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EIS studies of valinomycin-mediated K^+ transport through supported lipid bilayers

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Abstract

Membranes capable of emulating biological systems were attached to gold electrodes via self-assembled monolayers (SAM) of mercaptopropionic acid molecules. The resulting supported bilayer lipid membranes (s-BLMs) doped with valinomycin were used to study the kinetics of K^+ transport by electrochemical impedance spectroscopy in a wide frequency range and for varying K^+-ion concentration in solution. Experimental data were modeled according to a set of fundamental microscopic equations describing the kinetic pathway for the K^+/valinomycin system. The experimentally observed dependences of the membrane conductance on concentrations of the transported ion and the ionophore confirm the theoretical propositions. Derived values for the rate constants of association and dissociation of the permeating valinomycin–K^+ complex agree with literature data.

Keywords: EIS; Ion transport; Lipid bilayers; Valinomycin

1. Introduction

Membranes supported on solid electrodes provide a natural environment for the study of biomolecules in a functionally active state. The experimental models of biomembranes are simple convenient systems that exhibit membrane mimetic behavior [1]. There is an increasing interest in the design of new stable lipid layers on solid supports for application in biosensors as well as for fundamental structural studies [2]. This research effort resulted in the development of supported lipid membranes (s-BLMs) having a number of advantages: (i) ease and reproducibility of preparation, (ii) long-term stability, (iii) formation in the absence of solvent, and finally (iv) formation on electrically conductive supports [3–5].

Classical examples of these systems are the self-assembled monolayers of alkanethiols (SAMs) covalently attached to gold surfaces, that provide a hydrophobic surface on top of which it is possible to deposit a phospholipid monolayer obtained from different deposition methods like Langmuir–Blodgett technique, painted method and vesicle fusion [6–8].

A second alternative is to replace the alkanethiol monolayer by a thiolipid monolayer and then to deposit a second phospholipid monolayer on top [2,9–11].

A different simple approach involves the non-covalent self-organization of a phospholipid monolayer on freshly formed surfaces of hydrophilic metals [12–14].

In this work, a self-assembled mercaptocarboxilic acid monolayer formed directly on gold surfaces was employed. This method provides an adequate substrate for the deposition, on top of this monolayer, of stable self-assembled lipid bilayers by phospholipid vesicle fusion.

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Mercaptopropionic acid was employed to obtain a negatively charged surface. At the pH of the experiments the carboxylic group is dissociated having a negative charge. Subsequent addition and fusion of lipid vesicles generates electrostatic fixation of the bilayer.

In this study ion transport across lipid membranes doped with the antibiotic valinomycin were examined using electrochemical impedance spectroscopy (EIS). As a cation carrier for potassium transport across cell membranes, valinomycin is of considerable biological importance [15]. It is a cyclodepsipeptide, cyclo(L-Val-D-Hyl-D-Val-L-Lac)$_3$ that contains a single 36-member ring. This structure confers on the carrier the ability to form lipid-soluble ionic complexes [16]. Comparable systems showing linear potentiometric responses toward potassium ion concentration were proposed as a novel kind of biosensor [17].

The main goal of this work is to demonstrate the applicability of EIS to provide quantitative data about the permeation process, in the form of electrical, chemical and physical parameters of this system, as previously informed for analogous ion-transport mechanisms [18,19]. With this purpose we attempt the derivation of a theoretical impedance transfer function that describes properly K^+ transport through supported membranes containing the carrier valinomycin.

2. Experimental

2.1. Chemicals

Egg yolk phosphatidylcholine (eggPC) and 3-mercaptopropionic acid (MPA) were purchased from Sigma. Dimethyldioctadecylammonium chloride (DODAC) and valinomycin were obtained from Fluka. Tris buffer, consisting of 10 mM Tris was adjusted to pH 7.4 by titration with HCl.

All aqueous solutions were prepared with Millipore water (specific resistance 18 MΩ cm^{-1}). All solutions were degassed with nitrogen before use.

2.2. Sample preparation

Polycrystalline gold electrodes (0.28 cm^2 apparent area) were subjected to the same pretreatment procedure before each experiment [6]. The exposed gold surface was mechanically ground down to a mirror finish with 1.0 and 0.3 μm alumina powder, rinsed in triply distilled water and finally subjected to a potentiodynamic cycling within the 0.4–1.6 V potential range (vs. SCE) at 0.1 V s^{-1}, in 0.5 M H_2SO_4 until a stable voltammetric profile was obtained.

Following the surface pretreatment the electrode was modified with a self-assembled monolayer of MPA that results covalently attached by immersion into 10 mM MPA ethanolic solution for 10 min and finally the electrode was rinsed thoroughly with the buffer solution. MPA was employed to obtain a negatively charged surface. When the carboxylic group is dissociated at the pH of the experiments it supports a negative charge. Subsequent addition and fusion of positively charged lipid vesicles generates electrostatic fixation of the bilayer.

2.3. Bilayer deposition by fusion of vesicles

Lipid vesicles were prepared by mechanical dispersion using a chloroform solution of a lipid mixture containing 80% EggPC and 20% positively charged DODAC. This last species confers positive charge to the vesicles. For this purpose thin lipid films were obtained by solvent evaporation under nitrogen stream to avoid phospholipid oxidation. The dry film was manually shaken in a buffer solution (10 mM Tris, pH 7.4) with added valinomycin dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 6 μM.

The bilayer was formed at room temperature by dipping the negatively charged electrode in the vesicle dispersion for 1 h and then it was rinsed again with the buffer to remove the remaining dispersion. For the experiments the buffer solution was replaced by 0.001 M, 0.005 M, 0.01 M, 0.05 M and 0.1 M KCl or NaCl solutions in Tris 10 mM buffer pH 7.4.

In order to control the deposition procedure each step was checked by EIS, measuring the respective capacitance values.

2.4. Electrochemical measurements

A conventional three-electrode cell, consisting of the modified Au electrode, a large-area Pt counter electrode, and a saturated Ag/AgCl reference electrode connected to the working volume with a Luggin capillary, was used for the electrochemical measurements. The cell was positioned in a grounded Faraday cage.

Impedance measurements were performed using a FRA Solartron SI 1254 device coupled to a PAR 273 potentiostat. Spectra were recorded over a frequency range of 65 kHz–3 mHz with a perturbation signal amplitude of 10 mV.

Data analysis was performed according to proper transfer function derivation and identification procedures by using complex non-linear least squares (CNLS) fitting based on the Levenberg–Marquardt algorithm.

3. Theoretical treatment

Permeation through the membrane is preceded by the formation of a 1:1 valinomycin–K^+ complex at the interface, with the K^+ ion situated inside the ring-shaped valinomycin molecule. Although this complex is electrically charged, it crosses the membrane. At the opposite membrane surface dissociation of the complex is followed ion release into the aqueous phase [20].

The kinetics of K^+ transport across both membrane/aqueous solution interfaces can be described by finite interfacial reaction rate constants that are assumed to have