Kinetics of Base Catalyzed Chemiluminescence Reaction of Spiro[adamantane -1,3′-(4′-(*m*-hydroxyphenyl)-4′-methoxy-1′,2′-dioxetane)]

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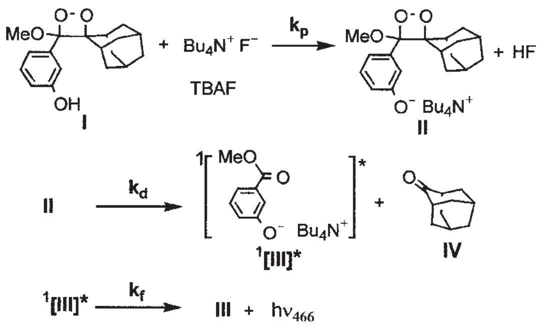
Our study of the kinetics of deprotonation and decomposition of spiro[adamantane-1,3′-(4′-(*m*-hydroxy-phenyl)-4′-methoxy-1′,2′-dioxetane)] (**I**) showed kinetic parameters for decomposition of tetrabutylammmonium salt of **I** to be $\Delta H^{\ddagger} = 85.4 \pm 1.1\,\mathrm{kJ\,mol^{-1}}$; $\Delta S^{\ddagger} = 16.0 \pm 3.5\,\mathrm{J\,mol^{-1}\,K^{-1}}$.

Most bioluminescence occurs via thermal decomposition of luciferin-derived-1,2-dioxetanes bearing negatively charged chromophores to yield two carbonyl compounds, one of which is an electronically excited singlet.[1] Such bioluminescence proceeds smoothly at room temperature with an extremely high luminescence quantum yield.[1] Thermolysis of 1,2-dioxetane derivatives with electronically neutral substituents, however, proceeds at elevated temperature but with the poor luminescence quantum yields.[1,2] The 1,2-dioxetane derivatives of biomimetic chemiluminescence thus possess negatively charged chromophores.[3–6] Mechanisms for generation of excited singlet state light emitter from 1,2-dioxetanes, chemiexcitation, remain controversial.[5–13] The basic requirement for a 1,2-dioxetane derivative to emit light with a high chemiluminescence quantum yield is that the energy difference between the transition state of dioxetane fragmentation and products in an electronic ground state must exceed the excitation energy of one of the fragments, i.e., the light emitter.[1b,2,14] Although the activation energy of biomimetic chemiluminescence is the most basic physicochemical data, no kinetic data for thermal fragmentation of 1,2-dioxetanes bearing negatively charged chromophores has been reported because the reaction is too fast to measure conventionally.



Scheme 1.

We therefore studied the kinetics of thermolysis of tetrabuylammmonium salt (**II**) of spiro[adamantane-1,3′-(4′-(*m*-hydroxyphenyl)-4′-methoxy-1′,2′-dioxetane)] (**I**), one of the most successful biomimetic luminescence systems. **II** is widely used for diagnostic clinical analysis and molecular biology as a chemiluminescent probe,[15] and chemiexcitation mechanism of its chemiluminescence reaction has been studied extensively by Adam et al.[13a–c]

II was generated by deprotonation of **I** with tetrabutylammonium fluoride (TBAF) in acetonitrile. A 0.82 mM solution of **I** in acetonitrile and 11.8 mM TBAF solution were mixed in a stopped flow spectrophotometer (Otsuka Electronics RA-2000) to record spectra at 0.8 s intervals using a photodiode array detector. Spectral change in the reaction mixture involved 2 steps (Figure 1). The spectrum recorded at $t = 0$ is identical to the **I** spectrum. The spectrum recorded after the first 0.8 s is **II** (Figure 1), changing eventually to the spectrum of tetrabutylammonium *m*-carbomethoxyphenolate (**III**). The initial fast spectral change completed within 0.8 s was recorded in 16 ms (Figure 1, inset). Both spectral changes in rate processes from **I** to **II** and **II** to **III** have isosbestic points suggesting no measurable intermediates in either rate process in ms time resolution.

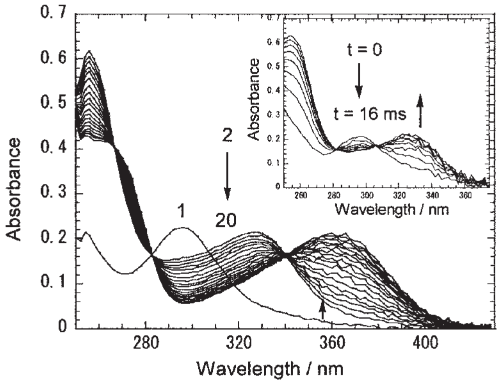


Figure 1. Time-dependent spectral change in the reaction of **I** with TBAF in CH_3CN at 25 °C (time interval, 1–6: 0.8 s; 7–11: 1.6 s; 12–20: 3.2 s). Inset: Spectral chage during initial 16 ms.

The proton transfer process from **I** to the fluoride anion was followed by decreasing absorbance at 290 nm due to **I** (Figure 2, solid line). A simulation curve based on the first-order kinetic equation (Figure 2, broken line) with $k_p^{obs} = 7.34 \pm 0.02\,\mathrm{s^{-1}}$ overlaps the experimental one well. A nonlinear plot of pseudo first-order rate constant k_p^{obs} against the TBAF concentration (Figure 2, inset) indicates that the process is expressed by a combination of a few elementary processes.

