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CYPRIDINA BIOLUMINESCENCE VII. CHEMILUMINESCENCE IN MICELLE SOLUTIONS — A MODEL SYSTEM FOR CYPRIDINA BIOLUMINESCENCE

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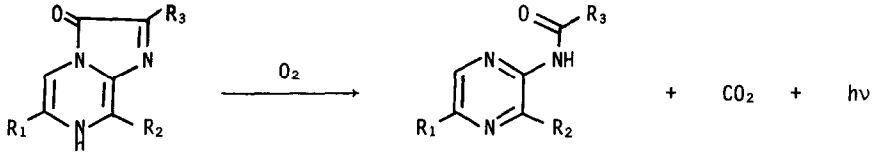
Cypridina bioluminescence is produced in aqueous solution by oxidation of Cypridina luciferin (substrate) with molecular oxygen in the presence of Cypridina luciferase (enzyme), which may be classified as one of the dioxygenases (1). Luciferin also chemiluminesces strongly in aprotic solvents such as diglyme or DMSO without enzyme (2), and the mechanism of the bioluminescence is assumed to be identical with that of the chemiluminescence with the exception that luciferin does not produce light in aqueous solution without enzyme (3,4). We have assumed (3) that the role of the enzyme would be simply to give luciferin a suitable hydrophobic environment (5), which may be expected to be in a cave of the enzyme; luciferin (I) then reacts spontaneously with molecular oxygen to produce oxyluciferin (II) in a singlet



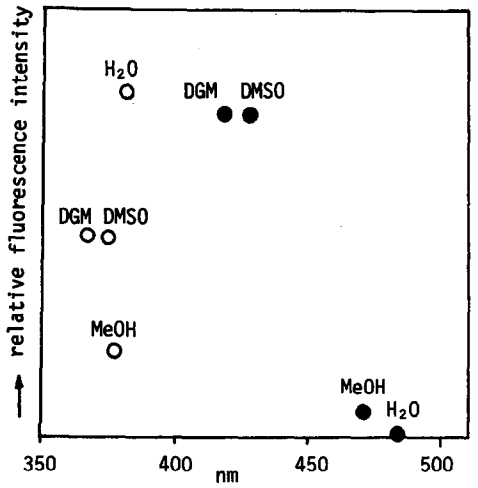
I, III, V II, IV

I and II: R_1 = 3-indolyl, R_2 = $-(CH_2)_3NHC(=NH)NH_2$, R_3 = $-CH(CH_3)CH_2CH_3$
III and IV: R_1 = phenyl, R_2 = H, R_3 = CH_3
V: R_1 = 3-indolyl, R_2 = H, R_3 = CH_3

excited state, which then gives light. If this assumption is true, major roles of the enzyme would be: (a) enhancement of fluorescence intensity of oxyluciferin (II) and (b) enhancement of reaction rate of luciferin (I) with molecular oxygen. These are verified by the following observations. In the case of bioluminescence, the fluorescence quantum yield of oxyluciferin must be high, since the bioluminescence quantum yield is high (6), and the luminescence spec-

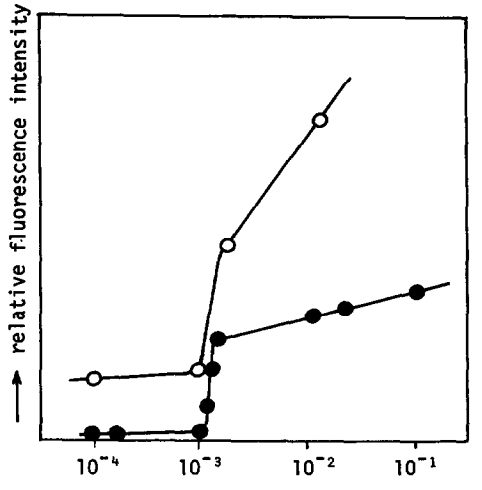
trum should be identical with the fluorescence spectrum of oxyluciferin (3). However, while fluorescence of oxyluciferin (II) in aprotic solvents is strong, in aqueous solutions it is very weak (Fig. 1) and its maximum is not identical with the bioluminescence spectrum (λ_{max} 450 nm). We have explained this contradiction as assuming the presence of an enzyme-substrate complex, in which the emitter, oxyluciferin in excited states, is in environment similar to that in aprotic solvents (4). Indeed, Shimomura et al. (7) reported that addition of purified enzyme to an aqueous solution of oxyluciferin (II) enhances the fluorescence intensity of oxyluciferin and the fluorescence spectrum becomes identical with the bioluminescence spectrum of luciferin; a 1:1 complex between oxyluciferin and luciferase being formed. It is also observed (3) that luciferin analog (III), but not luciferin (I) itself, can chemiluminesce in aqueous solution in the presence of hydrogen peroxide and ferric ions (but not with molecular oxygen) (8), since the corresponding acylaminopyrazine (IV) shows strong fluorescence in aqueous solutions as well as in aprotic solvents (3). Luciferin (I) and the analogs,(III) and (V), react with molecular oxygen in aprotic solvents, but not in water.

