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Sensitized chemiluminescence with long alkyl chain energy donors and acceptors in micellar media

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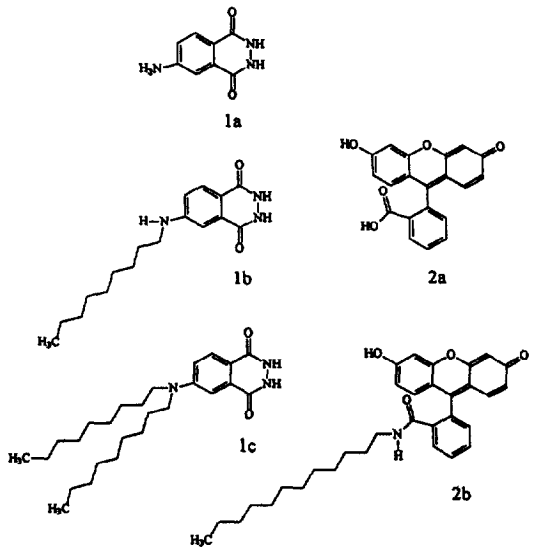
Abstract

The light reaction of nonyl- and dinonyl isoluminol with sodium hypochlorite is reported in cationic cetyltrimethylammonium chloride micelles in the presence of fluorescein and *N*-dodecylfluorescein as sensitizers. The chemiluminescence quantum yields and intensities of 4-dinonylaminophthalic hydrazide **1c** are four times more than that of 4-nonylaminophthalic hydrazide **1b** and twenty four times more than that of isoluminol due to better binding of energy donor and acceptor to the micelle.



Keywords: Isoluminol; Micelle; Chemiluminescence quantum yield

1. Introduction

Chemiluminescence (CL) in organized molecular assemblies usually results in increased quantum yields and/or CL intensities, depending on the mechanism of the light reaction and the photophysics of the resulting excited state. Lucigenin [1–4], lophines [5] and luminols [6–11] have been employed as luminescent probes in these media (micelles, lamelar and vesicular aggregates, phospholipid aggregates etc.). Besides being a tool for structural studies of organized molecular assemblies [12], applications of CL in analytical chemistry are now becoming very important [13–15]. In employing the term 'CL in organized molecular assemblies' we tend to forget that such reactions occur both within the

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aggregate and the bulk aqueous phase, so that the CL profiles obtained in such studies are combinations of light reactions in both media. As light reactions occurring within the aggregate—mainly in the Stern region—are more efficient, it was desirable to anchor the CL substrate in the aggregate and, to this effect, long alkyl isoluminols [10,16] lucigenins [4,17,18] biacridylidenes [19] and acridinium long alkyl carboxylates [20] have been synthesized and shown to be strongly CL in micellar media. Energy transfer, on the other hand, can be very rewarding in enhancing CL in organized systems, due to the close proximity of energy donor and acceptor imposed by the inclusion in the micelle and a dramatic increase in CL has been reported for fluorescein sensitized luminol CL in CTAC micelles [6] while differences between CL and fluorescence energy transfer in the same system have also been established [9]. An obvious next step, therefore would be anchorage of both the CL substrate and the energy acceptor in the micelle and this is the object of the present work where we report the chemiluminescence of mono- and disubstituted isoluminol derivatives (**1b**, **1c**) in cetyltrimethylammonium chloride (CTAC) micellar media in the presence of long alkyl substituted fluorescein derivative (**2b**). Derivatives of isoluminol (4-aminophthalic hydrazide) were employed, mainly because alkylation of the amino group in isoluminol increases the quantum efficiency to about the same value as that of luminol, whereas such substitution on luminol decreases the quantum efficiency substantially, due to steric hindrance [21].

