Intramolecular Fluorescence Self-Quenching of Fluoresceinamine

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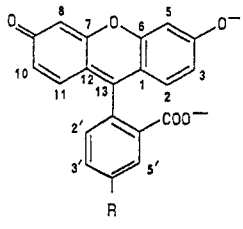
Abstract: In this paper, the intramolecular self-quenching of fluoresceinamine has been investigated. The ubiquitous use of fluorescein dyes as labels and indicators requires a thorough understanding of their properties. This fluorescein derivative has a quantum yield of 0.015 and exhibits none of fluorescein's indicator properties until the amine's electron lone pair is made unavailable for electron transfer through covalent or electrostatic binding process. A series of acyl and sulfonyl halides react with fluoresceinamine to produce highly fluorescent products with the spectral and indicator properties of fluorescein. A secondary quenching mechanism can be imposed on the molecule in derivatives formed by reaction with nitrobenzoyl chlorides. In these cases the degree of quenching is influenced by the nitro group position. Complexation of fluoresceinamine with a cationic surfactant, cetyltrimethylammonium bromide, also regenerated the fluorescence. The ability to regenerate the quenched fluorescence provides a remarkable example of electron density acting as a switch in the control of fluorescence. Fluoresceinamine can be quenched systematically by two different processes, offering the potential for studying quenching mechanisms and their distance and spatial requirements.

Although numerous examples exist[1] of fluorescence quenching by excited-state electron transfer from inter- and intramolecular amines, only recently has the reversal of this quenching by electronic mediation been observed. Huston et al.[2] demonstrated that the fluorescence of bis[[[2-(dimethylamino)ethyl]methylamino]methyl]anthracene is dramatically enhanced by amine chelation with zinc chloride. The fluorescence is reputedly quenched in this compound by exciplex formation made very efficient by the high effective concentration of the intramolecular amine group. When chelated to a zinc ion, the nitrogen lone pair participates in bonding and is unable to donate its electrons to the excited state of the anthracene. Blough and Simpson[3] observed the intramolecular quenching of a fluorophore by a nitroxide radical and were able to demonstrate the direct relationship between the loss of the radical and increased fluorescence yield. In both cases, the fluorescence is quenched electronically and is then restored by complexation or free-radical reaction.

Fluorescein[4] (**1**) is used in many applications because of its pH sensitivity and high fluorescence quantum yield (ϕ). For example, in cytology it is used in the underivatized form to label cells[5] or as an ester that is hydrolyzed to **1** by intracellular enzymes[6] or by photoactivation.[7] For applications that require immobilization,



1 R = H
2 R = NH_2
3 R = SCN
4 R = CH_2=CHCONH

derivatized fluoresceins, such as fluoresceinamine (**2**) or fluorescein isothiocyanate (**3**, FITC), are frequently employed.[8] These derivatives contain a reactive substituent on the phenyl ring that can engage in facile coupling reactions. Because these dyes have been used with such success, it has been assumed that the derivatization reactions had little effect on the indicator properties of the parent molecule, provided the xanthene ring remains unsubstituted. As an example, in the preparation of fiber optic chemical sensors for the measurement of pH,[9] derivatization of **2** with acryloyl chloride produces the *N*-fluoresceinylacrylamide (**4**), which can be immobilized by copolymerization with acrylic monomers. The high quantum yield and pH sensivitity of fluorescein were observed in **4** when immobilized in the polymer, and the diminished fluorescence quantum yield of **2** originally went unnoticed. Although the low quantum yield of fluoresceinamine has been observed previously,[10] the variability of the quantum yield upon derivatization has not been studied. The ubiquitous use of this dye as a pH indicator and its application in cell labeling studies make it imperative that the chemical and physical properties be clarified.[7,9a,e]

We now report that although fluoresceinamine has a markedly quenched fluorescence, the conversion of its amine to an amide

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(4) Fluorescein exists as a fluorescent xanthen-3-one tautomer formed in nonacidic aqueous solution or a nonfluorescent lactone tautomer existing in acidic pH or nonaqueous solvents. As this paper addresses the quenching of the xanthen-3-one tautomer, we present only this fluorescent dianionic structure.

