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Fluorescent properties of hydrogen-bonded ellipticine: A special effect of fluoride anion

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Abstract

Fluorescence properties of ellipticine, a plant alkaloid, was studied in the presence of hydrogen-bond acceptors, such as F^-, CH_3COO^-, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and *N*-methylimidazole in different solvents. Global analysis of the spectrophotometric titration data proved that ellipticine deprotonation in the ground state occurred only in the case of the interaction with two F^- anions in acetonitrile. The fluorescence band of the 1:1 complex of anions showed large Stokes shift implying significant proton displacement upon excitation along the hydrogen-bond. The lack of ellipticine-DBU complex emission was rationalized by quick proton transfer in the excited state. The kinetics and mechanism of the excited ellipticine quenching were also revealed.

Keywords: Ellipticine; Fluorescence; Hydrogen bonding; Proton transfer; Quenching

1. Introduction

Ellipticine (Scheme 1) is a pyridocarbazole type plant alkaloid [1], which exhibits high cytotoxic activity against various tumor cells [2,3]. Many of its analogues have been synthesized in an attempt to improve the antitumor efficiency and to understand the biological function at the molecular level [4]. As a result of the intense research efforts, several ellipticine derivatives have reached the phase II of clinical trials [4]. The origin of their biological activity is fairly complex [5] and is related to the intercalation into DNA [6], inhibition of enzymatic processes, participation in redox reactions as well as binding to mitochondrial membranes [7].

Despite the importance of ellipticine (EH) in the biomedical field, no systematic studies have been performed to unravel the effect of hydrogen-bond acceptors on its photophysical properties, even though the N–H group in the five-member ring of EH is expected to provide a suitable hydrogen-bonding site. The main goal of the present paper has been to examine how the interaction with anions and organic nitrogen compounds influence the absorption and fluorescence characteristics as well as the kinetics and mechanism of the excited state relaxation of EH in solvents of different polarity.

2. Experimental details

Ellipticine, also called 5,11-dimethyl-6H-pyrido[4,3-b] carbazole (Fluka, fluorescence grade), 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) (Fluka) and solvents (Aldrich, HPLC grade) were employed without further purification. Tetrabutylammonium salts (Aldrich) were dried in vacuum and stored in a desiccator; *N*-methylimidazole (MIm) (Aldrich) was distilled prior to use. The UV–vis absorption spectra were recorded with a Unicam UV 500 spectrophotometer in quartz cuvette of 10 or 50 mm optical path. The reference cell contained additive with the same concentration as the sample solution. The number of linearly independent absorbing or emitting species was determined by matrix rank analysis using the program MRA 3.11 developed by Peintler et al. [8] In the course of the evaluation of the spectrophotometric titrations, the global fit of all data with the program PSEQUAD [9] provided the binding equilibrium constants as well as the concentrations and the molar absorption coefficients of the complexes. The softwares were downloaded from Dr. Gábor Peintler's home page: http://www.staff.u-szeged.hu/~peintler/index.htm. Corrected fluorescence and excitation spectra were obtained on a

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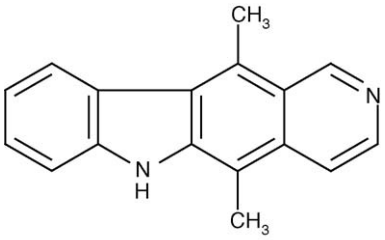

Ellipticine (EH)

Scheme 1.

Jobin-Yvon Fluoromax-P photon-counting spectrofluorometer. The fluorescence decays were measured with a Picoquant time-correlated single-photon counting apparatus equipped with Hamamatsu R3809U-51 microchannel plate photomultiplier. The samples were excited by a diode laser at 400 nm or a light emitting diode at 340 nm. Data were analyzed by a non-linear least-squares reconvolution method using Picoquant FluoFit software.

