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Quenching of pH-Responsive Luminescence of a Benzoindolizine Sensor by Ultrafast Hydrogen Shift

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Abstract

Fluorescent-sensor design requires consideration of how photochemical dynamics control properties of a sensing state. Transient absorption (TA) spectroscopy reveals an ultrafast net [1,3] H shift following excitation of a protonated methoxy benzoindolizine (bi) sensor in solution. These photochemical dynamics explain a quenched pH-responsive fluorescence shift and dramatically reduced fluorescence quantum yield relative to other (e.g. methyl) bi compounds that do not tautomerize. Calculations predict the energetic and structural feasibility for rearrangement in protonated bi compounds, such that interaction between the pi-network and strongly electron-donating methoxyl must lower the barrier for suprafacial H or H⁺ shift across an allylic moiety. As bi compounds broadly exhibit pH-responsive emission shifts, chemical interactions that modulate this electronic interaction and suppress tautomerization could be used to facilitate binding- or surface-specific acid-responsive sensing.

Keywords

Benzoindolizine; Femtochemistry; Fluorescent probes; Hydrogen transfer; Time-resolved spectroscopy

The design of fluorescent molecular sensors requires consideration of how photoinduced structural dynamics control the photophysics of a sensing state.^[1, 2] For example, sensors that operate via excited-state proton transfer (ESPT)^[3] require the analyte to disrupt or create H⁺ transfer networks between donating and accepting groups. For sensors based on fluorescent proteins,^[4–6] intrinsic ESPT within the fluorophore alters the emission gap; interactions with an analyte quench ESPT and alter the sensor's fluorescence. In contrast, fluorescent sensors for anion detection involves ESPT directly to the analyte.^[7, 8] A related consideration is how secondary interactions (in addition to those with an analyte) affect sensor photophysics and can thus be used to limit sensing to specific environments, such as surfaces, interfaces, chemical hosts, or binding pockets. Here we report quenching of pH-responsive emission of a methoxylated benzoindolizine through a novel mechanism: ultrafast, net [1,3] H shift. Through comparison with the photophysics of a methylated analogue, we illustrate that this rearrangement is controlled by electronic interactions

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between the core bi chromophore and strongly electron-donating methoxyl group – an effect that could be modulated through binding interactions for location-specific sensing.

We previously reported the synthesis of 6-amino-8-cyanobenzo[1,2-*b*]indolizines (bi) that exhibit pH-responsive fluorescence.^[9] The emission peak for a methylated derivative (biMe, Chart 1) dissolved in methanol (MeOH) blueshifts from 510 to 442 nm upon adding trifluoroacetic acid (TFA), with an increase in emission quantum yield (QY) from 0.10 to 0.24. In contrast, a 2-methoxylated derivative (biOMe) exhibits no significant fluorescence shift and a drop in emission QY from 0.10 to < 0.01 with decrease in pH. Figure 1 plots normalized absorption and fluorescence spectra of protonated biMe and biOMe (biMeH⁺ and biOMeH⁺, respectively) in MeOH. A modest absorption redshift from biMeH⁺ to biOMeH⁺ [$E_{\text{Abs}} = 584 \text{ cm}^{-1}$] is attributed to a stronger electronic push-pull effect across biOMeH⁺. However, the large difference in Stokes' shifts [4403 vs. 6445 cm^{-1} , ($E_{\text{Stokes}} = 2042 \text{ cm}^{-1}$)] cannot be attributed to increased charge separation in excited biOMeH⁺, as emission from both exhibits weak solvatochromism [$E_{\text{Solv}} = 371 \text{ vs. } 506 \text{ cm}^{-1}$; Figure S1].

