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Fluorescence Quenching of Auramine in Fluid Solutions: A Femtosecond Spectroscopy Study

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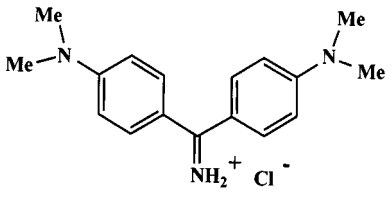
The quenching of Auramine fluorescence in ethanol is studied by two ultrafast spectroscopy techniques. The gain band, probed by transient absorption spectroscopy, vanishes in a few picoseconds, while a transient absorption band rises and the ground-state repopulation is delayed. In up-conversion experiments, nonexponential wavelength-dependent fluorescence decays are observed. The average decay times increase with the wavelength and the reconstructed instantaneous spectrum exhibits a few hundred-wavenumber red shift and a broadening while its intensity drops. The previously proposed relaxation model, involving a barrierless internal twisting motion toward a transient dark state, is further examined. In particular, the extinction coefficients of the transient state are extracted from the differential absorption spectra. The band is found to lie in the same wavelength range as the dimethylaniline cation radical. This result is discussed as a possible support for an internal twisting process involving a charge shift.

KEY WORDS: Fluorescent probe; ultrafast spectroscopy, barrierless twisting; charge shift.

INTRODUCTION

Auramine is a yellow, cationic, dimethylamino-substituted diphenylmethane dye, the structure of which is shown in Scheme I. It is weakly fluorescent in water and in low-viscosity solvents and highly fluorescent in viscous solvents or in the presence of DNA and polymeric acids [1–3].



Scheme I.

Various uses of Auramine, as a fluorescent probe for investigating the structure and function of proteins [4–6] or the physical aging of polymers [7,8] and as a fluorochrome in vitro biological applications [9], have been reported. However, the radiationless process which quenches Auramine fluorescence when the molecule is free from local strain is not yet completely understood. Oster and Nishijima [3] proposed that in fluid solvents the dominant photoinduced process is an internal conversion process via the rotational diffusion of the phenyl rings. In previous studies of Auramine in solvents of different viscosity, we demonstrated that its photophysical behavior is very similar to that of triphenylmethane (TPM) dyes [10,11], which are generally considered as model compounds for barrierless internal twisting [12,13]. There are still very few studies of Auramine despite of its many applications, whereas TPM dyes have been investigated

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extensively by fast spectroscopy in the last decades (see references in Refs. 10 and 13–15). In the present paper, we reexamine Auramine photophysics in ethanol by ultrafast spectroscopy. We proposed previously that the fluorescence quenching is due to fast photoinduced barrierless internal twisting accompanied by charge shift [10,11]. Here we focus our interest on the spectral characterization of the transient state formed in the relaxation pathway and discuss its formation and deactivation mechanism.

EXPERIMENTAL

Time-resolved transient absorption and gain spectra were measured by the pump-probe technique using a 700-fs, 20-μJ, pump-pulse at 425 nm and a continuum probe produced by focusing a 300-fs, 200-μJ, 710-nm pulse in a 1-cm water cell. The 425- and 710-nm synchronized pulses were generated by the same dye laser system using a nano- to subpicosecond-pulse shortening method [16]. The differential absorption spectra were measured in a two-beam probe arrangement as in Ref. 17. Measurements were carried out at the magic angle and accumulated over 500 laser shots. The samples were recirculated in a 1-mm flow cell. The FWHM of the pump-probe cross-correlation function was estimated to be about 1.5 ps. The transient spectra were corrected for the group velocity dispersion of the probe pulse.

Fluorescence decays were measured by the fluorescence up-conversion technique using the second harmonic of a 80-fs, 10-nJ, 840-nm, cw, Ar^+ laser-pumped, Ti:sapphire laser (Tsunami) as the pump pulse and the fundamental frequency as the gating pulse in a setup described elsewhere [18]. The pump beam was focused onto a 1-mm cell containing the Auramine solution. The sample holder was moved back and forth, perpendicularly to the excitation beam, to prevent heating of the sample. The fluorescence decays were measured under magic angle conditions. The up-converted signal was accumulated for 1 s, for each time-delay step. Decay measurements were performed using typical step sizes of 20 fs. The instrumental response was estimated to be roughly 150 fs (FWHM) from the cross-correlation function of the gating and pump pulses. Steady-state spectroscopy was studied by means of a Cary 210 spectrophotometer and an Hitachi–Perkin Elmer fluorimeter.

