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Electrochemiluminescent detection of acetylcholine using acetylcholinesterase immobilized in a biomimetic Langmuir–Blodgett nanostructure

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Abstract

The performances of bioelectronic devices mainly depend on the properties of the bioactive sensing layer and on the quality of its association to the transducer. The achievement of new biospecific organised ultrathin films, directly interfaced with the transducer and inserting biomolecules in a functional and orientated position, may open an original way in the development of new biomimetic optoelectronic devices. This study deals with the possibility to detect acetylcholine using acetylcholinesterase immobilized at the surface of a miniaturised biomimetic nanostructure by means of an electrochemiluminescent device. The highly organised proteo-lipidic nanostructure has been designed using interfacial liposome fusion and Langmuir–Blodgett techniques. By inserting a non-inhibitor monoclonal antibody in a functional position, this nanostructure is able to sequester acetylcholinesterase (AChE) in a suitable orientation and to maintain the enzyme activity for several months. This molecular assembly has been intimately associated with a performant optical screen-printed choline sensor based on luminol electrochemiluminescence. The linear range for acetylcholine extends over more than two decades, with a detection limit of 4×10^{-7} M.

Keywords: Langmuir–Blodgett films; Monoclonal antibody; Acetylcholinesterase; Protein orientation; Electrochemiluminescence detection; Biooptoelectronic device

1. Introduction

Trends of the ongoing research in nanoscience and nanobiotechnology fields concern, among others, the controlled elaboration of nanoscale systems with the final aim to be able to detect single molecules or aggregate of molecules. In this way, bioinspired systems, like nanostructures mimicking cell membranes, constitute an outstanding model to devise 'intelligent structures' that will be integrated to new bioelectronic devices. Hence, the concept of using biomolecules as elementary structures to develop self-assembled superstructures of predefined geometry receives considerable attention. New and/or improved properties could be achieved through the control of matter, molecule-by-molecule, nanostructure-by-nanostructure. Notably, the ability of amphiphilic biomolecules such as lipids, to self-assemble into lipidic structures mimicking the cell membranes, appears suitable for designing biomimetic membrane models after association of bio-sensitive elements.

In this context, the development of functional proteo-lipidic nanostructures corresponding to highly organised molecular assemblies with orientated biological recognition

Abbreviations: AChE, acetylcholinesterase; a.u., arbitrary unit; ChOD, choline oxidase; DEAE, diethyl aminoethyl; LB, Langmuir–Blodgett; ECL, electrochemiluminescence; PVA-SbQ, poly(vinyl alcohol) bearing styrylpyridinium groups; PBS, phosphate buffered-saline

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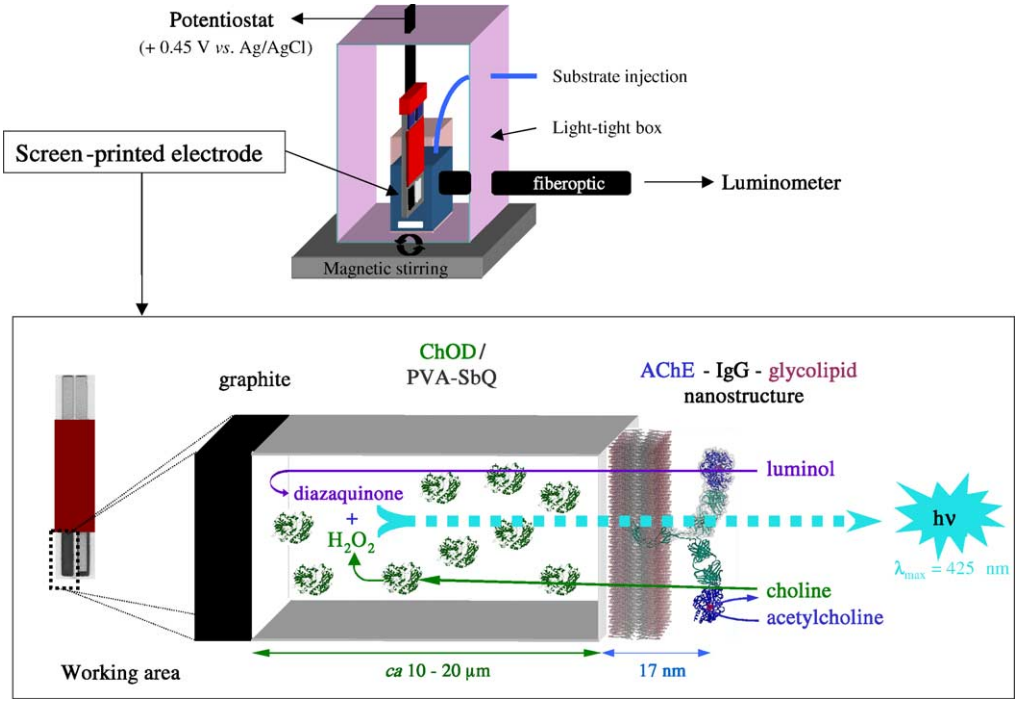

