Monatshefte für Chemie 119, 703—710 (1988)

Monatshefte für Chemie
Chemical Monthly

Chemiluminescence in Model Membrane Structures. Chemiluminescence of Lucigenin in the Presence of $Mg(OH)_2$ and Benzyl Alcohol. Temperature Effects

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(Received 2 April 1987. Accepted 15 May 1987)

The quantum yields of the lucigenin light reaction in didodecyldimethylammonium bromide (*DDAB*) are affected by the presence of $Mg(OH)_2$; a 35% increase is observed in lamellar and a 65% increase in vesicular aggregates. The system is insensitive to benzyl alcohol. The quantum yields in *DDAB* versus those in water, as a function of temperature show a slope change in the region of the phase transition in lamelar aggregates. This effect is far less pronounced in vesicular aggregates. In contrast to the *DDAB* aggregates, anionic sodium dimethyldidodecylphosphate (*SDDP*) sonicated aggregates are associated with lower quantum yields and no apparent slope change in the region of the phase transition.

(Keywords: Chemiluminescence; Lucigenin; Membranes)

Chemilumineszenz in Modell-Membranstrukturen. Die Chemilumineszenz von Lucigenin in der Gegenwart von $Mg(OH)_2$ *und Benzylalkohol. Temperatureffekte*

Die Quantenausbeuten der Lichtreaktion von Lucigenin in Didodecyldimethylammoniumbromid (*DDAB*) werden durch die Gegenwart von $Mg(OH)_2$ beeinflußt. Es wird 35% Verstärkung in lamellaren und 65% Verstärkung in vesicularen Aggregaten beobachtet. Das System ist Benzylalkohol gegenüber unempflindlich. Eine Gegenüberstellung der Quantenausbeuten in *DDAB* bzw. in Wasser als eine Funktion der Temperatur zeigt eine Änderung des Anstiegs im Bereich des Phasenüberganges bei lamellaren Aggregaten. Dieser Effekt ist in vesicularen Aggregaten nicht so ausgeprägt. Im Gegensatz zu den *DDAB*-Aggregaten sind die beschallten anionischen Natriumdimethyldodecylphosphat (*SDDP*)-Aggregate durch geringere Quantenausbeuten und keine offensichtlichen Anstiegsänderungen im Bereich des Phasenüberganges gekennzeichnet.

Introduction

Working with the 10,10′-dimethyl-9,9′-biacridinium nitrate (lucigenin) light reaction we have shown [1, 2] that chemiluminescence in micellar media, among other effects (a) results in increased quantum yields and (b) light emission from the primary emitter is demasked, whereas said emission in homogeneous media is masked by energy transfer to other species. These effects were further intensified [3] in didodecyldimethylammonium bromide (*DDAB*) bilayer lamelar aggregates, a membrane mimetic agent thermodynamically more stable than micelles. As there are chemical factors affecting the rigidity and stability of biological membranes, we have sought to modify the rigidity and stability of the above membrane mimetic agents by the presence of additional factors, expecting changes in the quantum yields of the light reaction taking place in the membrane mimetic agent. Indeed, the light reaction in *DDAB* lamelar 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 [7]. Steroid sex hormones (estrone and 17a-ethylnylestradiol) [8] resembled cholesterol causing a steep rise in quantum yields at low concentrations, followed by a decline at higher estrogen concentrations. Employing progesterone as the addend, the light reaction was studied in lamelar and vesicular *DDAB* aggregates [9] and appreciable differentiations were observed regarding the reaction's quantum yields in the two media. As it has been shown that magnesium ions and benzyl alcohol affect the fluidity of both biological and model membranes in opposite directions [10], the quantum yields are now reported as functions of addend concentration.

Temperature is another important factor influencing the stability and fluidity of membranes and membrane mimetic agents. In an effort to correlate the chemiluminescence quantum yields in said media with the stability and fluidity of the media, the ratios Q_{DDAB}/Q_{H_2O} (Q_{DDAB}: the quantum yield in the oriented system; Q_{H_2O}: the quantum yield in water) are also reported as a function of temperature. Finally, for comparison, the reaction was studied in anionic sodium dimethyldidodecyl phosphate (*SDDP*) as well.

Experimental

Surfactants: (i) Didodecyldimethylammonium bromide (*DDAB*), Kodak, was employed without further purification.

(ii) Sodium dimethyldidocyl phosphate (*SDDP*). The free acid was prepared as described earlier [11], m.p. 52–53 °C (Ref. [12, 14], 51–52 °C, 56–58 °C). The sodium salt was obtained as described [13], *Karl Fisher* determination showed 0.6% H_2O, m.p. 250 → 265 (Ref. [14] 243 → 250). The structure was confirmed by elemental analysis, IR and NMR spectroscopy [^{1}H NMRin CD_3OD: δ, 0.89 (CH_3, t), 1.29 ($(CH_2)_{10}$, m), 3.83 (CH_2O, apparent q)].