3. Results and discussion

3.1. Effect of fluoride anion

The absorption spectrum of EH proved to be very sensitive to fluoride anion in nonhydroxylic solvents. In apolar and moderately polar media, such as toluene and CH_2Cl_2, the structured EH absorption markedly changed when the F^- concentration was gradually increased. The results of the spectrophotometric titration (Fig. 1) clearly show the appearance of isosbestic points and the emergence of a new band in the 400–450 nm range. The insets exhibit the absorbance growth at a specific wavelength and the solid lines represent the best fit, which is obtained by the simultaneous evaluation of the experimental data above 310 nm with the program PSEQUAD [9]. The spectral changes can be described well assuming the formation of a complex with 1:1 stoichiometry. The logarithm of the hydrogen-bonding equilibrium constant ($\log K_1$), reported in Table 1, is significantly lower in CH_2Cl_2 than in toluene because the stronger solvent–solute interaction in the more polar medium weakens the hydrogen bonding between EH and F^-.

It is obvious from Fig. 1 that an entirely different behaviour is obtained in acetonitrile. In the presence of low (<0.5 mM) F^- concentration the spectral changes resemble that found in less polar solvents implying 1:1 hydrogen-bonded complex formation. However, further addition of F^- results in the evolution of a broad band in the 450–550 nm domain, which is formed via interaction of F^- with the hydrogen-bonded complex. The lack of isosbestic point and the shape of the titration profile displayed in the inset of Fig. 1C are in accord with the two stepwise equilibria. The solid line in the inset presents the fitted function corresponding to $\log K_1 = 2.40$ and $\log K_2 = 3.47$ values for the logarithm of the equilibrium constants of the two consecutive equilibria, respectively. As it is expected for a hydrogen-bonded complex, K_1 data diminish with increasing solvent polarity (Table 1). The more than one order of magnitude higher K_2 value evidences that F^- exhibits larger affinity to the hydrogen-bonded complex than to EH. The calculated spectra of the light absorbing species are compared in Fig. 2. The position of the bands assigned to EH and 1:1 complex barely alter in solvents of different polarity but no vibronic structure appears in acetonitrile. The remarkably red-shifted absorption spectrum stemming from the interaction of the 1:1 complex with F^- closely resembles that of deprotonated ellipticine, which is produced in the presence of 4.3×10^{-4} M sodium methoxide in

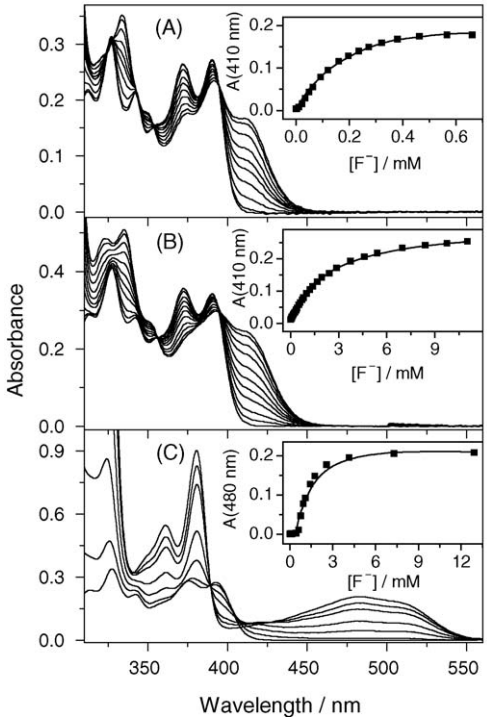

Fig. 1. Alteration of the absorption spectra on addition of increasing amount of F^- in toluene (A), CH_2Cl_2 (B) and CH_3CN (C); [EH] = 78.9 μM. Insets present the absorbance variation at 410 nm (A), (B) and at 480 nm (C); the solid lines refer to the best fit.

Table 1
Logarithm of the ground state binding constants and the rate constants of the excited ellipticine quenching

	Solvent	$\log K$	k_q (10^9 M^{-1} s^{-1})
F^-	Toluene	3.80	Negligible
	CH_2Cl_2	2.62	5.5
	CH_3CN	2.40[a]	6.3
		3.47[b]	
CH_3COO^-	CH_3CN	2.24	4.0
DBU	Toluene	2.14	4.4
	CH_3CN	0.83	2.0
MIm	Toluene	1.32	Negligible
	CH_3CN	Negligible	1.7[c]
			0.39[d]

[a] 1:1 Complex.
[b] 2:1 Complex.
[c] Forward reaction of the reversible quenching.
[d] Back reaction of the reversible quenching.

