

Solvent-Cage Effect (Viscosity Dependence) as a Diagnostic Probe for the Mechanism of the Intramolecular Chemically Initiated Electron-Exchange Luminescence (CIEEL) Triggered from a Spiroadamantyl-Substituted Dioxetane

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Abstract: The excitation step of the intramolecular CIEEL generation in the triggered cleavage of spiroadamantyl-substituted dioxetane has been studied. The electron back-transfer (BET) process versus the direct chemiexcitation of the phenolate-anion emitter have been considered as mechanistic alternatives. The observed solvent-cage effect on the CIEEL generation, manifested by the increase of the singlet chemiexcitation yield at increased viscosity, provides evidence that the BET process operates in the intramolecular CIEEL mechanism.

Introduction

Chemically initiated electron-exchange luminescence (CIEEL),¹ a phenomenon of light emission derived from electron-transfer chemistry,^{2,3} constitutes a general chemiluminescent process. This phenomenon was originally discovered by Schuster for diphenoyl peroxide⁴ and in the meantime documented for α -peroxy lactones⁵ and appropriate dioxetanes.⁶ The CIEEL generation may result from both inter- and intramolecular electron transfer, the latter has been proposed in the case of the firefly bioluminescence.⁷ The intramolecular CIEEL is of particular interest for modern chemiluminescent bioassays^{8,9} developed for clinical applications, in which CIEEL-active dioxetanes are used. The most efficient CIEEL systems^{10,11} for the latter purpose utilize thermally persistent spiroadamantyl-substituted dioxetanes with a properly protected phenolate ion. Also diisopropyl-substituted derivatives synthesized recently¹²

have revealed remarkable thermal persistence and CIEEL efficiency. The CIEEL of these dioxetanes may be generated at will by treatment with an appropriate reagent (trigger) to release the phenolate ion; the choice of the trigger depends on the nature of the protective group. The rational design⁸ of more effective CIEEL systems requires a detailed knowledge of the mechanism for the intramolecular CIEEL process, the purpose of our present study.

Recently we have reported model studies on the phenolate-initiated intramolecular CIEEL process, in which we have examined the reaction kinetics of the silyloxy-substituted spiroadamantyl dioxetanes chemically triggered by fluoride ions¹³ and of the phosphate-protected ones enzymatically triggered by alkaline phosphatase¹⁴ and elucidated the solvatochromic effects on the CIEEL versus the photoexcited fluorescence of the authentic CIEEL emitter.¹⁵ However, the most fundamental question on the CIEEL mechanism remained open. Scheme 1 illustrates the mechanistic alternatives for the intramolecular CIEEL process of the spiroadamantyl-substituted dioxetane **1**. The CIEEL is generated by the cleavage of the

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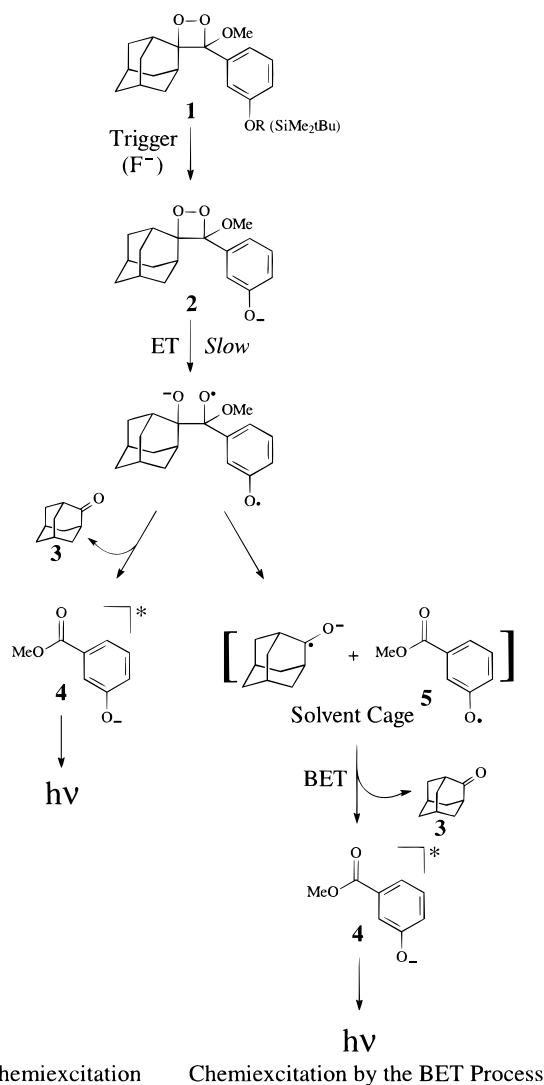
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Scheme 1



