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# Effect of Nicotine on the Chemiluminescence of Lucigenin in Model Membrane Structures

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The chemiluminescence of the classical lucigenin light reaction is adversely affected by nicotine in didodecyldimethylammonium bromide bilayer lamelar aggregates. The ratio of chemiluminescence quantum yields in the oriented medium, with and without nicotine, is equal to $e^{-0.571[\text{Nicotine}]}$ whereas in the homogenous medium, the same ratio is equal to $e^{-0.214[\text{Nicotine}]}$. Furthermore, the appearance of the primary emitter's (*N*-methylacridone) emission in the chemiluminescence spectrum, already observed in oriented systems, is strongly supressed by nicotine.

*(Keywords: Chemiluminescence; Lucigenin; Membranes; Nicotine)*

*Der Effekt von Nikotin auf die Chemilumineszenz von Lucigenin in Modellmembranen*

Die Chemilumineszenz der klassischen Lucigenin-Lichtreaktion wird von Nikotin in Didodecyldimethylammoniumbromid-Lamellaraggregaten geschwächt. Das Verhältnis der Chemilumineszenzquantenausbeuten ist in orientiertem Medium — mit oder ohne Nikotin — $e^{-0,571[\text{Nikotin}]}$, in homogenem Medium jedoch $e^{-0,214[\text{Nikotin}]}$. Außerdem wird die Emission des Primäremitters (*N*-Methylacridon) im Chemilumineszenzspektrum von Nikotin stark unterdrückt.

## Introduction

Working with the 10,10′-dimethyl-,9,9′-biacridinium nitrate (lucigenin) light reaction, we have shown earlier[1,2] that chemiluminescence in micellar media (a) results in altered (increased) quantum yields, (b) in cases in which energy transfer from the primary excited product to other species in homogeneous media masks the primary emitter's fluorescence

in the chemiluminescence spectrum, in micellar media, this emission is clearly seen in the chemiluminescence spectrum from the very beginning of the light reaction due to seclusion of the primary emitter in the *Stern* region of the micelle and (c) chemiluminescence, or at least this light reaction in micellar media can form the basis of just another method for measuring the critical micelle concentrations of surfactants.

In extending this work from cetyltrimethylammonium bromide (*CTAB*) and sodium lauryl sulfate (*SDS*) micellar media to the more stable didodecyldimethylammonium bromide (*DDAB*) bilayer lamelar aggregates, a closer membrane mimetic agent, the above (a) and (b) effects were strongly intensified[3].

It was thought at this point that if the stability and rigidity of the *DDAB* membrane mimetic agent versus that of micelles is responsible for this intensification and as there are chemical factors that influence the rigidity and stability of biological membranes, the presence of such factors in our membrane mimetic agents might modify the quantum yields and the chemiluminescence spectroscopy in such media, eventually leading to methods for the determination of such factors directly incorporated in the membrane.

Indeed, the above (a) and (b) effects were even further intensified when the lucigenin light reaction was carried out in *DDAB* bilayer lamelar aggregates in the presence of cholesterol leading to very high chemiluminescence quantum yields and distinct chemiluminescence spectra[4], while the opposite was observed in the presence of vitamin C[5,6]; vitamin P on the other hand was ineffective as nicotinamide while the quantum yields were raised by almost a factor of 2 in the presence of sodium nicotinate at high concentrations[6].

Encouraged by the above results which we tentatively attribute to modification of the model-membrane structure by cholesterol and vitamins C and P, we now wish to report the chemiluminescence of lucigenin in *DDAB* lamelar aggregates in the presence of nicotine.

## Experimental

### *Reagents*

(—)-Nicotine (Fluka) was purified by distillation and was kept in a light-proof container. Lucigenin (Aldrich) and *DDAB* (Kodak) were employed without further purification.

The samples were aqueous lucigenin ($10^{-5}\,M$) solutions with or without *DDAB* ($10^{-2}\,M$) and were prepared by stirring for 1 h, followed by centrifuging for 1.5 h at 3 500 r.p.m. The stock solutions were kept in the dark at over 20 °C for no longer than one week and the appropriate amount of nicotine (to cover the concentration range 0–2%) was added prior to the chemiluminescence measurement followed by stirring for 1 h.

*Chemiluminescence Measurements*

These were obtained with an Aminco "Chem-Glow" photometer-integrator with the timer circuits disconnected; the $I$—$t$ diagrams were recorded with the aid of a H.P. 7044 A recorder. The light reactions were carried out on addition of NaOH (5 $N$, 30 µl) and $H_2O_2$ (3.5%, 30 µl) to the lucigenin solutions (200 µl) described above. Each experiment was followed by a blank (in the absence of nicotine), the light integrals thus obtained were corrected for self-absorption and the corrected ratios of each such pair of light integrals were plotted versus nicotine concentration. As there was some scattering of the experimental points (more pronounced in the case of the oriented system), the ratios of the light integral pairs versus nicotine concentration were fed to a suitable computer and the exponential curve fits thus obtained are shown in Fig. 1 (plot 1 is the result of 16 such pairs while 2 is the result of 46 pairs).

