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Impedance Spectroscopy of Ion Channels in Tethered Lipid Bilayers*

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The efficient analysis of the function of ion channels is an important task in many different fields ranging from (bio)analytics and drug-screening to nanoelectronics. Here, the modulation of the channel activity of a synthetic ligand gated ion channel by a specific antibody is measured in tethered lipid bilayers by impedance spectroscopy. Tethered single lipid bilayers with exceptionally high electrical resistances suitable for the detection of a few channels are presented. [DOI: 10.1380/ejssnt.2005.203]

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I. INTRODUCTION

Ion channels are crucial components of all living cells where they control ion fluxes across the cell membrane. They play essential roles in cellular processes such as maintenance of the transmembrane potential difference, signal transduction and osmoregulation and are therefore directly or indirectly targeted by many clinically used drugs [1]. In addition to their biological importance, ion channels are particularly interesting in the field of electrical biosensors because they provide an intrinsic amplification of ligand binding. The large flux of ions (up to 10^8 ions per second) induced by a single molecular interaction is the basis of a highly sensitive detection of any ligand that modulates the ion channel activity.

Tethered lipid bilayers are ideally suited for reconstituting membrane proteins at surfaces, in a functionally and mechanically stable form, where they can be interrogated with several surface-sensitive techniques [2, 3]. The modulation of the electrical resistance of tethered lipid bilayers containing ion channels by a biological function, such as ligand binding, has been demonstrated by different groups [4–6].

In this paper we present an example of immunosensing provided by a synthetic ligand gated ion channel (SLIC) incorporated in tethered single lipid bilayers [7]. SLICs change their transmembrane ion conductance by selectively binding an analyte, here an antibody. We then report on the formation of tethered single lipid bilayers with exceptionally high electrical resistances [8]. These new tethered lipid bilayers allow the detection of a few channels and open the way to single-channel experiments on this highly stable and versatile platform.

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II. ION CHANNEL MEASUREMENTS IN TETHERED LIPID BILAYERS

A. Principles of tethered lipid bilayers

The design of a prototypical tethered lipid bilayer on a gold electrode which is suitable for measuring channel activity by impedance spectroscopy is illustrated in Fig. 1. Phospholipid bilayers chemically bound to the gold support are obtained by self-assembly of sulfur containing synthetic lipids. These thiolipids are phospholipids comprising at their polar head groups a hydrophilic spacer, which is terminated by a -SH group (Fig. 1) [2, 4, 9, 10]. The hydrated spacer decouples the lipid bilayer from the gold surface. The resulting aqueous phase between the electrode surface and the lipid bilayer has been designed to accommodate extracellular parts of transmembrane proteins. The bilayers can be tuned to an appropriate fluidity and composition. G protein coupled receptors and ion channels have been reconstituted in a functional active form into these gold supported lipid bilayers [5, 9, 11]. Several reviews report on the formation of tethered lipid bilayers and the reconstitution of transmembrane proteins [3, 12, 13].

On gold the following surface sensitive techniques can be used to characterize the proteolipid layer: (i) infrared spectroscopy provides details about the structural organization of the lipid and polypeptide components [14, 15], (ii) surface plasmon resonance delivers the surface coverage and the concentration of each component in the supported molecular layer [16], (iii) impedance spectroscopy reveals the degree of integrity of the bilayer.

B. Principles of impedance spectroscopy

In a typical impedance spectral measurement a small-amplitude sinusoidal voltage is applied between two electrodes at successive frequencies and the current response is measured. The frequency-dependent impedance Z is usually described by complex numbers, whose real and imaginary parts are derived from the currents in phase and 90° out of phase with the applied ac voltage, res-

