Substituent Effects on the Chemiluminescent Properties of Coelenterazine Analogues

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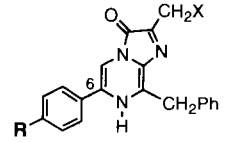
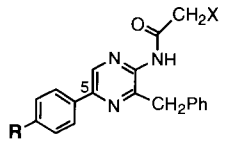
Chemiluminescence maxima and quantum yields of coelenterazine analogues possessing a substituent R [= CF_3, F, H, OCH_3, OH, $N(CH_3)_2$] at the *para*-position on the 6-phenyl group were measured in DMSO. The result indicates that the variation of the electronic properties of R of these analogues caused the small change of the efficiency of chemical generation of a singlet excited light-emitter (Φ_S).

Coelenterazine **1** is the luminescent substrate of a photo-protein, aequorin isolated from the jellyfish *Aequorea victoria*[1] and is used as the luciferin of various bioluminescent marine organisms.[2] During aequorin bioluminescence reaction, **1** is oxidized to coelenteramide **2** and CO_2 with O_2 in a polypeptide environment and the singlet excited state of **2** bound to apoprotein is formed with a high efficiency.[3,4] Coelenterazine **1** also exhibits the chemiluminescent property in an aprotic solvent such as DMSO, although the chemiluminescence efficiency (Φ= 0.002 in DMSO)[5] is lower than that of aequorin bioluminescence (Φ= 0.2).[4] It has been predicted that the bio- and chemiluminescence reaction of **1** follows the mechanism as shown in Scheme 1.[6] Decomposition of the dioxetanone intermediate **I** is an important process for generating an singlet excited molecule.[7] To explain the high efficiency of the aequorin bioluminescence, the chemically initiated electron exchange luminescence (CIEEL) mechanism has been applied to the process generating a singlet excited molecule.[8] According to this mechanism, the electron transfer from the 3-benzyl-5-(4-hydroxyphenyl)pyrazinylamino anion moiety to the dioxetanone part may accelerate the decomposition of **I**, resulting in the generation of the singlet excited amide anion **II** with high efficiency.[9] In order to clarify a role of an electronic property of the 5-aryl-3-benzylpyrazinylamino anion moiety in the dioxetanone **I** in determining the efficiency of the generation of **II**, we studied the chemi-luminescence properties of a series of coelenterazine analogues **3a-e** possessing a substituent R [= CF_3, F, H, OCH_3, OH, $N(CH_3)_2$] at the *para*-position on the 6-phenyl group in DMSO.

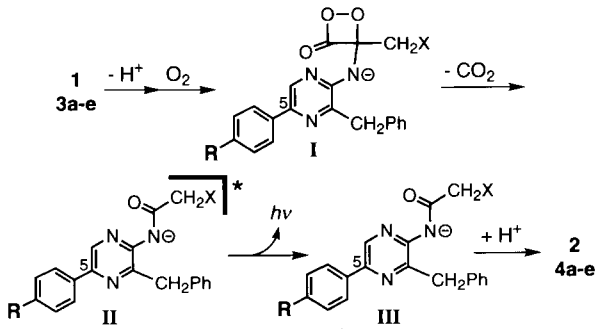


1: R = OH, X = C_6H_4OH
3a: R = CF_3, X = H
3b: R = F, X = H
3c: R = H, X = H
3d: R = OCH_3, X = H
3e: R = $N(CH_3)_2$, X = H



2: R = OH, X = C_6H_4OH
4a: R = CF_3, X = H
4b: R = F, X = H
4c: R = H, X = H
4d: R = OCH_3, X = H
4e: R = $N(CH_3)_2$, X = H

Coelenterazine analogues **3a-e** were synthesized by the coupling of the corresponding coelenteramine analogues[10] with pyruvic aldehyde according to the reported procedure.[11] Mixing a MeOH solution (100 μl) of **3a-e** (1.0 x 10^{-3} mol l^{-1}) with



Scheme 1.

DMSO (2.0 ml) in a quartz cuvette at 25 °C under air showed a blue light emission. The chemiluminescence maxima (CL_{max}) of **3a-e** are summarized in Table 1. The CL_{max} values of **3a-e** match the fluorescence maxima of the amide anions (**III**) of coelenteramide analogues **4a-e** in DMSO containing 1.0 mol l^{-1} NaOH aqueous solution (0.5% v/v), indicating that each coelenterazine analogue gave the singlet excited amide anion **II** in accord with the proposed mechanism (Scheme 1). The fluorescence maxima of the amide anions of **4a-e** showed a slight bathochromic shift with increasing the electron-donating ability of the substituent R in the coelenteramide analogues. This bathochromic shift of the amide anion of **4a-e** is smaller than that of the CT fluorescence of the neutral form of **4a-e**.[10]

Table 1. Chemiluminescence maxima (CL_{max}) and quantum yields (Φ_{cl}, Φ_r, Φ_{fl}, and Φ_S) for chemiluminescence reactions of coelenterazine analogues **3a-e** in DMSO under air

compounds	CL_{max}	quantum yield			
(R)	/ nm	Φ_{cl}	Φ_r	Φ_{fl}	Φ_S
3a (CF_3)	454	0.0006	0.78	0.05	0.015
3b (F)	467	0.0015	1.00	0.12	0.012
3c (H)	462	0.0015	0.93	0.18	0.008
3d (OCH_3)	473	0.0018	0.95	0.21	0.009
3e ($N(CH_3)_2$)	479	0.0015	0.96	0.19	0.008

