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Chemiluminescence in Model Membrane Structures Chemiluminescence of Lucigenin in the Presence of Estrogens

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The chemiluminescence quantum yields of the lucigenin light reaction in 10^{-2} *M* didodecyldimethylammonium bromide (*DDAB*) lamelar aggregates are affected by the presence of estrone and 17α-ethynylestradiol. A rise in quantum yields is observed at ca. $1 \cdot 10^{-4}$ *M* estrogen concentration as compared with the homogeneous (aqueous) medium, followed by a dramatic drop as the estrogen concentration increases. Unexpectedly, the smecticity of the lamelar aggregate is destroyed by estrone concentration as low as 10^{-3} *M* and 17α-ethynylestradiol concentrations as low as $2 \cdot 10^{-4}$ *M*.

(Keywords: Chemiluminescence; Estrogens; Lucigenin; Membranes)

Chemilumineszenz in Modell-Membranstrukturen
Die Chemilumineszenz von Lucigenin in Gegenwart von Estrogenen

Die Chemilumineszenzquantenausbeuten der Lucigenin-Lichtreaktion in 10^{-2} *M* Didodecyldimethylammoniumbromid (*DDAB*)-Lamellaraggregaten wird bei Gegenwart von Estron und 17α-Ethinylestradiol beeinflußt. Es wird eine Erhöhung der Quantenausbeuten bei ca. $1 \cdot 10^{-4}$ *M* Estrogengehalt gegenüber einem homogenen (wäßrigen) Medium beobachtet, bei Erhöhung der Estrogenkonzentration erfolgt jedoch eine drastische Erniedrigung der Quantenausbeuten. Die Smektizität der Lamellaraggregate wird bei Estronkonzentrationen von etwa 10^{-3} *M* und 17α-Ethinylestradiolkonzentrationen von etwa $2 \cdot 10^{-4}$ *M* unerwarteterweise zerstört.

Introduction

Working with the 10,10′-dimethyl-9,9′-biacridinium nitrate (lucigenin) light reaction we have shown[1,2] that chemiluminescence (CL) in micellar media, among other effects (a) results in altered (increased) quantum

yields and (b) in cases where energy transfer from the primary emitter to other species masks the primary emission. In micellar media this emission is clearly seen in the CL spectrum from the very beginning of the light reaction due to isolation of the primary emitter in the *Stern* region of the micelle.

In extending this work to the more stable didodecyldimethylammonium bromide (*DDAB*) bilayer lamelar aggregates, a better membrane mimetic agent, the above (a) and (b) effects were further intensified[3]. We have generally attributed the increased CL efficiencies to the lower polarity of the *Stern* region (versus that of water) in which the critical decomposition of the intermediate dioxetane partly takes place and we have sought to modify the structure of the lamelar aggregate by the presence of factors influencing the rigidity and stability of biological membranes. Indeed, the light reaction in *DDAB* aggregates was more efficient in the presence of $10^{-4}\,M$ cholesterol[4], less efficient in the presence of vitamin C[5, 6], the results with vitamins P were inconclusive[6], while nicotine caused a dramatic drop in quantum yields[8]. As steroid sex hormones are of great importance for their role on human cancer cells[9, 10] and as a lot of work has been reported elucidating the mechanism of oxidation and reduction of said steroids by chemical means or by X and γ-ray radiation[11, 12, 13], we now wish to report the CL of this system in the presence of estrogens (estrone and 17α-ethynylestradiol).

Experimental

The light reactions were carried out as described earlier[8] in an Amino "chemglow" photometer with the timer circuitry disconnected, on addition of NaOH (30 μl, 0.05 N) and H_2O_2 (30 μl, 3%) to a pre-centrifuged 250 μl aqueous solution 0.01 M in *DDAB* and $10^{-5}\,M$ in lucigenin, plus estrogen, covering the concentration range $0\text{–}8 \cdot 10^{-4}\,M$. Each measurement was repeated in the absence of estrogen and the ratios of the light integrals Q_{estrogen}/Q, corrected for self-absorption at $\lambda = 500$ nm (from the mean transmittance at the beginning and the end of the light reaction, taking into account the light path in the CL reaction vessel) were curve fitted with the aid of a computer versus estrogen concentration. Similar plots of Q_{estrogen}/Q versus estrogen concentration in the absence of *DDAB* were obtained with a modified technique to account for the insolubility of the estrogens in aqueous media.