Spectral change from **II** to **III** + **IV** (Figure 1) was found to be accelerated by light irradiated to record absorption spectra, where light from the light source was irradiated on the sample cuvette without using monochrometer. Decomposition of **II** to form **III** was thus followed by increasing absorbance at 366 nm due to **III** (Figure 3) at 25 °C using a photomultiplyer detector where monochromatic light at 366 nm was irradiated on the sample cuvette. Its time course obeyed the first-order kinetic equation. The simulation curve with $k_d = (4.64 \pm 0.16) \times 10^{-2}\,\mathrm{s^{-1}}$ overlapped the experimental curve

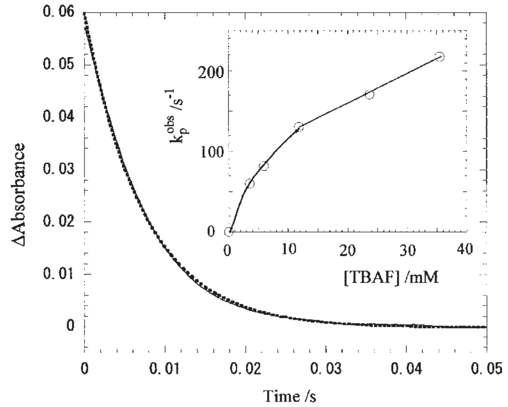


Figure 2. Time course of absorbance at 290 nm due to **I** in the deprotonation of **I** with TBAF in CH_3CN at 25 °C (solid line). The broken line is first-order kinetic simulation. Inset: Plot of k_p^{obs} against [TBAF].

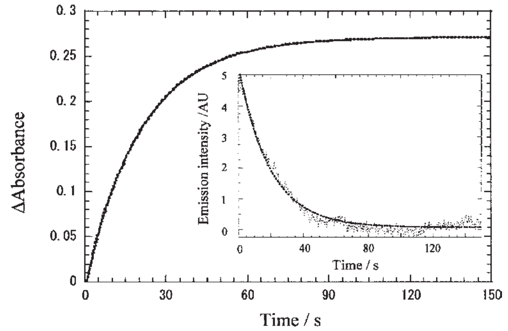


Figure 3. Increase of absorbance of **III** at 366 nm in CH_3CN at 25 °C. Inset: Decrease of visible light emission intensity. Solid line, experimental; broken line, simulation curve.

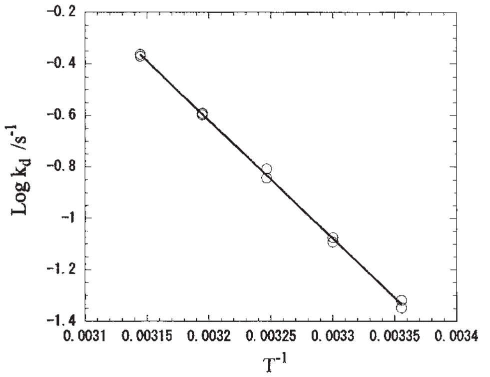
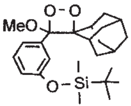
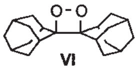
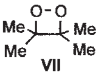


Figure 4. Arrhenius plot of themal decomposition of **II** in CH_3CN.

well. Since k_d was thus found to be identical to experimental error to k_d determined by following the decrease of visible light emission from the excited singlet state of **III** ($(4.76 \pm 0.37) \times 10^{-2}\ s^{-1}$), monochromatic light used to measure increasing **III** absorption at 366 nm had no measurable effect on the decomposition rate. An Arrhenius plot for k_d at 25–45 °C dropped on a linear line (r = 0.9994). Activation parameters for thermolysis of **II**, $\Delta H^{\ddagger} = 85.4 \pm 1.1\ kJ\ mol^{-1}$ and $\Delta S^{\ddagger} = 16.0 \pm 3.5\ J\ mol^{-1}\ K^{-1}$, are compared with some dioxetanes in Table 1. $\Delta H^{\ddagger}$ for **II** thermolysis is ca 30 kJ mol^{-1} less than that for dioxetanes having electronically neutral substituents. Greater k_d for dioxetanes bearing negatively charged substituents compared to dioxetanes bearing electronically neutral substituents is well known and explained by electron transfer from the substituent to dioxetane ring lowering transition state energy with the lack of $\Delta H^{\ddagger}$ measurement. We verified that **II** decomposition faster than **V–VII** is due to a potential energy barrier lower than that for **V–VII**.

Table 1. Kinetic parameters and excited singlet yield (Φ_S)[a]

	$\Delta H^{\ddagger}$ kJ mol^{-1}	$\Delta S^{\ddagger}$ J K^{-1} mol^{-1}	Φ_S
II	85.4±1.1[b]	16.0 ± 3.5[b]	0.45[c] 0.55[d]
 **V**	116[c]	-12[c]	
 **VI**	141	12 ± 8	0.02
 **VII**	112.5±0.4	15.5 ± 1.3	0.0025

[a]Reference 2. [b]This work. [c]Reference 3. [d]Reference 13e.

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