Auramine [4,4′-(imidocarbonyl)bis(*N*,*N*-dimethylaniline-monohydrochloride)] was purchased from Aldrich (80%) and purified by several sublimations to eliminate a fluorescent impurity which could be detected when Auramine solutions were excited near 350 nm. UV-spectroscopy-grade ethanol (Merck) was used as the solvent. The Auramine concentration was chosen to be $2 \times 10^{-4}\,M$ for pump-probe experiments and 5×10^{-4} M for fluorescence up-conversion experiments. The experiments were carried out at room temperature.

RESULTS

The ground state absorption of Auramine in ethanol exhibits a maximum at 432 nm. An estimation of its extinction coefficient leads to $\varepsilon = 27{,}200\ M^{-1}\ cm^{-1}$. From Strickler and Berg's expression [19] the excited-state radiative lifetime is found to be about $\tau_{rad} = 6.1$ ns [20]. These values are in good agreement with those reported by Oster and Nishijima in glycerol ($\varepsilon = 27{,}400\ M^{-1}\ cm^{-1}$ and $\tau_{rad} = 4.3$ ns) [3]. The uncorrected fluorescence spectrum was found to be broad and lie in the 430- to 650-nm range, with a maximum around 490–500 nm. By extrapolating Oster and Nishijima's curve of $1/\phi_{Fl}$ versus T/η for Auramine in ethanol at room temperature, i.e., for a viscosity of $\eta = 1.08$ cP, we roughly estimated a fluorescence yield ϕ_{Fl} of 4×10^{-3} [20].

The time-resolved differential absorption spectra (ΔD) of Auramine in ethanol after subpicosecond excitation at 425 nm are shown in Fig. 1. The steady-state absorption and fluorescence spectra are also drawn to show the regions where the bleaching of the sample absorption and the gain are expected. At short delays, ΔD is negative in the whole spectral range, due to a dominant bleaching below 460 nm and a dominant gain above 470 nm. A fast decrease in the negative ΔD band is observed in the gain region, and after a few picoseconds ΔD becomes positive between 470 and 550 nm. Simultaneously $|\Delta D|$ increases in the bleaching region and a

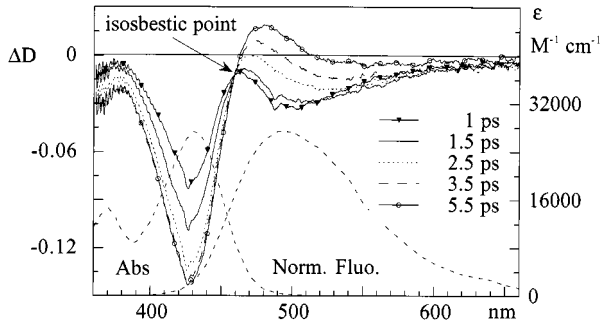

Fig. 1. Time-resolved differential absorption spectra [$\Delta D(\lambda,t)$; left scale] of Auramine in ethanol after subpicosecond excitation at 425 nm for 1- to 5.5-ps pump-probe delays. Ground-state absorption (extinction coefficient, ε_a; right scale) and uncorrected fluorescence spectra (normalized).

temporary isosbestic point is evidenced at 459 nm, with $\Delta D \approx -0.011$. At longer delays (not given in the figure) both the transient absorption and the bleaching bands decay. The kinetics $\Delta D(t)$ are shown in Fig. 2, at 439 nm, in the bleaching band, and at 479 nm, where the initial gain signal gives way to a transient absorption band. The experimental data (markers) were fitted with a two-exponential function convoluted with the pump-probe correlation function, which fitted itself to a Gaussian (1.5 ± 0.5-ps FWHM). The best fits (lines) led to time components of 1.75 and 20 ps at 439 nm and of 2.75 and 32 ps at 479 nm.

