauka

Protonuotų ir neutralių 9-(4-dietilaminostiril)-akridino (DSA) molekulių sužadintos būsenos relaksacija buvo tirta pikosekundinės sugerties žadinimo ir zondavimo bei laikinės fluorescencijos metodais. Tų molekulių fluorescencijos savybės labai priklauso nuo aplinkos rūgštingumo, todėl jos gali būti taikomos kaip neinvaziniai pH jutikliai. Tačiau fluorescencijos savybės priklauso ir nuo kitų aplinkos parametrų. Darbo tikslas buvo išsiaiškinti abiejų formų DSA molekulių sužadintos būsenos relaksaciją, kad būtų galima suprasti fluorescencijos ypatumus.

Tyrimais parodyta, kad abiejų formų molekulių sužadintos būsenos relaksuoja per kelias tarpines būsenas, tačiau jų relaksacijos mechanizmai visiškai skirtingi. Išskirtos trys protonuotų molekulių tarpinės būsenos, kurių susidarymo ir relaksacijos spartos stipriai priklauso nuo tirpiklio klampos. Tarpinių būsenų spektroskopinės savybės ir relaksacijos ypatybės parodė, kad greitą sužadintosios būsenos relaksaciją ir mažą fluorescencijos našumą lemia molekulės konformaciniai pokyčiai, vykstantys esant sužadintai būsenai.

Neutralių molekulių relaksacijos metu realizuojasi dvi fluorescuojančios tarpinės būsenos. Jų relaksacijos trukmės yra 8 ± 2 ps ir 100 ± 10 ps ir nepriklauso nuo tirpiklio klampos. Tai rodo, kad neutralių molekulių sužadintos būsenos relaksacija nėra susijusi su molekulių konformaciniais pokyčiais. Aptarti keli relaksacijos mechanizmai susiję su sužadintos būsenos fotoredukavimu, tripletinės būsenos susidarymu bei šuoliais tarp $\pi\pi^*$ ir $n\pi^*$ būsenų. Tačiau kol kas tikslus relaksacijos mechanizmas išlieka neaiškus.

Apibendrinant, mūsų rezultatais paaiškinamas protonuotos ir neutralios DSA molekulių formų fluorescencijos efektyvumo skirtumas. Jais parodoma, kad protonuotos formos fluorescencija nėra tinkamas parametras naudojant šias molekules kaip pH jutiklius. Tačiau neutralios formos fluorescencija, kuri yra žymiai stipresnė ir nepriklauso nuo aplinkos klampos, yra geras rūgštingumo indikatorius.