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The effect of the ionophore valinomycin on biomimetic solid supported lipid DPPTE/EPC membranes

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Abstract

Self assembled monolayers and bilayers are produced on a flat glass surface, bound by a thiolipid onto bare gold. 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphothioethanol (DPPTE) is used as the molecule binding to the electrode surface. The lipid λ-α-Phosphatidyl-Choline-β-Oleoyl-g-Palmitoyl (POPC) and the lipid mixture eggphosphatadiylcholine (EPC) are used as spacer lipids with the aim of achieving solid-supported artificial lipid membranes. With the aim of creating and investigating more natural systems, ion carrier proteins such as valinomycin are introduced into the DPPTE/EPC system. The direct influence on the membranes as well as the effects of different ionic solutions on the proteins is shown.

Keywords: Solid supported biomimetic lipid bilayer membranes; Thiolipids; Peptides; Ionophores; Valinomycin; POPC; DPPTE; EPC; Egg-PC; Electrochemical impedance spectroscopy

1. Introduction

Biological membranes have to serve multiple functions. As a plasma membrane, they separate different cells and permit free flow of material between them. The cell nutrition and other materials are transported trough the membrane by special channels and carrier molecules. Cell membranes can, due to their preferred order direction, be categorised as smectic liquid crystals [1–4]. The stability of membrane systems derives from multiple effects. First, dissociation of protons from the lipid headgroups results in a negatively charged membrane surface which adsorbs conversely charged ions out of the aqueous solution. Thus, an electrically charged double layer is formed on each side of the membrane. This field can be modelled after the Gouy–Chapman model of an electrically charged diffusive bilayer [5]. Some of the positively charged ions like calcium can adsorb on the membrane and increase its rigidity by forming chelates with the lipids. Further structural integrity of the membranes derives from the hydration of the membrane where water molecules up to a thickness of 7 Å form a dense hydrobond network around the membrane [6].

The interactions of a great variety of membrane molecules make it difficult to measure complete real cell systems. To get an overview of how complex systems work, each part of these systems has to be investigated separately. Proteins, incorporated into membranes, can act as transporters for polar and ionic molecules and atoms which would otherwise not be capable of passing the membrane. Proteins have the advantage of being amphiphilic and can thus anchor within a membrane and float within it. They alter the thermodynamic properties of the membrane due to electrostatic force interactions. Conversely, their own enzymatic capability is dependent on the very lipid system of the membrane [7]. Proteins can be divided into two groups. The first group regulates the transport across the membrane via uncatalysed diffusion control [8]. The second group actively transports ions through a membrane. The activation is often very ion-specific. Valinomycin is a peptide actively transporting ions through membranes and has been reported to be highly potassium ion selective [9,10]. The valinomycin activity and selectivity in a DPPTE/EPC bilayer membrane system has been investigated in this research.

Stability of these bilayer systems is an issue in research [11,12], incorporation of valinomycin has been shown to be an option to observe the membrane integrity [13]. Valinomycin is a rather small

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peptide with the chemical formula of C54H90N6O18. This peptide is a cyclodepsipeptid antibiotic produced from *Streptomyces fulvissimus*, a structural relative of the enniatines [14].

Binding lipid bilayer to a solid gold substrate via thiol groups, by simultaneously retaining its biomimetic properties, has seen a vast increase in interest over recent years [15–33].

Spacer lipids with different headgroups are added to fill the area on the gold where no thiolipids have anchored as well as to form the surface distal second layer creating a membrane. It is theoretically possible to construct biomimetic systems that are highly analyte specific by utilising the specificity found naturally in cellular biology [34,35]. Practically, problems still prevail. First of all, a sufficiently flexible and defect free lipid bilayer has to be formed on a solid surface, such that ion channel activity can actually be measured. Secondly, to be biomimetic, the lipid membrane has to be in a chemo-physical state in which incorporated biological molecules such as peptides function as if in a real natural system. And finally, water must be incorporated on the inner surface of the membrane to facilitate the incorporation of transmembrane proteins. The incorporation of isolated peptides into supported bilayer membranes has been found to be impossible, only peptides integrated in vesicles could easily be transferred into existing membrane systems. The utilisation of solid supported bilayers on gold facilitates the benefit of direct in-situ electrochemical measurements of the properties of defined amounts of peptides in natural solutions.

2. Experimental section

2.1. Electrodes

Standard microscope glass slides were cleaned following a procedure already published elsewhere [36]. Gold electrodes for electrochemical measurements were made by the thermal evaporation of 5 nm of chromium followed by 5 nm of gold (purity > 99.99%) onto these slides at a pressure of $2 * 10^{-4}$ Pa to produce an electrode of 39 mm^2. The electrode-covered slides were annealed for 2 min at 600 °C and finally cleaned in piranha, a mixture of 1/3 hydrogen peroxide and 2/3 concentrated sulphuric acid.

