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ARTICLE

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Impedance analysis of phosphatidylcholine membranes modified with valinomycin

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Abstract The effect of the ion carrier valinomycin on the electrochemical features of the phosphatidylcholine membrane was investigated by electrochemical impedance spectroscopy. Phosphatidylcholine and valinomycin were chosen for the study because they fulfil essential functions in lively organisms. The experimental impedance values obtained in the presence of different amounts of carrier, studied with several potassium ion concentrations, were used for the research ability of valinomycin to form a 1:1 potassium ion complex on the lipid bilayer/electrolyte solution interface. Based on derived mathematical equations, the heterogeneous equilibrium constant (K_h), association rate constant of the complex (k_R) and dissociation rate constant of the complex (k_D) were calculated. The result of the investigation is the proposal of a new method for the determination of the parameters used to describe the chemical reaction at the interface between a carrier molecule from the membrane and a monovalent ion from the aqueous phase.

Keywords Bilayer lipid membrane · Impedance spectroscopy · Phosphatidylcholine · Valinomycin

Introduction

The classical carrier model for ion transport is based on the idea that a membrane-bound carrier molecule transports a substrate by binding it on one side of the membrane and crossing over to the other side where the substrate is released. The carrier is then free either to pick up another passenger or to return to the original side. The model can be taken literally as in its application to the ion carriers (Hladky 1979; Benz and Lauger 1976; Benz 1978), or formally as when it is used to describe variable conformation pores (Lauger 1980). Electrochemical experiments with lipid membranes in the presence of valinomycin carried out by different methods have led to the conclusion that valinomycin behaves like a carrier in the above sense (Benz et al. 1973; Lauger 1972; Benz et al. 1977; Benz and Cros 1978; Kemp and Wenner 1972). Usually the time dependence of the electric current across the membrane or of the membrane voltage is followed by an external stimulus such as a voltage-jump, a charge-pulse, a T-jump or a photo-generated concentration-jump [see (Lauger et al. 1981) for a review]. The time resolution, which has so far been achieved under favourable conditions, is of the order of 2 μs [V-jump (Knoll and Stark 1975)], 40 ns [charge-pulse technique (Benz and Zimmermann 1980)] and 10 μs [T-jump (Awiszus and Stark 1988)]. In particular, electric relaxation techniques (the voltage-jump or charge-pulse method) may be used to determine individual rate constants (association of ion and carrier in the interface k_R, dissociation of the complex k_D, translocation of the complex k_{MS} and translocation of free carrier k_S) and equilibrium constant (heterogeneous equilibrium constant K_h and equilibrium constant in the aqueous solution K).

In order to characterize ion transport across lipid membranes, de Levie et al. (Rangarajan et al. 1979; de Levie 1979) developed a theoretical model for transport impedance. This model is restricted to the permeation of membrane-soluble ions across an ultrathin lipid bilayer separating two aqueous phases. However, in the literature, impedance results obtained with lipid bilayers containing pores or carriers that enable ion transport were analyzed either in terms of equivalent circuits

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(Gritsch et al. 1998; Alonso-Romanowski et al. 1995) or in terms of the model of de Levie (Steinem et al. 1996, 1998).

Impedance spectroscopy is highly useful in membrane research because it yields information on both conductivity and dielectric constant of the membrane itself as well as information about the electric properties of the inner and outer surfaces of the membrane, e.g. the capacitance and resistance of the interfacial region (Steinem et al. 1997). We have utilized electrochemical impedance spectroscopy to study the formation of a 1:1 valinomycin-K^+ complex in the membrane/electrolyte solution interface. That heterogeneous reaction was described by mathematical equations and was further verified experimentally. Adequate equations allow us to calculate such parameters as: association rate constant of the complex, dissociation rate constant of the complex and heterogeneous equilibrium constant.

Theory

The heterogeneous reaction taking place in the membrane/electrolyte solution interface between an ion M^+ from the aqueous phase and a carrier molecule S from the membrane may be described by the rate constants of association (recombination) and dissociation being k_R and k_D, respectively. The reaction mechanism can be formally written as:

$$M^+ + S \underset{k_D}{\overset{k_R}{\Leftrightarrow}} MS^+ \tag{1}$$

Then:

$$K_h = \frac{k_R}{k_D} \tag{2}$$

where K_h denotes the heterogeneous equilibrium constant ($cm^3\ mol^{-1}$).

Denoting the volume concentrations of the complex M^+ and the free carrier S by $c^b{}_{MS}$ and $c^b{}_S$ (expressed in mol cm^{-3}) and the ion activity by a_M (expressed in mol cm^{-3}), the heterogeneous equilibrium constant may then be written in the form:

$$K_h = \frac{c^b_{MS}}{c^b_S \cdot a_M} \tag{3}$$

Membrane component concentrations can be related to its surface area by multiplying volume concentrations by the lipid bilayer thickness. The heterogeneous equilibrium constant can then be expressed as:

$$K_h = \frac{N_{MS}}{N_S \cdot a_M} \tag{4}$$

where: N_{MS}-surface concentration of the complex (mol cm^{-2}), N_S-surface concentration of the free carrier (mol cm^{-2}).