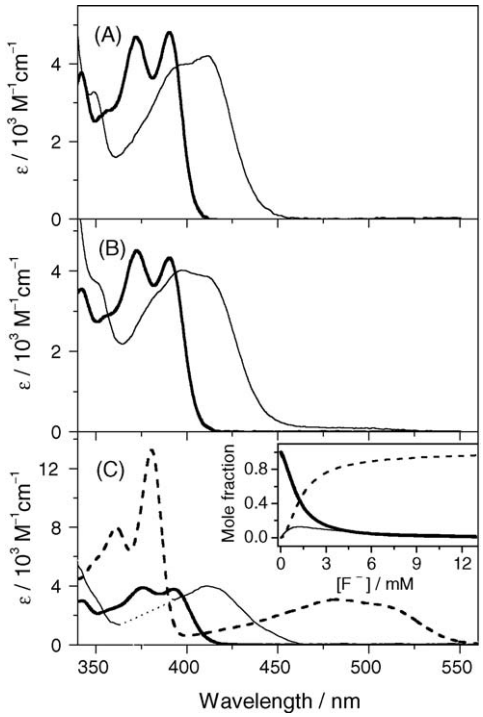

Fig. 2. Molar absorbances and mole fractions at the equilibrium (inset) calculated from the global analysis of the spectrophotometric titrations in toluene (A), CH_2Cl_2 (B) and CH_3CN (C); EH (heavy line), 1:1 hydrogen bonded complex (thin line) and deprotonated ellipticine (dash line).

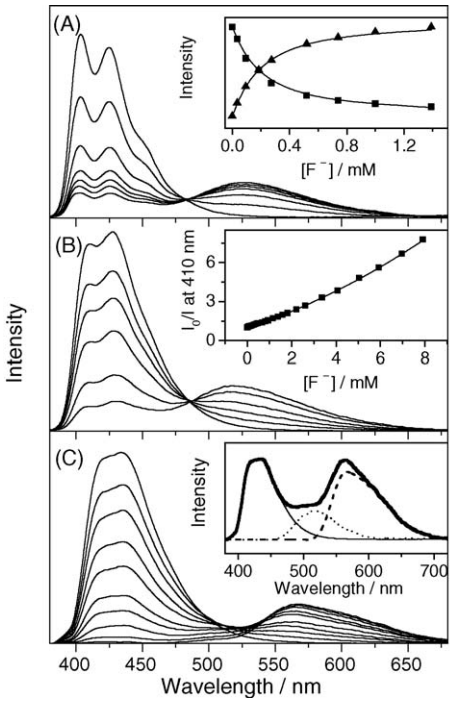

Fig. 3. Change of the fluorescence spectrum of ellipticine on addition of F^- in toluene (A), CH_2Cl_2 (B) and CH_3CN (C). *Insets:* (A) fluorescence intensity variation at 404 nm (■) and 530 nm (▲); solid line refer to the calculated function; (B) Stern–Volmer plot of the results obtained at 410 nm; (C) resolution of the fluorescence spectrum recorded in the presence of 1.75 mM F^- in acetonitrile (heavy line). Spectra of the components: EH (thin line), E^- (dash line) and hydrogen-bonded complex (dotted line).

acetonitrile. Therefore, we conclude that the binding of two F^- to EH leads to the removal of a proton from the N–H group resulting in ellipticine anion (E^-) and HF_2^-. The high bond energy in HF_2^- (38.6 kcal mol^{-1} in the gas phase [10]) and the solvation energy of the formed ions permit of this reaction in acetonitrile. The fluoride induced deprotonation of H-bond donor systems has been clearly elucidated by Fabbrizzi and co-workers in a recent series of papers [11,12]. Deprotonation of pyrrole-based receptors following the reaction with 2 equivalent of fluoride has been discussed [13,14]. The inset in Fig. 2C displays the calculated mole fractions of the various light-absorbing species in the function of the F^- concentration. It is apparent that the mole fraction of the 1:1 complex never exceeds 0.13 and barely decreases in a broad concentration domain.