2.2. Electrochemical cell

The as-prepared slides were mounted in an electrochemical cell depicted in Fig. 1. The slide was heated to 37 °C from below with pumped warm water, temperature controlled by a thermocouple. Sealed with a rubber ring, a Teflon cylinder was mounted atop the slide. The setup was screwed tight in a grounded brass frame. Counter electrode (CE) and first reference electrode (RE1) were connected to a 2-mm gold (> 99.99%) wire (Advent Research Materials Ltd, Oxford, UK) immersed in the respective cell solution. Working electrode (WE) and second reference electrode (RE2) were connected to the part of the gold electrode that showed out of the measurement cell on the bottom.

2.3. SAM preparation

2 mg DPPTE (Avanti Polar Lipids, Alabaster, U.S.) were dissolved in 1 ml chloroform and 1 ml ethanol in a Petri dish. A lipid monolayer was allowed to self assemble on the gold electrode as the slide was immersed in that solution over 60 min and the measurement cell was subsequently assembled atop the slide. The cell was rinsed five times in 0.1 M KCl without completely drying the gold electrode and impedance was measured. The system was then allowed to settle another 60 min

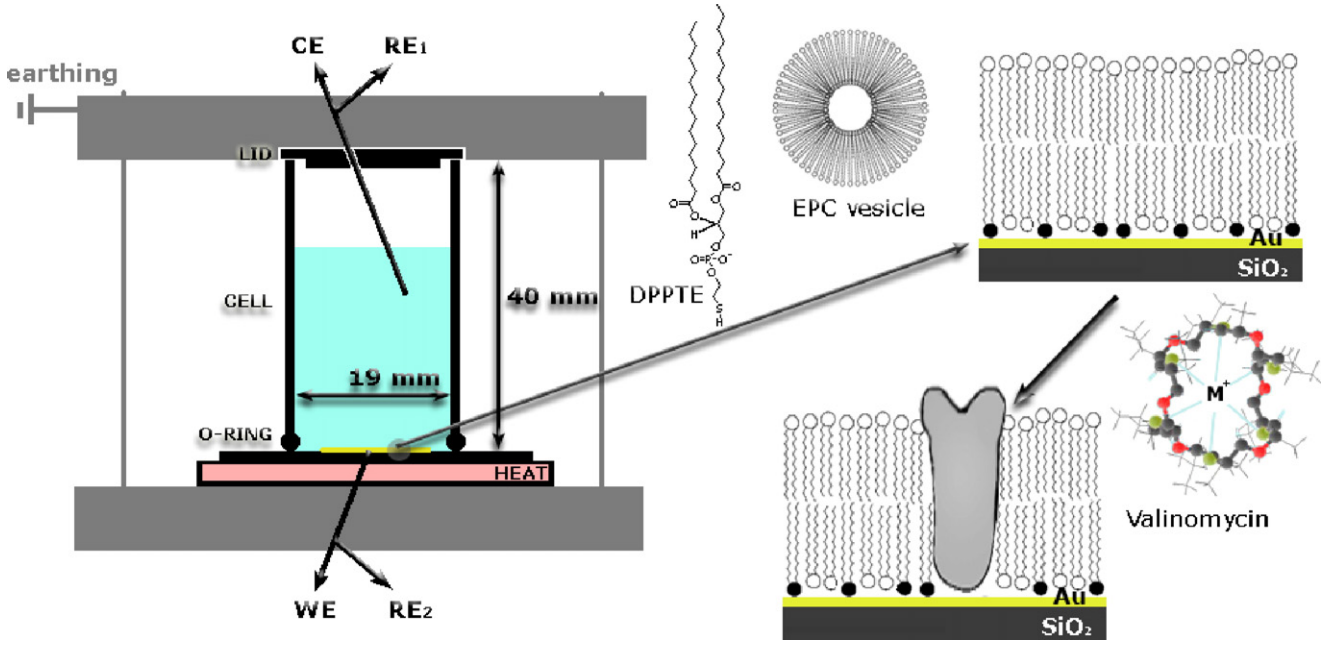

Fig. 1. The completely mounted electrochemical cell and the steps of producing a solid supported lipid bilayer membrane with the ionophore valinomycin. From left to right: Addition of DPPTE forming a binding monolayer on the gold surface and of EPC vesicles settling onto this monolayer to form a solid-supported bilayer membrane. Then, the ionophores, dissolved in EPC vesicles (here represented by the chemical structure of the valinomycin molecule) are introduced into the electrochemical cell.

before the cell was rinsed once again and another impedance measurement was made. Using this technique, not only the chemically bonded molecules build a layer on the surface, but additionally some unbonded lipid molecules floating around the surface. To remove these floating lipids and to be sure to have only bonded DPPTE molecules on the gold surface, the cell was rinsed five times with ethanol and than again five times with 0.1 M KCl solution. This way, it can be observed how dense the formed layer really is using a calculation described elsewhere [37].

