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Chemiluminescence at liquid–liquid interfaces. Enhanced chemiluminescence of lucigenin and long alkyl lucigenins.

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Abstract

The chemiluminescence quantum yields of lucigenin and long alkyl lucigenins in two phase solvent systems (water/organic solvent) are increased by a factor as high as 12 while the chemiluminescence (CL) signals are increased by a factor as high as 80, compared to those in water. The role of the orientation of the CL substrate imposed by the interface is discussed. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Chemiluminescence; Interface; Lucigenins

1. Introduction

The classical lucigenin (10,10′-dimethyl-9,9′-biacridinium nitrate) light reaction is four times more efficient in micellar [1,2] and 20 times more efficient in bilayer lamelar aggregates [3]. A generally accepted mechanism for this reaction [4] involves the decomposition of an intermediate 1,2-dioxetan at the 9,9′- position leading to excited N-methylacridone and the micellar chemiluminescence (CL) enhancement has been attributed [2] to the more efficient decomposition of this dioxetan in the less polar microenvironment of the Stern region. The variation of the CL profiles in said membrane mimetic agents in the presence of factors affecting the fluidity of such aggregates (cholesterol, hormons, vitamines, ions etc) has been employed as a tool in structural studies of said systems [5], while the enhanced CL in micellar media has been employed extensively in analytical applications [6].

In order to gain full advantage of the enhanced CL in oriented systems, further enhancement was sought through better binding to the micelle and lucigenins with long alkyls in place of the methyl groups have been synthesized [7] and shown to be very efficient in micellar media [8] and the same is true with long alkyl isoluminols [9], long alkyl acridinium derivatives [10] and long alkyl biacridylidenes [11]. Despite the lack of a well defined polarity gradient characteristic of the organized molecular assemblies so far employed, replacement of said assemblies by two-phase solvent systems seemed at this point promising and the CL reactions were performed in hexane/water, methylacetate/water, ether/water, long alkyl alcohols, aldehydes and ketones/water as well as in purely aqueous media for comparison purposes.

2. Experimental details

2.1. Reagents

Lucigenins **1a–d** (Scheme 1) were synthesized as published elsewhere [7]. Distilled water was employed for the preparation of solutions; working solutions were freshly prepared and were used on the day of their preparation.

2.2. Chemiluminescence measurements

The CL quantum yields were obtained using a 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°C. The light-generating reactions were started by injecting 400 μl of a 1 : 1 mixture of sodium hydroxide (1 M) and hydrogen peroxide (0.88 M) to a mixture of aqueous lucigenin (500 μl, 10^{-6} M) and organic solvent (500 μl). The light intensity-time integrals and the CL signals thus obtained were compared with those in homogeneous media.

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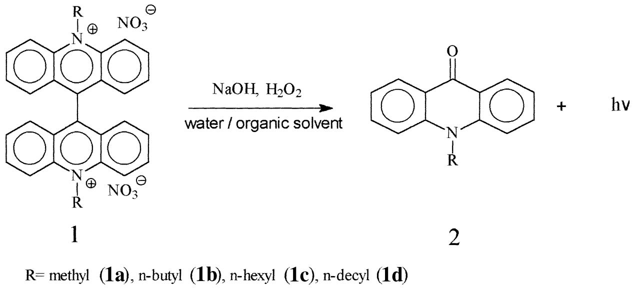

Scheme 1. The light reaction of lucigenins **1a–d**

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} [1.5 ml lucigenins **1a–d**, 1×10^{-6} M and 1.5 ml of a 1 : 1 mixture of sodium hydroxide (1 M) and hydrogen peroxide (0.88) M]. 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.

