Viscosity Dependence of the Chemically Induced Electron-Exchange Chemiluminescence Triggered from a Bicyclic Dioxetane

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Abstract: The viscosity dependence for the excitation step of the chemically initiated electron-exchange luminescence (CIEEL) triggered from a novel bicyclic furan-annelated dioxetane has been studied. The observed small but mechanistically significant enhancement of the CIEEL efficiency with increasing viscosity is rationalized in terms of the free-volume model, in which the molecular bond-rotation process that leads to the ground-state product is frictionally impeded and the chemiexcitation yield is higher.

Introduction

Chemically initiated electron-exchange luminescence (CIEEL),[1] a chemiluminescence process derived from electron-transfer chemistry,[2,3] is a general phenomenon which was originally discovered by Schuster for diphenoyl peroxide[4] and abundantly reported for α-peroxy lactones[5] 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] in which CIEEL-active dioxetanes are used. The most popular CIEEL systems,[10,11] developed for clinical bioassay applications, utilize thermally persistent monocyclic spiroadamantyl-substituted dioxetanes with a properly protected phenolate ion. Also the novel triggerable bicyclic dioxetanes[12] possess remarkable thermal persistence and appreciable CIEEL efficiency and thereby qualify for chemiluminescence bioassays.

A pertinent feature of the phenolate-ion-initiated CIEEL process is the intramolecular electron transfer (ET) from the phenolate moiety to the antibonding σ^* orbital of the peroxide bond, concomitant with O−O bond cleavage; however, a detailed mechanism of excited-state generation in this chemiluminescent process still needs to be established.[13] Recently, we have provided a valuable experimental probe, namely, the viscosity effect on the CIEEL efficiency, to distinguish between the two main mechanistic alternatives for the CIEEL generation of the monocyclic dioxetane,[14] namely, direct chemiexcitation by concerted dioxetane cleavage through charge transfer and a stepwise electron-transfer process, in which chemiexcitation arises from electron back-transfer (BET) between the primary solvent-caged radical fragments as the key step. The basic concept of the BET mechanism for the CIEEL process[1,4,7] rests on the observed chemiexcitation in the electron transfer for chemically[3] and electrochemically[2] generated ion−radical pairs. If the BET process operates in the CIEEL generation, its efficiency should be subject to a solvent−cage effect; e.g., it should obey a viscosity dependence. In our previous study on the monocyclic dioxetane, we have found an increase of the chemiluminescence efficiency on increasing viscosity,[14] which is consistent with the BET mechanism.

Presently, we have investigated the viscosity dependence of the CIEEL-active bicyclic dioxetane **1**[12] (Scheme 1), for a rigorous scrutiny of the CIEEL mechanism. The reasons for

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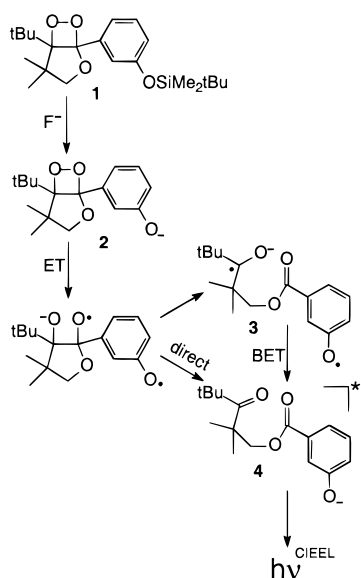   (11) (a) Schaap, A. P.; Chen, T.-S.; Handley, R. S.; DeSilva, R.; Giri, B. P. *Tetrahedron Lett.* **1987**, *28*, 1155−1158. (b) Schaap, A. P.; Handley, R. S.; Giri, B. P. *Tetrahedron Lett.* **1987**, *28*, 935−938.
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Scheme 1

Figure 1. Viscosity dependence of the singlet chemiexcitation yield (Φ_{S_1}, ■) the fluoride-ion-triggered ($[n\text{-}Bu_4NF] = 8.5 \times 10^{-3}$ M) CIEEL cleavage of the dioxetane **1** ($[\mathbf{1}] = 6.5 \times 10^{-9}$ M) and the fluorescence quantum yield (Φ^{fl}, ●) of the authentic methyl *m*-oxybenzoate ion ($[\mathbf{4}] = 1.1 \times 10^{-5}$ M); the error bars represent the range of four measurements for each data point.