2. Experimental details

2.1. Reagents

Isoluminol **1a** was recrystallized from warm methanol. *n*-Nonyl isoluminol **1b** and di-*n*-nonyl isoluminol **1c** were synthesized as published elsewhere [16]. *N*-dodecylfluorescein **2b** was synthesized from fluorescein **2a** as follows. Fluorescein (1.0 g, 3 mmol) and *n*-dodecylamine (0.61 g, 3.3 mmol) were heated to 160°C in 50 ml DMF for 8 h. The solvent was removed under vacuo and the brown crude product was purified on silica gel (methanol). Chem. yield: 1.32 g (90%). Mp.: decomposition over 250°C; ^1H-NMR (d4-methanol); $\delta = 8.01$ (pseudo singlet, 1H), 7.72 (m, 3H), 7.19 (d, J = 7.6 Hz, 1H), 6.58 (m, 5H), 3.18 (t, 2H, NCH_2), 1.51 (t, 2H), 1.33 (m, 18 H), 0.88 (t, 3H, CH_3). $^{13}C-NMR$ (d4-methanol): $\delta = 163.6$ (CO), 154.5, 136.0, 130.9, 130.3, 126.2, 125.6, 114.2, 111.7, 103.5, 38.9 (NCH_2), 33.0, 30.7, 30.6, 30.5, 30.4, 30.3, 27.9, 27.4, 23.7, 14.4 (CH_3).

Hexadecyltrimethylammonium chloride (CTAC) was recrystallized from acetone. Distilled water was employed for the preparation of solutions; working solutions were freshly prepared and were used on the day of their prepara-

Table 1
CL quantum yields and intensities of the isoluminols **1a–c** in the presence of fluoresceins **2a–b** at various CTAC concentrations

Compound	CTAC × 10^4 (M)	Fluorescein **2a**		Fluorescein amide **2b**	
		$\Phi_{CL} \times 10^3$ (einstein mol^{-1})[a]	CL intensity (arb. units)	$\Phi_{CL} \times 10^3$ (einstein mol^{-1})[a]	CL intensity (arb. units)
Isoluminol	0	2.65	40	1.48	40
	4	2.96	40	2.36	51
	8	2.79	45	2.48	69
	10	2.72	42	3.90	70
	20	2.47	39	2.16	64
	30	3.00	46	1.78	58
	50	1.90	50	1.43	38
n-nonyl-isoluminol	0	1.16	19	2.94	61
	4	1.80	32	1.63	68
	8	0.52	24	1.88	80
	10	1.00	30	4.28	172
	20	5.84	261	8.08	447
	30	8.00	350	9.05	478
	50	7.65	379	8.57	382
n-dinonyl-isoluminol	0	...*	...*	...*	...*
	4	1.27	89	2.22	139
	8	7.31	318	9.18	524
	10	12.26	545	16.76	872
	20	25.2	875	34.06	1334
	30	33.3	1393	41.44	1615
	50	32.4	1372	36.86	1461

*1c insoluble.
[a]Corrected values.
Φ_{CL} of **1a** in water: 1.41×10^{-3}; Φ_{CL} of **1a** in CTAC: 9.87×10^{-4}; Φ_{CL} of **1b** in water: 9.40×10^{-4}; Φ_{CL} of **1b** in CTAC: 1.79×10^{-3}.

tion. Sodium hypochlorite (Eau de Labaraque) solutions were titrated with sodium thiosulphate prior to use.

2.2. Chemiluminescence measurements

The CL quantum yields were obtained using an LKB 1250 Bio-Orbit luminometer with the timer circuitry disconnected. The cell's jacket was thermostatically controlled with the aid of a constant temperature bath-circulator and the temperature was maintained at 25.0°C. The light-generating reactions were started by injecting sodium hypochlorite solutions (25 μl, 3%) into the sample (250 μl, 5×10^{-5} M isoluminols **1a–c**, 5×10^{-5} M fluoresceins **2a–b**, 1×10^{-2} M NaOH, and $0–50\times10^{-4}$ M CTAC). The light intensity-time integrals thus obtained were compared with the luminol standard [22] which served as an absolute photon source and allowed calculation of the quantum yields based on isoluminols **1a–c**. The quantum yields obtained were corrected for the photomultipliers spectral response, by multiplying the values measured by a factor equal to $(1+R)/(1+0.45\ R)$ where R is the ratio of the peaks at 425 and 530 nm in the CL spectrum.

2.3. Spectra

Chemiluminescence spectra were obtained on a Jasco FP-777 spectrofluorimeter with the excitation source off, employing wide slits and a scanning rate of 2000 nm min^{-1} [2.5 ml isoluminols **1a–c**, fluoresceins 5×10^{-5} M, 250 μl sodium hypochlorite (3%)]. Fluorescence spectra were recorded on the same instrument at a scan speed of 200 nm min^{-1} with an emission slit width of 3 nm. Absorption spectra were recorded on a Jasco V-500 spectrophotometer.