3. Results and discussion

The CL of the lucigenin derivatives **1a–d** was studied in two-phase solvent systems such as water/hexane, water/methylacetate, water/ether, water/alcohols, water/aldehydes and water/ketones with long alkyl chains and was compared with the light reaction in water. The maximum chemiluminescence intensities and the chemiluminescence integrals of the lucigenins **1a–d** are shown in Tables 1 and 2, respectively and in Figs. 1–4 for water/hexane, water/methylacetate and water/ether. It should be noted at this point that reaction in water lasted for a few minutes while those in the binary systems were over in a few seconds. Also reactions in chlorinated solvents/water such as chloroform/water, dichloromethane/water and carbontetrachloride/water as well as reactions in benzene/water and toluene/water were less rewarding in comparison to those in water and the same is true for reactions in water/long alkyl chain aldehydes and water/long alkyl chain ketones. On the other hand reactions in water/long chain alcohols were again more efficient (Fig. 5) and associated with stronger signals (Fig. 6). Here,

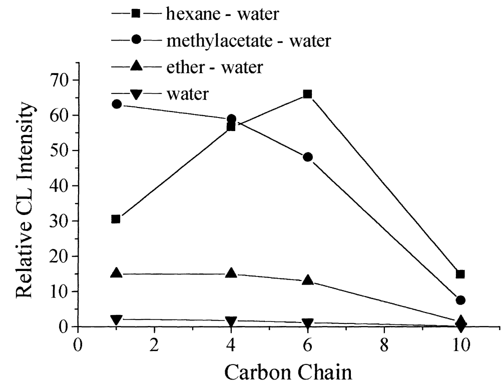

Fig. 1. CL intensities versus alkyl chain length.

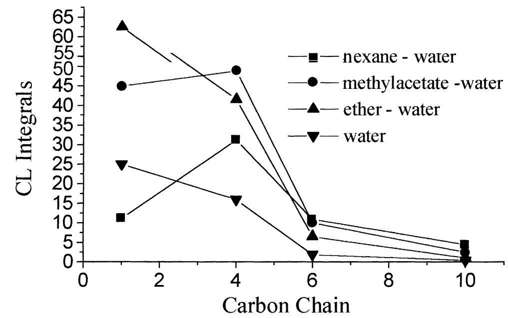

Fig. 2. CL integrals versus alkyl chain length.

Table 1
Ratio of CL intensities in binary systems (water/organic solvent) over CL intensities in water of lucigenins **1a–d**

1	Hexane	Ether	Methylacetate	*n*-butanol	*n*-pentanol	*n*-hexanol	*n*-octanol	*n*-dodecanol
a	13.0	6.3	26.8	1.5	1.9	1.6	0.6	0.2
b	29.2	7.6	30.5	3.2	2.7	1.9	1.8	1.2
c	48.9	9.3	35.5	6.2	4.4	3.4	2.6	2.7
d	74.0	7.5	37.0	8.6	6.6	4.0	2.8	2.6

Table 2
Ratio of CL integrals in binary systems (water/organic solvent) over CL integrals in water of lucigenins **1a–d**

1	Hexane	Ether	Methylacetate	*n*-butanol	*n*-pentanol	*n*-hexanol	*n*-octanol	*n*-dodecanol
a	0.5	2.5	1.8	2.0	1.2	0.8	1.0	0.5
b	2.0	2.6	3.1	0.6	0.6	0.4	0.8	1.0
c	5.9	3.5	5.4	4.5	3.5	2.9	0.7	2.4
d	11.0	2.5	6.3	16.9	8.0	3.1	1.2	1.0

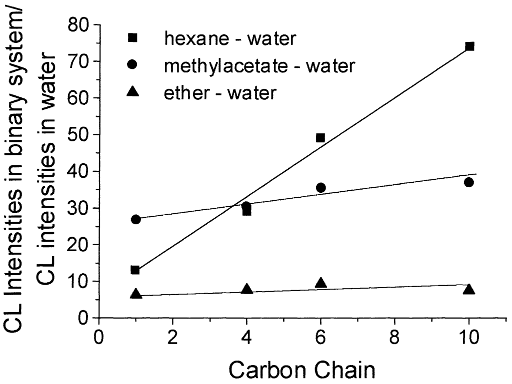

Fig. 3. Ratio of CL intensities in binary systems over intensities in water as a function of alkyl chain length.

