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Fluorescence Depolarization Kinetics of Neutral and Charged 2-(3'-Pyridyl)oxazole

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Abstract — The results of measurements of the starionary and time-resolved flurescence anisotropy and of the AM1 and INDO/S calculations were used for conformational analysis of a neutral molecule of the 2-(3'-pyridyl)oxazole series and of its *N*-ethyl cation. Most probably, the excitation of the quasi-planar rotamers of the cation, in contrast to the neutral molecule, is accompanied by a ~90° turn of its Et–py fragment with the formation of a twisted charge-transfer conformation. According to the data obtained for ethanol and glycerol solutions and for the poly(methyl methacrylate) matrix at 20°C, the efficiency of the intramolecular relaxation is independent of the viscosity.

Structural relaxation of fragments of 2-(3'-pyridyl)-oxazole cations was suggested as one of the pathways of deactivation of their singlet excited state [1]. This follows from the abnormally high Stokes shift of the fluorescence (up to 200 nm) and by a considerable (by a factor of 3.2–3.4) increase in the rate constant of their radiative deactivation (k_f) in normal alcohols with a decrease in temperature from 20 to –153°C, which is not characteristic of the initial neutral molecules. Relaxation of this type depends on temperature and on the viscosity and polarity of the medium. A continual model with the emission occurring from any molecular state with different extents of relaxation of the fragments is assumed to be applicable to this case [2].

If a mutual turn of fragments of an excited fluorophore is accompanied by a change in the angle between the directions of the radiative transition moment, the method of time-resolved fluorescence anisotropy allows direct detection of this intramolecular motion against the background of slower fluorescence depolarization caused by thermal rotation of the molecule as a whole. This specific feature of intramolecular dynamics is widely used in studies of the conformational flexibility of proteins [3]. The goal of this study was to check the hypothesis of structural relaxation of the compounds in question using the method of time-resolved fluoresence anisotropy for its detection in combination with quantum chemical calculations.

According to the calculations, the first excited state of **I** and **Ia** is of the $\pi \rightarrow \pi^*$ type with the charge transfer (0.32 and 0.91 e, respectively) from the phenyloxazole fragment to the Py (or Et–Py) fragment. The difference $\mu_e - \mu_g$ for planar conformers of **I** and

Absorption (λ_a^{max}) and fluorescence (λ_f^{max}) maxima, fluorescence quantum yields (φ_f), fluorescence lifetimes (τ_f), oscillator strengths of transitions (f), and differences of the dipole moments ($\mu_e - \mu_g$) in excitation of the planar [0° (*N-trans*), 180° (*N-cis*)] and twisted (90°) forms of **I** and **Ia**, and the differences $\mu_e - \mu_g$ obtained by the method of solvatochromic shifts (*) [4]

Comp. no.	Solvent	λ_a^{max}, nm	λ_f^{max}, nm	φ_f	τ_f, ns	f			$\mu_e - \mu_g$, D			
						0°	90°	180°	0°	90°	180°	*
I	Ethanol	310	378	0.63	0.6	0.851	0.809	0.858	5.6	1.9	5.7	5.0
	Glycerol	312	388	0.23	0.4							
Ia	Ethanol	332	529	0.l5	2.7	0.199	0.0004	0.188	20.4	23.7	19.9	12.0
	Glycerol	336	518	0.21	3.3							

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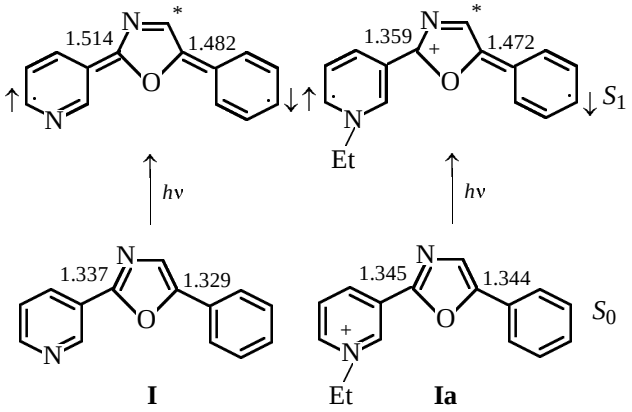
Ia ranges from 5.6 to 20.4 D (see table). The electron density redistribution upon excitation can be illustrated by the following mesomeric structures (figures denote the bond orders at the central oxazole ring):