2.4. Fluorescence quantum yields

The fluorescence quantum yields of fluoresceins **2a–b** in CTAC solutions were calculated by comparison with that of a fluorescein aqueous solution in the presence of 0.01 M NaOH which is equal to 0.92 [23]. It was found that the fluorescence quantum yields ratio of fluorescein **2a** to **2b** was 1.35 [0.62 and 0.46 at the best CTAC concentration for chemiluminescence measurements (C, 2×10^{-3} M)], whereas the ratio of fluorescence intensities of **2a** to **2b** was 1.57.

3. Results

The CL of the substituted isoluminol derivatives *N*-*n*-nonyl isoluminol **1b** and di-*n*-nonyl isoluminol **1c** were studied in CTAC micelles in the presence of fluorescein **2a** or *n*-dodecyl fluorescein **2b** and were compared with the isoluminol light reaction in said media. The CL quantum yields and the maximum CL intensities of the isoluminols **1a–c** in the presence of fluoresceins **2a–b** at various CTAC concentrations are shown in Table 1 and Figs. 1 and 2. It should be noted that

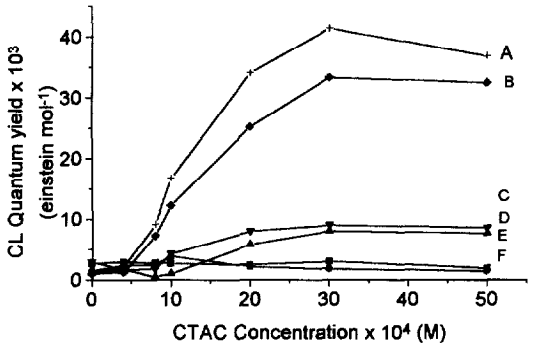

Fig. 1. CL quantum yields of isoluminols **1a–c** in the presence of fluoresceins **2a, b** as a function of CTAC concentration. **A**: dinonylisoluminol/fluorescein amide; **B**: dinonylisoluminol/fluorescein; **C**: nonylisoluminol/fluorescein amide; **D**: nonylisoluminol/fluorescein; **E**: isoluminol/fluorescein amide; **F**: isoluminol/fluorescein.

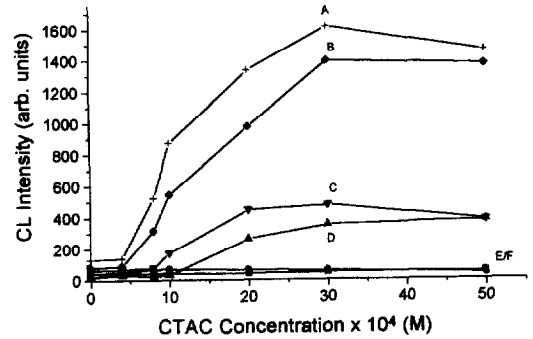

Fig. 2. CL intensities of isoluminols **1a–c** in the presence of fluoresceins **2a, b** as a function of CTAC concentration. **A**: dinonylisoluminol/fluorescein amide; **B**: dinonylisoluminol/fluorescein; **C**: nonylisoluminol/fluorescein amide; **D**: nonylisoluminol/fluorescein; **E**: isoluminol/fluorescein amide; **F**: isoluminol/fluorescein.

CL measurements of **1a** and **1b** in water without energy acceptors were also obtained and found to be 1.41×10^{-3} and 9.40×10^{-4} whereas for CTAC solutions were 9.87×10^{-4} and 1.79×10^{-3} einstein mol^{-1}, respectively (Table 1, footnotes). Notable is also the bathochromic effect of substitution on the isoluminol absorption shown in Fig. 3, while the spectral distribution of the sensitized CL as a function of CTAC concentration is shown in Fig. 4.

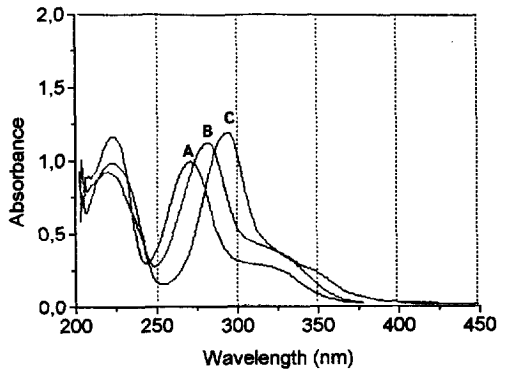

Fig. 3. Absorption spectra of isoluminols **1a–c** in alkaline sodium hydroxide in CTAC micellar media. A: isoluminol, **1a**; B: *n*-nonylisoluminol, **1b**; C: di-*n*-nonylisoluminol **1c**.