FIG. 1. Solvent Effects on the Fluorescence of Oxyluciferin (II) and its Analog (IV)



DGM = diglyme; DMSO = dimethyl sulfoxide

FIG. 2. Fluorescence Intensity of Oxyluciferin (II) in Micelles



Consideration of these factors led us to the expectation that if micelles of a suitable surfactant are present, luciferin might be adsorbed into micelle interior formed by the hydrophobic portion of the surfactant, and produce light in aqueous solution, as it does in the presence of the enzyme. In Figure 2, dependence of the fluorescence intensity of oxyluciferin on the concentration of a cationic surfactant, cetyltrimethylammonium bromide (CTABr), and an anionic surfactant, sodium lauryl sulfate (NaLS), is presented. A sudden increase in fluorescence intensity is observed when the surfactant concentrations become above 10^{-3} mol/l; this limiting concentration being related to the critical micelle concentration. Partitioning of oxyluciferin into the hydrocarbon interior of the micelles seems to be occurring with either cationic or anionic surfactants.

Then, chemiluminescence of luciferin (I) and its analog (V) was measured in these micelle solutions. As shown in Table 1, luminescence is observed in the micelle solutions of CTABr and neutral surfactant Tween #60; the reaction being much faster in the former than in the latter.

TABLE 1. Chemiluminescence in Micelle Solutions

	(V)		luciferin (I)	
	L.Yield*	k (sec^{-1})†	L.Yield*	k (sec^{-1})†
0.01M Tween #60 in 0.1M phosphate pH 7.0	450	very slow	470	very slow
0.01M CTABr in 0.1M phosphate pH 7.0	830	7.6×10^{-4}	260	11.9×10^{-4}
" in 0.1M Tris pH 9.0	14,000	6.7×10^{-4}	1,600	31.6×10^{-4}
" in 0.1M carbonate pH 9.0			430	55.0×10^{-4}
0.01M NaLS in 0.1M phosphate pH 7.0	0	•	0	
0.1M phosphate only pH 7.0	0		0	
diglyme contg. 0.5% 0.1M acetate pH 5.6	100,000	0.25×10^{-4}	100,000	17.6×10^{-4}
luciferase in 0.1M phosphate§ pH 7.0	6,000	¶	500,000	¶

* light yield (arbitrary unit) † pseudo-first order rate const. at 25°

§ containing 0.1M NaCl ¶ depend on the concentration of the enzyme

A very contrasting result is obtained with the micelles of NaLS, which has no ability to produce luminescence with luciferin (I) or its analog (V). The reason would be either that NaLS anion forms micelles with highly charged negative surfaces, which prevent luciferin anion to enter micelle interior, or that in the micelles luciferin cannot react with molecular oxygen. As the fluorescence spectra of oxyluciferin (II) change markedly with the solvents (Fig. 1),

it is surprising that both of luminescence spectrum of luciferin (λ_{max} 450 nm, FWHM 3800 cm^{-1}) and fluorescence spectrum of oxyluciferin (λ_{max} 450 nm, FWHM 3800^{-1}) in CTABr micelle solutions are superimposable to those in the enzyme solutions (luminescence of I: λ_{max} 450 nm, FWHM 3000 cm^{-1}; fluorescence of II: λ_{max} 450 nm, 3500 cm^{-1}) (9). The reaction rate is pseudo-first order to luciferin (I) or analog (V) in the micelle solutions.

Spectral and kinetic behaviors of the luminescence indicate that the micelle solutions have Cypridina luciferase-like activity, although the reaction rate and the quantum yield of luminescence are far low compared to the bioluminescence.

REFERENCES AND FOOTNOTES

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2. T. Goto, S. Inoue, and S. Sugiura, Tetrahedron Letters 3873 (1968).
3. T. Goto, Pure Appl. Chem. 17, 421 (1968).
4. T. Goto, S. Inoue, S. Sugiura, K. Nishikawa, M. Isobe, and Y. Abe, Tetrahedron Letters 4035 (1968).
5. The role of the enzyme would not only be to make the hydrophobic environment, but also positive abstraction of a proton from luciferin to produce luciferin anion, which initiates the reaction with oxygen (3).
6. F. H. Johnson, O. Shimomura, Y. Saiga, L. C. Gershman, G. T. Reynolds, and J. R. Waters, J. Cell. Comp. Physiol. 60, 85 (1962).
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8. Luciferin analog (III) chemiluminesces in water also by oxidation with hydrogen peroxide and ferri- or ferro-cyanide, with t-butyl hydroperoxide and ferric ions, ferri- or ferro-cyanide, or with hydrogen peroxide and bromine.
9. FWHM = full band width between half-maximum intensity points of the spectrum. Accuracy will be ± 100 cm^{-1}.