The fluorescence up-conversion decays of Auramine in ethanol at selected wavelength across the steady-state spectrum are shown in Fig. 3. The decays are found to be nonexponential and strongly dependent on the detection wavelength. They could be fitted to a two-exponential decay function convoluted with the up-conversion setup response function. Within the 465- to 600-nm range, the two time components were found to vary from 0.7 to 1.9 ps and from 2.7 to 4.6 ps, respectively, while the weighted average lifetime increased gradually with the wavelength, from 1.2 to 2.9 ps. In all cases, the fluorescence rises within the time resolution of the experiments (150 fs). Using the spectral reconstruction method of Maroncelli and Fleming [21], the instantaneous fluorescence spectra were obtained at different time delays. The spectrum exhibits a slight dynamic shift to the red and a broadening while the intensity drops. These time-resolved changes (first moment, FWHM, integrated intensity) are plotted in Fig. 4.

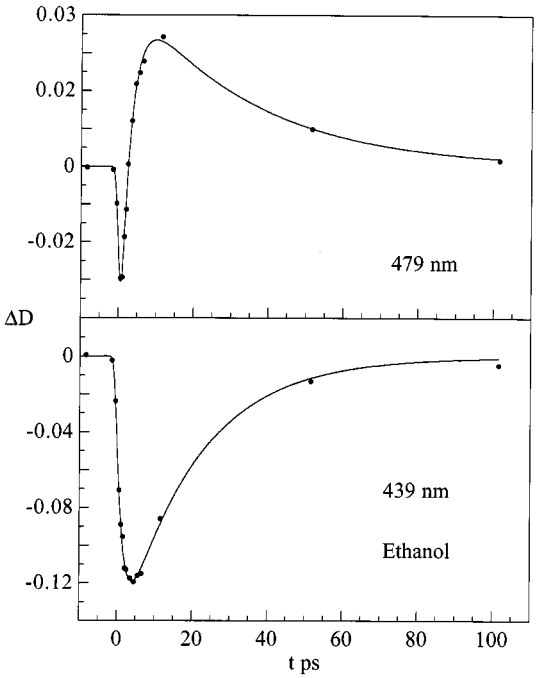

Fig. 2. Kinetics of $\Delta D(\lambda,t)$ measured at 439 and 479 nm for Auramine in ethanol: experimental data (markers) and best fit (line) with a two-exponential function convoluted by the pump-probe correlation function fitted as a Gaussian of FWHM of about 1.5 ps. The fit leads to time constants of 1.75 and 20 ps at 439 nm and 2.75 and 32 ps at 479 nm.

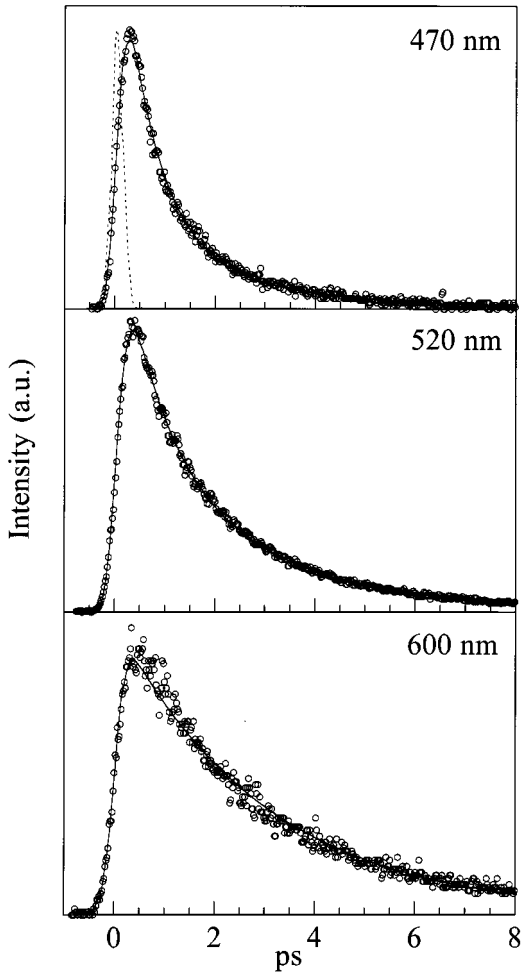

Fig. 3. Fluorescence decays of Auramine in ethanol at 470, 520, and 600 nm measured by up-conversion after 80-fs excitation at 420 nm. The apparatus response function (150-fs FWHM) is shown with the decay at 470 nm.

