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STUDIES ON THE BACTERIAL LUCIFERASE REACTION: ISOTOPE EFFECTS ON THE LIGHT EMISSION. IS A "CIEEL" MECHANISM INVOLVED?

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Introduction

Bacterial luciferase, an FMN dependent enzyme catalyses the conversion of a long chain aldehyde to the corresponding fatty acid, with the concomitant emission of light. In the last years progress was achieved in the elucidation of the mode of activation of O_2, which involves formation of a flavin C(4a)-hydroperoxide (1,2). In a parallel presentation (3) it is shown, that emission of light goes along with formation of a flavin C(4a)-hydroxide, the latter is proposed to be the primary emitting chromophore. Several important questions still await elucidation: What is the mechanism of oxidation of the aldehyde? What is the mechanism of population of the excited emitter chromophore, i.e. how is the energy resulting from oxidation of the aldehyde converted into excitation?

Results and Discussion

Studies with deuterated aldehydes

We have reinvestigated the effects of 1-deuterated aldehydes on the rate of the luciferase reaction. Contrary to previous results (4), we have found nearly identical values for the isotope effects with octanal, decanal and dodecanal (Table 1). Very littel isotope effect ($\sim$5 %) was found on the quantum yield using either 1-protio or 1-deutero aldehydes. Similar to the

earlier report, the onset of light in the bioluminescence reaction is distinctly biphasic, with a rapid rise at the onset followed by a slower increase (Fig. 1); the rapid onset is strongly dependent on the concentration of aldehyde.

Table 1: Rates and isotope effects in the bioluminescence reaction

Aldehyde	k_{decay} (s^{-1})	k_{rise} (s^{-1})	$\frac{k_{decay}(H)}{k_{decay}(^2H)}$	$\frac{k_{rise}(H)}{k_{rise}(^2H)}$
Octanal	0.00146	n.d.	1.45	n.d.
(1-^{2}H)-Octanal	0.0010	n.d.		
Decanal	0.010	0.143	1.5	1.5
(1-^{2}H)-Decanal	0.0067	0.095		
Dodecanal	0.0015	0.037	1.55	1.4
(1-^{2}H)-Dodecanal	0.00097	0.026		

The rates were obtained using purified luciferase flavin-hydroperoxide (1). The values for k_{rise} and k_{decay} were obtained at 2° and at -4° respectively under the conditions described in the Legend of Fig. 1.

Schuster (5) has proposed a CIEEL (Chemically Induced Electron Exchange Luminescence) mechanism for the chemical generation of excited states. Such a mechanism can be formulated (Scheme 1, see also Mager and Addink (6)) for the breakdown of the proposed flavin peroxyhemiacetal intermediate to yield an electronically excited product upon electron transfer back to the flavin. According to Schuster one can expect a dependence of the rate of light production and decay on the redox potential of an activator, which in this case would be the reduced 4a,5-dihydroflavin hydroperoxide. To test this we have investigated the rates of decay of the bioluminescence as related to the redox potential of different FMN analogues used in the reaction. Fig. 2 shows that a correlation does exist and that, as required by the CIEEL theory, with low potential flavins a higher rate of the process is observed. In the case of bacterial luciferase, mechanisms can be formulated in which the 4a,5-dihydro-flavin nucleus acts as an activator thus initiating the reaction. If, alternatively, the bioluminescence reaction is governed primarily by a Bayer-Villiger type mechanism (7),