NMR spectra of both protonated compounds in CD₃CN reveal a loss of C-ring aromaticity to yield a methylene (T1 or T2) – not amino (N) – tautomer.^[9] Only one protonated species of either compound is observed; however, ¹H signals from the amino, C-7, and C-9 sites disappear for CD₃OD solutions, indicating facile H/D exchange at each of these sites (Figure S2). Quantum-chemical computations illuminate the relative energies of protonated tautomers in their ground (S_0) and first electronically excited (S_1) states, as well as their vertical absorption and emission gaps (Table 1). The structures and relative energies of S_0 and S_1 N, T1 and T2 are similar for biMeH⁺ and biOMeH⁺: T1 has the lowest ground-state energy for both systems. In contrast, T2 has the lowest energy in the excited state for both protonated compounds and the smallest emission gap. Our calculations reveal that conversion from T1 to T2 is downhill energetically for both excited, protonated compounds. In contrast, the N tautomer has the highest calculated excited-state energy, and conversion of T1 or T2 to N would increase the emission gap.

Our computational results suggest that the different emission Stokes' shifts for biOMeH⁺ and biMeH⁺ may be due to emission from different tautomers. We therefore used ultrafast spectroscopy to probe photochemical dynamics of both systems to look for signs of excited-state tautomerization. TA spectra of biMeH⁺ excited at 360 nm are presented in Figure 2. A negative feature peaks below 500 nm near the peak position of dispersed fluorescence (Figure 1) and is ascribed to $S_1 \rightarrow S_0^*$ stimulated emission (SE). The difference in fluorescence and SE peak positions arises from overlap of the latter with excited-state absorption (ESA) that spans the visible and dominates spectra at the shortest wavelengths probed. The SE position redshifts from 440 to 480 nm and ESA above 500 nm decays in the 20 ps that follow excitation (Figure 2(a), Figure S3). Spectra do not change appreciably between 50 ps and 1 ns (Figure 2(b)), indicating that biMeH⁺ relaxes to a long-lived state. A single-exponential fit to the SE position vs. time yields a spectral relaxation time of $3.7 \pm 0.4 \text{ ps}$ (Figure S4). Notably, TA spectroscopy of biMe and biMeH⁺ are distinctly different (Figure S5).

Figure 3 presents the TA spectroscopy of biOMeH⁺ excited at 360 nm. At 200 fs the TA spectrum consists of SE peaked at 450 nm flanked by absorption below and above 420 and 510 nm, respectively. The SE position shifts dramatically from 450 to 560 nm within a few picoseconds, resulting in a spectrum dominated by ESA below 500 nm. Time-resolved spectra collected between 0.15 and 21 ps were fit using principle-component global analysis (PCGA)^[10] subject to a simple kinetic model ($A \rightarrow B \rightarrow C$); this procedure extracts characteristic spectra associated with transient photochemical species and the timescales associated with their appearance and decay. This analysis yielded (exponential) kinetic interconversion lifetimes of 0.21 and 2.6 ps (Figure 3(a)). The short-lived spectral component obtained from this analysis, *A*, is similar to the TA spectrum of unrelaxed biMeH⁺ (Figure 2(a), Figure S6); intermediate component *B* is similar to *C* but blueshifted. SE and ESA of excited biOMeH⁺ decay with a 295-ps lifetime, with residual absorption below 500 nm remaining after SE disappears (Figure 3(b), Figure S7). TA spectroscopy of biOMe and biOMeH⁺ likewise differ qualitatively (Figure S9).

Our analyses of TA spectra from biMeH⁺ and biOMeH⁺ indicate initial preparation of excited species with similar emission gaps. The SE band for excited biMeH⁺ red shifts modestly with time, whereas that of biOMeH⁺ evolves dramatically. Although the SE peak position of neither species matches the corresponding fluorescence maximum due to overlapping ESA, the differences in emission energy from the two methods roughly agree ($E_{SE} = 2868$ vs. $E_{FL} = 2544$ cm⁻¹).

