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Advances in lipid film based biosensors

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ABSTRACT

Since first report on formation stable free standing bilayer lipid membranes (BLM) in 1962 by Mueller et al. [1] these systems became very popular in modeling various membrane processes at molecular level, such as ionic transport, ligand-receptor interactions or lipid-protein interactions. Despite of advantage of BLM in respect of variation in lipid composition and their modifications by receptors and proteins, the work with these systems was rather difficult due to their limited stability. The situation has been considerably improved by introduction of supported bilayer lipid membranes (sBLM) in 1980 by Thompson et al. [2] for possible applications in biosensors. Recent advances in stabilization of supported lipid membranes, appearance of novel nanomaterials such as carbon nanotubes has increased the number of publications on this topic. This review summarizes latest achievements in the field of biosensors utilizing sBLMs.

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1. Introduction

The biomembrane is the basic cell structure that serves not only as the barrier that separate intracellular and extracellular environment, but contain macromolecules that provide various function important for living organisms, such as transport of ions and other compounds, receptor functions, immunity response and others. The lipid environment protects the biopolymers against degradation. This extremely thin (approx. 5 nm) lipid matrix with incorporated receptors is unique biosensor developed by nature. Since discovery of model bilayer lipid membranes (BLM) by Mueller et al. [1], there have been attempts to use them in biosensing applications. However,

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these free standing BLM were fragile and not suitable for long-term use. Important step toward practical applications of lipid films took place in 1980 when Thompson et al. [2] introduced supported bilayer lipid membranes (sBLM) formed on a polyamide polymer substrate. sBLMs can be formed on various substrates, such as metals, polymers, microfiltration membranes, carbon nanotubes, graphen, porous silicon, etc. They can be modified by proteins or artificial receptors, enzymes, antibodies, channel formers and carriers that serve as signal transducers or receptors for detection of analyte. In contrast with BLM, sBLM are more stable and in certain cases can be stored at low temperature (4°C) for several days without lost of stability and functionality. In addition, many physical techniques can be applied for study the properties of sBLMs [3]. Recent advances in stabilization of lipid bilayer have resulted in preparation of sBLM based biosensors for detection of a variety of analytes. Lipid membranes represent an appropriate biocompatible structure for the development of new types of biosensors with fast response (on the order of a few seconds) and high sensitivity (i.e., nanomolar detection limits) that may be used in health diagnosis and in field applications for food analysis and environmental monitoring. These biosensors are cost efficient, easy-to-use and are good alternative to expensive and time consuming standard analytical methods (i.e., chromatography or mass spectroscopy).

This review describes methods of preparation of stabilized sBLMs and summarizes novel trends in biosensors based on supported lipid films. Recent achievements in nanotechnology offer the route to develop selective and sensitive biosensors based on sBLMs that are easy to prepare and can be used for detection various compounds important for health diagnosis, such as cholesterol, D-dimers, antibodies and for monitoring toxicants in foods and environment (i.e., carbofuran in fruits and vegetables [4], etc.).

2. Methods for preparation biosensors based on sBLMs

2.1. Free standing BLMs and supported BLMs

The structure of biomembrane is rather complex and involved lipid bilayer into which the integral proteins (for example glycophorin, Na,K ATPase, bacteriorhodopsin etc.) are incorporated. The peripheral proteins, such as cytochrome c (cyt c) are localized at the membrane surface (Fig. 1). Red blood cells contain also spectrin net at the surface of cytoplasmic side that plays important role in maintaining the cell shape. Outer part of the membrane contains glycocalix, that is composed of sugars covalently connected with

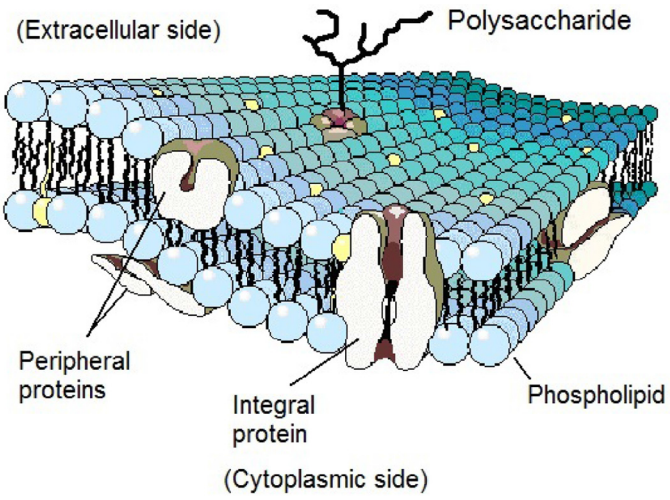

Fig. 1. The model of the structure of biomembrane [3].