2.4. Lipid bilayer membrane

2.4.1. Kinetic experiments

Large unilamellar vesicles (LUVs) were formed from DPPTE and POPC (Avanti Polar Lipids, U.S.). A 1:1 mixture of DPPTE and POPC containing an overall of 16.4×10^{-7} mol lipids was prepared in 1200 µl 0.1 M KCl and vortexed to ensure full dispersion of the lipid in the KCl solution. The resultant dispersion was extruded 15 times through a Avestin Inc. (Mannheim, Germany) LiposoFast Basic extruder containing membranes with 50 nm diameter pores to form unilamellar vesicles of approximate diameter < 50 nm [38]. The changes in layer density were observed in the capacitive element from impedance experiments. Subsequent measurements (impedance scan duration of 2 min for the 10 first experiments) were made to reveal the adsorption kinetics of this system.

2.4.2. Biomimetic systems

In order to get to more natural system, LUVs were produced from the lipid mixture EPC (Lipid products, U.K.). It is also well known that temperature has a crucial influence on lipids phase transitions [39]. and thus the gold surface temperature was adjusted to natural living conditions (37 °C), well above the EPC phase transition temperature of around 1 °C. 5 mg EPC dissolved in a chloroform/methanol 1:1 mixture were dried under a stream of nitrogen and then hydrated in 0.1 M KCl solution and vortexed. The resultant dispersion was extruded 15 times as described above to form desired vesicles. These were injected into the electrochemical cell containing a DPPTE SAM formed on the gold electrode as described above. The changes in layer density were observed in the capacitive element from impedance experiments.

2.5. Peptide preparations

Since a direct introduction of up to 2.5 wt.% peptides (Valinomycin, Sigma Aldrich, Milwaukee, U.S.) dissolved in KCl solution into the cell containing a DPPTE/EPC bilayer did not introduce significant changes in impedance results, 2.25×10^{-7} mol valinomycin were dissolved in 10^{-5} mol of EPC and adequately vortexed. If the ion channel was already dissolved in lipid, it should more easily settle into the membrane. The ion channel should ideally introduce a second time constant since it reacts differently in an electric field and permits potassium cations to pass the membrane at a certain voltage.

2.6. Electrochemical measurements

EIS was performed on an Autolab PGSTAT12 Electrochemical Interface. Modelling was made by an equivalent circuit as follows. A perfect interface, without peptides, was modelled using a standard $R_S(R_2C_1)$ equivalent circuit, where R_S was the series resistance of the system, mainly resistance of the solution, cables and attached peripheral instruments, $R_{(2)}$ was the interfacial resistance and $C_{(1)}$ the capacitance of the interface. Index numbering of each impedance arc is done by increasing time constant $\tau=RC$ with $|Y_0|$ as the capacitance. The distinctiveness in the obtained values made a more refined criterion unnecessary. The system was verified on an appropriate test circuit to reproduce data within 1%. The equivalent circuit, $R_S(R_2(R_3C_2)C_1)$, for membranes containing ionophores was more complex. Fig. 2a shows this circuit. The arrows indicate the flip-flop mechanism of the protein that occurs when a transportable ion has entered the molecule and is transported through the membrane. This behaviour can be treated like an ion-channel where molecules can diffuse freely through proteins like for example gramicidin [40–43]. The interpretation of the circuit components and especially the capacitors was more difficult for these systems. R_S again resembled the resistance of the solution, cables and attached peripheral instruments. R_2 was the resistance of the film giving evidence of the ionic transport

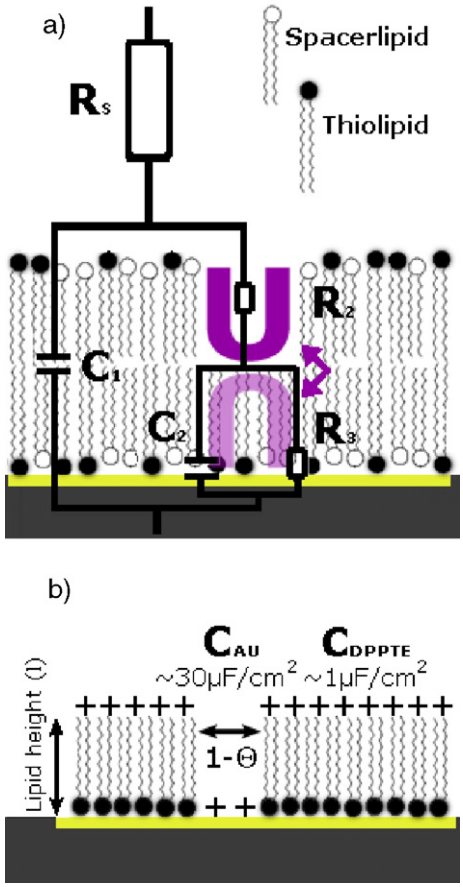

Fig. 2. Equivalent circuits for a dielectric layer on gold and with embedded valinomycin (a) and representation of a real, non-perfect film showing the coverage Θ.

properties because it directly showed at which rate the proteins carried ions through the membrane; this value will decrease for a good transport. R_3 was merely an indication of the roughness of the gold surface and will increase with decreased roughness. C_1 can be regarded as the capacitance of the dielectric on gold, while C_2 represented the interface between the gold electrode and the ion-channel. In this case, a Bode plot will reveal multiple time constants as two separate slopes in the magnitude and two different peaks in the phase shift plot. An unbiased sinusoidal 0.1 V ac potential was swept from 100 kHz–0.01 Hz and the current response of the system was registered. Impedance fitting of these results was done using the Autolab fitting software and Boukamp's program "Equivcrt" [44]. This DPPTE/EPC/valinomycin system and variations, including POPC, 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) and outer membrane protein F, were also subjected to intense UV radiation to obtain information of the stability of these membranes presented elsewhere [45].