DISCUSSION

Formation of a Transient State

As discussed previously [10,22] the observation of a temporary isosbestic point in time-resolved differential absorption spectra for a nonzero ΔD value (Fig. 1) is a strong indication that at least two species, in addition to

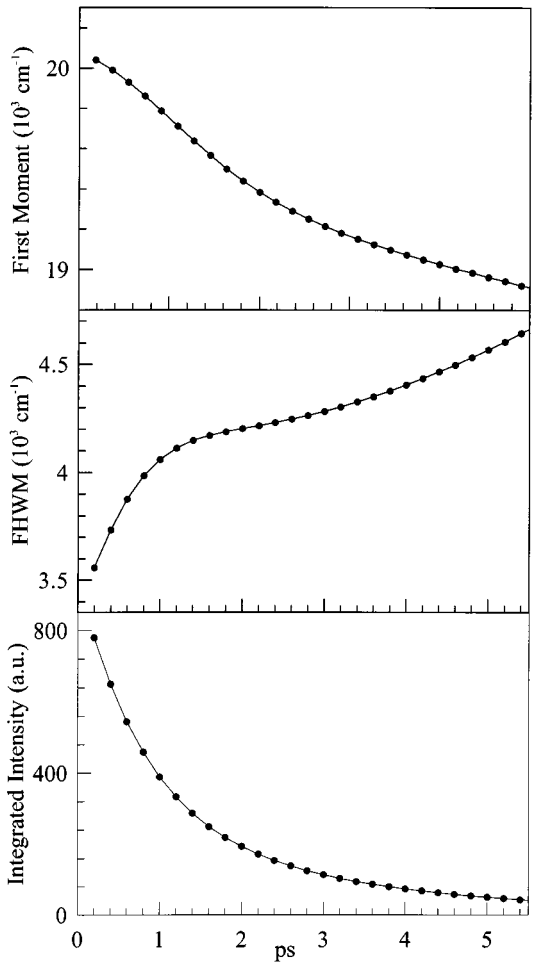

Fig. 4. Evolution of the reconstructed instantaneous fluorescence spectra of Auramine in ethanol under 80-fs excitation at 420 nm: (top) red shift of the first moment; (middle) broadening of the full width at half-maximum (FWHM); (bottom) drop of the integrated intensity.

the ground state, are present during this time interval. This observation, together with the rise of a transient absorption band while the gain signal decays, demonstrates a precursor–successor relationship between the initially emissive excited state and a transient state. Assuming the coexistence of these two states after excitation, with a constant concentration while the temporary isosbestic point is observed [$c_1(t) + c_2(t) = c^*$, the initial excited state concentration], the differential absorption can be written

$$\Delta D\,(\lambda,t) = [\,\{\varepsilon_g^1(\lambda) - \varepsilon_a(\lambda)\}c_1(t) + \{\varepsilon_g^2(\lambda) - \varepsilon_a(\lambda)\}c_2(t)]L \qquad (1)$$

where L is the cell length, ε_a the ground-state extinction coefficient, $\varepsilon_g^1 = \varepsilon_u^1 - \varepsilon_e^1$ the difference of the absorption ε_u^1 and stimulated emission ε_e^1 coefficients of the initial state, and $\varepsilon_g^2 = \varepsilon_u^2 - \varepsilon_e^2$ that of the transient state. The observed temporary isosbestic point indicates that at this particular wavelength ε_g^1 is equal to ε_g^2. The apparent increase in the bleaching signal while the reaction proceeds is attributed to the decay of the initial excited-state population, the absorption of which overlaps that of the ground state with $\varepsilon_u^1 < \varepsilon_a$. The fit of the ΔD kinetics at 439 and 479 nm with a two-exponential function supports a mechanism involving the fast decay of the initial excited state into a transient state with a 2- to 3-ps reaction time, followed by the decay of the latter back to the ground state with a 20- to 30-ps reaction time. The discrepancy between the long time components of the two measured kinetics (see Results) could be due to errors arising from the weakness of the signal at 479 nm and the scarcity of the data points (Fig. 2), whereas the difference between the short components may be real. This short component corresponds to the decay of the initial fluorescent state, and as a matter of fact, wavelength-dependent decays are also and clearly observed in the fluorescence upconversion measurements (Fig. 3). The fluorescence average decay times (1.2–2.9 ps) are in close agreement with the short components of the ΔD kinetics.