Apparently, upon excitation of the neutral compound, the interaction between the central and side fragments of the molecule becomes somewhat stronger, whereas excitation of the cation makes stronger only the phenyl–oxazole bond. The dependence of the energy of formation of structures **I** and **Ia** on the dihedral angle θ between the Py (or Et–Py) and oxazole moieties in the S_0 and S_1 states is shown in Fig. 1. The conformation of the molecules with θ 0° corresponds to that shown in the scheme (planar *N-trans* conformer). For both compounds in the ground electronic state, the planar conformers are the most stable. The barrier to *N-trans* → *N-cis* (preferential conformation) transformation in **Ia** is 17.0 kJ mol^{-1}, which is consistent with the experimental value for styrenes, 16–20 kJ mol^{-1} [5]. As follows from the Boltzmann distribution of molecules with respect to energy at 20°C, the mean twisting angle in the cation in the S_0 state is 12.2°. Thus, the calculations suggest that, in solution, the neutral compound is an equimolar mixture of quasi-planar *cis* and *trans* rotamers, and the charged compound is a mixture with a high content of quasi-planar *cis* rotamers. In the excited state, the energy profiles of structures **I** and **Ia** differ significantly. The preferential conformation of the excited neutral molecule remains planar, whereas for the cation it becomes twisted (θ 90°). The radiative transition from the twisted state in a vacuum is largely forbidden (see table).

Figure 2 shows how the angle α between the directions of the radiative transition moments in cation **Ia** in the Franck–Condon state and in the state characterized by the turn of the Et–Py fragment by angle θ depends on angle θ. It is seen that, in excitation of the

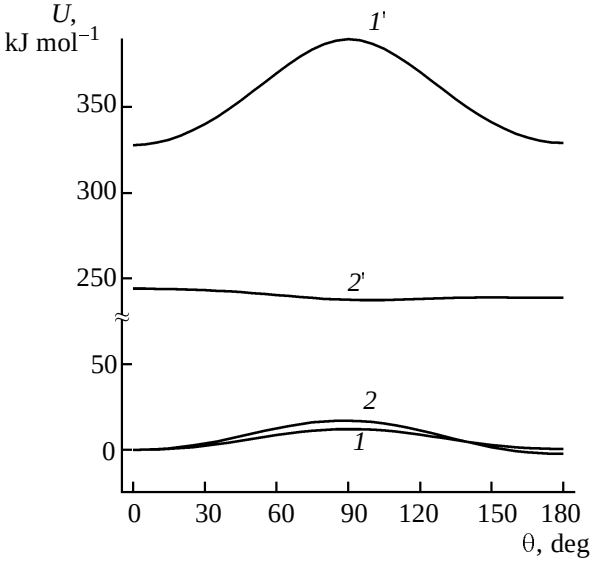


Fig. 1. Potential energy of (*1*, *1*') **I** and (*2*, *2*') **Ia** in the (*1*, *2*) S_0 and (*1*', *2*') S_1 states in a vacuum as a function of the dihedral angle θ between the Py (or Et–Py) and oxazole moieties.

trans conformer, an increase in θ to ~75° does not cause an appreciable increase in α, whereas in the excitation of the *cis* form, on the contrary, α is highly sensitive to changes in θ. Thus, it is justified to use the method of time-resolved fluorescence anisotropy for detecting the structural relaxation in the compounds under consideration.

The Stokes shift of the fluorescence of cation **Ia** decreases from 197 to 182 nm on replacement of ethanol

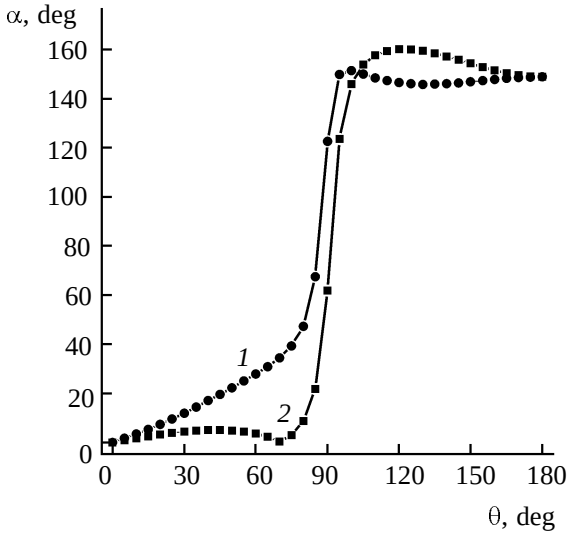


Fig. 2. Angle α between the directions of the radiative transition moments in the excited (*1*) *cis* and (*2*) *trans* rotamers of cation **Ia** as a function of the dihedral angle θ between the Et–Py and oxazole moieties.