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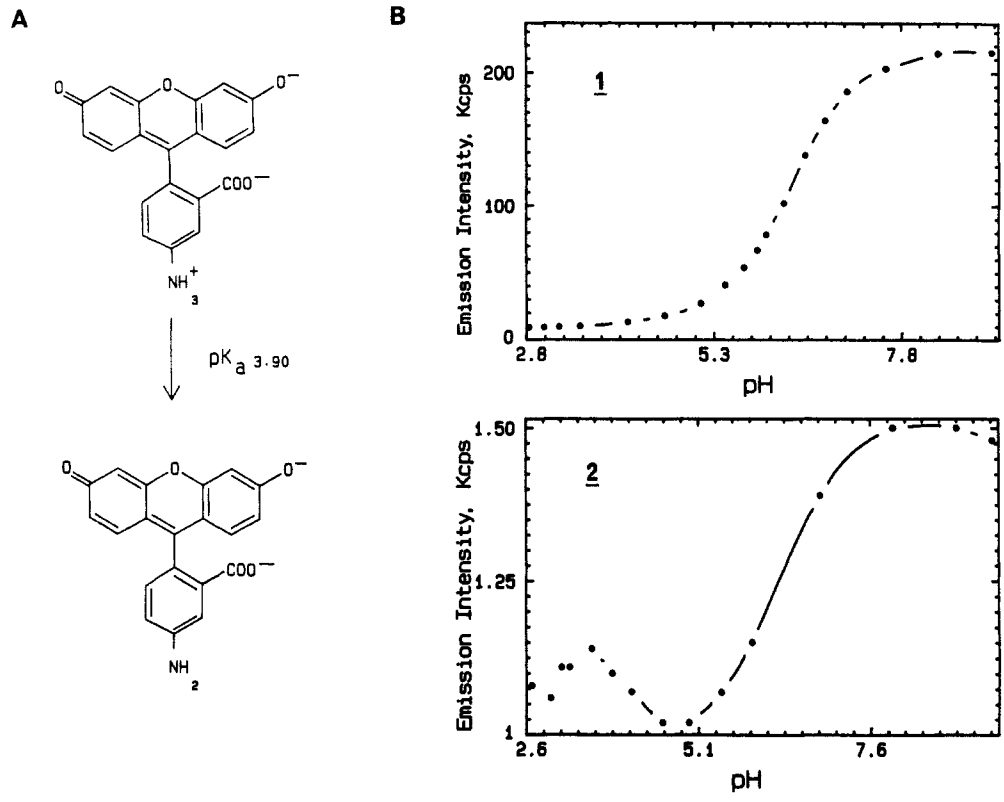

Figure 1. (A) Deprotonation of the ammonium ion restores electron density to nitrogen and initiates quenching of fluoresceinamine. (B) Fluorescence titrations of fluorescein and fluoresceinamine. Concentrations (10^{-5} M) and excitation energies were identical; λ_{exc} = 488, λ_{em} = 530.