this choice are the following: (a) Contrary to the monocyclic dioxetane,[14] cage escape is not possible for bicyclic dioxetane **1**; (b) the CIEEL emitters triggered from mono-[14] and bicyclic dioxetanes possess the same chromophore, i.e., the *m*-oxybenzoate ion moiety; (c) as we have recently established,[12b] no exciplex is involved in both CIEEL processes (Scheme 1). The relevant question is, however, whether the CIEEL intensity triggered from **1** is subject to a viscosity effect, as we have observed for the monocyclic dioxetane. Herein, we report our results on the viscosity dependence of the CIEEL process for the bicyclic furan-annelated dioxetane **1** and compare it with that of the monocyclic spiroadamantyl-substituted dioxetane.[14]

The CIEEL intensity, $i^{\text{CIEEL}} = \Phi^{\text{CIEEL}}v$, is a product of the reaction rate v and the CIEEL yield, $\Phi^{\text{CIEEL}} = \Phi_{S_1}\Phi^{fl}$, in which Φ_{S_1} and Φ^{fl} are the singlet chemiexcitation yield and the fluorescence efficiency of the CIEEL emitter. The required experimental data for Φ_{S_1} versus viscosity (η) were obtained by measurement of the Φ^{CIEEL} and Φ^{fl} values as a function of viscosity, as described previously.[14] The viscosity was varied by changing the amount of the viscous Ph_2CH_2 in benzene–Ph_2CH_2 mixtures. This solvent combination was used originally with success for the viscosity studies on triplet–triplet energy transfer[15] and autoxidation kinetics.[16]

Results and Discussion

The experimental results for the excitation efficiency (Φ_{S_1}) of dioxetane **1** and the fluorescence quantum yield (Φ^{fl}) of the authentic CIEEL emitter **4** are shown in Figure 1. It is fortunate that the fluorescence yield of the methyl *m*-oxybenzoate anion (**4**), the CIEEL emitter, does not depend on the Ph_2CH_2 concentration, as manifested by the constant values $\Phi^{fl} = 0.220 \pm 0.014$ over the entire concentration range (Figure 1). The same behavior was observed for the *m*-oxybenzoate anion triggered from the monocyclic dioxetane.[14]

The experimental data in Figure 1 disclose a regular enhancement of the chemiexcitation yield (Φ_{S_1}) with an increase in the viscosity. The observed enhancement (ca. 1.4 times) of Φ_{S_1} for a 4-fold viscosity increase is considerably smaller than for the
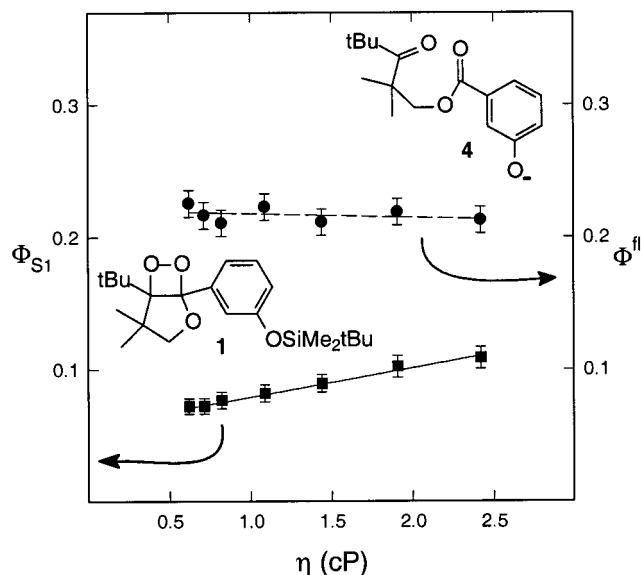
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monocyclic spiroadamantyl-substituted dioxetane (ca. 2.6 times for the 4-fold viscosity increase[14]) but mechanistically significant. What molecular features are responsible for this small (ca. 40%) but definitive viscosity effect in the triggered CIEEL process of bicyclic dioxetane **1**? It certainly cannot be cage escape in the BET step, as was previously established for the monocyclic dioxetane.[14]