intermediate dioxetane phenolate ion **2**, initiated by the intramolecular electron transfer (ET) from the phenolate moiety to the antibonding σ^* orbital of the peroxide bond (Scheme 1), concomitant with O—O bond cleavage. This initial ET process is expected to be slow (rate-limiting) because energy is required to remove an electron from the phenolate-ion functionality¹⁶ and put it into the peroxide bond.¹⁷ As one can see from Scheme 1, the formation of the excited methyl *m*-oxybenzoate ion (**4**), the CIEEL emitter, may proceed either directly or by the electron back-transfer (BET) between the primary solvent-caged radical fragments. Consequently, for the elucidation of the intramolecular CIEEL mechanism, a distinction between the direct chemiexcitation channel and the chemiexcitation by the BET process is required.

Our recent observations¹⁵ of the hydrogen-bonding effects on the fluorescence versus the CIEEL emission spectra of the oxybenzoate ion **4** in protic media have been rationalized in terms of the BET mechanism for the CIEEL generation. The oxybenzoate radical **5**, a species with negligible hydrogen bonding compared to the ground-state ion **4**, was proposed as immediate precursor to the singlet-excited emitter **4** (Scheme 1) to account for the lack of solvatochromism (blue shift in

protic solvents) in the CIEEL emission. However, the validity of the BET mechanism was questioned for the intermolecular CIEEL process; for example, alternative to the BET mechanism, in the intermolecular CIEEL reactions of diphenoyl peroxide and anthracene endoperoxide,^{18,19} a charge-transfer complex was proposed, in which the excited activator (the chemiluminescence emitter) is generated simultaneously with the peroxide cleavage.¹⁸

How can one distinguish between the direct and the BET excitation channels? If the BET process is responsible for the CIEEL generation (Scheme 1), its efficiency should be subject to a solvent-cage effect through the viscosity dependence. In contrast, no significant viscosity effect should be expected on the direct chemiexcitation channel. Thus, the viscosity effect on the chemiexcitation efficiency may serve as an experimental diagnostic tool to differentiate between the mechanistic alternatives shown in Scheme 1.

Viscosity Dependence of the Chemiexcitation Process

CIEEL and Chemiexcitation Yields. The CIEEL intensity i^{CIEEL} is expressed by eq 1, in which ν is the reaction rate, Φ^{CIEEL}

$$i^{\text{CIEEL}} = \Phi^{\text{CIEEL}} \nu = \Phi^{\text{fl}} \Phi_{\text{S1}} \nu \quad (1)$$

$$N_{\text{photons}} = \int_0^{\infty} i^{\text{CIEEL}} dt = \Phi^{\text{CIEEL}} \int_0^{\infty} \nu dt = \Phi^{\text{CIEEL}} [\mathbf{1}] \quad (2)$$

$$\Phi^{\text{CIEEL}} = \frac{N_{\text{photons}}}{[\mathbf{1}]} \quad (3)$$

$$\Phi_{\text{S1}} = \frac{\Phi^{\text{CIEEL}}}{\Phi^{\text{fl}}} \quad (4)$$

$$\Phi_{\text{S1}} = \Phi_{\text{S1}}^0 + \Phi_{\text{S1}}(\eta) \quad (5)$$

$= \Phi_{\text{S1}} \Phi^{\text{fl}}$ represents the CIEEL yield, and Φ_{S1} and Φ^{fl} are the singlet chemiexcitation yield and the fluorescence efficiency of the CIEEL emitter. Data on the CIEEL yield are available through the measurements of the total amount of light (N_{photons}) emitted in the complete dioxetane decomposition. The expression for N_{photons} , *i.e.* the area under the CIEEL intensity curves, is given by integration of $i^{\text{CIEEL}}(t)$ over the reaction time (eq 2), in which $\int_0^{\infty} \nu dt$ represents the total concentration $[\mathbf{1}]$ of the decomposed dioxetane; it follows that Φ^{CIEEL} is experimentally defined by eq 3. The required data on Φ_{S1} versus viscosity (η) may be obtained from eq 4 by measurement of the Φ^{CIEEL} and Φ^{fl} values as a function of viscosity. In the general case of the CIEEL process (Scheme 1), the singlet excitation yield (Φ_{S1}) is the sum of the contributions of the viscosity-independent direct (Φ_{S1}^0) and the viscosity-dependent BET [$\Phi_{\text{S1}}(\eta)$] chemiexcitation channels (eq 5).