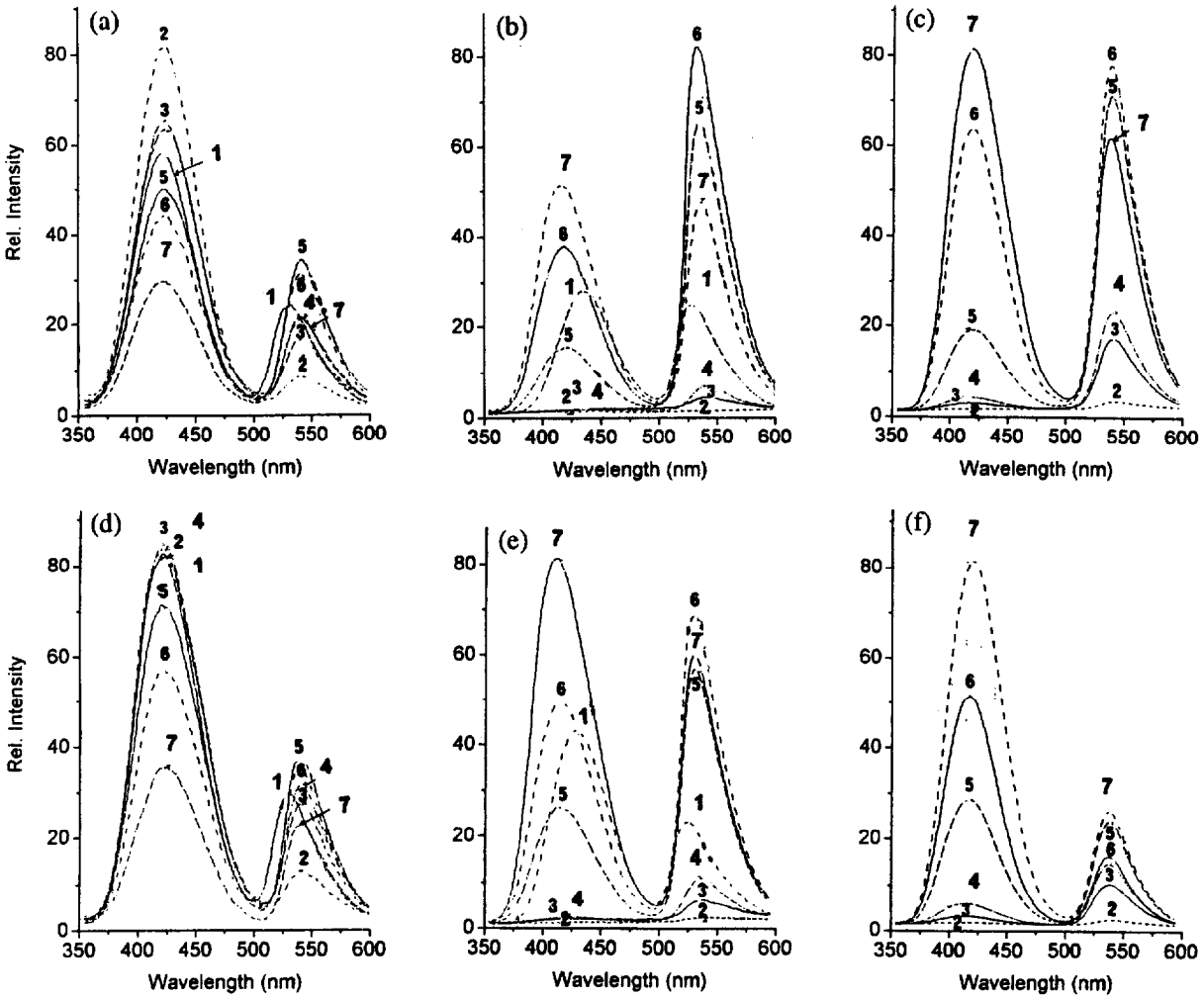

Fig. 4. CL spectra of the isoluminols **1a**, **1b** and **1c** in the presence of fluorescein **2a** (A, B, C) and fluorescein amide **2b** (D, E, F) at various CTAC concentrations. **1** without CTAC; **2:** 4×10^{-4} M; **3:** 8×10^{-4} M; **4:** 10×10^{-4} M; **5:** 20×10^{-4} M; **6:** 30×10^{-4} M; **7:** 50×10^{-4} M.