Excitation, fluorescence and CL spectra were run on an Aminco-Bowman spectrophotofluorimeter, while absorption spectra were run on a Hitachi 220 spectrophotometer.

Results and Discussion

The effect of estrone and 17α-ethynylestradiol on the chemiluminescence of lucigenin was studied for comparison purposes, both in homogeneous and oriented systems. In the *DDAB* oriented system the

estrogens are solubilized at the interface, but on addition of the sodium hydroxide necessary for the light reaction the solubility of the estrogens in the aqueous phase increases and a partition between the oriented phase and the bulk phase should be considered. Lucigenin, on the other hand, is repelled from the micellar interface owing to its charge and it seems 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

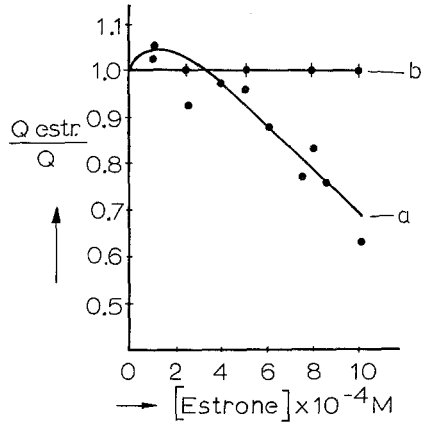

Fig. 1. Q_{estrone}/Q as a function of estrone concentration. *a* in *DDAB* aggregates; *b* in aqueous homogeneous solution

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 estrogen, in the same system under the same conditions. The effects of the estrogens on the lucigenin CL are now reported separately for estrone and 17α-ethynylestradiol.

(i) *Estrone:* The effect of estrone on the chemiluminescence of lucigenin in *DDAB* bilayer lamelar aggregates is shown in Fig. 1 *a* where the ratio Q_{estrone}/Q is plotted versus estrone concentration, the law being $Q_{\text{est}}/Q = 1 + 0.464\,[\text{est}] - 0.0179[\text{est}]^2 + 9.46 \cdot 10^{-4}[\text{est}]^3$. A similar plot, Fig. 1 *b*, in homogenous aqueous solution, follows the law $Q_{\text{est}}/Q = 1$ showing that estrone is not involved in, or in any way affect the light reaction. A small rise at ca. $1 \cdot 10^{-4}\,M$ estrone is reminiscent of a similar rise in the presence of $1 \cdot 10^{-4}\,M$ cholesterol[4] and could be attributed to the steroid incorporated in the aggregate, followed by a decline as the increased concentrations of the addend further modify the structure of the membrane mimetic agent.

Although the smecticity of the aggregate is not affected by estrone concentrations in the range shown in Fig. 1, and although, in any case the Q_{est}/Q ratios are corrected for self-absorption, it is important to notice the abrupt drop of the oriented system's optical density at an estrone concentration of ca. $9 \cdot 10^{-4} M$ (Fig. 3 *a*), indicating that at estrone concentrations of over $9 \cdot 10^{-4} M$ the oriented system is no longer the same.