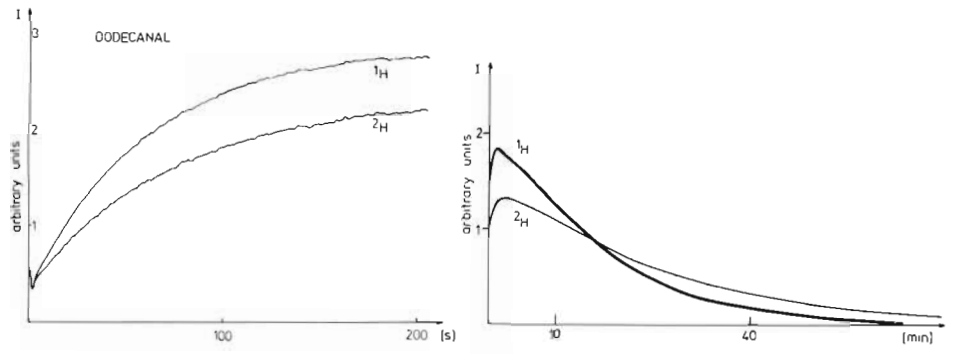


Fig. 1) Course of luminescence rise (left hand side), and of luminescence decay (right hand side) observed upon reaction of luciferase flavin-C(4a)-hydroperoxide with 1(^{1}H)- or with 1(^{2}H)-dodecanal

Sephadex purified luciferase hydroperoxide (1) in 0.01 M phosphate buffer, pH 7.0, and 0.35 M NaCl was reacted at 2° in the stopped-flow spectrophotometer with dodecanal (left). The fluorescence decay (right) was measured in a conventional cuvet at -4° under the same conditions.

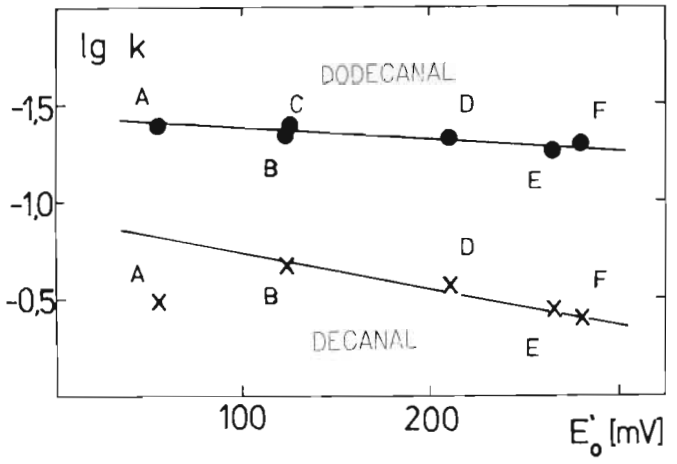
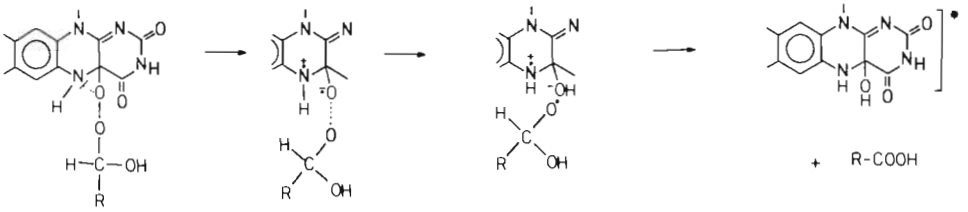


Fig. 2) Dependence of Luminescence decay rate from the FMN redox potential

The luminescence emission was measured in a dithionite assay (8) at aldehyde final concentrations of 10^{-5} M, and with 6.7×10^{-7} M luciferase. The data shown are the average of at least 5 measurements. The FMN derivatives used were (redox potential in V): A) 4-Thio-FMN (-0.055); B) 2-Thio-FMN (-0.122); C) 7,8-Dichloro-FMN (-0.125); D) FMN (-0.210); E) 6-OH-FMN (-0.265); and F) 1-Deaza-FMN (-0.280)

Scheme 1) CIEEL Mechanism for the bacterial luciferase reaction



then a reverse dependence of the reaction rate from the flavin redox potential might be expected (flavins with high redox potential should yield "stronger" peroxides and with them aldehyde oxidation might be expected to be faster).

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