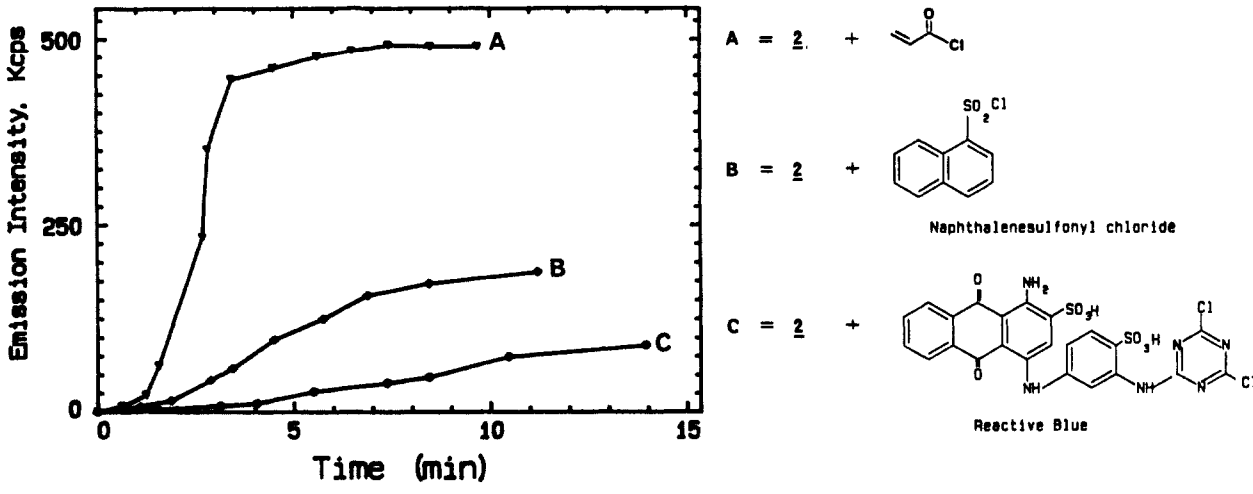

Figure 2. Reactivity of fluoresceinamine with three acyl halides. Equimolar solutions were mixed in 0.05 M phosphate buffers, pH 7.5, and stirred while the production of fluorescence was monitored by an optical fiber in the solution, with the instrumentation described previously.[9b]

restores to the molecule the full fluorescent indicator properties of fluorescein.[11] This observation provides a striking example of the ability to turn a fluorescent molecule off or on by changing the electronic influence of the nitrogen electron lone pair.

Results and Discussion

The pH titrations of fluorescein and fluoresceinamine as a function of fluorescence emission intensity are shown in Figure 1. The changes in the titration curve of fluoresceinamine reflect the influence of the nitrogen electron pair on the fluorescence of the molecule. In solutions below pH 3.2, the amine is fully protonated and the fluorescence intensity is equal to that of fluorescein, both dyes displaying the low intrinsic fluorescence observed in acidic pH solutions. Fluorescein is typically used for its pH sensitivity beginning at pH 4.0, where the molecule starts to form the highly fluorescent dianionic structure, the xanthen-3-one tautomer. Fluoresceinamine, on the other hand, shows a decrease between pH 3.2 and 4.6, due to protonation of the aromatic amine.[12] Once deprotonated, the nitrogen electron pair is available to quench the fluorophore. After this drop, the molecule shows a fluorescence titration curve parallel to that of fluorescein but with markedly quenched fluorescence relative to **1**. The degree of initial quenching that follows the deprotonation of the ammonium ion might be much more apparent if it occurred

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Table I. Relative Fluorescence Emission Intensities of Fluorescein and Derivatives

no.	R	concn (m × 10^{-6})[a]	λ_{max} (nm)	I_0 (K_{cps})[b]	ϕ
1	H	5.00	490	607	0.85[18]
2	NH_2	4.27	486	10.5	0.015
3	SCN	4.44	490	541	0.76
	Amide				
4	$CH_2CHCONH$	5.68	490	544	0.76
5	C_6H_5CONH	5.40	490	566	0.79
6	$(CH_3)_2CHCH_2CONH$	4.77	490	585	0.82
7	p-$CH_3OC_6H_4CONH$	3.39	490	625	0.88
	Nitrobenzamide Derivatives				
8	o-$NO_2C_6H_4CONH$	4.76	492	31.5	0.044
9	m-$NO_2C_6H_4CONH$	5.00	492	160	0.224
10	p-$NO_2C_6H_4CONH$	5.16	492	11	0.015

[a] Concentrations in 100 mM phosphate buffer, pH 7.5, were determined with an IBM 9400 UV/vis spectrometer with an extinction coefficient (490 nm) of 8.8 × 10^4. [b] Fluorescence intensities (I_0) were determined with λ_{ex} = 490 and λ_{em} = 520 nm, in units of 10^3 counts per second (K_{cps}) after normalization to concentration of fluorescein.

at a pH where the quantum yield of the fluorophore was higher.

To test these initial observations, the amide or sulfonamide derivatives of fluoresceinamine were prepared by reaction with a series of acyl or sulfonyl halides, respectively. In these derivatives, electron withdrawal by oxygen of the carbonyl or sulfonyl group makes the amide nitrogen a much poorer source of electrons than the amine nitrogen and, therefore, decreases the intramolecular resonance with the π-electron system of the ring as well as the participation in excited-state transfer processes. A typical amide derivatization, such as the conversion of **2** to **4**, causes a fluorescence enhancement in excess of 50-fold (Table I). The titration curve of *N*-fluoresceinylacrylamide resembled that of fluorescein, in both quantum yield and pH sensitivity. The derivatization reactions can be monitored by following the rate of fluorescent generation (Figure 2), affording a measure of the reactivity of the acyl or sulfonyl halides.[13] Reaction with less electrophilic species, such as sulfonyl chlorides or triazine chloride, produced a less rapid recovery of fluorescence.

