LUMINESCENCE DETECTION OF BIOLOGICAL REACTIONS

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Abstract A highly sensitive and specific fiberoptic biosensor based on bioluminescence or chemiluminescence reactions has been developed in our group. The sensor is made of a fiber optic bundle associated with a polyamide enzyme membrane connected to a luminometer, enabling the emitted light to be processed. Contrary to other optical devices, this one does not require a light source or monochromators, making the system simple and attractive for remote analysis. It can be associated with flow injection analysis and allows unpretreated samples to be assayed. ATP and NAD(P)H could be easily determined at the picomole level using the bioluminescent enzyme systems from the firefly and from marine bacteria, respectively. Enzyme activities, especially dehydrogenases, could also be monitored with the same device. A reagentless design, involving a loosely embedded co-reactant continuously released in the bound enzyme microenvironment, enabled 30-35 assays to be performed with the same loading.

INTRODUCTION

Since the discovery by the end of the nineteenth century in Lyon by the French physiologist Raphaël Dubois that it was possible to produce light by mixing two compounds extracted from a light emitting beetle, bioluminescence reactions were found to occur in numerous species especially from marine origin. In the last decades the related enzyme reactions involving luciferases were extensively studied, especially in the group of Mac Elroy\textsuperscript{1} enabling an ultrasensitive test for ATP to be widely available in biochemistry laboratories.

An increasing interest appeared for optical transduction in recent years but little attention was devoted to bio- or chemiluminescence\textsuperscript{2,5}! Our main goal was to develop a fiberoptic biosensor involving light emitting reactions with different types of luciferases. Two systems were selected: the first one was the monoenzyme system from the firefly \textit{Photinus pyralis} specific for ATP and the second one was the bienzyme system found in marine bacteria specific for NAD(P)H.

Luciferase (EC 1.13.12.7) from the firefly \textit{Photinus pyralis} catalyzes the reaction:
firefly luciferase / Mg$^{2+}$

\[
\text{ATP} + \text{LH}_2 + \text{O}_2 \longrightarrow \text{AMP} + \text{PPi} + \text{Oxyluciferin} + \text{CO}_2 + \text{hv}
\]

The presence of luciferin LH$_2$ and Mg$^{2+}$, which serve as co-reactants, and of molecular oxygen enables the enzymic reaction to take place and the light emission to occur. The intensity of emitted light ($\lambda_{\text{max}} = 560$ nm) is proportional to the ATP concentration in a wide linear dynamic range.

For NAD(P)H monitoring, the enzymatic systems from *Vibrio harveyi* and *Vibrio fischeri* were used. In this case, two consecutive reactions take place involving the specific enzymes NAD(P)H : FMN oxidoreductase (EC 1.6.8.1) and luciferase (EC 1.14.14.3). The light emission intensity at $\lambda_{\text{max}} = 490$ nm is directly related to the NAD(P)H concentration.

oxidoreductase

\[
\text{NAD(P)H} + \text{FMN} \longrightarrow \text{NAD(P)}^+ + \text{FMNH}_2
\]

NAD(P)H, which is the target analyte, is oxidized, whereas the coenzyme FMN is reduced into FMNH$_2$ which is a substrate for luciferase.

*bacterial luciferase*

\[
\text{FMNH}_2 + \text{R-CHO} + \text{O}_2 \longrightarrow \text{FMN} + \text{R-COOH} + \text{H}_2\text{O} + \text{hv}
\]

This second enzyme requires both molecular oxygen and a long chain aldehyde, generally decanal.

II DESIGN AND PERFORMANCE OF THE LUCIFERASE-BIOSENSOR

The principle is shown in Fig. 1. The bioactive membrane with the selected enzyme system for the light emitting enzyme reaction (LER) is associated with the waveguide, here a fiberoptic bundle connected to the photomultiplier of a luminometer used for signal processing. The bioactive membrane was prepared through direct enzyme covalent immobilization, using preactivated polyamide membranes from Pall Co., USA. In brief, simply wetting the membrane with a few microliters of concentrated enzyme solution enables any bioactive membrane to be prepared in a few minutes.