Barrierless Reaction

We previously reported a detailed analysis of the fluorescence decays in ethanol and decanol [11]. The nonexponential character of the decays and the strong viscosity effect on the lifetimes led us to conclude that the probed photoreaction proceeds along a barrierless, or low-energy activated, excited-state surface similar to that reported for TPM dyes, involving a diffusive rotation of the phenyl groups. In a barrierless reaction we probe directly the motion of the population on the excited-state surface and thus the measured kinetics may be dependent on the observation window set by the probe wavelength. The short time component in the blue wing of the fluorescence spectrum (470 nm; Fig. 3), i.e., the dynamic red shift of the spectrum (Fig. 4), is interpreted by the motion of the excited-state population out of the Franck–Condon region. To explain the increase in the fluorescence lifetime in the red wing (600 nm; Fig. 3) accompanied by the drop in intensity (Fig. 4), we proposed that the radiative rate decreases while the reaction proceeds, i.e., as the molecule twists [11]. As a matter of fact, one might think that the drop in intensity could be due to a large nonradiative rate decreasing the excited-state population, this rate fading as the molecule twists, to account for the longer fluorescence lifetimes in the red. Nevertheless, in such a view one would expect fast components in the

ground-state repopulation kinetics, which are not observed. Recent studies of temperature effects together with theoretical calculations based on a modified BFO theory confirmed this proposal [23]. The observed broadening of the fluorescence spectrum (Fig. 4) is thus likely to be due to the spread of the population on the excited state, which leads to a broader distribution of geometries along the reaction path. The observation of an isosbestic point at 459 nm in the time-resolved differential absorption spectra for 1 to 5.5 ps (Fig. 1) while the fluorescence (thus, the instantaneous gain) spectrum shifts and broadens can be explained by the conjunction of three factors: (i) most of the fluorescence shift is over in the 1- to 5.5-ps interval, (ii) the 1.5-ps resolution of the absorption experiment obscures any fast spectral evolution, and (iii) at 459 nm a large contribution of excited-state absorption overlaps the gain signal and further hides what remains of the shift. This also probably means that the absorption extinction coefficients of both the initially excited state and the transient state do not evolve much in this time range.

The fluorescence quenching of Auramine is thus interpreted to be due to the fast formation of a transient state of twisted geometry and low radiative rate, a transient dark photoproduct.

Transient Extinction Coefficients

To characterize this photoproduct further, we tentatively extracted its extinction coefficient from the present differential absorption in a visual way previously described in Ref. 17. The incident excitation energy was 22 μJ/pulse at 425 nm and the energy transmitted by the sample was 9 μJ/pulse, thus the number of photons absorbed was 28×10^{12}. Assuming an excited volume of about 1 mm^3 and neglecting the number of photons absorbed by the excited state during the pump-pulse propagation into the sample containing 1.2×10^{14} molecules/mm^3, one roughly estimates that 23% of the molecules were excited. With the assumption that at the end of the excitation pulse (Δt = 1ps) only the initially excited state is present ($c_2 = 0$), one obtains from Eq. (1) the spectral distribution of ϵ_g^1 given by Eq. (2).

$$\varepsilon_g^1 = \varepsilon_u^1 - \varepsilon_e^1 = \varepsilon_a + \Delta D(1\text{ps})/\{cL(c_1/c)\} \qquad (2)$$

In fact the calculations were made with $c_1/c = 0.27$ for reasons explained below. The calculated spectrum is shown in Fig. 5. The negative part indicates the region where the instantaneous stimulated emission coefficient ε_e^1 is dominant. Assuming complete conversion of the initial excited-state population into the twisted dark state, one estimates its cross section ε_g^2 from the differential