Choice of Solvents for the Viscosity Studies. For the viscosity variation, variable compositions of mixtures of a fluid (benzene) and a viscous (diphenylmethane) solvent have been used in this work. The choice of the solvent components for such viscosity studies is restricted for several reasons. Since highly polar media will affect ion pairing, one should use solvents of low polarity to avoid this. Hydrogen bonding in protic media would also complicate the cage-effect studies; therefore, aprotic solvents are preferable. The major requirement of the proper solvent choice comes from the Marcus free-energy relationship for the electron transfer (eq 6).^{20,21} Besides the

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$$\Delta G_{\text{BET}}^* = \Delta G_0^* \left(1 + \frac{\Delta G^0}{4\Delta G_0^*} \right)^2 \quad (6)$$

$$\Delta G_0^* = \frac{e^2}{4} \left(\frac{1}{2r_D} + \frac{1}{2r_A} - \frac{1}{r_{\text{DA}}} \right) \left(\frac{1}{n^2} - \frac{1}{\epsilon} \right) \quad (7)$$

standard free-energy difference ΔG^0 for the electron transfer, the so-called "intrinsic" activation barrier, ΔG_0^* , is also involved. The latter value is associated with the bond-length changes and the reorganization energy of the solvent, which for most cases of the electron transfer between organic molecules is considered as the most important term.^{21b} According to Marcus theory,^{20,21} ΔG_0^* may be expressed by eq 7, in which e is the charge of the electron, r_D and r_A are the electron-donor and -acceptor radii, r_{DA} represents their collision diameter, and ϵ is the dielectric constant of the solvent and n is its refractive index. As one can see from eq 7, the variation of the solvent composition may significantly affect the electron-transfer (BET) process through the changes of the solvent reorganization energy, especially when the values of $(1/n^2 - 1/\epsilon)$ differ substantially for the solvent components. To avoid this, the $(1/n^2 - 1/\epsilon)$ value should be as close as possible for the chosen low and high viscosity components of the reaction medium. The selected benzene and diphenylmethane (Ph_2CH_2) solvents meet all the requirements mentioned above, namely both are aprotic solvents of low polarity with the $(1/n^2 - 1/\epsilon)$ value of ca. 0.004 each. The same solvent combination was previously used for the study of the viscosity effect on the triplet-triplet energy transfer²² and the autoxidation kinetics.²³

Experimental Section

Materials. 3-(2'-Spiroadamantane)-4-methoxy-4-(3''-butyldimethylsilyloxy)phenyl-1,2-dioxetane (**1**) was prepared as previously reported.¹³ Methyl 3-butyldimethylsilyloxybenzoate was kindly made available by Dr. M. Schulz.²⁴ As fluoride ion source, a 1 M stock solution of tetrabutylammonium fluoride in tetrahydrofuran (THF) [Aldrich] was used. The solvents benzene and diphenylmethane (Fluka) were distilled from EDTA (50 g/L) under a nitrogen-gas atmosphere at reduced pressure.

CIEEL Measurements. The CIEEL-intensity measurements in benzene- Ph_2CH_2 solutions were performed on a Mitchell-Hastings photometer²⁵ as described previously.¹³ Small portions (1.6% of volume) of the THF/DMSO solutions [1:1] of ammonium fluoride (the fluoride-ion source) were added to the benzene- Ph_2CH_2 mixtures to trigger the decomposition of the dioxetane **1** (Scheme 1) in these media.