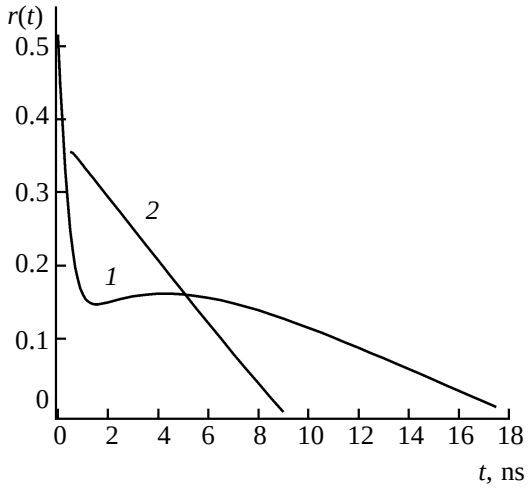


Fig. 4. Fluorescence depolarization kinetics of (*1*) **Ia** and (*2*) **I** in glycerol at 20°C. Excitation wavelengths 331 and 313 nm; observation wavelengths 525 and 400 mn. (*1*) $r(t) = 0.54\exp(-t/0.55) + 0.8\exp(-t/5.67) - 0.83\exp(-t/3.1)$ and (*2*) $r(t) = 1.92\exp(-t/2.6) - 1.36\exp(-t/1.7)$.

by glycerol. For the neutral molecule of **I**, the same quantities are 68 and 76 nm. The fluorescence lifetimes (τ_f) of the cation in these solvents are 2.7 and 3.3 ns; the functions of universal interaction $f(\varepsilon, n) = (2n^2 + 1)(\varepsilon - 1)/[(n^2 + 2)(\varepsilon + 2)] + (n^2 - 1)/(n^2 + 2)$ (where ε is the dielectric permittivity and *n*, refractive index) of ethanol and glycerol are close (0.2887 and 0.263); only their viscosities differ essentially (1.2 and 1412 cP at 20°C). Therefore, the decrease in the Stokes shift of **Ia** may be due to the fact that, in highly viscous glycerol at 20°C, relaxation occurs at a lower rate and within the lifetime of the excited state does not occur to the same extent as in ethanol. This assumption is supported by the fact that, in the case of cationic ethyl derivative of 2-(3-quinolyl)-1,3-benzothiazole, similar replacement of the solvent causes the Stokes shift of the fluorescence to decrease from 160 to 132 nm [6]. In this case, the relaxation of the bulkier ethylquinolinium fragment should be expected to be more sensitive to the solvent viscosity. Thus, measuring the fluorescence depolarization kinetics in glycerol seems to be preferable from the viewpoint of resolving power of the apparatus.

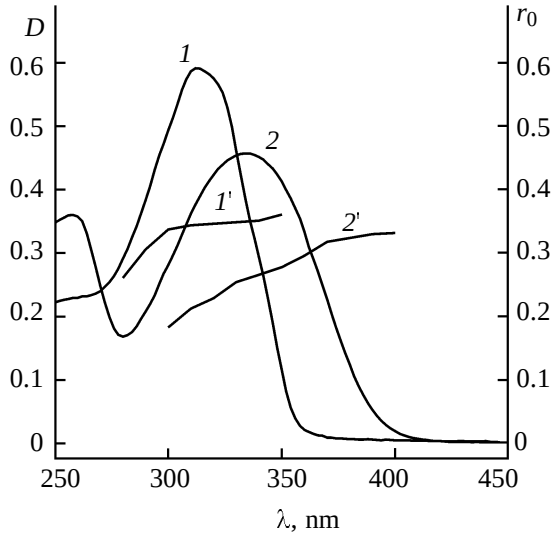


Fig. 3. (*1, 2*) Absorption and (*1', 2'*) polarization spectra of (*1, 1'*) **I** and (*2, 2'*) **Ia** in glycerol at 20°C. Excitation wavelengths 313 and 331 nm.

The polarization spectra of **I** and **Ia** in glycerol, recorded within the first long-wave absorption bands of these species, contain no minima with negative limiting emission anisotropy r_0 (Fig. 3). This fact allows us to rule out the probability of simultaneous excitation of two mutually orthogonal oscillators of the long-wave absorption, which could complicate interpretation of the kinetics.

The fluorescence depolarization kinetics of **I** and **Ia** in glycerol is shown in Fig. 4. Both times of the rotational correlation of the neutral molecule are shorter than those of the cation. This may be due to slower rotation of the cation because of its larger volume and weight. The depolarization kinetics of **Ia** in ethanol, glycerol, and poly(methyl methacrylate) involves also a fast component with the correlation time of ~0.5 ns,

observed in the first 1.5–2.2 ns after the excitation. It can reflect the changes in the molecular conformation proper.