We therefore interpret TA data as follows: the modest SE shift observed for biMeH⁺ is consistent with vibrational relaxation or dynamic solvation of long-lived S₁ T1.^[11, 12] In contrast, the dramatic spectral evolution observed for biOMeH⁺ on a 210 fs timescale reflects substantial structural changes in the excited state that we ascribe to T1-to-T2 tautomerization by effective H transfer. We note that excited-state tautomerization by H⁺ transfer can occur on timescales ranging between < 100 fs to picoseconds depending on the size of the excited-state tautomerization barrier, which is intimately associated with chemical structure.^[13] Spectral dynamics in Figure 3(a) do not exhibit isosbestic behavior and can only be fit by adding an intermediate component, *B*. Non-isosbestic behavior can be due to vibrational relaxation or dynamic solvation in the excited state that is concurrent with or follows tautomerization; we expect that both molecules should exhibit such relaxation, and indeed similar relaxation timescales (3.7 ± 0.4 and 2.6 ± 0.3 ps) are extracted from our spectral analyses for excited biMeH⁺ and biOMeH⁺. Alternatively, *B* could correspond with a short-lived intermediate structure. The difference in excited-state lifetimes on ps-to-ns timescales further supports that these species emit from different tautomeric structures. Finally, the residual absorption below 500 nm following decay of excited biOMeH⁺ (magnified in Figure S7) is consistent with an S₀ T2-T1 difference spectrum given the relative energies of the calculated vertical excitation gaps; hence, some ground-state T2 is likely produced as a result of this photoinduced process.

Various controls clarify the tautomerization mechanism. biOMeH⁺ fluoresces similarly in solvents that can serve as H⁺ donors, acceptors or neither (Figure S1), implying that tautomerization does not require solvent-assisted proton transfer (PT).^[14] Furthermore, biOMeH⁺ fluorescence reveals no changes in band shape or position across a large biOMe

concentration range (2.5–240 μM) with excess TFA (Figure S11). If dimers facilitate tautomerization (by intermolecular PT), we would expect fluorescence similar to biMeH^+ at low concentrations.^[14] Finally, a large local TFA concentration would be required for a reliable intermolecular PT network to facilitate tautomerization.

We also recognize that there should be some ion-pair interaction between biOMeH^+ and the trifluoroacetate counterion (TFA^-) in organic solvents,^[15, 16] such that tautomerization might occur by PT via TFA^- . However, the TFA^- is more likely to interact with the iminium group, as natural population analysis (NPA)^[17] of computed structures reveals that the iminium carries most of the formal positive charge in S_0 biOMeH^+ (Table S16). Furthermore, we expect different distributions of ion-pair types (e.g. contact, solvent-separated, free) in various media;^[15, 18] presumably only contact pairs would enable ultrafast PT back to TFA^- , while solvent-separated pairs would have different behavior. Hence PT via TFA^- does not seem to be a plausible pathway for ultrafast tautomerization. Finally, significant differences in the TA spectroscopy (Figure S9) and fluorescence of biOMe and biOMeH^+ indicate that the dynamics are not due to excited-state quenching by PT to TFA^- to create excited biOMe .

We conclude that S_1 tautomerization of biOMeH^+ is an intramolecular process. As C-7 and C-9 are at the ends of an allylic moiety, S_1 T1-to-T2 interchange occurs by *net* [1,3] H shift. Orbital-symmetry correlations predict that photoinduced suprafacial [1,3] H shift is allowed,^[19] but this rearrangement could alternatively involve photoinduced C-to-C PT coupled with electronic rearrangement within the allylic moiety. Calculated minimum-energy structures support the feasibility of either mechanism (Figure 4): the C-ring remains roughly planar in the ground state, with the C-H bond at C-7 oriented 38° relative to the plane of the ring. C-7 moves above the plane in excited T1, such that the C-H bond is oriented almost perpendicular to the rest of the C-ring and proximal to the pi-cloud of the neighboring bond between C-8 and C-9. This leaves the H (or H^+) poised for suprafacial transfer to C-9. Thus, the two-step dynamics observed spectroscopically can be interpreted as a rapid net H shift (210 fs) followed by relaxation of nascent S_1 T2 (2.6 ps).

