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A chemiluminescent Langmuir–Blodgett membrane as the sensing layer for the reagentless monitoring of an immobilized enzyme activity

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ABSTRACT

In the nanotechnology field, the concept of using biomolecules as an elementary structure to develop self-assembled entities has received considerable attention. Particularly, the ability of amphiphilic molecules like lipids to self-organize into bilayers can be exploited to provide biomimetic membrane models. Langmuir–Blodgett (LB) technology, based on the transfer of an interfacial film onto a solid support, offers the possibility to prepare lipid bilayers suitable for biomolecule immobilization and achievement of nanoscale-organized sensing layers, tailored for the design of miniaturized biosensors. With the aim of immobilizing enzymes in a defined orientation at the surface of LB bilayers, an original strategy has been previously developed in our group. This approach combines two techniques based on molecular self-assembly properties: liposome fusion at an air/buffer interface and Langmuir–Blodgett technology. It allows the functional insertion of a non-inhibitory antibody in lipid bilayers, further used to anchor a soluble enzyme at the surface of the lipid membrane. When associated with an electrochemiluminescent (ECL) sensor, this molecular assembly allows the design of a biomimetic sensor able to closely integrate the recognition and transduction events. However, sensor's performance not only depends on bioactive sensing layer properties, but also on the additional introduction of luminol in the reaction medium which delays ECL reaction. This work explores the potentiality of two neosynthesized amphiphilic luminol derivatives to form a lipid bilayer serving as a matrix used for both antibody insertion and ECL detection in order to develop a new sensing layer allowing a reagentless detection. As a model, choline oxidase activity has been detected. After enzyme immobilization at the surface of the luminol derivative LB bilayer by the way of specific recognition of a non-inhibitory antibody, *in situ* catalytic generation of hydrogen peroxide is able to trigger ECL reaction in the sensing layer interfaced with an optoelectronic device leading to a reagentless detection of choline oxidase activity.

1. Introduction

Biological membranes play a central role in the cell life. Such highly organized supramolecular structures are a key component of the way living cells are able to maintain and organize their function. Unlocking the secrets of those membranes provides important lessons that are valuable in guiding the construction of devices to be used for nanotechnological applications [1]. In this context, the direct bioelectronic interface between nanostructures mimicking cell membranes and electronic devices lies at the heart of nanobioscience, offering a direct way of following biocatalytic reactions by studying a very limited number of molecules.

Natural membrane organization builds upon the self-association properties of biological macromolecules. Using such properties, biomimetic membranes can be reconstituted *in vitro*. The self-assembly ability of amphiphilic biomolecules like lipids, to spontaneously organize into nanostructures mimicking living cell membranes, has emerged as a suitable concept for the development of biomimetic membrane models [2].

Langmuir–Blodgett (LB) technology allows building up bilayer stacks as membrane leaflet, by transferring a monomolecular lipid film formed at an air/water interface onto a flat solid support, with an accurate control of the thickness and of the molecular organization [3–6]. Based on the self-assembly properties of amphiphilic biomolecules at the air/water interface, this technique offers the possibility to prepare lipid bilayers, as supports suitable for immobilization of biomolecules [7–9] like enzymes [10–19]. As recently

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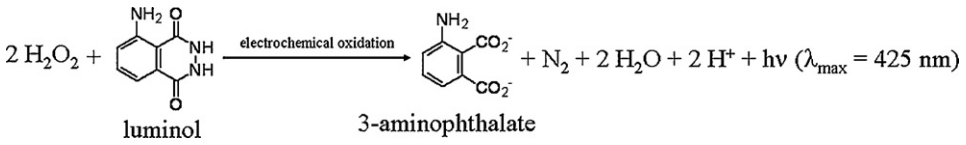


Scheme 1. Luminol electrochemiluminescence (ECL) reaction in the presence of hydrogen peroxide (H_2O_2).

reviewed [20], several papers report on the association of proteins with lipid LB films.

With the aim of immobilizing enzymes in a defined orientation, we previously designed an organized proteo-lipidic LB membrane by inserting a non-inhibitory monoclonal immunoglobulin G (IgG) directed against the soluble monomer of acetylcholinesterase in the lipid bilayer, able to bind the enzyme in a functional orientation [21]. The structural stability allowed to detect the enzyme activity over a long period [22]. The enzyme kinetics obtained with such a biomimetic membrane perfectly fitted with heterogeneous biocatalytic behaviour representative of cellular enzymatic catalysis [21].

Besides, the detection of hydrogen peroxide by luminol electrochemiluminescence (ECL) measurements has proven to be efficient to develop optoelectronic sensors [23]. Luminol electrochemiluminescence reaction, described by Sakura [24], is presented in Scheme 1. The oxidation of luminol by application of an adequate potential triggers luminol electro-generated chemiluminescence with inherent high sensitivities and wide linear working ranges [25,26]. Based on this principle and in combination with appropriate enzymatic reactions, some ECL biosensors with low detection limits and wide linear detection ranges were reported for instance for choline measurements [26], which allowed the development of low-cost disposable or reusable optical biosensors involving oxidases [25]. Reagentless sensors have been developed by an original immobilization of luminol on the surface of a carbon screen-printed electrode. The polymeric form has been obtained after electropolymerization of luminol considered as an aniline-like monomer [27].