The pH-dependent absorption spectra of the amide derivatives were similar to spectra of fluorescein. The hypsochromic shift of fluoresceinamine suggests a small ground-state interaction between the xanthene ring (acceptor) and amine (donor), although no spectral broadening was observed. No exciplex emission was observed in a solvent study of fluoresceinamine, consistent with the requirement that the exciplexes be able to assume a conformation permitting in-line interaction of the nitrogen lone pair with the aromatic π-system.[1b] This conformation is not possible in **2** because it does not contain methylene groups between the xanthene and phenyl rings. The geometrical requirements for exciplex emission are much more stringent than for fluorescence quenching where the distance between the two groups is a more important factor.[1b]

The ^1H NMR spectra provide evidence that the nitrogen electron density in **2** is localized in the phenyl ring and thus proximal to the xanthene structure. Figure 3 contains the ^1H NMR spectra of the aromatic regions of fluorescein, fluoresceinamine, *N*-fluoresceinylacrylamide, and *N*-fluoresceinylbenzamide (**5**). In the case of fluoresceinamine, the phenyl protons are shielded and shifted to lower frequency; the nitrogen electron pair interacts with the phenyl ring, and the proximal fluorophore is rendered nonfluorescent. The three fluorescent compounds (**1**, **4**, and **5**) display phenyl proton resonances (2′, 3′, and 4′) that are deshielded and shifted downfield relative to those of fluorescein.

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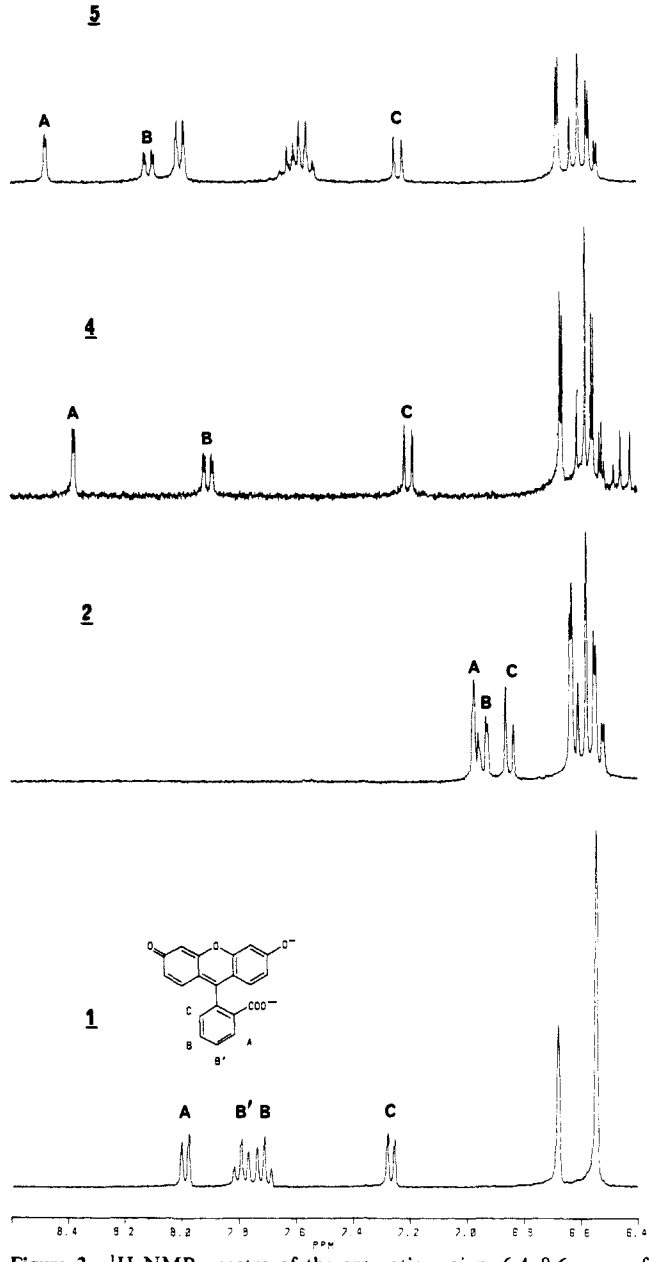