Solutions: DDAB was added to water and the mixture was stirred at room temperature for 72 h to achieve complete dissolution of the surfactant (0.01 *M*). Magnesium hydroxide or benzyl alcohol was then added to cover the concentration range $0–50 \cdot 10^{-4}$ *M*, this mixture was stirred for additional 0.5 h and then divided into two portions. One portion was centrifuged (3 500 r.p.m., 1.5 h); this solution will from now on be refered to as "*lamelar*". The second portion was sonicated (M.S.E. sonicator, 0.5 h) and then centrifuged (3 500 r.p.m., 0.5 h); this solution will from now on be refered to as "*vesicular*". In the case of *SDDP* the procedure followed was that leading to vesicular aggregates. Small volumes from a stock solution of lucigenin (10^{-3} *M*) were then added to the solutions to reach a lucigenin concentration of 10^{-5} *M* and the resulting solutions were stirred for 0.5 h prior to their employment in the chemiluminescence measurements. It should be noted here that unsonicated *SDDP* solutions were too opaque to be employed in chemiluminescence measurements.

Chemiluminescence Measurements: These were carried out in an Aminco "Chem-glow" photometer with the timer cirquitry disconnected, on addition of NaOH (30 μl, 0.01 *N*) and H_2O_2 (30 μl, 3%) to 250 μl of the above solutions. Each measurement was repeated in the absence of additive (Mg^{++} or benzyl alcohol) and the light integrals Q_{add} and Q were corrected for self absorption at $\lambda = 500$ nm. This correction was based on the mean transmittance at the beginning and the end of the light reaction, taking into account the radius of the reaction vessel representing the mean light path. The ratios of Q_{add}/Q were then plotted versus the addend concentration.

Chemiluminescence as a Function of Temperature: The chemiluminescence measurements were (in this case conducted in the chemiluminescence chamber earlier described [2]) equipped with a self constructed suitable cell. Temperature was maintained by circulation through the cell's jacket, of liquid from an external constant temperature bath and was monitored with the aid of a bimetallic thermometer inserted in the reaction mixture. The light integrals in the presence and in the absence of addend were corrected for self absorption as described above. The reactions were in this case performed by squirting NaOH (1.2 ml, 0.1 *N*) and H_2O_2 (1.2 ml, 3%) to 10 ml of the above solutions.

Spectra: Excitation and fluorescence spectra were run on an Aminco-Bowman SPF spectrophotofluorometer. Chemiluminescence spectra were run on the same instrument with the excitation source off. Absorption spectra were recorded on a Hitachi 220 spectrophotometer. IR spectra were obtained on a Perkin-Elmer 283 spectrophotometer and NMR spectra were obtained with the aid of a Varian CFT 20 (80 MHz) instrument.

Results and Discussion

The lucigenin-NaOH-H_2O_2 light reaction leads to electronically excited N-methylacridone (*NMA*). De-excitation of this primary emitter results in light emission with λ_{max} ca. 430 nm plus energy transfer to other species (lucigenin included) with subsequent emission at ca. 500 nm. In cationic oriented systems such as the *DDAB* aggregates employed here, the lucigenin cation is expected to be repelled from the positively charged interface and it has been argued [1–3] that a non ionic intermediate of the light reaction migrates to the *Stern* region of the aggregate resulting in

NMA emission from this region. Later reports [15], however, show that although cationic, lucigenin binds to cationic micellar and vesicular aggregates. Magnesium ions, on the other hand are not primarily expected to enter the aggregate's *Stern* region as such due to electrostatic repulsion by the positively charged head groups and lack of a hydropholic moiety. Yet, magnesium as magnesium hydroxide does enter in the *Stern* region (a) because it is sparingly soluble in water (less that $2 \cdot 10^{-4}$ *M*) and (b), because magnesium hydroxide is highly soluble in ammonium salts [16] as is the case with the *DDAB* polar heads. Indeed, in the course of our experiments, we were able to solubilize magnesium hydroxide in *DDAB* in concentrations much higher than $2 \cdot 10^{-4}$ *M*. As the chemiluminescence efficiency is affected by the fluorescence efficiency of the emitting species, the fluorescence of *NMA* in the presence of $Mg(OH)_2$ and benzyl alcohol was studied both in homogeneous and oriented systems; the quenching observed was insignificant, and the same is true for the quenching of *NMA* by phosphate in the case of *SDDP* aggregates. Finally any effect of the hydroxide anion (due to the high normalities of the NaOH employed) on the structure [2, 10] of the oriented phase is automatically nullified, as the parameter reported here is the ratio of quantum yields with and without additive in the same system under the same conditions.