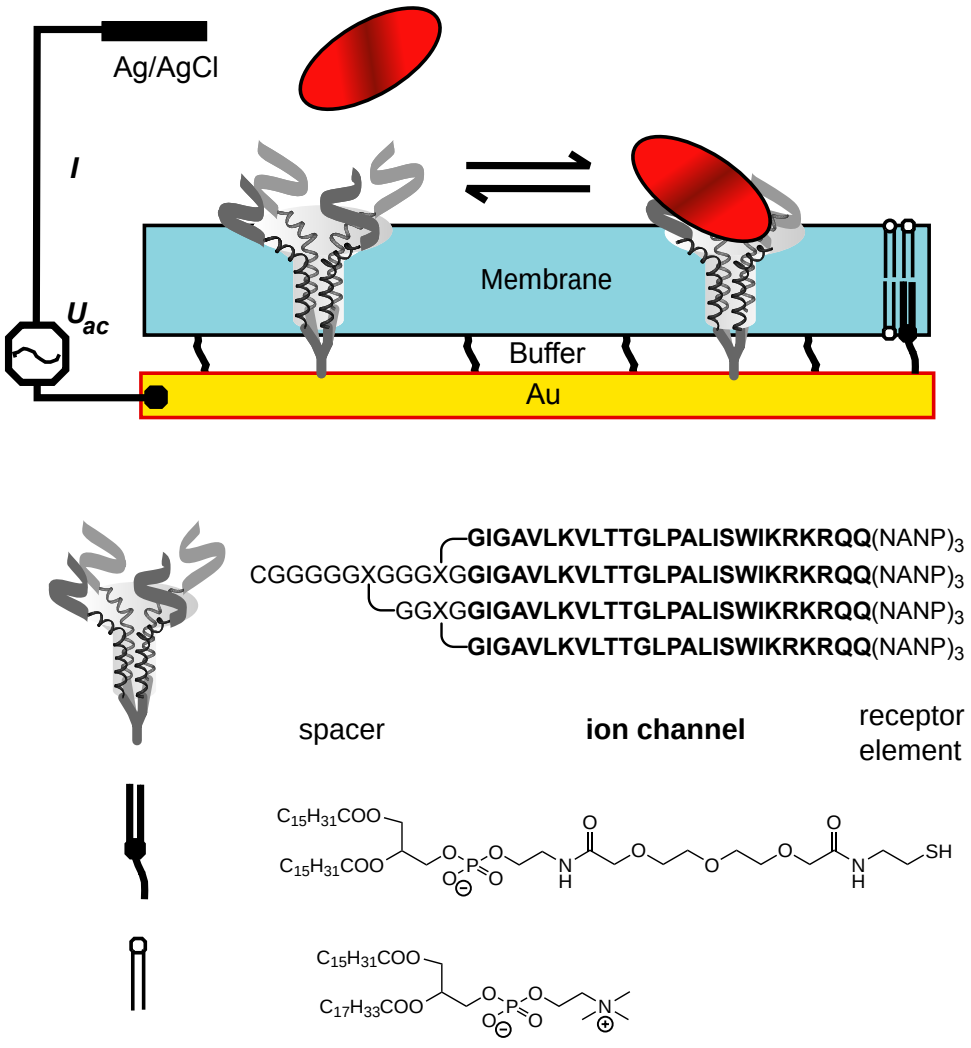

FIG. 1: Schematic view of a two-electrode electrochemical cell with an Ag/AgCl counter electrode and a gold working electrode coated with a SLIC-containing lipid bilayer, which is tethered to the gold electrode via synthetic thiolipids. The gating of an ion channel by the binding of an antibody molecule is schematically illustrated. U_{ac} and I are the applied ac voltage and the resulting current, respectively. The aminoacid sequence of SLIC in one-letter-code and the chemical structure of a thiolipid and the phospholipid POPC are shown below the schematic.

pectively [17, 18]. An impedance spectrum can often be fitted by an equivalent electrical circuit composed of resistors and capacitors. Tethered lipid bilayers are classicaly modelled by the equivalent circuit shown in Fig. 2A, where C_M and C_I describe the dielectric properties of the lipid membrane and the interfaces, respectively. The resistances R_M and R_S stand for the resistance of the lipid membrane and of the electrolyte solution, respectively. More complicated models consisting of a larger number of circuit elements or using non-ideal circuit elements such as the constant phase angle element have been proposed but will not be considered here [7]. There are hardly needed for layers with a resistance higher than 10^5 Ω [19]. Similarly, the very high charge transfer resistance measured at gold electrodes in the absence of faradic reactions has no marked influence on the impedance spectrum and can be omitted.

The membrane capacitance C_M describes the dielectric properties of the electrically insulating lipid layer sandwiched between two conducting phases, namely the electrode and the electrolyte. In practice, the layer capacitance is used to characterize the bilayer structure. A capacitance value of 0.6 ± 0.1 μF/cm^2 is found for the tethered bilayer, which is similar to values reported for unsupported membranes such as black lipid membranes and is indicative of a high degree of integrity [19]. The interfacial capacitance C_I is in the range of 10 μF/cm^2 and indicates hydration of the spacer region. The membrane resistance critically depends on electrically conducting pathways in the lipid layers. Its value is influenced by the ion channel to lipid ratio and varies typically between 10^3 to 10^5 Ω when measured at high ionic strengths, which is considerably lower than for black lipid membranes. The recent increase of the resistance of tethered lipid bilayers to values above 10^8 Ω will be discussed in Section IV. The modulation of the electrical resistance is used to detect the binding of an analyte that modulates the channel activity and to quantify its concentration in the aqueous