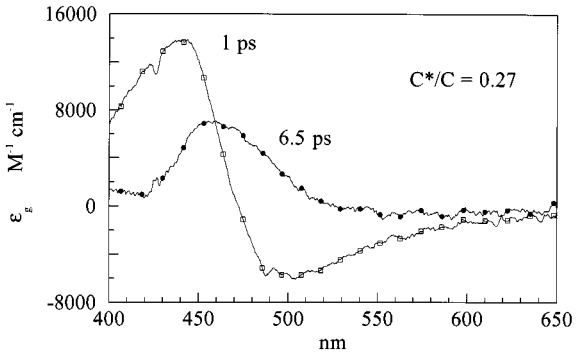

Fig. 5. Excited-state extinction coefficient ε_g extracted from the differential absorption spectra of Auramine in ethanol, immediately after subpicosecond excitation at 425 nm (1 ps) and after decay of the gain signal (6.5 ps). ε_g is the difference of the instantaneous stimulated emission ε_e and the absorption coefficient ε_u of the excited state. The spectrum at 6.5 ps gives the absorption spectrum of the transient dark state.

absorption spectrum measured at Δt = 6.5 ps ($c_1 = 0$, $c_2/c = 0.27$) by using Eq. (3).

$$\varepsilon_g^2 = \varepsilon_u^2 - \varepsilon_e^2 = \varepsilon_a + \Delta D\ (6.5\text{ps})/\{c\ L\ (c_2/c)\} \qquad (3)$$

We had to choose at least 0.27 for c_2/c in order not to have negative extinction coefficients in the bleaching region. The discrepancy with the above-mentioned 0.23 value is accountable for our quite inaccurate estimation of the pumped volume. The result is shown in Fig. 5; it directly gives the extinction coefficient ε_u^2 since the transient photoproduct is assumed to have a very low radiative rate, i.e., to be nonemissive ($\varepsilon_e^2 = 0$). As expected, the spectrum of ϵ_u^2 crosses that of ε_g^1 at 459 nm. It is interesting to note that the band lies mainly in the same wavelength range as that of the dimethylaniline cation radical, with a maximum around 450–470 nm [24,25]. On the other hand, the extinction coefficient at the maximum ($\approx$ 7000 M^{-1} cm^{-1}) is of the same order of magnitude as that reported for the dimethylaniline cation radical in frozen glassy solutions (4600 M^{-1} cm^{-1} [24,25]) and in a mixed solvent, CH_3CN:H_2O (4:1, v/v), at room temperature (4700 M^{-1} cm^{-1} [26]).

Comparison with TPM Dyes

Scheme I shows one possible resonance form of Auramine, but one can also set the positive charge on the central carbon, as in TPM dyes. An intramolecular charge shift from the electron-donor dimethylamino substituent to the positively charged central carbon site was previously thought to contribute to the photoreaction [10,11]. The present observation may thus support an internal twisting process involving a charge shift,

although the photoproduct absorption spectrum extracted from the differential absorption spectra of the TPM dye ethyl violet did not show this characteristic band [14]. In ethyl violet or crystal violet, there are three substituted anilino groups and some conjugation may remain on two of them if the third one rotates, and the spectroscopy of the twisted photoproduct may be different from that obtained with Auramine. Another similarity, however, with the TPM dyes is the short lifetime of the transient dark photoproduct and its sensitivity to the solvent viscosity. In a recent study of a julolidine derivative of crystal violet it was suggested that the molecule deactivates to the ground state via a conical intersection [27]. Further motion, involving rotation of a second phenyl group, on the excited-state surface before reaching the conical intersection was proposed to explain the viscosity effect on the photoproduct deactivation time. This proposal might also apply to Auramine excited-state photophysics.

CONCLUSION

The nonradiative process responsible for the fluorescence quenching of Auramine in ethanol has been reexamined by ultrafast spectroscopy. From both transient absorption and fluorescence up-conversion data, it is confirmed that the photoinduced process leads within a few picoseconds to an unstable dark state, which relaxes back to the ground state. The process was previously attributed to a barrierless rotational diffusion motion of the dimethylamino-substituted phenyl rings, leading to a state of twisted geometry. The extinction coefficients of the twisted state are tentatively extracted from the differential absorption spectra once the gain signal has vanished. The absorption spectrum lies in the same wavelength range as the dimethylaniline cation radical. The result is discussed as possible support for our previous proposal that the twisting process involves a charge shift.

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