To rationalize the observed viscosity behavior for **1** (Scheme 1), it is helpful to consider the MOs of the CIEEL emitter.[12b] The AM1-based calculations have revealed that the $\pi \rightarrow \pi^*$ excitation of this CIEEL emitter constitutes an electronic transition between HOMO and LUMO+1. For the subsequent discussion, it is important to note that the HOMO coefficients are maximal on the phenolate ion functionality, while in the LUMO+1 they are concentrated on the ester moiety[12b] (cf. Supporting Information). For the BET process, this implies that the electron transfer from the ketyl radical to the phenoxyl group of the intermediate **3** should result in the *ground-state* product **4** ($k_{S_0}^{\text{BET}}$), while the electron transfer to the ester moiety should generate the electronically excited **4***, i.e., chemiexcitation ($k_{S_1}^{\text{BET}}$). The reason for the latter is that the ester–ketyl/phenoxyl diradical species generated from **3** is cross-conjugated and represents the first π,π^*-excited state of the phenolate product **4**. Consequently, the chemiexcitation yield (Φ_{S_1}), as expressed by eq 3, is a result of the competition between these excited-state ($k_{S_1}^{\text{BET}}$) and ground-state ($k_{S_0}^{\text{BET}}$) BET channels.

What role does the medium viscosity play in this BET process? Scheme 2 reveals that the initial conformation of the diradical **3** formed on dioxetane cleavage has the ketyl-radical functionality in proximity to the ester carbonyl group. This geometrical arrangement is predestined for electron transfer ($k_{S_1}^{\text{BET}}$) to afford the electronically excited phenolate product **4***; subsequent emission (hv^{CIEEL}) leads to its ground state **4**. Rotations about the methylene bonds in diradical **3** competes with this chemiexcitation BET process ($k_{S_1}^{\text{BET}}$) in that it separates spatially the ketyl-radical and ester-carbonyl functionalities and thereby makes improbable the electron jump between them. At the same time, during such conformational motion, the ketyl-

radical fragment approaches the phenoxyl group, which corresponds to the conformation **3′** in Scheme 2. Electron transfer ($k_{S_0}^{BET}$) from the ketyl radical to the phenoxyl moiety in **3′** should generate preferably the *ground-state* phenolate ion product **4** (cf. Scheme 2) since HOMO coefficients are maximal on the phenolate group.[12b] Expectedly, the molecular rotation in the **3 → 3′** conformational change (competitive to the chemiexcitation BET, $k_{S_1}^{BET}$) is retarded in a more viscous medium and, consequently, the chemiluminescence yield should increase because more of the conformation **3** than **3′** is populated. This was observed experimentally (Figure 1)! Although the viscosity effect is small (ca. 40%) over the narrow viscosity change (ca. 4-fold) investigated here, for such molecular rotations the viscosity retardation is expected to be small.[17,18]

The frictional model[19,20] should apply for the observed viscosity dependence since the available "free volume" in the medium will determine how readily conformational changes through bond rotation will take place and thereby influence the chemiexcitation yield (Φ_{S_1}). The latter is given by eq 1, in which

$$\Phi_{S_1} = \frac{k_{S_1}^{BET}}{k_{S_0}^{BET} + k_{S_1}^{BET}} \tag{1}$$

$$\eta = A \exp(V_0/V_f) \tag{2}$$

$$k_{S_0}^{BET} = k_0 \exp(-\alpha V_0/V_f) \tag{3}$$

$$k_{S_0}^{BET} = k_0(A/\eta)^\alpha \tag{4}$$

$$\frac{k_{S_0}^{BET}}{k_{S_1}^{BET}} = \frac{1 - \Phi_{S_1}}{\Phi_{S_1}} = \frac{k_0}{k_{S_1}^{BET}}(A/\eta)^\alpha \tag{5}$$

$$\ln\frac{1 - \Phi_{S_1}}{\Phi_{S_1}} = \text{const} - \alpha\ln\eta \tag{6}$$

the rate constant $k_{S_0}^{BET}$ needs to be expressed as a function of solvent viscosity (η). According to the free-volume approach,[19a] molecular motion (rotations, translations) in a liquid medium is possible when sufficient free volume (V_f) is available, i.e., when the V_f per molecule is larger than some "critical" value V_0. The fluidity (η^{-1}) is proportional to the probability factor [$\exp(-V_0/V_f)$] for the motion of molecules in the liquid medium, and, hence, the free-volume dependence of the viscosity may be expressed by eq 2, in which A is a proportionality factor. In contrast to the translational motion of a molecule, rotations about bonds involve only a portion of the molecule and, thus, only a fraction αV_0 ($\alpha < 1$) of V_0 is required. Therefore, the rate constant $k_{S_0}^{BET}$ for the ground-state BET process is given by eq 3 (in which k_0 is a preexponential factor), an expression which