Fluoride anion markedly altered the fluorescent behaviour of EH as well (Fig. 3). Fluorescence quenching and a concomitant rise of the emission in the long-wavelength range were detected. Matrix rank analysis using the program MRA 3.11 [8] confirmed that only one fluorescent component other than EH emission existed in toluene and CH_2Cl_2, whereas three emitting species were found in acetonitrile. Fig. 3C presents the resolution of the fluorescence spectrum recorded in the presence of 1.75 mM F^- in acetonitrile (heavy line). The longest-wavelength band is assigned to E^- emission because it can be also obtained by the selective excitation of E^- at 490 nm. Subtraction of E^- and EH fluorescence from the overall spectrum gives a band, which resembles the hydrogen-bonded complex emission in toluene and CH_2Cl_2. The substantial Stokes shift in all solvents indicates a considerable change in the structure of the 1:1 complex upon light absorption. This is probably due to the acidity enhancement of EH in the excited complex, which brings about photoinduced proton displacement along the hydrogen bond. The clear isoemissive points in Fig. 3A and B corroborate that the basicity of F^- is not large enough in apolar and moderately polar solvents to induce complete proton transfer in the excited 1:1 complex. E^- is formed only via the interaction of the hydrogen-bonded complex with F^- in acetonitrile, where the reaction is facilitated by the high stability of HF_2^- and the solvation of the product anions in the polar medium.

Time-resolved fluorescence measurements proved that only static quenching occurred in toluene because 9.1 ± 0.3 ns fluorescence lifetime was measured in the EH band irrespective of F^- concentration. No interaction occurred with the excited EH because the low F^- concentration made the pseudo-first order quenching process too slow to compete with the first order decay of the excited species. The fluorescence of the hydrogen-bonded complex also decayed monoexponentially with a lifetime slightly longer (12.9 ± 0.3 ns). The instantaneous rise of the signal corroborated the lack of excited hydrogen-bonded complex formation via the interaction of excited EH with F^-. In accord with these findings, the simultaneous evaluation of the spectrofluorimetric titration data gave the same log K value as that derived from the alteration of the absorption spectra (Table 1). The inset in Fig. 3A illustrates the accuracy of the fit of the fluorescence intensity variation at 404 and 530 nm.

In spite of the similar spectral characteristics of the fluorescence in toluene and CH_2Cl_2 (Fig. 3A and B), time-resolved measurements implied different fluorescence quenching mechanism. In CH_2Cl_2 the reciprocal fluorescence lifetime of EH grew linearly with F^- concentration, and the slope gave $k_q = 5.5 \times 10^9\ M^{-1}\ s^{-1}$ for the rate constant of the reaction between excited EH and F^-. As expected for simultaneous dynamic and static quenching, the Stern–Volmer plot of the EH emission showed an upward curvature (inset to Fig. 3), which could be described well with the following function:

$$\frac{I_0}{I} = (1 + K_1[F^-])(1 + k_q\tau_0[F^-]) \quad (1)$$

Using $\tau_0 = 14.4$ ns for the fluorescence lifetime of EH in CH_2Cl_2 and k_q derived from time-resolved measurements, $\log K_1 = 2.67$ was obtained in fair agreement with the value calculated from the absorbances (Table 1). The excited hydrogen-bonded complex did not react with F^- because its lifetime was constant ($\tau_{HB} = 23.7 \pm 0.5$ ns) within the limits of the experimental errors. The lack of the reaction in toluene and CH_2Cl_2 seems to indicate that the relatively weak solvent–solute interactions in apolar and moderately polar solvents cannot promote the E^- and $HF_2{}^-$ formation even in the excited state.

In acetonitrile, the EH fluorescence lifetime was also shortened by F^- and $k_q = 6.3 \times 10^9\ M^{-1}\ s^{-1}$ was derived for the quenching rate constant. Selective excitation of E^- above 470 nm provided 10.7 ns for its fluorescence lifetime, whereas the excitation at 340 nm gave no rise for the E^- emission implying that the singlet-excited E^- was formed predominantly via light absorption of its ground state. As seen in Fig. 1C, EH is converted almost completely into E^- in the F^- concentration range where even a diffusion-controlled reaction of excited EH with F^- would be much slower than the decay rate of the excited EH. Thus, no excited state reaction is feasible.

