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Luminol chemiluminescence selectively stimulated with self-assembled molecular aggregates of fluoroalkylated end-capped *N*-(1,1-dimethyl-3-oxobutyl) acrylamide oligomer

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Abstract

Fluoroalkylated end-capped *N*-(1,1-dimethyl-3-oxobutyl) acrylamide oligomer is a unique host molecule accommodating luminol and potentially enhancing light emission yield in its chemiluminescence with hydrogen peroxide-potassium ferricyanide in an aqueous solution.

Keywords: Chemiluminescence; Fluorinated oligomer; Molecular aggregate; Luminol; Lucigenin

Considerable attention has been focused on partially fluorinated macromolecules, particularly fluoroalkylated end-capped macromolecules, due to their unique properties, such as biological activity and surface-active properties imparted by end-capped fluoroalkyl segments setting them apart from ordinary fluorinated polymers [1,2]. Fluoroalkylated end-capped amphiphilic oligomers have been demonstrated to form self-assembled molecular aggregates with aggregations of the end-capped fluoroalkyl segments in aqueous and organic media [3], indicating that well-known chemiluminescent compounds such as luminol and lucigenin are potential guest molecules for fluorinated molecular aggregates to enhance chemiluminescence intensity. It would be interesting to develop this chemiluminescence into an enzymatic model for luciferase-catalyzed bioluminescence. Given the development of such biomimetic chemiluminescence using partially fluorinated macromolecules, we were interested in the chemiluminescence of luminol in the presence of self-assembled aggregates of fluoroalkylated end-capped oligomers. We report here that fluoroalkylated end-capped acrylamide oligomers, particularly fluoroalkylated end-capped *N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomer [R_F-$(DOBAA)_n$-R_F], as a powerful enhancer of luminol-H_2O_2-$K_3Fe(CN)_6$ chemiluminescence.

A series of fluoroalkylated end-capped oligomers were prepared by known methods [4–7]. In a typical chemiluminescence study, aqueous solutions of luminol (200 nmol/ml, 200 μl) and $K_3Fe(CN)_6$ (400 μmol/l; 50 μl), R_F-$(DOBAA)_n$-R_F methanol solution (2 g/l, 450 μl) and 0.2 mol/l sodium carbonate buffer

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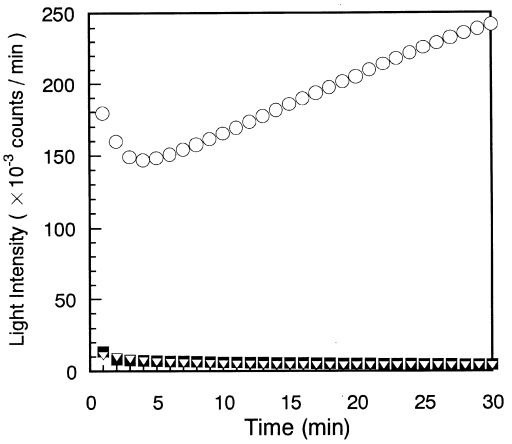
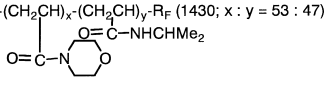
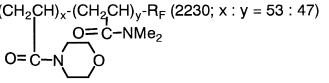
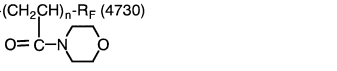


Fig. 1. Time-course of chemiluminescence of luminol in the presence of fluorinated oligomer.
○: $^{R_F\text{-}(CH_2\text{-}CH)_n\text{-}R_F}_{O=\ CNHCMe_2CH_2(C=O)Me}$, $R_F = C_3F_7OCF(CF_3)CF_2OCF(CF_3)$;
▽: $^{\text{-}(CH_2\text{-}CH)_n\text{-}}_{O=\ CNHCMe_2CH_2(C=O)Me}$; ■: in the absence of oligomer.