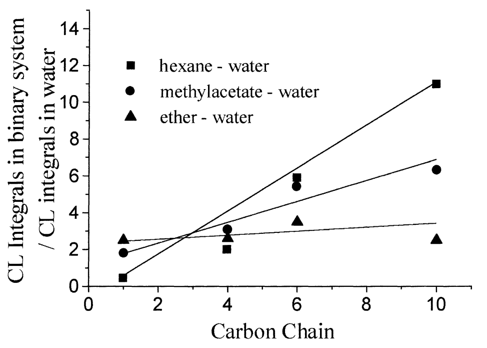

Fig. 4. Ratio of CL integrals in binary systems over CL integrals in water as a function of alkyl chain length.

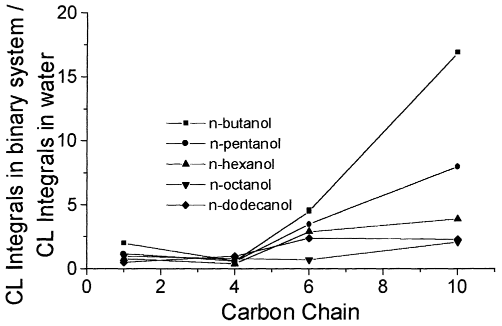

Fig. 5. Ratio of CL integrals in binary systems (alcohols/water) over CL integrals in water as a function of alkyl chain length.

however, it should be remembered that the lower members of the long chain alcohols herein employed are slightly soluble in water and that water /alcohol solutions are known to give rise to more efficient CL [12,13].

As the lucigenins of the present work are soluble both in water and at the solvents' interface, in order to avoid masking of the interface emission by the bulk emission, the concentrations were lowered to 10^{-6} M as dictated by the results of Fig. 7. Lucigenins with alkyls longer than methyl are less efficient than lucigenin (N-methyl) in homogeneous aqueous solution [8,9] a trend counterbalanced and even reversed in micellar media [8]. Here, in the two-phase solvent system, both CL intensities and light integrals are much higher than in purely aqueous medium, with all carbon chain lengths (Figs. 1 and 2). Also when the two-phase system is compared with bulk water for each chain length, a dramatic increase is observed for longer chains, as high as 80 fold for the CL intensities and 12 fold for the CL integrals (Figs. 3 and 4). A probable orientation at the interface is that shown in Fig. 8. The long alkyls in combination with the charges on nitrogen are expected to increase the concentration at the interface (Figs. 3 and 4) where the decomposition of the intermediate dioxetan will take place in a less polar microenvironment. Even in the absence of long alkyls, lucigenins will also tend to concentrate at the interface and as lucigenin (N-methyl) is non-polar with polar ends, a curved configuration like the one in Fig. 8 would tend to

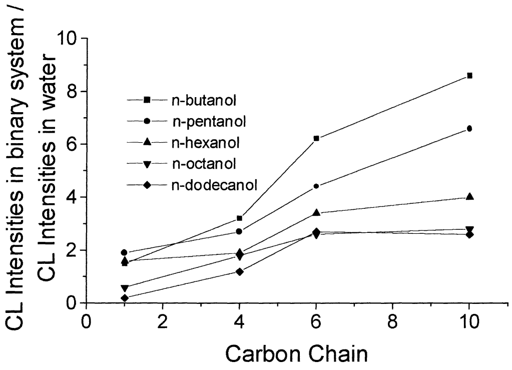

Fig. 6. Ratio of CL intensities in binary systems (alcohols/water) over intensities in water as a function of alkyl chain length.

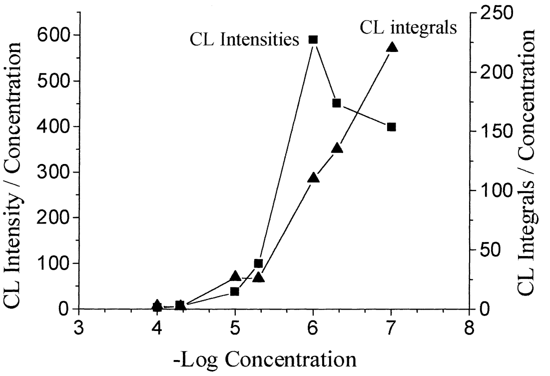

Fig. 7. CL integrals and intensities over concentration of the N-hexyl homolog as a function of concentration.