We also found a stable, excited-state tautomer in which the extra H resides at C-8 (Figure 4, Int). This structure's energy lies between that of excited T1 and T2 (Table 1), such that H shift might rather occur by a two-step transfer (C-7 to C-8 to C-9) that would likewise be consistent with the kinetic model. However, the calculated SE gap for Int is quite small due to its relative instability in S_0 . Hence, this mechanism does not match our observables.

The driving force for the shift is presumably greater conjugation and lower S_1 energy in T2. However, our calculations predict the same relative tautomer energies for S_1 biMeH^+ , which does not tautomerize. Fluorescence spectra from a series of biXH^+ , where X are substituents with electron-donating strengths somewhat weaker than methoxyl, fluoresce like biMeH^+ (Figure S12). These discrepancies indicate that the strongly donating methoxyl must uniquely lower the tautomerization barrier. Higher-level calculations are required to chart the H-shift coordinate and how the electronic push-pull effect introduced by methoxyl lowers the energetic barrier.

In sum, we used ultrafast spectroscopy and quantum-chemical calculations to elucidate the photochemistry underlying disparate photophysics of biMeH⁺ and biOMeH⁺ in solution. Dramatic TA spectral evolution observed for biOMeH⁺ results from an ultrafast, net [1,3] H shift not active in biMeH⁺ (a Jablonski diagram in Figure S10 summarizes and contrasts these pathways). Although photoinduced suprafacial [1,3] H shift is predicted to be symmetry-allowed it is virtually unreported in the literature; biOMeH⁺ and analogues may present possibilities for further fundamental exploration of this mechanism – or, alternatively, a likewise unusual C-to-C PT. We note that this rearrangement quenches a pH-dependent emission shift observed with other bi compounds. We hypothesize that chemical interactions or binding that modulate the donating strength of a methoxyl or hydroxyl substituent could suppress the H shift; thus, the photophysics of biOMeH⁺ could be useful for surface- or binding-specific acid-responsive tagging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

6-amino-8-cyanobenzo[1,2-*b*]indolizines (bi) and their protonated amino (N) and methylene (T1, T2) tautomers.

Figure 1. Normalized absorption (solid) and dispersed fluorescence (dashed) of biMeH^+ and biOMeH^+ in MeOH. Fluorescence excitation – 370 nm.

Figure 2. TA spectroscopy of biMeH⁺ in MeOH following 360 nm excitation (a) ~ 0 – 56 ps (b) ~ 56 – 1000 ps

Figure 3.

TA spectroscopy of biOMeH⁺ in MeOH following 360 nm excitation (a) ~ 0 – 21 ps (b) ~ 21 – 1000 ps. Insets: Experimental data at selected probe wavelengths (symbols) compared with global fits (solid lines) from three- and two-state interconversion models in (a) and (b), respectively.

Figure 4. Optimized structures of S_0 T1, and S_1 T1, a C-8 protonated intermediate (Int), and T2. Relevant carbon atoms are numbered (c.f. Scheme 1).

Table 1

Calculated Energies, Absorption and Emission Gaps/Wavelengths of N, T1, T2, and C-8 Protonated (Int) Tautomers of biMeH⁺ and biOMeH⁺ in MeOH (DFT/TDDFT at CAM-B3LYP/6-31G*/PCM). All values are relative to S₀ T1 for each compound.

Tautomer	biMeH ⁺				biOMeH ⁺			
	N	T1	Int	T2	N	T1	Int	T2
S ₀ (eV)	0.68	0	1.49	0.19	0.67	0	1.50	0.236
S ₁ (eV)	3.51	3.14	2.99	2.71	3.55	3.08	2.99	2.73
S ₀ → S ₁ * (eV/nm)	3.25/381	3.80/326	2.15/576	3.23/384	3.29/377	3.71/334	2.08/595	2.72/382
S ₁ → S ₀ * (eV/nm)	2.56/484	2.44/509	0.55/2259	1.72/723	2.63/472	2.38/521	0.51/2421	1.76/704