The effect of additive on the chemiluminescence of lucigenin in lamelar and vesicular aggregates is shown in Figs. 1 and 2, respectively. The conclusions drawn from the plots are as follows. (a) Magnesium hydroxide has no effect on the chemiluminescence of lucigenin in homogeneous aqueous media. (b) Benzyl alcohol has no effect on the light reaction, both in homogeneous aqueous media and in *DDAB* aggregates lamelar as well as vesicular. (c) Magnesium hydroxide gives rise to increased chemiluminescence in lamelar *DDAB* aggregates and even more so, in vesicular *DDAB* aggregates. Other conclusions will have to be discussed in connection with the plots of chemiluminescence versus temperature.

It is well accepted that temperature affects the rigidity and stability of both phospholipid [17] membranes and membrane mimetic agents such as the *DDAB* aggregates of this work [18, 19]. In an effort to connect the chemiluminescence efficiency in these systems with the stability and rigidity of the aggregates, the chemiluminescence of lucigenin was studied in *DDAB* aggregates as a function of temperature and the results are shown in Fig. 3. Here, the ratio Q_{DDAB}/Q_{H_2O} (Quantum yield in the oriented system over quantum yield in the homogeneous aqueous system) decreases as the temperature increase gives rise to higher fluidity and it is interesting to note that the slope changes in the region around 16 °C (a) which is the region of the phase transition temperature [18] for this system. It follows that in this case increased chemiluminescence is associated with

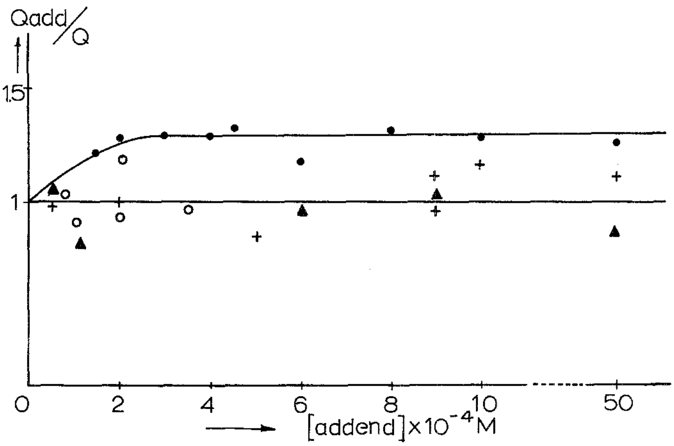

Fig. 1. Ratios of chemiluminescence quantum yields in the presence and in the absence of additive as a function of additive concentration. *a* In *DDAB* lamelar aggregates: $Mg(OH)_2$ (●); benzyl alcohol (+). *b* In aqueous solutions: $Mg(OH)_2$ (○); benzyl alcohol (▲)

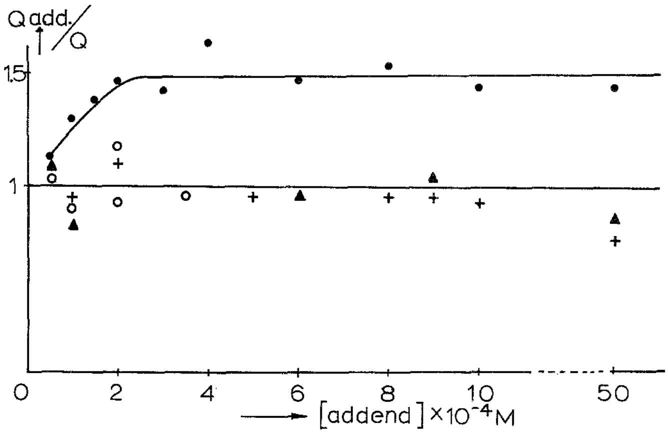

Fig. 2. Ratios of chemiluminescence quantum yields in the presence and in the absence of additive as a function of additive concentration. *a* In *DDAB* vesicular aggregates: $Mg(OH)_2$ (●); benzyl alcohol (+). *b* In aqueous solutions: $Mg(OH)_2$ (○); benzyl alcohol (▲)

increased rigidity. The effect is more pronounced in the lamelar system and such a differentiation is expected. Indeed, the destruction of a system of superimposed lamelae should be more drastic than any effect on the vesicular system.