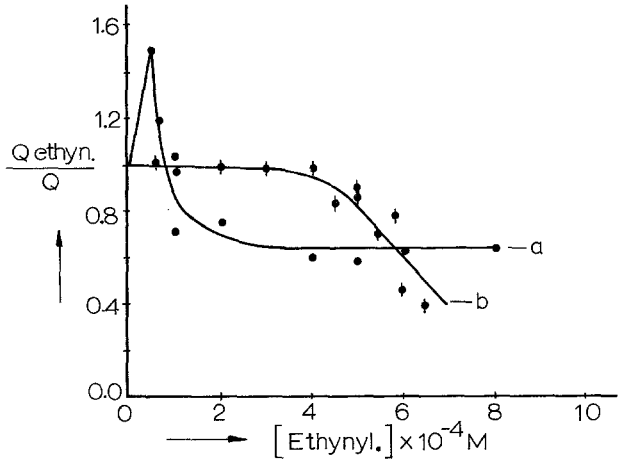

Fig. 2. $Q_{ethynylestradiol}/Q$ as a function of ethynylestradiol concentration. *a* in *DDAB* aggregates; *b* in aqueous homogeneous solution

(ii) *17 α-Ethynylestradiol:* Unlike estrone, 17 α-ethynylestradiol does affect the light reaction even in the homogeneous (aqueous) system the law being $Q_{eth}/Q = 1 - 0.082\,[\mathrm{Eth}] + 0.047\,[\mathrm{Eth}]^2 - 7.5 \cdot 10^{-3}\,[\mathrm{Eth}]^3$, shown diagrammatically in Fig. 2 *b*. Although a competition reaction of the lucigenin light reaction's intermediates with 17 α-ethynylestradiol unsaturated side chain cannot be ruled out at this stage, quenching of the primary emitter's (*N*-methylacridone) fluorescence by 17 α-ethynylestradiol is certainly at least partly responsible for the shape of curve (b) in Fig. 2. Indeed, appreciable quenching of the *N*-methylacridone's fluorescence is observed on addition of increasing amounts of 17 α-ethynylestradiol to saturated aqueous alkaline solutions of *N*-methylacridone in the range of 17 α-ethynylestradiol concentrations of Fig. 2. (Saturated *N*-methylacridone solutions have to be employed as its solubility in water is very low.)

In the *DDAB* oriented system, the ratio Q_{eth}/Q versus 17 α-

ethynylestradiol concentration rises abruptly and then declines, the decline following the law $Q_{eth}/Q = [eth]/1.7[eth] - 5 \cdot 10^{-5}$, as shown in Fig. 2*a*. Here, the peak at 17 α-ethynylestradiol concentration ca. $0.5 \cdot 10^{-4} M$ is more pronounced than that in the presence of estrone. Regarding the shape of the curve at 17 α-ethynylestradiol concentrations over $1.5 \cdot 10^{-4} M$ it should be noted that the optical density of the oriented system also falls abruptly (Fig. 3*b*), reflecting a dramatic change in the

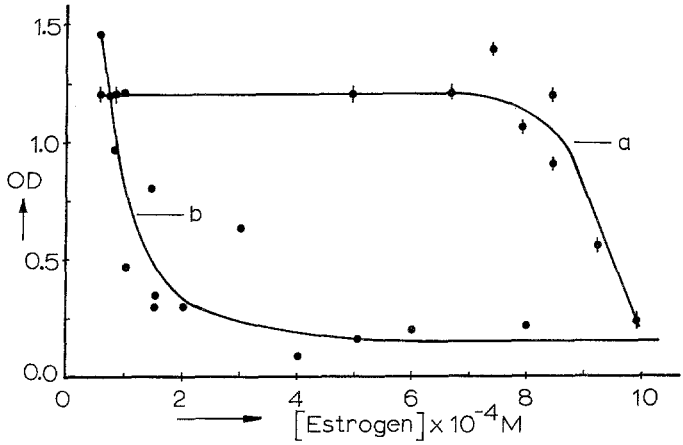

Fig. 3. Optical density of the *DDAB*-oriented system after addition of the reagents as a function of estrogen concentration. *a* estrone; *b* ethynylestradiol

smecticity of the aggregate and although the curves of Figs. 1 and 2 are corrected for self-absorption, the changes in the system itself render any further discussion unsafe.

In conclusion, the presence of the estrogens employed here affects the CL efficiency of the lucigenin light-reaction in *DDAB* bilayer lamelar aggregates, due, as we believe to a modification of the structure of the membrane mimetic agent.

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