Fig. 1. Schematic representation of the biomimetic optoelectronic device for ECL acetylcholine detection and of sequential reactions occuring at the surface of the screen-printed electrode. The graphite working electrode is coated with a ChOD/PVA-SbQ membrane, covered by the AChE–IgG-glycolipid ternary nanostructure. The potential poised at +0.45 V vs. printed Ag/AgCl reference electrode allows to trigger luminol ECL.

sites is of great interest. Associated with microelectronic and optoelectronic devices, they should lead to the design of new bioelectronic hybrids whose performance mainly depend on the bioactive sensing layer properties. The achievement of new biospecific membranes as organised ultrathin films at the nanoscale level, directly interfaced with the transducer and inserting biomolecules in a functionalised and orientated position, must open a new way in the development of miniaturised bioelectronic devices and other nanoanalytical tools.

This study reports on the association of a biomimetic proteo-lipidic nanostructure, specially designed to retain acetylcholinesterase monomers (AChE) in an orientated position [1], with a performant electrochemiluminescent (ECL) device [2] (Fig. 1). The aim is to achieve a new biomimetic optoelectronic device devoted to acetylcholine detection by the way of luminol electrochemiluminescence, according to the following sequential reactions:

$$\text{acetylcholine} + H_2O \xrightarrow{\text{AChE}} \text{acetate} + \text{choline} \quad (1)$$

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

$$\text{luminol} + 2H_2O_2 \xrightarrow[+0.45\,\text{V versus Ag/AgCl}]{\text{graphite}} \text{3-aminophtalate} + N_2 + 3H_2O + h\nu(\lambda_{max} = 425\,\text{nm}) \quad (3)$$

AChE catalyses the production of choline from acetylcholine (Eq. (1)); choline is oxidised in betaine and hydrogen peroxide by choline oxidase (ChOD) in the presence of oxygen (Eq. (2)); hydrogen peroxide is finally involved in the ECL reaction of luminol and gives rise to a light signal (Eq. (3)) proportional to choline and to acetylcholine concentrations in definite ranges.

The highly organised proteo-lipidic nanostructure retaining AChE has been obtained using an adapted combination of liposome fusion at an air–buffer interface and Langmuir–Blodgett (LB) technologies [3]. Langmuir–Blodgett (LB) technique allows to build up lamellar lipid stacking by transferring a monomolecular film formed at an air–water interface onto a solid support, with an accurate control of the thickness and of the molecular organisation. Based on the self-assembly properties of amphiphilic biomolecules at the air–water interface, this technique offers the possibility to prepare ultrathin layers suitable for biomolecule immobilisation. In our adapted procedure, the mixed proteo-lipidic interfacial film is formed by interfacial disruption of proteo-lipidic liposomes [4]. Using this approach, we have succeeded in inserting a non-inhibitor monoclonal antibody (directed against the hydrophilic AChE monomer) into a glycolipidic matrix. After transfer of the mixed monolayer, we have previously demonstrated that the antibody, adopting a preferential functional position in a bilayer structure, is able to sequester AChE in an orientated position at the surface of the lipidic membrane. The enzyme activity retained on the AChE–IgG-glycolipid molecular assembly is stable for several months [1].