In order to achieve molecular recognition and signal transduction in a single step, the LB biomimetic membrane described above was directly interfaced with an efficient ECL choline biosensor [28]. Such an association leading to the intimate contact between the biomimetic structure and the biochemical signal transducer, gave direct access to the intrinsic enzyme behaviour [21]. However, the performance of such sensors will mostly depend on both the bioactive sensing layer properties and the additional introduction of soluble luminol to trigger ECL reaction [28], resulting in a delay for diffusion of this reactant through the lipid bilayer. The possibility to insert an amphiphilic luminol derivative directly into the lipid bilayer and usable for ECL detection could give the opportunity to develop reagentless nanosensors.

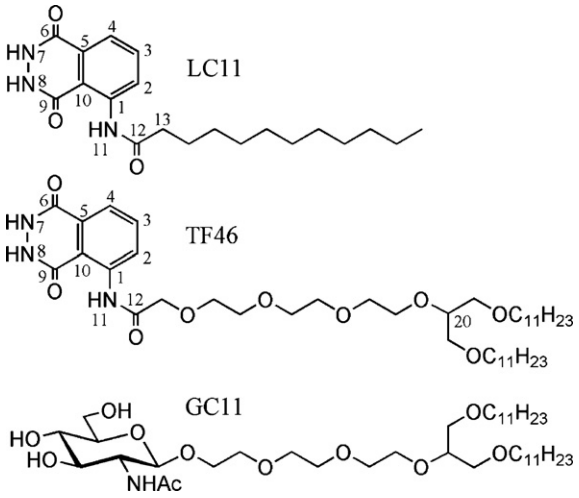
For this purpose, two amphiphilic luminol derivatives were synthesized in our group (Scheme 2). Their potential for ECL measurement has been demonstrated first [29]. In the meantime, their interfacial behaviour and the morphologies of pure or mixed monolayers with the same neoglycolipid (glycolipid GC11) used to develop the previous biomimetic membrane [21] were investigated by surface pressure-molecular area isotherms and Brewster angle microscopy [30].

In this work, we have explored the potentiality of these luminol derivatives to form a LB lipid bilayer serving as a matrix for both the insertion of a non-inhibitory antibody (IgG) specific to the C-terminal extremity of choline oxidase (ChOD) and ECL detection.

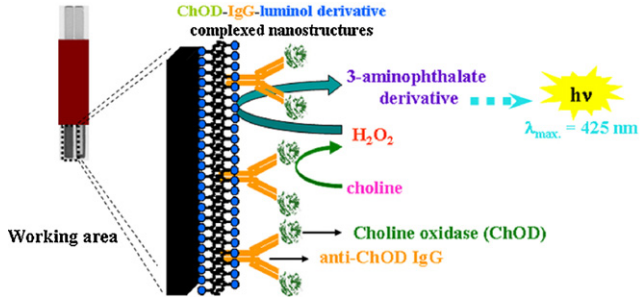
ChOD catalyzes the following reaction:

$$\text{choline} + H_2O + 2O_2 \xrightarrow{\text{ChOD}} \text{betaine} + 2H_2O_2$$

After oriented immobilization of ChOD at the surface of the luminol derivative LB bilayer, the enzyme activity has been detected by



Scheme 2. Chemical structures of amphiphilic luminol derivatives (LC11, TF46) and glycolipid (GC11).



Scheme 3. Reagentless detection principle of choline oxidase activity by ECL reaction triggered directly in the sensing layer.

electrochemiluminescence. This was made possible by the enzymatically generated H_2O_2, which directly triggers the ECL reaction in the sensing layer interfaced with a potentiostatically controlled optoelectronic device (Scheme 3).

2. Experimental

2.1. Materials

Luminol (3-aminophthalhydrazide), choline chloride, choline oxidase (from *Arthrobacter globiformis*), bovine serum albumin (BSA, fraction V) and hydrogen peroxide were purchased from Sigma–Aldrich Chimie (St Quentin Fallavier, France) and used without further purification. Anti-ChOD immunoglobulin G (IgG) directed against the C-terminal extremity of choline oxidase was produced by Covalab (Villeurbanne, France). Solvents such as pyridine, dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were purchased from Acros Chemicals (Geel, Belgium) and distilled before use. Glycolipid, 10-undecyloxymethyl-3,6,9,12-tetraoxatricosyl 2-acetamido-2-deoxy-β-D-glucopyranoside (GC11) was synthesized as previously reported [31]. Two luminol derivatives, *N*-(1,4-