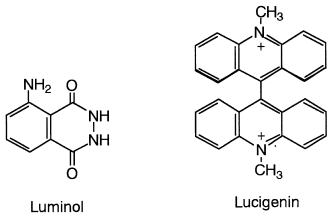
(pH 11.7; 300 μl) were mixed in a polyethylene tube placed in a chemiluminescence detector (Luminescence Reader BLR-201, Aloka Co. Ltd, Japan) at 37°C. After 1 min, chemiluminescence was initiated by adding an aqueous H_2O_2 solution (100 μmol/l, 100 μl) by an injector to record the light emission time course.

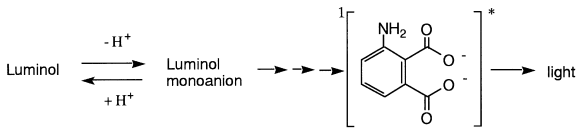
Table 1
Light intensity of luminol in the presence of a series of fluoroalkylated end-capped oligomers

Oligomer ($\overline{Mn}$) [a]	Light intensity (x 10^{-3} counts/30min)
R_F-$(CH_2CHCOOH)_n$-R_F (9200)	600
R_F-$(CH_2CMe)_n$-R_F (12300) $CO_2CH_2CH_2SO_3H$	700
R_F-$(CH_2CH)_n$-R_F (9040) $CO_2CH_2CH_2N^+Me_3Cl^-$	800
R_F-$(CH_2CH)_x$-$(CH_2CH)_y$-R_F (1430; x : y = 53 : 47) 	1000
R_F-$(CH_2CH)_x$-$(CH_2CH)_y$-R_F (2230; x : y = 53 : 47) 	1400
R_F-$(CH_2CH)_n$-R_F (4730) 	1600
R_F-$(CH_2CH)_n$-R_F (7900) $O=C-NHCMe_2CH_2(C=O)Me$	5700

[a] $R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$.

The fluoroalkylated end-capped DOBAA oligomer (R_F-$(DOBAA)_n$-R_F) was found to enhance dramatically the light emission yield, while the corresponding nonfluorinated DOBAA oligomer (Mn = 6900) decreased the light yield (Fig. 1). Relative chemiluminescence light yields measured after all luminol was consumed were determined to be 1:0.4:65 (absence of polymer: presence of nonfluorinated DOBAA oligomer: presence of R_F-$(DOBAA)_n$-R_F). These observations indicate that the perfluoroalkylated end-capped structure is essential in oligomers enhancing luminol chemiluminescence light yield.





To study the effect of the fluoroalkyl structure on luminol chemiluminescence, we tested several fluoroalkylated end-capped oligomers under similar conditions (Table 1). Fluoroalkylated end-capped oligomers containing carboxy, sulfo, and trimethylammonium segments were found to be less effective than fluoroalkylated end-capped acrylamide oligomers in enhancing chemiluminescence. Among these R_F-$(DOBAA)_n$-R_F, a polyacrylamide, was most effective. We reported that fluorinated acrylamide oligomers such as fluoroalkylated end-capped acryloylmorpholine, *N, N*-dimethylacrylamide and *N*-isopropylacrylamide oligomers form molecular aggregates in aqueous solutions through (a) the interaction between end-capped fluoroalkyl segments and (b) the intermolecular hydrogen bonding of amide segments. These aggregates act as hosts of calcium ions as a guest molecule [8], but corresponding nonfluorinated acrylamide oligomers do not [8]. In contrast, the calcium binding of fluoroalkylated end-capped acrylic acid oligomers reportedly is almost the same as the theoretical binding one [9]. The acrylamide moiety in fluoroalkylated end-capped oligomers is a segment very important to the formation of molecular aggregates with suitable host moieties, and these fluorinated aggregates are essential to enhancing luminol chemiluminescent light yield.