*Corrections for Self-Absorption*

These were necessary as (a) the samples were slightly coloured and (b) the small bubbles produced in the course of the reaction render the samples somewhat opaque and are especially annoying in the case of surfactant solutions. Each light integral was, therefore, multiplied by a correction factor which was the reciprocal of the mean transmittance (calculated from the transmittance at the beginning and at the end of the reaction) based on the radius of the reaction vessel, at $\lambda = 500$ nm.

*Spectra*

Absorption spectra were run on a Cary 17 spectrophotometer; excitation and fluorescence spectra were recorded on an Aminco-Bowman spectrophotofluorometer calibrated with a quartz "pen ray" lamp and are uncorrected. Chemiluminescence spectra were recorded on the same instrument, with the excitation source off, employing fast scanning rates and wide slits.

## Results and Discussion

The effect of (—)-nicotine on the lucigenin light reaction was studied for comparison purposes both in homogeneous and oriented systems. In the *DDAB* oriented system, nicotine, being water soluble is apparently partitioned between the bulk and the micellar phase of the aggregate and this is verified by the differentiation of the nicotine absorption at ca. 260 nm, in hexane, ethanol, water and *DDAB*. Lucigenin on the other hand is repelled from the micellar interface owing to its charge; it seems therefore that a non-ionic intermediate of the light reaction migrates to the *Stern* region, resulting as shown earlier[1,2] in *N*-methylacridone (*NMA*) emission from this region. Finally, any effect of the hydroxide anion on the structure[2,7] of the oriented system is automatically taken into account as the parameter reported here is the ratio of quantum yields with and without nicotine in the same system under the same conditions.

The quantum yields of the lucigenin chemiluminescence (based on lucigenin employed) were found to drop slightly on addition of nicotine in the concentration range 0–2%, the expression being $Q_{Nic}/Q = a \cdot e^{b[Nic]}$, where $Q_{Nic}$ is the quantum yield in the presence of nicotine, $Q$, the quantum yield without nicotine, $a \equiv 1$ (in the absence of nicotine $Q_{Nic} = Q$ and $Q_{Nic}/Q = 1$), $b = -0.214$ and $[Nic]$ is the

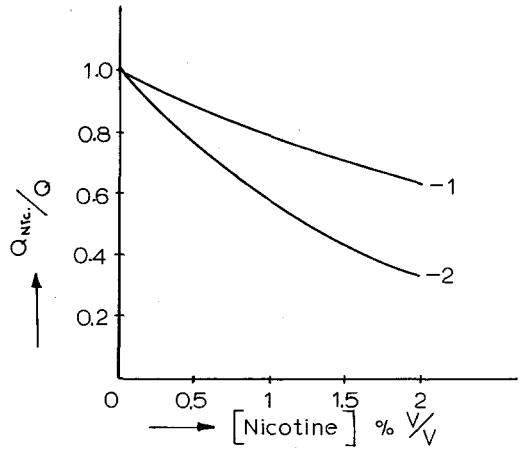


Fig. 1. Exponential curve fits of the chemiluminescence quantum yields of the lucigenin light reaction in the presence of nicotine ($Q_{Nic}$) over those in the absence of nicotine ($Q$) versus nicotine concentration *1* in aqueous solution, *2* in aqueous 0.01 *M DDAB* solution

concentration of nicotine expressed as % *v/v*. A similar exponential relationship was obtained in the *DDAB* oriented system, but here the drop in quantum yields caused by the presence of nicotine was much more pronounced with a value for $b$ equal to $-0.571$; these results ase shown diagrammatically in Fig. 1.

The fluorescence and chemiluminescence spectroscopy of the light reactions is shown in Figs. 2–5. In Fig. 2, which is the luminescence spectroscopy of the well known luciginin light reaction in aqueous solution, the fluorescence of lucigenin before the reaction (2.1) is replaced by the fluorescence of *NMA* (the primary emitter) at ca. 430 nm, towards the end of the reaction (2.2), while the chemiluminescence spectrum (2.3), is not identical with the fluorescence spectrum of the emitter (as it ought to be since both emissions arise from de-excitation of the same molecule), due to energy transfer. In Fig. 3 which is the luminescence spectroscopy in aqueous solution in the

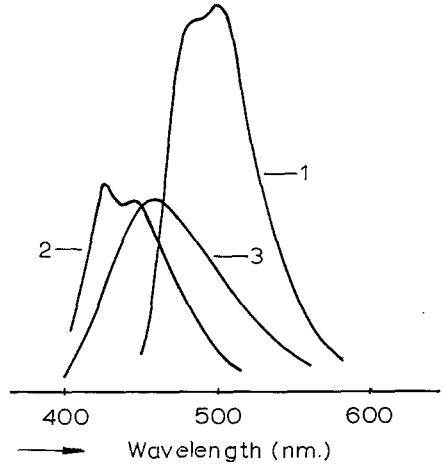


Fig. 2. Luminescence spectra of the lucigenin light reaction in aqueous solution. *1* Fluorescence spectrum of lucigenin before the reaction; *2* fluorescence spectrum of the reaction mixture at the end of the light reaction (excitation $\lambda_{max} = 360$ nm); *3* chemiluminescence spectrum