Figure 3. ^1H NMR spectra of the aromatic region, 6.4–8.6 ppm, of fluorescein (**1**), fluoresceinamine (**2**), *N*-fluoresceinylacrylamide (**4**), and *N*-fluoresceinylbenzamide (**5**). The labeled peaks correspond to the protons on the phenyl ring.

Table II. ^1H NMR Frequencies (δ) of Phenyl Protons on Fluorescein and Derivatives.

	^1H		
compound	2′	3′, 4′	5′
1	7.26	7.72, 7.79	7.99
2	6.87	6.93	6.97
3	7.25	7.85	8.21
4	7.21	7.92	8.39
5	7.24	8.12	8.47
10	7.27	8.11	8.47

The phenyl proton shifts of fluoresceinamine derivatives can also be correlated with the unquenching of fluorescence by the mechanism of excited-state electron transfer from the nitrogen electron pair. The predictive value of the NMR spectra has provisional utility. Although all of the amides (Table I) had ^1H NMR spectra with this corresponding shift of the phenyl protons, not all formed the highly fluorescent products.

The choice of substituent on the acyl chloride can impose a second quenching mechanism on the molecule. Formation of the

N-fluoresceinyl-*p*-nitrobenzamide produced a molecule with markedly quenched fluorescence. Since the ^{1}H NMR spectrum confirmed the structure of the amide (Table II), the original quenching mechanism of electron transfer from the amine has been relieved and a secondary one must be operative. It is well-known that nitrobenzenes can quench fluorophores via the strong magnetic fields surrounding their atomic cores, which promote spin decoupling of electrons and triplet formation;[14] in this phenomenon, contact between the quencher and fluorophore is a requirement for the quenching mechanism to occur. It is plausible that the intramolecular geometry of the nitrobenzoylated fluoresceinamine derivative makes the interactive decoupling pathway between the xanthene and the nitro groups very efficient. In the three nitrobenzoylated derivatives, different degrees of quenching were observed (Table I), suggesting that the quenching efficiency is influenced by the position of the nitro group.[15,16]

Fluorescence intensity of **2** could also be restored by addition of the cationic surfactant, cetyltrimethylammonium bromide (CTAB). Micellar-enhanced fluorescence is used to improve sensitivity in fluorescence-based assays.[17] For example, Habashy et al.[18] observed a 3-fold enhancement of the fluorescence of acyclic azines when in a concentrated solution of CTAB. They attributed this result to the azine molecule residing in a solvation site of the host micelle with limited rotational freedom leading to higher fluorescence intensity. In our experiment addition of the nonionic polyoxyethylenesorbitan monolaurate (Tween 20) to fluoresceinamine produced a similar 3-fold enhancement, while addition of CTAB to fluoresceinamine produced a dramatic 27-fold enhancement of fluorescence. Other reasons have been suggested for decreases in radiationless relaxation by addition of a surfactant to a homogeneous dye solution such as shielding from quenchers and decreased polarity.[17c] The very sizable increase of fluorescence observed by addition of CTAB suggests that a different mechanism is operative with the cationic surfactant and that the intramolecular quenching of **2** may be relieved by noncovalent interactions.

In conclusion, the fluoresceinamine molecule presents another example of electronically mediated fluorescence where a quenching process is reversed, by either covalent bonding or electrostatic processes. Studies of quenching mechanisms and their distance and spatial requirements are well suited to this molecule. The ready preparation and purification of a wide variety of derivatives offers a facile means for investigating quenching substituents. When used for applications in cytology or sensor chemistry, fluoresceinamine must be derivatized at the amine to display the highly fluorescent pH-sensitive indicator properties typical of fluorescein. Once derivatized, the molecule can be used as an indicator with the provision that all derivatives will not yield the same degree of fluorescence.