Spectral Measurements. The fluorescence and the CIEEL spectra were recorded on a Perkin-Elmer LS 50 spectrofluorimeter. To enable a comparison of the fluorescence and the chemiluminescence spectra with different intensities, these spectra were normalized by adjusting the maximum intensities to the same value. This procedure was done with the normalization option as implemented in the Perkin-Elmer Fluorescence Data Manager (FLDM) set of programs. The fluorescence quantum yields were measured by the conventional procedure versus quinine bisulfate ($[\text{QBS}] = 1.19 \times 10^{-6} \text{ M}$) in 1 N H_2SO_4 as the fluorescence standard ($\Phi_{\text{QBS}}^{\text{fl}} = 0.55$).²⁶ The solutions of the oxyben-

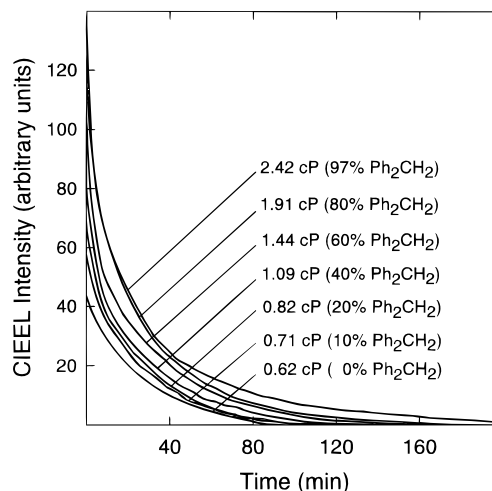


Figure 1. CIEEL decay of the fluoride-ion-triggered ($[\text{n-Bu}_4\text{NF}] = 8.33 \times 10^{-3} \text{ M}$) dioxetane **1** ($[\text{I}] = 6.0 \times 10^{-9} \text{ M}$) in aerated benzene-diphenylmethane mixtures at 26 °C as function of viscosity.

zoate ion **4** in benzene and Ph_2CH_2 for the spectral measurements were prepared by addition of small portions (0.08% of volume) of its stock solution in DMSO, obtained by the reaction of the methyl 3-silyloxybenzoate with an excess of fluoride ions.

Viscosity Measurements. The viscosity measurements of the benzene-diphenylmethane mixtures (0 to 97% Ph_2CH_2) were carried out with the help of an Ostwald capillary viscosimeter.²⁷

Results and Discussion

The viscosity dependence of the singlet chemiexcitation yield (Φ_{S_1}) was studied in benzene- Ph_2CH_2 mixtures by varying the amount of the viscous Ph_2CH_2 . It is fortunate that the fluorescence quantum yield (Φ^{fl}) of the methyl *m*-oxybenzoate anion (**4**), the authentic CIEEL emitter, does not depend on $[\text{Ph}_2\text{CH}_2]$ since it takes the values $\Phi^{\text{fl}} = 0.230 \pm 0.015$ over the entire concentration range.

The experimental data in Figure 1 show that a regular enhancement of the CIEEL emission responds to a sequential viscosity increase. From the areas under the CIEEL intensity curves (Figure 1), calibrated against the Hastings-Weber scintillation standard,²⁵ the required N_{photons} values were determined and the Φ^{CIEEL} quantities calculated according to eq 3. The chemiexcitation yields (Φ_{S_1}) were obtained as the quotient of Φ^{CIEEL} and Φ^{fl} (eq 4), for the latter the measured value of $\Phi^{\text{fl}} = 0.230 \pm 0.015$ was used. The plot of the singlet chemiexcitation yield versus viscosity is displayed in Figure 2. The viscosity dependence of the chemiexcitation yield suggests that the BET process (Scheme 1) operates in the CIEEL generation. A quantitative analysis of the observed viscosity dependence of the chemiexcitation yield for the BET process has been conducted in terms of the probabilistic model for the solvent-cage effect, similar to that which was previously developed for triplet-triplet energy transfer,²² which is given as Supporting Information.

An alternative to the BET chemiexcitation mechanism, which would also be subject to a viscosity effect, is exciplex formation in the CIEEL process (Scheme 2). The exciplex might form immediately from the dioxetane phenolate anion **2**, as well as from the primary solvent-caged reaction products **3** and **4**. In the latter case, a viscosity increase would promote exciplex formation between the solvent-caged species **3** and **4** by