Although not very clear from the plot, it appears that a slope change at around 5 °C is present in the vesicular system as well, in agreement with a

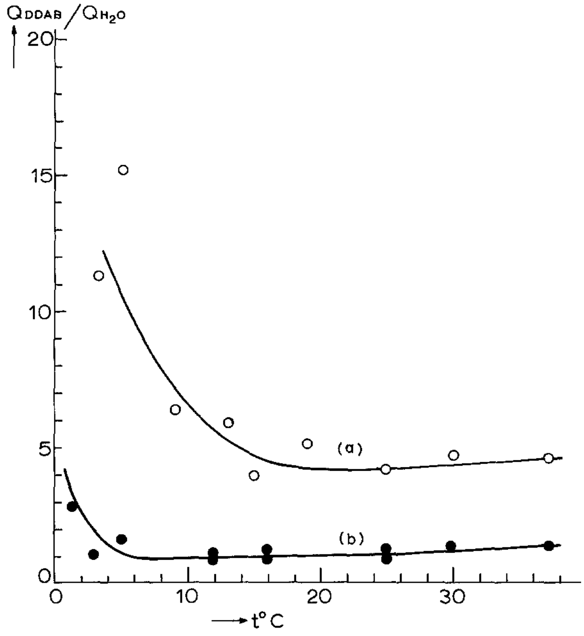

Fig. 3. Ratios of chemiluminescence quantum yields in *DDAB* aggregates and in water as a function of temperature. *a* Lamelar (○). *b* Vesicular (●)

report [19] giving 4.5 °C as the phase transition temperature of the vesicular aggregate.

The thermal experiments showed that on going from the rigid gel phase to the more disordered liquid crystalline state, the quantum yields of our reaction are diminished. Addition of magnesium ions, on the other hand, to phospholipid [17] membranes and anionic micelles increases the rigidity [10] due to contraction of the anionic polar heads [20]. With this reasoning one would expect the opposite effect in our cationic aggregates, followed by a reduction in quantum yields. Figures 1 and 2, however, show that this is not the case; addition of magnesium hydroxide gives rise to higher quantum yields. It should be emphasized again that magnesium cations cannot enter the aggregate, due to strong coulombic repulsion by the positively charged ammonium head-groups and will certainly not affect the aggregate's structure. As magnesium hydroxide is solubilized by the aggregate, it follows that this species enters the aggregate, perhaps as ion pairs, at least as far as the outer limits of the *Stern* region. There, in the unique environment of the *Stern* region, weak complexes or saltlike compounds with the polar heads would serve as bonds among the later, giving rise to effects similar to those produced by magnesium ions in anionic surfactants.

The results of the thermal experiments in *SPPD* vesicular aggregates (no dissolution was possible without sonication) are shown in Fig. 4. Lucigenin binds to *SPPD* far better than in *DDAB* due to its anionic character, yet, the quantum yields in this medium are lower than in *DDAB*. This is easily attributable to the low concentration of OH^- and HO_2^- in this medium due to electrostatic repulsion by the negatively charged phosphate head groups; is should be remembered that the medium should be strongly alkaline for an efficient lucigenin light reaction [21]. Although higher quantum yields have been reported for this light reaction in anionic sodium lauryl sulphate micelles [2], in our case higher sodium hydroxide concentrations resulted in salting out of the surfactant. In any case, quenching should be ruled out as a reason for the low chemiluminescence efficiency as we observed no quenching of the *NMA* fluorescence by phosphate ions. Unfortunately, excessive foaming of the surfactant reaction mixture at higher temperatures caused a large scattering of the experimental points so that meaningful results could not be obtained in this temperature region. This was particularly annoying as the phase transition temperature [22] for this vesicle (45 °C) falls in this region. Apparently, *SPPD* is not a very suitable membrane mimetic agent for the study of chemiluminescence in such media.

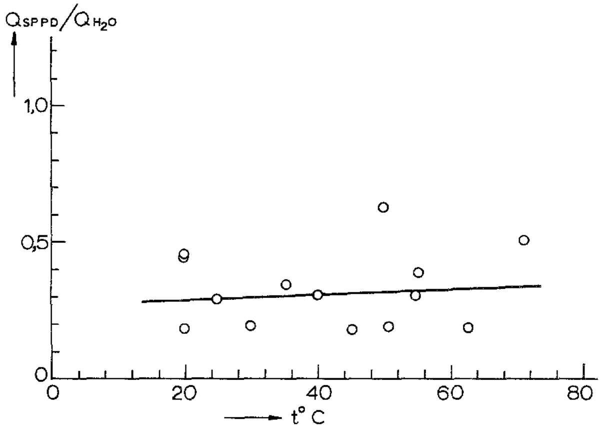

Fig. 4. Ratios of chemiluminescence quantum yields in *SDDP* vesicular aggregates and in water as a function of temperature

In conclusion, the results from the *DDAB* thermal measurements and from the $Mg(OH)_2$ experiments offer further evidence in support of our earlier suggestions [1–9] that chemiluminescence can be employed as a tool in the study of the structure of membrane mimetic agents and the effect of added factors in these structures.

Acknowledgement

The author wish to thank Mrs. *E. Sekeri-Papazoglou* for technical assistance in the experiments.

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