3. Results and discussion

3.1. Electrochemical impedance measurements

Experiments were made to estimate the capacitance of pure DPPTE. If this layer proved to be stable, the changes due to the addition of valinomycin to this system could be studied. First of all, the changes in film capacitance are of interest since they represent the film integrity [46]. The layers that form in a pure aqueous solution and on a gold electrode can be described with the Helmholtz model as a flat plate capacitor dielectric. If the gold surface is screened with an insulating dielectric lipid layer, the capacitance can be modelled as two capacitors in series. The capacitance itself changes due to the lipid film forming on the surface.

3.2. Adsorption kinetics

In real systems, the coverage of the gold electrode is never perfect, resulting in only partly covered electrodes. Following Jenkins et al., the overall coverage, Θ, can be derived from the two contributions to C_{meas}, the total measured area specific membrane capacitance: C_{DPPTE} from the lipid–liquid interface and C_{AU} from the interface gold–liquid [37]. Fig. 2b shows this coverage on an electrode. The actual capacitance measured is the combined capacitance of the perfect layer parts and the gaps in between these layers where the pure gold is in contact with the electrolyte.

3.2.1. DPPTE/POPC vesicles

The adsorption kinetics of DPPTE/POPC vesicles on bare gold, as investigated electrochemically in-situ by change in capacitance between sequential impedance scans, was in good comparison with those previously reported [36,47]. After an initial fast decrease in capacitance, attributed to the adsorption and spontaneous rupture of lipid vesicles on the surface, the decrease progresses more slowly for times longer than 5 min as seen in Fig. 3. This can be attributed to bilayer spreading and lipid self-organisation on the surface [48]. Consequently, the gold surface coverage increases fast in the beginning and levels out. Such a multi-step adsorption process is in agreement with reported surface Plasmon resonance investigations [42].

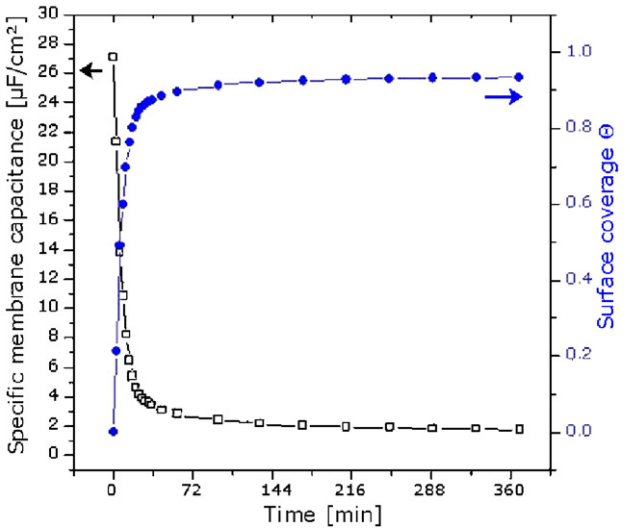

Fig. 3. Development of specific membrane capacitance (squares) and coverage (circles) over time for the addition of a DPPTE–POPC LUVs in 0.1 M KCl on bare gold.

3.2.2. DPPTE–SAM

While a bare gold electrode in 0.1 M KCl solution was measured to have a specific capacitance of 25.54 µF/cm^2, the specific capacitance of an as-produced DPPTE–SAM was found to be 1.27 µF/cm^2 which decreased to 1.07 µF/cm^2 while rinsing in potassium salt solution. This was attributed to improved surface kinetics allowing slightly more molecules bind to the gold surface forming a denser layer after rinsing in KCl. The surface kinetics of all these experiments showed a sharp drop in specific capacitance during the first ten minutes and a gradual decrease over the following 24 h, usually to a certain limiting value of about 1 µF/cm^2. In order to remove free-floating lipid molecules, the cell was rinsed with ethanol before switching the solution back to KCl giving an increase in specific capacitance to 3.08 µF/cm^2, a value derived from the less covered gold–liquid interface as only bonded molecules are left on the surface.