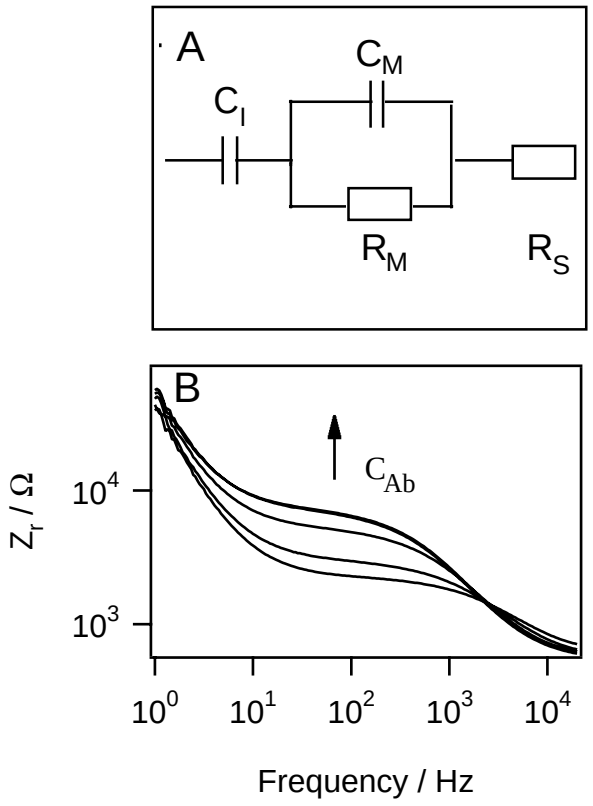

FIG. 2: (A) Equivalent circuit of a lipid bilayer tethered to a gold electrode. The lipid bilayer is described by a parallel combination of the resistance R_M and the capacitance C_M, while C_I and R_S represent the interfacial capacitance and the solution resistance, respectively. (B) Real part of the impedance spectra of a SLIC-containing lipid bilayer at various concentrations of antibody Sp3E9 (C_{Ab}, from bottom to top): 0, 10^9, 3×10^9, 10^8 and 3×10^8 M. (The curves for 10^{-8} M and 3×10^{-8} M overlap). The increase of the layer resistance as a function of the antibody concentration in the bulk solution is seen at frequencies between 10 Hz and 1 kHz. The conductive pathway can be closed reversibly and specifically by the antibody Sp3E9. Adapted from [7], with permission.

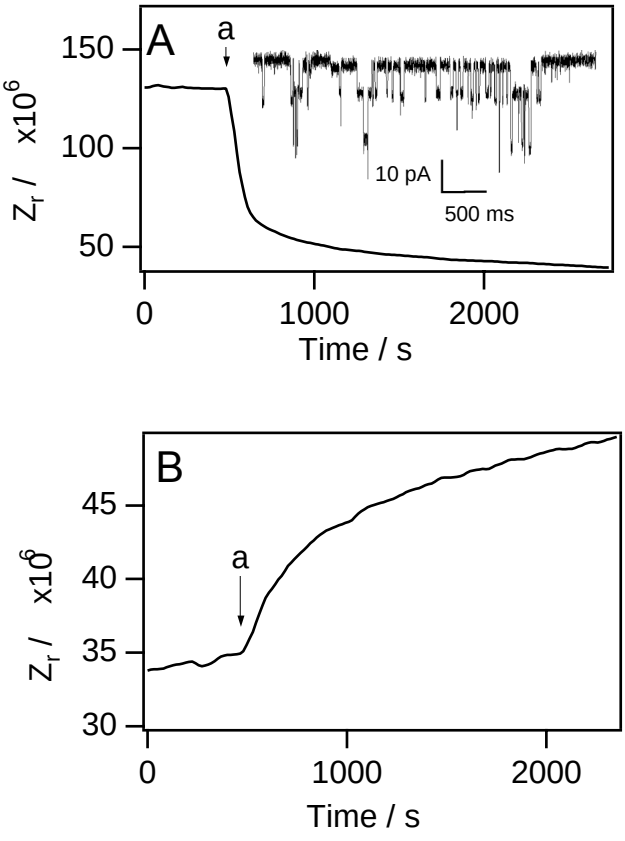

FIG. 3: (A) Real part of the electrical impedance (measured at 0.01 Hz) of a tethered single lipid bilayer after addition of 7×10^7 M (a) of SLIC to the aqueous phase. Single channel recordings on black lipid membranes were done at −30 mV in 1 M NaCl, 10 mM Tris-HCl, pH 7.4. The larger single channel events shown correspond to a conductance level of 90 pS at buffer conditions used for impedance spectroscopy. (B) Real part of the electrical impedance (measured at 0.01 Hz) of the tethered lipid bilayer containing SLIC after the addition (a) of 10^6 M of the antibody Sp3E9. Adapted from [8], with permission.

solution. Because the resistance is the parameter used for the presented ligand detection only the real part of the impedance is shown in this paper.