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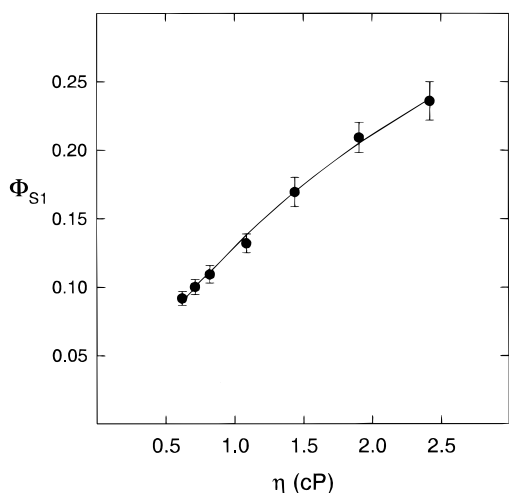
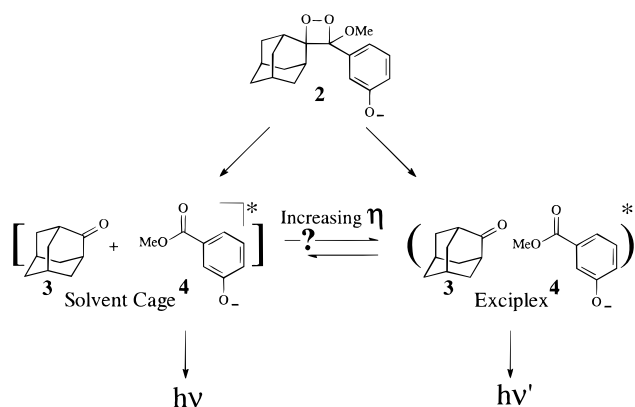


Figure 2. Viscosity dependence of the singlet chemiexcitation yield (Φ_{S1}) in the fluoride-ion-triggered CIEEL cleavage of the dioxetane **1** at the experimental conditions of Figure 1. The error bars represent the range of four measurements for each point on Figure 2.

Scheme 2



diminishing cage escape. To probe for such exciplex involvement, the CIEEL spectra of the dioxetane **1** were compared with the fluorescence spectra of the methyl *m*-oxybenzoate ion (**4**) in pure benzene and in pure diphenylmethane (Figure 3). As seen in Figure 3, the normalized CIEEL and fluorescence spectra coincide both in C_6H_6 and Ph_2CH_2 , which provides unequivocal evidence for the methyl *m*-oxybenzoate ion (**4**) as an authentic CIEEL emitter in both solvents. Consequently, no exciplex is involved in the CIEEL generation in these media. Therewith

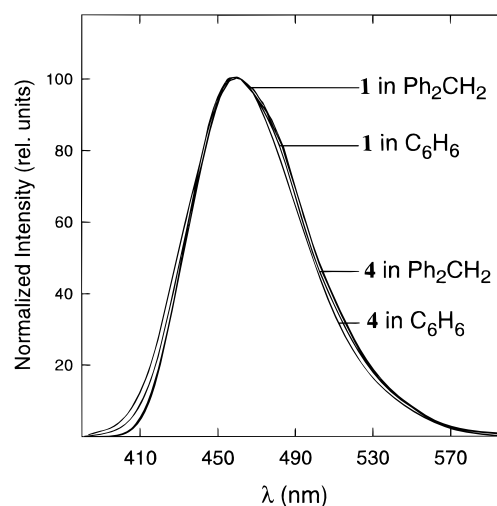


Figure 3. Normalized spectra for the fluoride-ion-triggered CIEEL emission of the dioxetane **1** ($[1] = 1.02 \times 10^{-4}$ M, $[n-Bu_4NF] = 8.33 \times 10^{-3}$ M) in benzene and diphenylmethane at 26 °C and fluorescence spectra of the authentic methyl *m*-oxybenzoate ion ($[4] = 1.05 \times 10^{-5}$ M, $\lambda_{ex} = 372$ nm).

we have convincingly established that the observed viscosity dependence of the chemiexcitation efficiency requires the solvent-cage effect as proposed for the BET excitation process in Scheme 1.

We conclude that the viscosity dependence of the chemiexcitation yield has proved to be a suitable diagnostic tool to substantiate the BET mechanism in the intramolecular CIEEL process examined herein. Whether the BET mechanism also operates in the questioned intermolecular CIEEL process^{18,19} requires a detailed study on the viscosity dependence of the chemiexcitation efficiency.

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Supporting Information Available: Quantitative analysis of the viscosity data in terms of the probabilistic cage-effect model (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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