3.2.3. EPC vesicles on DPPTE monolayer

Since the technique of external SAM formation produced a reasonably stable and dense layer of pure DPPTE, and in order to come back to more natural systems, a 0.1 M KCl solution containing extruded vesicles of EPC were introduced directly into the cell. The changes in specific capacitance can be seen in Fig. 4. After a rapid decrease of C_1 to 0.79 µF/cm^2 during the first 6 min, the EPC was allowed to settle on the surface for another 174 min until the specific surface capacitance had stabilised at a value of 0.69 µF/cm^2. Monitoring the variations in specific capacitance during the addition of EPC vesicles to a SAM allowed the kinetics of lipid film formation to be followed in-situ. The angular frequency reduced admittance (Y'/ω; $-jY''/\omega$) plot in the complex

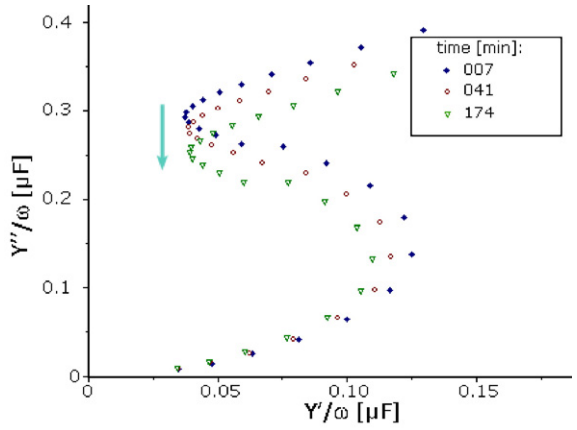

Fig. 4. Adsorption of EPC vesicles on a DPPTE monolayer bound to a gold surface. Time in minutes after the addition of the EPC into the cell.

plane was a good way to reveal the specific capacitance of systems with a dielectric interface directly in the intercept point of the graph with the imaginary axis.

3.3. Ion selectivity

2.25×10^{-7} mol of valinomycin were dissolved in 10^{-5} mol EPC and vortexed. This was done to dissolve the ionophores in lipid in order to make it easier for the peptide to get incorporated into the membrane. The ion carrier introduced a second time constant since it reacted differently in an electric field and permitted potassium cations to pass the membrane at a certain voltage. Consequently, fitting procedures were changed to the equivalent circuit drawn in Fig. 2a. It became obvious that an admittance complex plane plot did no longer easily reflect the reality of the system. Plotting the results in a Bode plot (|Z| and negative phase shift angle theta vs. log(frequency)), the second time constant becomes more obvious.

The interpretation became likewise difficult for these systems. The main aspect was no longer specific capacitance of the film to give an indication of electrode coverage, but the resistance of the film, R_2. Due to the capability of ionic charge conductance through the peptides, R_2 shows how well ions are being transported through the membrane, a low R_2 value thus reflect high ionic conductance through the ionophore The second time constant is clearly visible in Fig. 5 showing the impedance before the addition of valinomycin and after valinomycin-containing EPC vesicles were allowed to settle in the membrane for 71 min. Thus, it could be shown that valinomycin transports K^+-ions through a constructed biomimetic solid supported DPPTE/EPC membrane and is active in named system.

Besides making the lipid more natural, the introduction of ionophores into the lipid bilayer facilitates the in-situ detection of passage of specific ions. The type of ionophore used in this research, valinomycin, is an active ion carrier. Since valinomycin proved to be difficult to be incorporated into the membrane by only adding the dried peptide dissolved in salt solution, the valinomycin was dispersed, vortexed and extruded with the

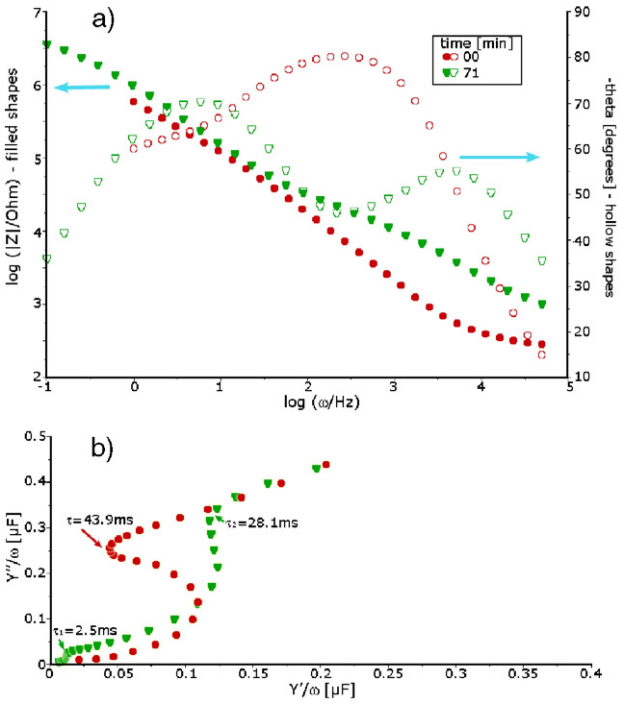

Fig. 5. a) Bode plot before (circles) and 71 min after (down triangles) the addition of valinomycin to the membrane system DPPTE/EPC. b) The same graphs represented in a complex plane plot.