Experimental Section

General Procedures. Unless noted otherwise, commercial solvents and reagents were used without further purification. Fluorescein, fluoresceinamine (isomers I and II), and the acyl halides were purchased from Aldrich Chemical Co. The concentrations of the fluoresceinamine derivatives were determined by absorption spectra with an IBM 9400 UV/vis spectrometer, with an extinction coefficient of 8.8×10^4. pH measurements were determined with an Altex 3500 Digital pH meter and a Fisher Acument pH meter, Model 815MP. ^{1}H NMR and ^{13}C NMR spectra were determined by a Bruker AM 300 spectrometer. Chemical shifts (δ) are reported relative to trimethylsilane. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartert; m, multiplet; and br, broad signal. Mass spectra were obtained with a Hewlett-Packard HP 5988 A GC–MS, at 70 eV, with a direct-insertion probe.

Fluorescence Measurements. Two different instrumentation systems, both employing fiber optics as light guides, were used for fluorescence measurements. The first system has been described[9b] previously and employs an argon ion laser (λ 488 nm) as the source. The excitation light is guided through the appropriate lenses and filters into an optical fiber (Corning Core Guide glass NA = 0.28). The fluorescence signal is then transmitted through the same fiber, reflected by a dichroic mirror, filtered with a short band-pass filter, and selected for emission wavelength with a Spex single-grating monochromator. The wavelength-selected light is focused on a photomultiplier tube and measured with a photon-counting detector (Pacific Instruments Model 126). The second system[19] has the capacity for excitation at variable wavelengths. The source consists of a 75-W high-pressure xenon arc lamp (Osram Co.), which provides a continuous spectrum from 190 to 750 nm, and a Spex 1680 0.22-m double monochromator for selecting the specified excitation wavelength. Again the light is focused into an optical fiber by the appropriate mirrors and lenses, with the returning fluorescence emission passing through the same fiber, and the light is guided into the entrance slit of the emission detection system, comprised of a second Spex 1680 0.22-m double monochromator with 300 lines/mm grating, a RCA 31034A-02 photomultiplier tube, and photometer. The data are finally acquired and displayed by a PC-AT, which also contains an AD (analog to digital) and DA (digital to analog) board to control the stepping motors used to move the excitation and emission spectrometers. All fluorescence measurements were carried out at room temperature.

Amide Derivatives of Fluoresceinamine. All amide derivatives formed after reaction of 2:1 stoichiometric quantities of acyl chloride with fluoresceinamine in dry acetone. The mixture was stirred between 2 and 18 h, until the product precipitated. Product was collected by filtration, washed with methylene chloride and acetone, and then dried by evaporation. The products were checked for purity with TLC (4:1, ethanol–CH_2Cl_2, silica plate) and were characterized by ^{1}H NMR, ^{13}C NMR, and low-resolution mass spectroscopy. All NMR spectra were taken at 50 °C in DMSO-d_6.

1: (included as reference) ^{1}H NMR δ 6.54 (s, 4 H), 6.68 (s, 2 H), 7.26 (d, 1 H), 7.72 (t, 1 H), 7.79 (d, 1 H), 10.18 (br, 1 H).

2: ^{1}H NMR δ 6.5–6.6 (m, 6 H), 6.87 (d, 1 H), 6.93 (m, 1 H), 6.97 (m, 1 H), 10.18 (br, 1 H); ^{13}C NMR 14 signals total (C4′, COO^-, amide, and derivative carbons reported only) δ 139.7 (C4′), 169.2 (COO^-); mp 223 °C dec.

4: ^{1}H NMR δ 5.82 (2d, 1 H), 6.33 (2d, 1 H), 6.4–6.7 (m, 1 H), 7.21 (d, 1 H), 7.92 (d, 1 H), 8.39 (d, 1 H), 10.8 (br, 1 H); ^{13}C NMR 17 signals total δ 145.8 (C4′), 168.4 (COO^-), 163.7 (amide) 131.5, 127.2 (vinyl); MS ($C_{23}H_{14}O_6N$) calcd 400.05, M^+ 400.4 (2%), $M^+ - CO_2$ 356.0 (12%); mp 237 °C dec.