It would be interesting to extend this experiment to

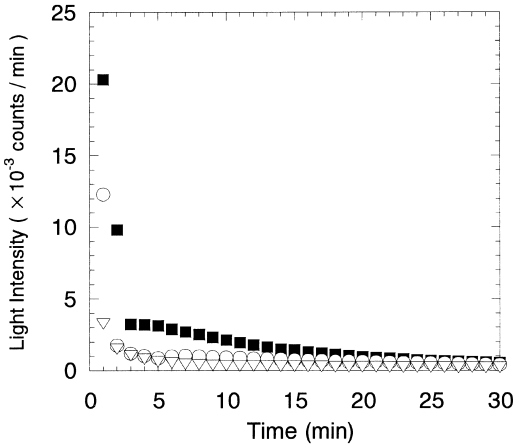


Fig. 2. Time-course of chemiluminescence of lucigenin in the presence of oligomer.

○: $^{R_F\text{-}(CH_2\text{-}CH)_n\text{-}R_F}_{O=CNHCMe_2CH_2(C=O)Me}$; $R_F = C_3F_7OCF(CF_3)CF_2OCF(CF_3)$;

▽: $^{\text{-}(CH_2\text{-}CH)_n\text{-}}_{O=CNHCMe_2CH_2(C=O)Me}$; ■: in the absence of oligomer.

lucigenin, another famous chemiluminescent compound. The light emission yield of the lucigenin chemiluminescence with H_2O_2 measured for 30 min at 37°C was, however, rather suppressed by R_F-(DOBAA)$_n$-R_F. (Fig. 2) and the corresponding nonfluorinated DOBAA oligomer, [-(DOBAA)$_n$-]. This finding suggests that our present fluorinated molecular assemblies formed by R_F-(DOBAA)$_n$-R_F recognize luminol rather than lucigenin preferentially as a guest molecule. We found that fluoroalkylated end-capped DOBAA oligomers form self-assembled aggregates that selectively recognize aromatic amine derivatives such as methylene blue and methyl orange bearing negatively charged groups. These aggregates do not, however, recognize lucigenin [10]. This suggests that these fluorinated aggregates accommodate luminol monoanion, an aromatic amine, as a guest molecule, but do not interact with lucigenin, a large aromatic quaternary ammonium ion.

To substantiate this, we have examined the liquid–liquid extraction behavior of an 1,2-dichloroethane solution (5 ml) of the fluoroalkylated end-capped DOBAA oligomer [2.0 g/l; $R_F = CF(CF_3)OC_3F_7$ (Mn = 6900)] for 0.2 mol/l sodium carbonate buffer (pH 11.7, 5 ml) containing luminol (0.02 mol/l). In spite of whole luminol molecules ($pK_a = 6$) in the monoanion forming in the aqueous medium, the $CF(CF_3)OC_3F_7$-containing organic phase was found to have an extraction ability (25%) toward the luminol monoanion. This fluorinated aggregate therefore recognizes luminol as a guest molecule potentially enhancing chemiluminescence light yield. Several factors determine chemiluminescence efficiency [11]. The polymer aggregation provides hydrophobic environment to the guest, luminol. The fluorescence quantum yield of the light emitter is often increased by changing the environment from hydrophilic to hydrophobic [12–15]. The fluorescence quantum yield of singlet excited sodium 3-aminophthalate, the light emitter of luminol chemiluminescence, was found to be scarcely affected by adding fluoroalkylated end-capped DOBAA oligomers, suggesting that fluoropolymers increase singlet excited state of 3-aminophthalate yield in the polymer bound luminol chemiluminescence.

Luciferin-luciferase bioluminescence quantum yields are generally greater by a few orders of magnitude than luciferin chemiluminescence. Our fluorinated polymer aggregates selectively include luminol to enhance the quantum yield of singlet-excited emitter formation such as luciferase. Our fluoroalkylated end-capped DOBAA oligomers are therefore promising in providing a hydrophobic environment mimicking luciferase bioluminescence and other enzymatic reactions.

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