EPC to form vesicles that were incorporated into the existing lipid bilayer upon interaction with the surface. The initial valinomycin concentration was 1.17 mol% of the total lipid (DPPTE/EPC) present. It should be mentioned that, since no experiments were made to investigate the incorporation rate of the ionophores in the membrane, the final concentration of peptides present in the lipid membrane remained unknown. Conduction of ions through the resulting biomimetic DPPTE/EPC membrane containing valinomycin was dominated by two effects; (i) bilayer defects and (ii) peptide activity. Since only (ii) showed ion selectivity, the presence of active ion carriers

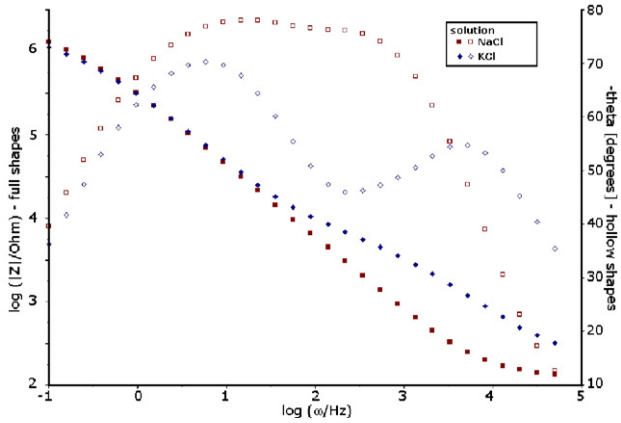

Fig. 6. Changes in impedance introduced by different ionic solutions. Since valinomycin conduction is very ion specific, two time constants can only clearly be distinguished when the peptide is active which is the case for solutions containing potassium ions; here: 0.1 M KCl.

could be seen by changes in the resistance of the film. The KCl solution was carefully rinsed five times with 0.1 M NaCl and with 0.1 M $CaCl_2$. As expected, valinomycin only permitted potassium cations through the membrane and the ionophore resistance R_2 dropped from 163 kΩ for a DPPTE/EPC membrane without ionophores fast to 8.7 kΩ (after 3 min) to a stable minimum value of 6.5 kΩ (after 71 min). Valinomycin is an ionophore that transports ions across lipid membranes with particular affinity for potassium. The arrangement of three repeating units of <L-valine, D-hydroxy-isovaleric acid, D-valine and L-lactic acid> forms an octahedral cavity with the optimum size for coordinating a potassium ion [49–51]. Since the resistance increased for sodium ions (30 kΩ) and even further for calcium ions (47.9 kΩ), it was shown that the ion transport was shut down by different salt solutions. Accordingly, the experiments proved the expectation that the second time constant should vanish or decrease significantly and the resistance of the surface should increase again as no charges were actively transported by the ionophore.

The experiments showed that, for NaCl, the ion transporting properties varied accordingly (Fig. 6). The second time constant was significantly reduced but still slightly noticeable. These effects intensified for $CaCl_2$ solutions due to the ion channels completely blocking the even larger calcium cations. A control experiment made on a similar DPPTE/EPC system without ionophores showed no selectivity towards any of the used salt solutions.

4. Conclusions

This paper demonstrates different ways of producing supported lipid bilayer membrane systems. The kinetics of formation of lipid bilayers on gold is measured by electrochemical impedance spectroscopy. Noticeably, it is illustrated that formed membranes retain their biomimetic nature. By addition of ionophores, the present system can be used to model ion transport properties of selected membranes. It is shown that potassium ion selective transport through the membrane was activated due to the incorporation of the peptide valinomycin into the membrane. This system blocked other ions and can therefore be considered to be a good model to test the ion transport capabilities of ionophores. A further application can be seen in biosensors created from arrays of the present system and with alternating lipid content, when potassium concentration is of crucial influence, such as in biological fluids. Since sensor tips should be replaced when the biological system of interest changes, it can initially be accepted that the present system is for one-time use only. Further materials engineering has to be carried out in order to make these sensors long-term stable and reliable. Mixtures of ionophores including gramicidin or alamethicin among others might prove to provide a biological equilibrium in which non-potassium ions will be conducted through the membrane without blocking valinomycin which remains potassium specific. Further experiments aim at the validation of seen effects using different lipid mixtures, ionophore concentrations as well as revelations of temperature dependency.