3.2. Effect of acetate anion in acetonitrile

Addition of CH_3COO^- to EH solution leads only to 1:1 complexation in acetonitrile and the association constant is close to that found for F^- (Table 1). As seen in Fig. 4A, no absorption emerges above ca. 440 nm because the low basicity of CH_3COO^- does not allow E^- anion production in the ground state. The acetate anion induced alteration of the EH fluorescence spectrum (Fig. 4B) is similar to that observed with F^- but the intensity of the hydrogen-bonded complex emission around 510 nm is significantly lower. Acetate ion is a slightly stronger base than F^- because the pK_a values for the conjugated acids, CH_3COOH and HF, are 4.75 and 3.2 in water, respectively [15]. Thus, the increase of the EH acidity upon excitation permits more efficient proton transfer to CH_3COO^-. Light absorption of the ground state complex is the dominant pathway of excited E^- formation because most of the EH molecules are hydrogen-bonded in the ground state at the CH_3COO^- concentration range where dynamic quenching can occur. From the acetate concentration dependence of the reciprocal fluorescence lifetime of EH $k_q = 3.9 \times 10^9\ M^{-1}\ s^{-1}$ is obtained for the rate constant of the excited EH quenching (inset to Fig. 4).

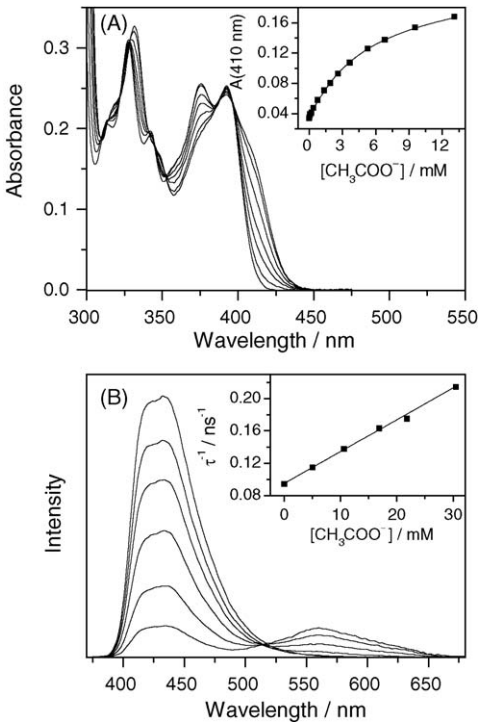

Fig. 4. Effect of CH_3COO^- on the absorption (A) and fluorescence (B) spectra of 78.9 μM ellipticine in acetonitrile. *Insets:* (A) absorbance change at 410 nm, (B) variation of the reciprocal fluorescence lifetime of EH vs. CH_3COO^- concentration. Solid line present the best fit.

3.3. Effect of nitrogen-heterocyclic compounds

Although 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is one of the most powerful organic bases, it exerts different influence on the spectral behaviour of EH than F^- anion. Solely 1:1 association takes place even in acetonitrile, whose equilibrium constant is more than one order of magnitude lower than the value measured for F^-. Despite its high basicity, DBU is not able to deprotonate EH in the ground state. Thus, we can conclude that pK_a of EH is higher than 24.34, the value reported for the conjugated acid of DBU in acetonitrile [16]. Moreover, $HF_2{}^-$ is much weaker acid than the protonated DBU and its pK_a is larger than 24.34 in acetonitrile because $HF_2{}^-$ does not donate proton to E^-.

In acetonitrile, the diminution of the fluorescence intensity of the EH band upon the gradual increase of DBU concentration is accompanied by the concomitant rise of the E^- emission centred at 564 nm (Fig. 5A). In contrast to the behaviour found in the presence of anions, no hydrogen-bonded EH–DBU complex fluorescence is detected because the radiative process is not able to compete with the rapid photoinduced proton transfer along the hydrogen bond, which generates the excited E^-. The decay time of EH emission matches the growing-in of the E^- signal giving further evidence for direct excited state proton transfer. The plot of the reciprocal decay parameter versus DBU concentration (inset in Fig. 5A) provides the rate constant of the EH quenching given in Table 1. The lifetime of the singlet-excited E^- agreed well with the 11.8 ± 0.4 ns value measured with F^- and CH_3COO^- reactants.