5: ^{1}H NMR δ 6.60 (m, 6 H), 7.24 (d, 1 H), 7.5–7.6 (m, 3 H), 8.0 (d, 2 H), 8.12 (2d, 1 H), 8.47 (d, 1 H), 10.8 (br, 1 H); ^{13}C NMR 19 signals total δ 145.5 (C4′), 168.4 (COO^-), 166.0 (amide), 127.2–145.5 (4 benzyl carbons); MS ($C_{27}H_{17}O_6N$) calcd 451.09, M^+ 451.3 (17.7%), $M^+ - CO_2$ 407.0 (11.2%); mp 225 °C dec.

6: ^{1}H NMR δ 1.0 (d, 6 H), 2.18 (m, 1 H), 6.6 (m, 6 H), 7.15, (d, 1 H), 7.82, (2d, 1 H), 8.29 (d, 1 H), 10.2 (br, 1 H); ^{13}C NMR 18 signals total δ 146.1 (C4′), 168.7 (COO^-), 171.4 (amide), 45.7 (CH), 25.7 (CH_2), 22.3 (CH_3); MS ($C_{25}H_{23}O_6N$) calcd 433.14, M^+ 433.1 (11.7%), $M^+ - CO_2$ 389.0 (18.9%); mp 214 °C dec.

7: ^{1}H NMR δ 3.84 (s, 3 H), 6.5–6.7 (m, 6 H), 7.11 (d, 2 H), 7.25 (d, 1 H), 8.0 (d, 2 H), 8.1 (d, 1 H), 8.49 (d, 1 H), 10.5 (br, 1 H); ^{13}C NMR 20 signals total δ 145.8 (C4′), 168.6 (COO^-), 165.5 (amide), 113.2–130.0 (4 benzyl carbons), 55.6 (OMe); MS ($C_{28}H_{19}O_7N$) calcd 481.10, M^+ 481.0 (2%), $M^+ - CO_2$ 437.0 (58.7%); mp 239 °C dec.

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(15) Fluoresceinamine is available in two isomers: isomer I, 5′-aminofluorescein; isomer II, 6′-aminofluorescein. All of the derivatives were made with isomer I. It was established that the fluorescence quenching behavior of isomer II was identical with that of isomer I. Therefore, the quenching is not due to the mesomeric effect of the amine. Reaction of isomer II with naphthalenesulfonyl chloride generated fluorescence similar to the reaction with isomer I. The unquenching of the fluorescence through amide formation is expected to be identical with the two isomers. However, the through-space quenching to nitro substituents could be affected by the choice of fluoresceinamine isomer where the degree of orbital overlap is a factor.

(16) It was suggested that the quenching in the nitrobenzoylated derivatives may be due to charge transfer where the xanthene moiety now acts as an electron donor. In this case the difference in quenching efficiency between the ortho and para derivatives and the meta derivative reflects differences in the resonance stabilization of the anion radical. Since the ortho and para derivatives are more easily reduced, they would necessarily produce more quenching of the fluorophore. The conformational requirements for a charge-transfer interaction may be possible in this derivative.

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8: ^{1}H NMR δ 6.5-6.7 (m, 6 H), 7.24 (d, 1 H), 7.77-7.94 (m, 4 H), 8.15 (2d, 1 H), 8.35 (br, 1 H); ^{13}C NMR 19 signals total δ 146.4 (C4'), 168.2 (COO^-), 164.6 (amide), 124.2-132.1 (4 benzyl carbons); MS ($C_{27}H_{16}O_8N_2$) calcd 496.07, $M^+ - CO_2$ 452.0 (39.4%); mp 220 °C dec.

9: ^{1}H NMR δ 6.5-6.7 (m, 6 H), 7.26 (d, 1 H), 7.87 (t, 1 H), 8.12 (2d, 1 H), 8.44 (m, 3 H), 8.82 (br, 1 H); ^{13}C NMR 19 signals total δ 146.1 (C4'), 168.3 (COO^-), 163.8 (amide), 124.2-132.1 (4 benzyl carbons); MS ($C_{27}H_{16}O_8N_2$) calcd 496.07, $M^+ - CO_2$ 452.0 (30.5%); mp 247 °C dec.