Acknowledgements

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References

[1] P.G. Gennes, J. Prost, The Physics of Liquid Crystals, 2nd ed., Clarendon Press, Oxford, 1993.
[2] S. Friberg, Lyotropic Liquid Crystals and the Structure of Biomembranes, Advances in Chemistry Series, vol. 152, ACS, Washington, 1976.
[3] G.H. Brown, J.J. Wolken, Liquid Crystals and Biological Structures, Academic Press, New York, 1980.
[4] B.A. Lewis, M. Engelman, Lipid bilayer thickness varies linearly with acryl chain length in fluid phosphatidylcholine vesicles, J. Mol. Biol. 166 (1983) 211–217.
[5] T.A.J. Payens, Ionized monolayers, the Gouy Chapman model, Philips Res. Rep. 10 (1955) 425–481.
[6] E. Sackmann, Physical Basis of Self-Organisation and Function of Membranes: Physics of Vesicles, Handbook of Biological Physics, vol. 1, Elsevier Science, Elsevier, Netherlands, 1995.
[7] A.G. Lee, Lipid–protein interactions in biological membranes: a structural perspective, BBA-Biomembranes 1612 (2003) 1–40.
[8] R.D. Burgoyne, M.J. Clague, Annexins in the endocytic pathway, Trends Biochem. Sci. 19 (1994) 231–232.
[9] M. Thompson, U.J. Krull, The electroanalytical response of the bilayer lipid membrane to valinomycin: membrane cholesterol content, Anal. Chim. Acta 141 (1982) 33–47.
[10] K. Cammann, Ion-selective bulk membranes as models, Top. Curr. Chem. 128 (1985) 219–258.
[11] H.T. Tien, A. Ottowa-Leitmannova, Membrane Biophysics as Viewed from Experimental Bilayer Lipid Membranes, Elsevier Science BV, Amsterdam, Netherlands, 2000.
[12] H.T. Tien, R.H. Barish, L.Q. Gu, A.L. Ottova, Supported bilayer lipid membranes as ion and molecular probes, Anal. Sci. 14 (1) (1998) 3–18.
[13] G. Favero, L. Campanella, A. D'Annibale, R. Santucci, T. Ferri, Mixed hybrid bilayer lipid membrane incorporating valinomycin: improvements in preparation and functioning, Microchem. J. 74 (2003) 141–148.
[14] D.W. Urry, Chemical basis of ion transport specificity in biological membranes, Top. Curr. Chem. 128 (1985) 175–218.
[15] D.P. Tieleman, Theoretical studies of membrane models: molecular dynamics of water, lipids and membrane proteins, PhD thesis, Rijksuniversiteit Groningen, Groningen, Netherlands, 1998.
[16] R.P. Richter, J.L.K. Him, A. Brisson, Supported lipid membranes, Mater. Today 6 (2003) 32–37.
[17] M. Stelzle, G. Weismueller, E. Sackmann, On the application of supported bilayers as receptive layers for biosensors with electrical detection, J. Phys. Chem. 97 (1993) 2974–2981.
[18] S. Terrettaz, T. Stora, C. Duschl, H. Vogel, Protein binding to supported lipid membranes: investigation of the cholera toxin–ganglioside interaction by simultaneous impedance spectroscopy and surface plasmon resonance, Langmuir 9 (1993) 1361–1369.
[19] G. Brink, L. Schmitt, R. Tampé, E. Sackmann, Self assembly of covalently anchored phospholipid supported membranes by use of DODA-Suc-NHS-lipids, BBA-Biomembranes 1196 (1994) 227–230.
[20] C. Duschl, M. Liley, G. Corradin, H. Vogel, Biologically addressable monolayer structures formed by templates of sulfur-bearing molecules, Biophys. J. 67 (1994) 1229–1237.
[21] A. Plant, M. Gueguetchkeri, W. Yap, Supported phospholipid/alkanethiol biomimetic membranes: insulating properties, Biophys. J. 67 (1994) 1126–1133.
[22] M. Kuhner, R. Tampé, E. Sackmann, Lipid mono- and bilayer supported on polymer films: composite polymer-lipid films on solid substrates, Biophys. J. 67 (1994) 217–226.
[23] H. Lang, C. Duschl, H. Vogel, A new class of thiolipids for the attachment of lipid bilayers on gold surfaces, Langmuir 10 (1994) 197–210.