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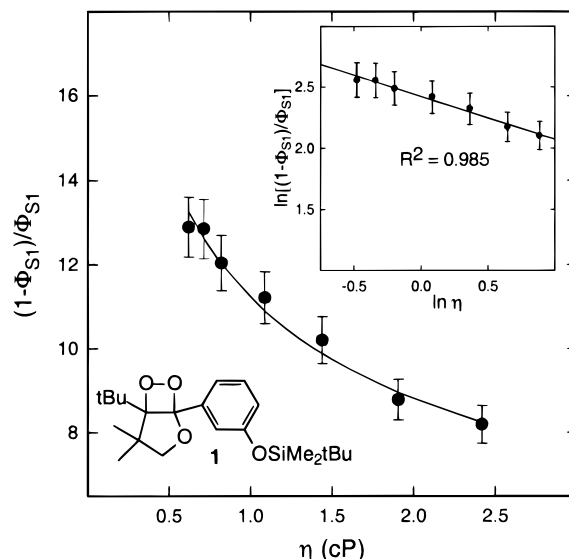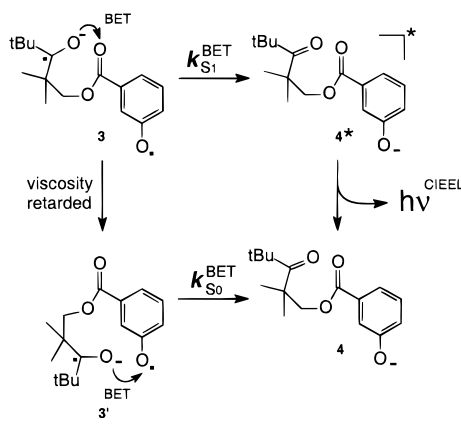

Figure 2. Plot of the ratio $(1 - \Phi_{S_1})/\Phi_{S_1}$ as a function of viscosity according to eq 5 in the fluoride-ion-triggered CIEEL cleavage of dioxetane **1**. The insert displays the double-logarithmic plot according to eq 6.

Scheme 2

was first applied to rationalize the viscosity dependence for the photoisomerization of stilbenes.[20] Substitution of eq 2 into eq 3 gives eq 4 for the viscosity dependence of the rate constant $k_{S_0}^{BET}$. Finally, the combination of eqs 1 and 4 leads to eq 5; its double-logarithmic form (eq 6) predicts a linear dependence of Φ_{S_1} on η.

The experimental viscosity dependence of the chemiexcitation yield is given in Figure 2, and the good linear fit of all the data points in the double-logarithmic plot according to eq 6 ($R^2 = 0.985$, cf. insert in Figure 2) manifests that the "free-volume" model is well-obeyed. The slope of the double-logarithmic plot provides $\alpha = 0.35$, which matches well the values (ca. 0.1−0.6) obtained for a number of viscosity-controlled rearrangement processes.[19,20] The fact that $\alpha < 1$ means that the motion of only a fraction of the molecule is subject to the viscosity effect, which in the present case corresponds to the bond rotations required to bring the ketyl-radical fragment in close vicinity to the phenoxyl-radical site (Scheme 2).

The present study demonstrates that the viscosity effect on the CIEEL generation triggered from the bicyclic furan-annelated dioxetane **1** is significantly lower than that observed from the monocyclic spiroadamantane-substituted dioxetane.[14] For the diradical derived from the bicyclic dioxetane, the conformational changes are retarded by the more viscous

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medium; for the radical pair generated from the monocyclic dioxetane, the more viscous medium slows down cage escape; expectedly, the latter should display a more pronounced viscosity effect in the CIEEL-triggered process.

Supporting Information Available: Text giving the Experimental Section . This material is available free of charge via the Internet at http://pubs.acs.org.

JA000408W