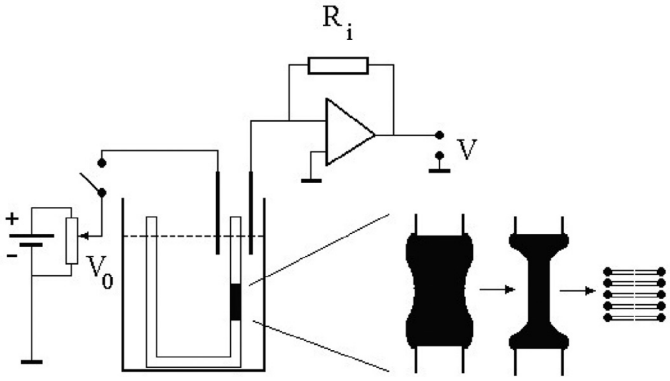

Fig. 2. The scheme of formation of BLM and the scheme of measurement current across BLM using current to voltage converter [3].

some lipids (glycolipids) and proteins (glycoproteins) [3]. The biomembrane is not only the barrier that separates the extracellular space from intracellular part of the cell, but plays several recognition, catalytic and signaling functions. Thanks to receptors incorporated into the lipid matrix, the cell can recognize physical and chemical signals, such as temperature, mechanical stress, presence of hormones, etc. Thus, it is unique sensing device developed by nature. It has been therefore attempts to mimics the biomembrane structure and properties in order to study membrane phenomena at molecular level and for biosensor development. The first successful attempt of formation stable bilayer lipid membrane (BLM) has been reported by Mueller et al. [1] in 1962. Due to amphiphilic nature of phospholipids, they spontaneously form the lipid bilayers in a water phase. In experiments by Mueller et al. the BLM has been formed from crude fraction of phospholipids in a circular hole of a relatively small diameter (0.8 mm) in a Teflon cup immersed in larger glass compartment filed by electrolyte (Fig. 2). Small amount of the phospholipids dissolved in hydrocarbon solvent (e.g. n-heptane or n-decane) can be placed to the hole using simple brush or Pasteur pipette.

Immersion of the drop of phospholipid to a hole resulted formation of relatively thick lipid film with a thickness >100 nm. The behavior of this thick film is determined by differences in hydrostatic pressures in its peripheral part (Plateau-Gibbs border) and in a central part that is relatively flat. According to Laplace law the differences in the hydrodynamic pressure between inner and outer phases is determined by equation:

$$\Delta p = \gamma(1/R_1 + 1/R_2) \quad (1)$$

where R_1 and R_2 are inner and outer radius of the surface curvature and γ is surface tension. In a central partof the membrane the radius of curvature is close to the infinity, i.e. $R_1 = R_2 \rightarrow \infty$. Therefore the pressure differences is close to the zero: $\Delta p = 0$. However, the pressure at the central part of Plateau – Gibbs border is lower then that in a water phase, i.e. $\Delta p < 0$. Therefore the solvent will move from thin-flat part of the BLM to the Plateau-Gibbs border. This will cause further thinning of the membrane (Fig. 2). This process can be observed also visually in reflected light. Thick films are colored, like oil films on a water surface. As soon as the films become thinner the black spots start to appear. From observation of thin films formation follows that the black spots forms non uniformly and not symmetrically. As soon as the films became thinner also the van der Waal's forces start to play considerable role in film formation. The resulted BLM is characterized by bilayer part surrounded by Plateau-Gibbs border (Fig. 2). The formation of BLM can be easily detected by measurement electrical properties of the film – conductance and capacitance. Since the first report on BLM formation the number