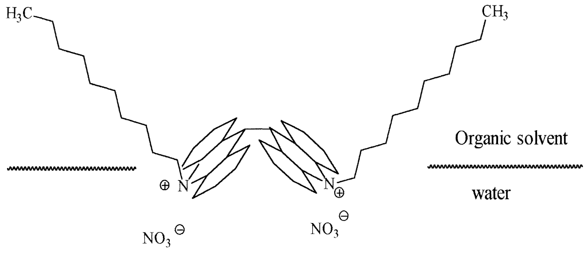

Fig. 8. Proposed orientation of the lucigenins at the interface.

stabilize it at the interface and explain the results of Fig. 1 and Fig. 2 where lucigenin is far more efficient in the systems of the present work than in water.

In conclusion a novel system is herein reported in which CL occurs at the interface of two non-miscible solvents with large polarity differences. The localization and orientation of the polar CL substrate at the interface is effected by the incorporation of long alkyls and we are presently also having good results with long alkyl isoluminols.

As CL emission comes mainly from the interface, a 'dish' luminometer or even a flow system would probably be more suitable for such studies and it should be noted that the interfacial system employed here should improve analytical applications of the lucigenin CL as it produces higher light integrals and intensities, it is simpler than micellar media and it offers larger volumes of polar and non polar solvents for the solubilization of factors interfering with the assay.

References

[1] C.M. Paleos, G. Vassilopoulos, J. Nikokavouras, Chemiluminescence of lucigenin in micellar systems: bioluminescence and chemiluminescence, in: M.A. DeLuka, W.D. McElroy (Eds.), Basic Chemistry and Analytical Applications, Academic Press, 1981, p. 729.

[2] C.M. Paleos, G. Vassilopoulos, J. Nikokavouras, J. Photochem. 18 (1982) 327.

[3] J. Nikokavouras, G. Vassilopoulos, C.M. Paleos, J.C.S. Chem. Commun. (1981) 1082.

[4] R. Maskiewicz, D. Sogah, T.C. Bruice, J. Am. Chem. Soc. 101 (1979) 5347.

[5] J. Nikokavouras, Chemiluminescence in Organized Molecular Assemblies: in: Trends in Photochemistry and Photobiology, J. Menon (Ed.), vol. 3, Council for Scientific Research Integration, Trivandrum, India, 1994, pp. 157–168.

[6] W.L. Hinze, N. Shrinivasan, T.K. Smith, S. Igarashi, H. Hoshino, Advances in Multidimensional Luminescence Spectroscopy, in: I.M. Warner (Ed.), J.A.I. Press, CT, 1991, pp. 149–206.

[7] K. Papadopoulos, J. Nikokavouras, J. Prakt. Chem.-Chem. Zeit. 335 (1993) 633.

[8] K. Papadopoulos, S. Spartalis, J. Nikokavouras, Anal. Chim. Acta 290 (1994) 179.

[9] K. Papadopoulos, A. Chantron, J. Nikokavouras, J. Hrbac, J. Lasovsky, J. Photochem. Photobiol. A: Chem., 1998, in press.

[10] K. Papadopoulos, S. Kamari, J. Nikokavouras, Proc. of the 17th Congress of the Greek Chemist Association, Patra, 1–5 December 1996, p. 647.

[11] K. Papadopoulos, S. Spartalis, J. Nikokavouras, K. Mitsoulis, D. Dimotikali, J. Photochem. Photobiol. A: Chem. 83 (1994) 15.

[12] K. Papadopoulos, J. Hadjianestis, J. Nikokavouras, J. Photochem. Photobiol. A: Chem. 75 (1993) 91.

[13] A. Larena, J. Martinez-Ureaga, Monatsh. Chem. 122 (1991) 697.