4. Discussion

In evaluating the above results, the mechanism proposed by Seitz for luminol is adopted here [24]. According to this, isoluminol **1a** reacts with sodium hypochlorite and is transformed into the corresponding endoperoxide. Decomposition of this product constitutes a strongly chemiluminescent path to 4-aminophthalate anion, the primary emitter.

At first glance, Figs. 1 and 2 reveal that substituted isoluminols **1b, c** are more efficient in the presence of fluoresceins **2a, b**. Furthermore, an abrupt increase of both CL quantum yields and intensities with increasing CTAC concentrations is observed. It should be kept in mind, however, that the diagrams of Figs. 1 and 2, are the net results of a number of factors often opposed to each other. To begin with, optimum results are expected at a combination of isoluminols, fluoresceins and CTAC concentrations for which Poisson statistics predict the maximum probability of one molecule of CL substrate and one molecule of sensitizer occupying the same micelle, which is in the order of 10^{-5} M for isoluminols and fluoresceins and in the order of 2×10^{-3} M for CTAC [9].

In addition the relative positions within the micelle of energy donor (isoluminol) and acceptor (fluorescein) play an important role. The fact that aminophthalate is the primary emitter is irrelevant as the energy transfer step is too fast compared to possible migration of 4-aminophthalate produced at the isoluminol inclusion site to its proper solubilization site [9]. So, the relative positions of isoluminols and fluoresceins in the Stern region of the micelle dictate the extent of energy transfer and these positions depend on the presence of 0, 1, or 2 chains on isoluminol and 0 or 1 chain on fluorescein. Of course, the better binding itself of the alkyl isoluminols and alkyl fluorescein to the micelle is the most important factor leading to the increased CL quantum yields and signals of Figs. 1 and 2 and it should be noted that a CL quantum yield of over 4% from isoluminol is very high indeed. This is so despite a number of adverse effects such as the lower fluorescence quantum yield of fluorescein **2b** compared to fluorescein **2a** by a factor of 1.35. Employment, therefore, of a fluorescein with the alkyl chain at a different site such as 5-alkylaminofluorescein, a goal towards which we are presently working, would raise the CL quantum yield

to over 5.5%. Furthermore the fluorescence quantum yields of dinonylaminophthalates are very low, e.g., less than 0.08 for the di-nonyl derivative in 1.5×10^{-3} M CTAC [10], let alone that CTAC is a quencher of the CL reaction of hydrazides due to free radical scavenging by CTA^+ and a quencher of the aminophthalate fluorescence [8] while hypochlorite is also a quencher of the aminophthalate fluorescence with a quenching constant Kq equal to 600 M^{-1} [8].

Spectroscopic study of the isoluminols **1a–c** herein reported reveals several differentiations. The absorption spectrum of disubstituted isoluminol **1c** in alkaline CTAC solution is shifted bathochromically up to 20 nm relative to unsubstituted isoluminol **1a** (Fig. 3). The fluorescence spectra of isoluminols **1a–c** were not unlike each other although the intensities for disubstituted isoluminol were substantially increased. The *N*-alkyl phthalate anions were the primary emitters of the light reactions in all cases. In CTAC micellar solutions in the presence of fluorescein, the well known picture appears once more and the chemiluminescence spectrum is a composite of the substituted aminophthalate and the fluorescein fluorescence spectra arising from energy transfer to fluorescein (Fig. 4).

In conclusion, on going a step forward from the combination of micellar catalysis in CL and sensitization, anchoring the CL substrate and the sensitizer to the micelle with the aid of long alkyl chains results in a dramatic increase in CL quantum yields and intensities even in the presence of a number of effects affecting negatively said CL parameters, an advance that should not pass unnoticed by analytical chemists. Furthermore, a comparison of CL parameters in Table 1, reveals that the disubstituted isoluminol **1c** is a better reagent for analytical purposes than isoluminol itself. The disubstituted derivative **1c** gives rise to stronger light intensities so that **1c** in CTAC micellar solutions and in the presence of fluoresceins produces a signal four times stronger than **1b** and twenty four times stronger than **1a**.

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