[24] A.L. Plant, M. Brigham-Burke, E.C. Petrella, D.J. O'Shannessy, Phospholipid/alkanethiol bilayers for cell surface receptor studies by surface plasmon resonance, Anal. Biochem. 226 (1995) 342–348.
[25] A. Nelson, Influence of biologically active compounds on the monomolecular gramicidin channel function in phospholipid monolayers, Langmuir 12 (1996) 2058–2067.
[26] B. Lindholm-Sethson, Electrochemistry at ultrathin organic films at planar gold electrodes, Langmuir 12 (1996) 3305–3314.
[27] E. Sackmann, Supported membranes: scientific and practical applications, Science 271 (1996) 43–48.
[28] M. Williams, S.D. Evans, T.M. Flynn, A. Marsh, P.F. Knowles, R.J. Bushby, N. Boden, Kinetics of formation of single phospholipid bilayers on self-assembled monolayer supports, as monitored by surface plasmon resonance, Supramol. Sci. 4 (1997) 513–517.
[29] N. Nakashima, Y. Yamaguchi, H. Eda, M. Kunitake, O. Manabe, Design of a lipid bilayer electrical device. Strong chemical structure dependence and molecular mechanisms on the phase transition-dependent electrical impedance responses, J. Phys. Chem., B 101 (1997) 215–220.
[30] Y. Cheng, N. Boden, R.J. Bushby, S. Clarkson, S.D. Evans, P.F. Knowles, A. Marsh, Attenuated total reflection Fourier transform infrared spectroscopic characterization of fluid lipid bilayers tethered to solid supports, Langmuir 14 (1998) 839–844.
[31] I. Burgess, M. Li, S.L. Horswell, G. Szymanski, J. Lipkowski, S. Satija, J. Majewski, Influence of the electric field on a bio-mimetic film supported on a gold electrode, Colloids surf., B Biointerfaces 40 (2005) 117–122.
[32] T. Neumann, Strategies for detecting DNA hybridisation using surface plasmon fluorescence spectroscopy, PhD thesis, Max Planck Institute for Polymer Research, Mainz, Germany, 2001.
[33] S. Boudard, B. Seantier, C. Breffa, G. Decher, O. Félix, Controlling the pathway of formation of supported lipid bilayers of DMPC by varying the sodium chloride concentration, Thin Solid Films 495 (2006) 246–251.
[34] A. Darszon, C.A. Vandenberg, M. Schoenfeld, M.H. Ellisman, N.C. Spitzer, M. Montal, Reassembly of protein–lipid complexes into large bilayer vesicles: perspectives for membrane reconstitution, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 239–243.
[35] J.T. Elliott, C.W. Meuse, V. Silin, S. Krueger, J.T. Woodward, A. Plant, in: J.F. Rusling (Ed.), Biomolecular Films, Design, Function, and Applications, Surfactant Sci. Ser. vol. 111, Marcel Dekker, New York, 2003, pp. 99–163.
[36] S. Lingler, I. Rubinstein, W. Knoll, A. Offenhäusser, Fusion of small unilamellar lipid vesicles to alkanethiol and thiolipid self-assembled monolayers on gold, Langmuir 13 (1997) 7085–7091.
[37] A.T.A. Jenkins, R.J. Bushby, N. Boden, S.D. Evans, P.F. Knowles, Q. Liu, R.E. Miles, S.D. Ogier, Ion-selective lipid bilayers tethered to microcontact printed self-assembled monolayers containing cholesterol derivatives, Langmuir 14 (1998) 4675–4678.
[38] E. Kalb, S. Frey, L.K. Tamm, Formation of supported planar bilayers by fusion of vesicles to supported phospholipid monolayers, BBA-Biomembranes 1103 (1992) 307–316.
[39] J.R. Silvius, Lipid–Protein Interactions, John Wiley, New York, 1982.
[40] D.A. Kelkar, A. Chattopadhyay, Monitoring ion channel conformations in membranes utilizing a novel dual fluorescence quenching approach, Biochem. Biophys. Res. Commun. 343 (2006) 483–488.
[41] A.T.A. Jenkins, N. Boden, R.J. Bushby, S.D. Evans, P.F. Knowles, R.E. Miles, S.D. Ogier, H. Schoenherr, G.J. Vansco, Microcontact printing of lipophilic self-assembled monolayers for the attachment of biomimetic lipid bilayers to surfaces, J. Am. Chem. Soc. 121 (1999) 5274–5280.
[42] A.B., Mamonov, M.G., Kurnikova, R.D., Coalson, Diffusion constant of K+ inside gramicidin: a comparative study of four computational methods, Biophys. Chem. in press doi:10.1016/j.bpc.2006.03.019.
[43] G.A. Woolley, B.A. Wallace, Model ion channels: gramicidin and alamethicin, J. Membr. Biol. 129 (1992) 109–136.
[44] B.A. Boukamp, A nonlinear least squares fit procedure for analysis of immittance data of electrochemical systems, Solid State Ionics 20 (1986) 31–44.
[45] L. Rose, A.T.A. Jenkins, in: T. Jenkins (Ed.), Abstracts from the 1st Mainz–Twente–Bath Joint Seminar, University of Bath, Bath, UK, 2003, p. 26.
[46] M.P. Breil, J.M. Mollerup, in: R. Dohrn (Ed.), Proc. 20th Europ. Symp. Appl. Thermodyn., VDI Gesellschaft fuer Verfahrenstechnik und Chemieingenieurwissenschaften GVC, Lahnstein, Germany, 2003.
[47] L.M. Williams, S.D. Evans, T.M. Flynn, A. Marsh, P.F. Knowles, R.J. Bushby, N. Boden, Kinetics of the unrolling of small unilamellar phospholipid vesicles onto self-assembled monolayers, Langmuir 13 (1997) 751–757.
[48] S.G. Boxer, Molecular transport and organization in supported lipid membranes, Curr. Opin. Chem. Biol. 4 (2000) 704–709.
[49] B. Hill, Ionic Channels of Excitable Membranes, Sinauer Associates, Sunderland, USA, 1992, pp. 305–312.
[50] K.N. Laves, M. Dobler, The crystal structure of a K+ complex of valinomycin, Helv. Chim. Acta 58 (1975) 432–442.
[51] H.W. Huang, C.R. Williams, Structure of valinomycin–K+ complex in solution by extended X-ray absorption fine structure, Biophys. J. 33 (1981) 269–273.