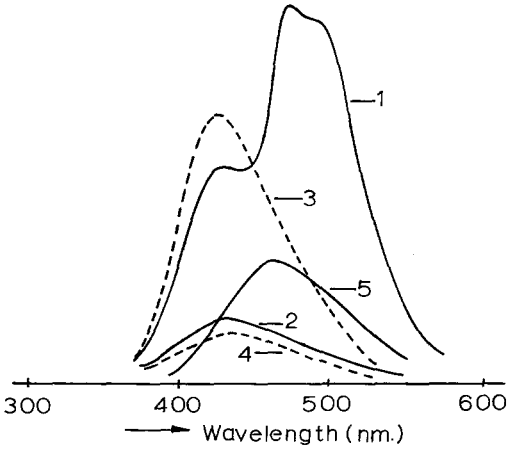


Fig. 3. Luminescence spectra of the lucigenin light reaction in aqueous solution in the presence of nicotine (1% *v/v*). *1* Fluorescence spectrum of lucigenin plus nicotine before the reaction; *2* fluorescence spectrum of the mixture after the light reaction; *3* fluorescence spectrum of aqueous nicotine; *4* fluorescence spectrum of aqueous nicotine after addition of NaOH and $H_2O_2$ (excitation $\lambda_{max} = 360$ nm); *5* chemiluminescence spectrum

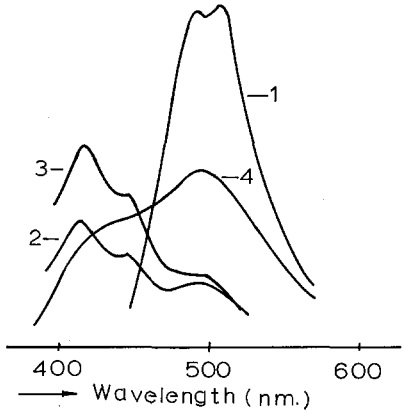


Fig. 4. Luminescence spectra of the lucigenin light reaction in *DDAB* aqueous solution. *1* Fluorescence spectrum before the reaction; *2* fluorescence spectrum after 15 min (sensitivity × 10); *3* fluorescence spectrum after 40 min (sensitivity × 10) (excitation $\lambda_{max} = 360$ nm); *4* chemiluminescence spectrum

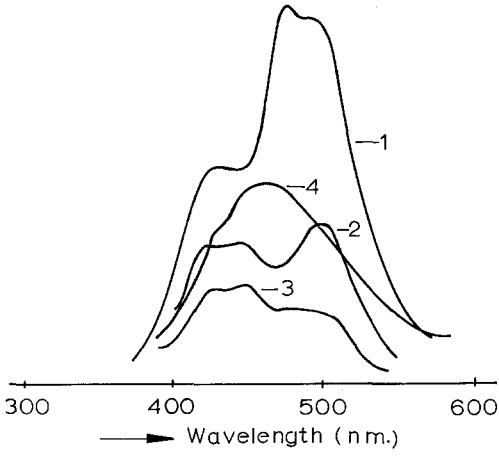


Fig. 5. Luminescence spectra of the lucigenin light reaction in *DDAB* aqueous solution in the presence of nicotine (1% *v/v*). *1* Fluorescence spectrum of the mixture before the reaction; *2* and *3* fluorescence spectra after 14 and 40 min, respectively, (excitation $\lambda_{max} = 360$ nm); *4* chemiluminescence spectrum

presence of 1% nicotine, the chemiluminescence spectrum is not affected by the presence of nicotine; yet, the fluorescence spectrum of the mixture before the light reaction also includes the fluorescence of nicotine which falls in the same spectral region as that of *NMA*, a peak which is quenched on addition of the reagents. Going from the aqueous

(homogeneous) medium to the *DDAB* aqueous (oriented) medium (Fig. 4), the emission of the primary emitter (*NMA*) appears in the chemiluminescence spectrum (4.4), due, as we have argued earlier[1–4] to restrictions of energy transfer, caused by the isolation of the primary emitter produced inside the aggregate. Of course, there is always energy transfer in the light reaction occuring in the bulk plus some energy transfer in the aggregate and this gives rise to the peak at ca. 500 nm. This differentiation of the chemiluminescence spectrum in the oriented system is almost completely ruined in the presence of 1% nicotine (Fig. 5.4), which apparently nullifies the effect of orientation, due, as we believe, to modification of the structure of the membrane mimetic agent, resulting in less chemiluminescence inside the aggregate.

In conclusion, the increased chemiluminescence efficiency of the lucigenin reaction in oriented media versus that in homogeneous media (which is further increased in the presence of cholesterol and vitamin P) is reduced in the presence of nicotine. This seems to be caused by structure modifications of the membrane mimetic agent in which a part of the light reaction takes place. Furthermore, the appearance of emission from *NMA*, the primary excited product of the reaction in the chemiluminescence spectrum in micellar and lamelar aggregates, attributed to seclusion of the primary emitter in said aggregates, vanishes in the presence of nicotine.

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