III. IMMUNOSENSING WITH SLIC

SLIC is a new synthetic ligand-gated ion channel made of several polypeptide chains [7]. Conceptually, a SLIC comprises a ligand-binding and a channel-forming region. As a ligand-binding region we have chosen a peptide, the repetitive NANP sequence which is recognized by the specific monoclonal antibody Sp3E9. The NANP sequence is the major B cell epitope of the circumsporozoite protein of *Plasmodium falciparum*, the cause of human malaria. The channel-forming part is built by 4 melittin peptide segments that are chemically attached to the branched spacer. The melittin sequence was chosen because of its well-known channel-forming properties. The sulfur-bearing peptide spacer has been designed to attach the molecule to a gold electrode and to present the 4 transmembrane helices symmetrically. A synthetic thiolipid containing a sulfur-terminated spacer of similar length as that of the SLIC and the phospholipid POPC were added sequentially to build a lipid bilayer tethered to a gold electrode. (Fig. 1). The proteolipid layer was characterized at every stage of formation by infrared spectroscopy, surface plasmon resonance and impedance spectroscopy [7].

The real part of the impedance spectrum of an ion channel-containing tethered lipid bilayer is shown in the Fig. 2B, where the membrane resistance can be measured from the plateau in the frequency range between 10 Hz to 1 kHz [7]. Here, the important finding is the increase of R_M in the presence of nanomolar concentrations of the antibody Sp3E9 in the bulk solution. Furthermore, the unspecific adsorption of IgG was negligible. The reported relative effect of Sp3E9 antibody binding is large: the membrane resistance increases by almost a factor of four at saturation. The absolute increase of approximately 4500 Ω is however limited by the number of defects in

the lipid layer. The values of R_M obtained after complete antibody binding are indeed very close to the limiting values measured for pure lipid bilayers prepared under the same conditions. The complete blocking of a fraction of the surface-bound SLIC is sufficient to account for the reported effect. Enhanced sensitivity is therefore expected with membranes having higher electrical resistances.

IV. IMPROVING THE ELECTRICAL RESISTANCE OF TETHERED LIPID BILAYERS

The electrical resistances of tethered membranes were recently improved by using flat gold [20] and optimizing the lipid composition [8]. We obtained lipid bilayers tethered to gold electrodes with resistances above 2×10^8 Ω (for 3.34 mm^2 electrodes) which are getting close to resistance values of freestanding membranes used for classical single-channel measurements in black lipid membranes and patch-clamp experiments [8]. In Fig. 3A the incorporation of SLIC in a tethered lipid bilayer is reflected in a drop of membrane resistance measured on the real part of the impedance at 0.01 Hz. The low frequency of the detection is a result of the high time constant RC of these highly insulating bilayers. Upon addition of SLIC in the aqueous phase the layer resistance decreases immediately and saturates in about 30 minutes. The slow kinetics allows interruption of the insertion process of the protein into the lipid bilayer by washing with buffer and thus keeping the number of SLIC molecules in the membrane as low as possible in a controlled way. Selective antibody binding to SLIC in the lipid bilayer increases the membrane resistance as a function of the concentration of the antibody Sp3E9 in the aqueous phase (Fig. 3B). Taken into account a conductance of 90 pS measured in freestanding black lipid membranes [21], the observed responses correspond to the incorporation of 200 open SLIC channels and the total closure of 95 channels in the tethered lipid bilayer. With the presently achieved signal-to-noise ratio it is possible to detect the closure of a few individual channels. An improvement of the time resolution of the technique is however necessary to measure the kinetics of opening and closing of single-channels.

V. CONCLUSION

The proof of principle of immunosensing by SLIC has been demonstrated with the specific detection of the antibody Sp3E9 in the nanomolar range. By modifying the ligand-binding part of SLIC the detection of other antibodies and DNA molecules should be possible. SLIC based tethered lipid bilayers could thus deliver a generic tool for ultrasensitive biosensing.

The formation of tethered lipid bilayers with exceptionally high resistances is an important step towards single-channel experiments on this highly stable and versatile platform. The function of ion channels could then be investigated more efficiently and in higher throughput than currently possible. This would have wide-ranging consequences on issues such as drug discovery. The ultimate sensitivity of measuring single ligand-binding event by simple electrical devices would open new possibilities in bioanalytics.

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