Some investigations on the chemical origin of the multicolor firefly bioluminescence

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Fireflies naturally emit multicolor light from green (≈530 nm) to orange, and even to red (≈635 nm). In order to explain the variation in the color of the bioluminescence, many hypotheses have been proposed to date. However, there is still no consensus on which hypothesis best describes the mechanism behind the multicolor bioluminescence. The relationship between the wide range of bioluminescent colors and the structure of the light emitter remain challenging problems. Experimental studies of the light emitters are hindered due to the extreme instability of $OxyLH_2$. Thus theoretical predictions preferably with *ab initio* methods are advantageous. In this section it is reviewed that all available theoretical data of us are used to study aspects of the six hypotheses regarding the $OxyLH_2$-based light emitters from fireflies.

Light emitting system in a deep sea shark: *Etmopterus spinax* (Squaloidea: Etmopteridae)

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The biochemistry of light emitting systems has been largely studied in invertebrates. Among vertebrates, only fishes are endowed with this capability and the mechanism of light emission has merely been investigated in bony fishes. Although less known, because rather rare and difficult to observe, cartilaginous fishes also contain bioluminescent members. Two families are in concern, Dalatiidae and Etmopteridae[1], information about the biochemistry of their luminous system are lacking.

In this work, we aim to describe for the very first time the chemiluminescent reaction involved in a shark species: *Etmopterus spinax* (Etmopteridae). *E. spinax* is a deep-sea species displaying a continuous blue luminescence on its ventral and lateral faces[2]. Classical cross-reactions with known luminous substrates, such as known imidazolopyrazines, do not produce light *per se*, suggesting a new luminous system in this species. Studies are now in progress to detect and purify the substrate of the reaction by biochemical methods.

This work was supported by an FRS-FNRS grant to M. Renwart. J. Mallefet is a researcher associate of the FRS-FNRS.

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Bioluminescent assays for monitoring of air pollution

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When compared with the other available tests measuring water toxicity the bioassay methods based on the luminescent bacteria, soluble and immobilized coupled system of NAD(P)H:FMN-oxidoreductase-luciferase (reagent "Enzimolyum") have certain advantages as for analysis speed, handling and cost. However the available bioluminescent methods were not yet applied to measure air pollution. So we aimed to determine the sensitivity of luminous bacteria and their enzymes to air samples differed by industrial pollution degree.

Air samples were collected in the clean (Akademgorodok, sample#1) and polluted (the coal power plant area, sample#2) districts of Krasnoyarsk city. The air samples were collected into liquid absorption medium (water, ethanol or acetone). The standard aspirating device performing 1.0 liter per minute was used. Chemical composition of the samples was analyzed with gaseous chromatograph (Agilent Technologies 7890A). To compare the sensitivity of assays the numbers of dilution of the samples necessary to remove toxic effect were considered.

Results are presented in the table 1.

The results indicate that water is the better than ethanol or acetone medium for air sample preparation because of its sufficient capacity to absorb organic compounds, absence of interfering effects on bioluminescent. The sensitivity of soluble and immobilized enzymes is 3–24 times higher than sensitivity of bacterial-based test. The immobilized reagent provides the reduction of the time required to complete the analysis (down to 7 minutes), easy-to-use, higher sensitivity (allowed dilutions is up to 16000), possibility to increase the volume of the sample up to 97% of the total one. Thus we showed the possibility to apply the bioluminescent bioassays based on immobilized reagent "Enzimolyum" for air pollution monitoring.

This work was supported by the Federal agency of science and innovations (contract No 02.740.11.0766), the Program of the Government of Russian Federation "Measures to Attract Leading Scientists to Russian Educational Institutions" (grant No 11. G34.31.058).

Table 1. Comparative characteristics of bioluminescent assays used to monitor air pollution

Type of bioassay		Soluble coupled system	Luminescent bacteria	Immobilized coupled system (Enzimolyum)
Number of components (simplicity)		5	2	3
Duration of assay, min		10	5–30	7
Sensitivity (number of dilutions), sample#1 / sample#2	Water	2000 / 3	700 / 3	16000 / 3
	Ethanol	700 / 1	1 / 1	250 / 3
	Acetone	2000 / 3	1 / 1	>2000 / 3
Storage conditions		2 months at +15 °C, 3 years at –18 °C	6 months at +5 °C, 1 year at –18 °C	2 years at +4 – +25 °C