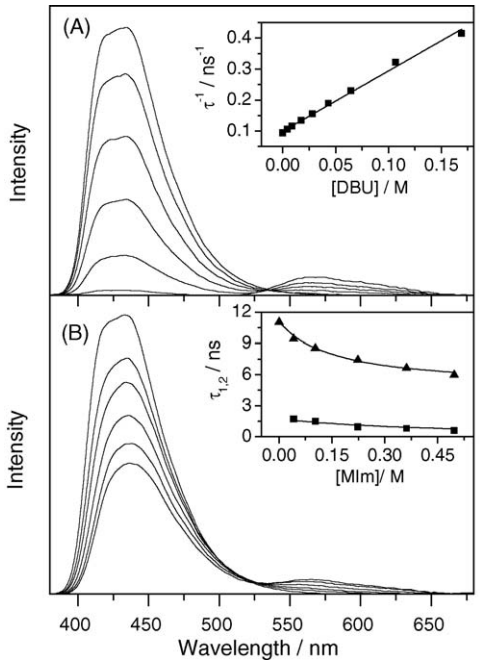

Fig. 5. Alteration of the fluorescence spectra on addition of DBU (upper segment) and MIm (lower segment) in acetonitrile. *Insets:* (A) the reciprocal fluorescence lifetime of EH vs. DBU concentration; (B) MIm concentration dependence of the fluorescence decay parameters τ_1 (▲) and τ_2 (■) at 415 nm. Solid lines display the results of the global fit.

In toluene, DBU does not alter the shape of the fluorescence spectrum of EH but induces both static and dynamic quenching via non-fluorescent excited hydrogen-bonded complex formation, as shown by the nonlinear character of the Stern–Volmer plot of the fluorescence intensity change. Time-resolved measurements proved that the dynamic quenching has a close to diffusion-controlled rate constant (Table 1). The apolar solvent does not stabilize the ion pair formed via proton transfer within the incipient excited hydrogen-bonded complex and the rapid proton back-transfer seems to be an efficient deactivation pathway.

Applying *N*-methylimidazole (MIm), a weaker base with $pK_a = 7.12$ in water [17], hydrogen-bonding causes merely 14 nm Stokes shift and negligible fluorescence quenching in toluene because the acidity of the excited EH is not high enough to bring about significant proton displacement within the complex. On the other hand, in acetonitrile, no interaction takes place in the ground state, but hydrogen-bonding with MIm in the excited state diminishes the intensity and modifies the shape of the EH fluorescence spectrum (Fig. 5B). The appearance of a weak E^- emission indicates that proton transfer can also occur within the excited complex. The dual exponential fluorescence decay at 415 nm suggests that the formation of the excited hydrogen-bonded complex is a reversible process and the concentration dependence of the decay parameters (inset in Fig. 5B) are described well with the following relationship:

$$\tau_{1,2} = \frac{2}{X + \tau_C^{-1} \pm \sqrt{(X - \tau_C^{-1})^2 + 4k_q k_{-q}[\mathrm{MIm}]}} \quad (2)$$

where $X = 1/\tau_0 + k_q[\mathrm{MIm}]$, τ_0 and τ_C denotes the lifetime of EH and the excited hydrogen-bonded complex, k_q and k_{-q} are the rate constants of the complex formation and the dissociation. The combined analysis of τ_1 and τ_2 data gave $\tau_C = 5.1$ ns and the calculated k_q and k_{-q} values are reported in Table 1.

4. Conclusions

The formation and deactivation mechanism as well as the fluorescent properties of the excited hydrogen-bonded complexes of ellipticine significantly changed for various hydrogen-bond acceptors in solvents of different polarity. Despite the low basicity of F^- and CH_3COO^-, the large Stokes shift of their hydrogen-bonded complex fluorescence evidenced considerable structural alteration upon excitation. The binding of a stronger base, MIm, to the excited EH proved to be reversible and weakly exothermic in acetonitrile indicating the lack of correlation between the energy of the excited hydrogen-bonded complex and the basicity for different type of hydrogen-bond acceptors. The complex of the strongly basic DBU did not fluoresce because fast proton transfer occurred along the hydrogen-bond. The 2:1 association of F^- with EH was favoured in contrast with 1:1 complexation in acetonitrile and the binding of two F^- had much higher deprotonation power than that of DBU. Several orders of magnitude change was observed in the ground state binding constants, but the rate constant of the excited EH quenching by the additives were close to diffusion controlled in acetonitrile.

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