10: ^{1}H NMR δ 6.5-6.7 (m, 6 H), 7.27 (d, 1 H), 8.11 (2t, 1 H), 8.23 (d, 2 H), 8.37 (d, 2 H), 8.47 (d, 1 H); ^{13}C NMR 19 signals total δ 146.8 (C4'), 168.5 (COO^-), 164.5 (amide), 124.5-127.5 (4 benzyl carbons); MS ($C_{27}H_{16}O_8N_2$) calcd 496.07, $M^+ - CO_2$ 452.0 (55.1%); mp 316 °C dec.

pH Titrations of Fluorescein and Fluoresceinamine. Dye solutions (10^{-5} M) were prepared with 5 mM HCl and titrated with 500 mM NaOH. pH and fluorescence intensity were monitored simultaneously with the Fisher Acumet pH meter and variable-excitation fluorescence spectrometer.

Titration of Fluoresceinamine with Surfactants. Solutions of 0.5 mM fluoresceinamine and 0.5 mM CTAB or Tween 20 were prepared with 100 mM phosphate buffer, pH 7.5. Aliquots of surfactant were added to fluoresceinamine solution, and the generation of fluorescence was monitored with a bare optical fiber in solution, with the instrumentation previously described.

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Determination of Exciton Hopping Rates in Ruthenium(II) Tris(bipyridine) Complexes by Picosecond Polarized Absorption Spectroscopy

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Abstract: Exciton hopping in excited state $Ru^{II}(bpy)_3$ has been studied in room-temperature solutions with picosecond polarized absorption spectroscopy. Initial excitation is to the metal-to-ligand charge-transfer state (460 nm), and the excited state is probed by polarized 355-nm light. In ethylene glycol the depolarization ratio exhibits a biexponential decay, consisting of 75 ps and 2.4 ns components. The two components of the decay are assigned, respectively, to exciton hopping and to rotational diffusion. The presence of a fast component shows that the optical electron is intrinsically localized on a specific ligand. The amplitudes of the fast and slow polarization decay components may be quantitatively understood in terms of photoselection theory. Exciton hopping in water and methanol solutions occurs much more rapidly than in ethylene glycol. These results are interpreted in terms of solvent-mediated electron-transfer theory.

The photophysics of tris(bipyridine)ruthenium(II), $Ru^{II}(bpy)_3$, have been extensively studied over the past decade. It has long been known that the lowest energy excited state of this complex is metal-to-ligand charge-transfer (MLCT) in nature. It was later established that in room-temperature solutions, the optical electron (i.e., the electron transferred from the metal) is localized on a single bipyridine ligand.[1-4] Since the issue of localization vs delocalization was settled, other questions, regarding the localization time scale and the role of the solvent in the localization process, have been widely debated in the literature. Recent interest has also focussed on the question of the time scale for transfer of the optical electron from one bipyridine ligand to another. This intramolecular electron-transfer process has generally been referred to as "exciton hopping". Since the electronic excitation remains on the same $Ru^{II}(bpy)_3$ molecule, this is a bit of a misnomer. However, in keeping with the precedents in the literature, we will refer to this excited state interligand electron transfer as "exciton hopping".

The results of several experimental studies have been interpreted in terms of initial delocalization of the optical electron. If the exciton is initially delocalized, localization may then occur in concert with solvent relaxation, which stabilizes the nascent dipole (the "solvent-induced localization" model). Magnetic circularly polarized luminescence, time-resolved luminescence, and nanosecond transient Raman results have been interpreted in terms of this model.[5-7] More recently, picosecond transient Raman results on $Ru^{II}(bpy)_3$ in water and in glycerol have also been interpreted to support this model.[8]

Other studies have yielded evidence in contradiction with the solvent-induced localization model. These studies have concluded that the optical electron is intrinsically localized on a single ligand upon absorption of the photon, so that solvent motions are not required to facilitate localization. Solvent dependent shifts in the absorption spectrum of $Ru^{II}(bpy)_3$ have been interpreted in terms of production of a dipole upon absorption of the photon, i.e., intrinsic localization.[9] This mechanism is also strongly supported by photoselection/polarized emission studies in frozen glasses.[4,10-12] Polarizations are obtained which are too large to be explained by a delocalized model. Further support for intrinsic localization also comes from picosecond transient Raman studies.[13] In these

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