The chemiluminescence quantum yields (Φ_{cl}) of **3a-e** were determined as relative values to that of luminol as a standard (Φ_{cl} = 0.028),[12] and the results were summraized in Table 1. The efficiency Φ_{cl} of coelenterazine analogue is a product of three efficiencies, those are the fraction of reacting molecules that pursue the correct chemical pathway (Φ_r), the efficiency of chemical generation of a singlet excited state (Φ_S), and the fluorescence quantum yield of the light emitter (Φ_{fl}) as shown in equation (1):

$$\Phi_{cl} = \Phi_r \times \Phi_S \times \Phi_{fl} \qquad (1)$$

The Φ_r values were determined by HPLC analysis of **4a-e** obtained after chemiluminescence reaction of **3a-e**, respectively. The Φ_{fl} values of the amide anions (**III**) of **4a-e** in DMSO-

NaOH were determined on the basis of the fluorescence efficiency of quinine sulfate in 0.1 M H_2SO_4 (Φ_{fl}= 0.55).[13] The Φ_S values were calculated by using the equation (1) and the results were summarized in Table 1. The Φ_{cl} values of **3b-e** are close to that of wild type coelenterazine **1** (Φ_{cl}= 0.002).[5] Analogue **3a** possessing an electron withdrawing group (R= CF_3) showed a slight decrease of the Φ_{cl} value. Analogue **3a** also showed a slight decrease of the Φ_r value, while **3b-e** quantitatively undergo chemiluminescence reaction. The fluorescence quantum yields Φ_{fl} for the amide anions of **4c-e** were ca. 0.2 and the Φ_{fl} values of the amide anions of **4a,b** were lower than those of **4c-e**. The estimated Φ_S values show a small change in the range of 0.008-0.015. In order to correlate the Φ_S values with the electronic property of the 5-aryl-3-benzylpyrazinylamino anion moiety, an energy level of HOMO and a negative charge on the N7 of the amino anion group of the core structure **5** possessing a substituent R were calculated by the PM3 semiempirical MO method[14] (Table 2). The energy level of HOMO varied in the

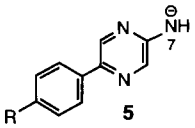


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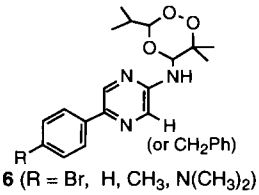
Table 2. PM3 semiempirical MO calculation of the electronic properties of **5** possessing a substituent R

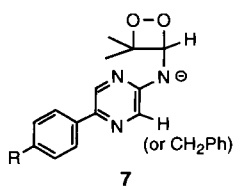
substituent (R)	energy level of HOMO / eV	negative charge on N7
CF_3	-3.76	-0.261
F	-3.47	-0.286
H	-3.32	-0.298
OCH_3	-3.33	-0.297
$N(CH_3)_2$	-3.39	-0.291

range of -3.32- -3.76 eV (Δ 0.44 eV) and the negative charge on the N7 in the range of -0.26- -0.30 (Δ 0.04). Therefore, these changes of the electronic properties of the anion moiety cause a small change of the Φ_S value. The chemiluminescent properties of **3a-e** are similar to those of 5-(5-aryl-2-pyrazinylamino)-1,2,4-trioxane derivatives **6** (R= Br, H, OCH_3, OH, $N(CH_3)_2$).[15] In the presence of a base, **6** chemiluminesced through the dioxetane intermediate **7**, yielding the singlet excited amide anion of coelenteramide analogues, and showed the small change of the Φ_S values (Φ_S= 0.0026-0.0067). On the other hand, the chemiluminescent character of 1,2-dioxetane derivative **8** is strongly dependent on the electronic character of the substituent R on the *para*-position of the phenyl group.[16] Especially, an electron donating $N(CH_3)_2$ group drastically increased the reaction rate and the Φ_S value. For generating an excited molecule, the substituent effect on the phenyl group directly linking the 1,2-dioxetane ring in **8** is larger than that on the 5-phenylpyrazinyl-amino anion moiety in **I** and **7**.

Aequorin bioluminescence shows the high quantum yield (Φ= 0.2),[4] indicating that the Φ_S value is larger than 0.2. By using the approach to change the electronic property of coelenterazine analogue, we could not obtain any coelenterazine analogue which chemiluminesces in DMSO with the similar Φ_S value to that of bioluminescence. Therefore, the present studies on the substituent effect does not assist the CIEEL mechanism during bioluminescence reaction of **1**. One of the explanation of the high Φ_S value of bioluminescence is that the reactivity of the dioxetanone intermediate **I** is regulated not only by the electronic



6 (R = Br, H, CH_3, $N(CH_3)_2$)



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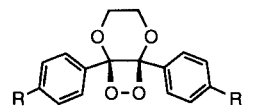
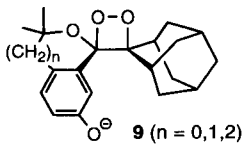


8 (R = H, CH_3, $N(CH_3)_2$)



9 (n = 0,1,2)

property of the 5-arylpyrazinylamino anion moiety in **I** (Scheme 1) but also by a conformation of the 5-arylpyrazinylamino anion moiety relative to the 1,2-dioxetanone ring. Recently, Matsumoto et al. showed that in the dioxetane derivatives **9** a conformation of the phenolate anion moiety relative to the dioxetane ring is important for determining a Φ_{cl} value.[17] During aequorin bioluminescence, a polypeptide environment of the apoprotein may regulate conformations of a reaction intermediate to increase the Φ_S value. To establish a role of a conformation of a reaction intermediate, the chemiluminescence character of coelenterazine analogues under organized environments are now under investigation.

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