In accordance with Eq. (1) describing a decrease in the anisotropy of an isotropic fluorophore solution due only to the photoselection (0.4) and angular shift of the absorption and emission dipoles [$\alpha(t)$ is the angle between these dipoles] [7], a decrease in the anisotropy in 1.5 ns due only to the fast component of the depolarization corresponds to the angle α of ~41°:

$$r(t) = 0.4\left(\frac{3\cos^2\alpha(t) - 1}{2}\right). \quad (1)$$

Here $r(t)$ is the fluorescence anisotropy as a function of time. In accordance with Fig. 2, this angle corresponds to the turn of the initially excited *trans* and *cis* forms of the cation by 88° and 76°, respectively. Taking into account the measurement errors, we can assume that this angle is close to 90°. The fluorescence depolarization kinetics of **I** and **Ia** in EtOH and poly(methyl methacrylate) are similar in character to that observed in glycerol, which suggests that the relaxation of the fragments at 20°C is not noticeably braked in the examined range of viscosities.

However, an alternative explanation is also possible: The fast component of the depolarization may also be caused by a turn of the radiation oscillator due to the relaxation of the reactive field of the solvent molecules surrounding the fluorophore [8]. The following factors count against such an interpretation. First, the decay correlation times of the fast component are approximately equal in glycerol, ethanol, and poly(methyl methacrylate), despite essentially different surrounding of the fluorophore. Second, the fast decay component is never present in the case of the neutral molecule (**I**). The fact that the fast components of the decay curves virtually coincide in ethanol, glycerol, and methyl methacrylate, despite essential differences in the viscosity, suggests that the microviscosity of the nearest surrounding of the fluorophore (the parameter relevant to our case) differs only slightly in these three media.

To understand better the kinetic aspects of the phenomenon in question, it would be appropriate to perform additional experiments in wide ranges of viscosity and temperature.

EXPERIMENTAL

The stationary and time-resolved fluorescence anisotropy were recorded in a synchrotron radiation beam of an S-60 synchrotron (Lebedev Physical Institute, Russian Academy of Sciences) with an installation for studying the kinetics of the decay of the intensity and anisotropy of the fluorescence of biological objects by single photon counting [9]. The experimental procedure is described in [10]. We successively measured the decay of the fluorescence intensity for the two components polarized parallel [$I_h(t)$] and perpendicular [$I_v(t)$] to the polarization of the excitation radiation, and also the shape of the excitation pulse. The fluorescence anisotropy was calculated as

$$r(t) = \frac{i_h(t) - i_v(t)}{i_h(t) + 2i_v(t)}, \quad (2)$$

where $i_h(t)$ and $i_v(t)$ are the intensities of the fluorescence decay for the same components after the deconvolution.

The optimal molecular geometry was calculated by the AM1 semiempirical method using the MOPAC-7.0 program in the approximation of the planar geometry (C_s symmetry). The potential energy of the conformers in the ground state was calculated similarly from the geometric data for the planar structure, with varying the angle between the planes of the phenyloxazole and pyridyl (pyridinium) fragments. The energies of molecules in the excited state, differences between their dipole moments in the excited and ground states ($\mu_e - \mu_g$), and $S_0 \rightarrow S_1$ transition moments were calculated by the INDO/S method (MOSF-4.2 program) taking into account 100 lowest-energy singly excited configurations. The bond orders were calculated from the contributions of the p_z orbitals of the directly bonded carbon atoms to the highest occupied and lowest unoccupied molecular orbitals.

3-(5-Phenyloxazol-2-yl)pyridine **I** and 1-ethyl-3-(5-phenyloxazol-2-yl)pyridinium toluene-4-sulfonate **Ia** were used without additional purification. The procedure for preparing **I** is described elsewhere [11, 12]. Cation **Ia** was prepared by refluxing **I** with ethyl tosylate in BuOH, which was followed by recrystallization of the product from EtOH/Et_2O. Ethanol was dehydrated by distillation from CaH_2. Chemically pure grade glycerol was used without additional purification. A poly(methyl methacrylate) film was prepared by evaporation of the solvent from a solution of ultrapure grade poly(methyl methacrylate) in acetone. The absorption and fluorescence spectra were recorded on a Shimadzu-3100 spectrophotometer and an Elyumin-2M spectrofluorimeter. The fluorescence quantum yields φ_f were calculated by comparing the areas under the corrected fluorescence spectra of the fluorophores in ethanol and quinine bisulfate in 1 N H_2SO_4 (φ_f 0.546) [13]. All the measurements were performed at 20°C.

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