IMMOBILIZATION OF BIOLOGICAL SPECIES ON BILAYER LIPID MEMBRANES

D. P. NIKOLELIS1 AND U. J. KRULL2
1 Department of Chemistry, University of Athens, Panepistimiopolis-Kouponia, 15771-Athens, GREECE
2 Department of Chemistry, Erindale Campus, University of Toronto, 3359 Mississauga Road North, Mississauga, ON L5L 1C6, CANADA

Abstract. This work reports how bilayer lipid membranes (BLMs) can be used as the host matrices for the immobilization of hydrolytic enzymes and antibodies and how can be designed to provide maximized transduction of the activity of these biological species. A transient current signal is obtained as a result of the selective membrane incorporated protein/stimulant (analyte) interaction. The time appearance or magnitude of the transient charging signal is related to the concentration of substrate or antigen, respectively. The mechanism of signal generation is explored in the present paper by being related to rapid reorganization of the double layer and BLM structure.

I. INTRODUCTION

Natural chemoreception offers a glimpse of a practical system of biosensors. Chemoreceptive membranes consist of a bimolecular layer of lipids, which are physically associated with proteins that have molecular recognition properties, i.e. receptors. The ion channel system that is found in biological organisms is associated with rapid, reversible, sensitive and selective chemical signalling, with unique features such as the amplification and transduction of chemical information into an electrical pulse by switching of channel conductivity.

The implementation of chemoreceptive processes for biosensor development based on the use of artificial lipid membranes has received significant attention. The preparation of planar bilayer lipid membranes (BLMs) was first reported three decades ago by Mueller et al. [1]; Del Castillo and coworkers [2] were the first to point out that BLMs could be used as the basis for the development of electrochemical biosensors. Recent reports about electrochemical biosensors based on BLMs demonstrate the potential of these promising devices for applications which make use of selective proteins that are not molecular receptors[3-5]. The essential idea is that a protein which can selectively bind to a specific organic or biochemical species can be
incorporated into an ordered lipid membrane assembly so that selective binding events can lead to changes in the structure or electrostatic fields of the membrane (transduction). Since an artificial BLM has a thickness of a few nm, the response time for chemical interactions can be on the order of seconds. External energy can be stored across a BLM as electrochemical potential; a single selective binding event can generate a discharge of this energy. This is an intrinsic amplification step which provides high sensitivity. Finally, BLMs provide a host matrix which is conducive to the maintenance of activity of many biochemically selective proteins.

We are interested in developing an analogue of the response of an ion-channel, where the signal would be modulated by the concerted action of selective binding interactions, and the method of transduction would be suitable for the use of a wide variety of different proteins without implementation of ion-channel proteins from natural sources. Our approach begins with the immobilization of active proteins directly onto a carefully defined lipid mixture at an air/electrolyte interface, followed by a film casting technique for BLM formation. Subsequent experiments have shown that selective chemical reactions can be transduced as time-dependent singular transient ion current signals. The electrochemical event is due to the rapid reorganization of the double layer and structure of a BLM, and provides the basis for the construction of switchable biosensors with rapid response times and reversibility.

II. MATERIALS AND METHODS

**Materials and Apparatus**

Egg phosphatidylcholine (PC) was used as the structural lipid agent for BLMs, and was used in mixture with dipalmitoyl phosphatidic acid (DPPA). Protein studies included the use of the enzymes acetylcholinesterase (AChE), urease, and penicillinase supplied from Sigma Chemical Co. (St. Louis, MO.), and antibody from rabbit antiserum to thyroxine (T4), which was supplied from UCB Bioproducts S. A. (Braine-l'Alleud, Belgium).

A Saran-Wrap™ film (10 µm thickness) with an aperture of 0.32 mm diameter was used to separate two identical plexiglass solution chambers (each with a volume of ca. 10 ml and an air/water interface of 3 cm²). The BLMs were located in the aperture and were supported in a 0.1 M KCl electrolyte solution. An external d.c. voltage of 25 mV was applied across the membrane between two Ag/AgCl electrodes. A digital electrometer (Model 614, Keithley Instruments, Cleveland, OH) was used as a current-to-voltage converter. The electrochemical cell and electronic equipment were isolated in a Faraday cage. All solutions were gently stirred and experiments were done at 25±1°C.