The sum of complex and free carrier surface concentrations is equal to the total carrier surface concentration in the bilayer N_T:

$$N_S + N_{MS} = N_T \tag{5}$$

Combining Eq. 4 with Eq. 5 the expression for the surface concentration of the complex is obtained:

$$N_{MS} = \frac{K_h \cdot a_M \cdot N_T}{1 + K_h \cdot a_M} \tag{6}$$

The total quantity of the carrier added to the solution forming the membrane can be represented in the form:

$$c_f V_f = c_m V_m + c_{aq} V_{aq} \tag{7}$$

where: c_f, c_m, c_{aq}—concentration of the carrier in the membrane-forming solution, the membrane and the electrolyte solution (mol cm^{-3}), respectively; V_f, V_m, V_{aq}—volume of the membrane-forming solution, the membrane and the electrolyte solution (cm^3), respectively.

The partition coefficient of the carrier γ_S may be written as:

$$\gamma_S = \frac{c_m}{c_{aq}} \tag{8}$$

Therefore, from Eqs. 7 and 8, the total carrier surface concentration can be expressed by the equation:

$$N_T = \frac{\gamma_S \cdot c_f \cdot V_f \cdot d}{\gamma_S \cdot V_m + V_{aq}} \tag{9}$$

in which: d—lipid bilayer thickness (cm).

Determination of membrane conductivity R_m^{-1} in terms of Ohm's Second Law yields:

$$R_m^{-1} = \frac{S}{d} \cdot \mu_{MS} \cdot \frac{N_{MS}}{d} \cdot F \tag{10}$$

here: S—membrane surface area (cm^2), μ_{MS}—mobility of the complex ($cm^2\ V^{-1}\ s^{-1}$), F—Faraday constant (C mol^{-1}).

If Eq. 6 is inserted in Eq. 10, the following expression for the membrane conductivity as a function of total carrier and/or electrolyte concentration is derived:

$$R_m^{-1} = \frac{S}{d^2} \cdot \mu_{MS} \cdot F \cdot \frac{K_h \cdot a_M \cdot N_T}{1 + K_h \cdot a_M} \tag{11}$$

The k_D value can be described by the equations determining the real and imaginary parts of transfer across interface impedance (Vetter 1961):

$$R_{it} = \frac{v^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_D} \cdot \frac{1}{1 + (\omega/k_D)^2} \tag{12}$$

$$\frac{1}{\omega \cdot C_{it}} = \frac{v^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_D} \cdot \frac{\omega/k_D}{1 + (\omega/k_D)^2} \tag{13}$$

here: R_{it}—resistance of the transfer across interface ($\Omega\ cm^2$), C_{it}—capacity of the transfer across interface

(μF cm^{-2}), v—stoichiometric coefficient of the complex, ω—angular frequency (s^{-1}); R, T, n, F are denoted as usual.

At low frequencies where ω is considerably smaller than k_D, the above formulae simplify to:

$$R_{it} = \frac{v^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_D} \tag{14}$$

$$\frac{1}{\omega \cdot C_{it}} = \frac{v^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_D} \cdot \frac{\omega}{k_D} \tag{15}$$

It results from Eqs. 14 and 15 that resistance of the transfer across the interface is frequency independent for the frequencies approaching zero whereas $1/\omega \cdot C_{it}$ increases proportionally to ω.

At high frequencies, where ω is considerably greater than k_D, Eqs. 12 and 13 reduce to:

$$R_{it} = \frac{v^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_D} \cdot \left(\frac{k_D}{\omega}\right)^2 \tag{16}$$

$$\frac{1}{\omega \cdot C_{it}} = \frac{v^2 RT}{n^2 F} \cdot \frac{1}{N_{MS} \cdot k_D} \cdot \frac{k_D}{\omega} \tag{17}$$

It means that the resistance and the capacity of the transfer across the interface approach zero: $1/\omega \cdot C_{it}$ decreases proportionally to $1/\omega$ and R_{it} decreases proportionally to the square of this value.

Experimental

Materials

The lipid bilayer was formed from Fluka production (Neu-Ulm, Germany) of egg lecithin (3-sn-phosphatidylcholine) and from valinomycin produced by Sigma (St. Louis, MO, USA). Lecithin was dissolved in chloroform to prevent oxidization and the solvent was evaporated in an atmosphere of argon. Valinomycin was added as a solution in chloroform (20 mg ml^{-1}) and the solvent was again removed by argon. Dried residues (phosphatidylcholine or phosphatidylcholine and valinomycin mixture) were dissolved in a hexadecane-butanol mixture (10:1 by volume). The forming solutions contained phosphatidylcholine (20 mg ml^{-1} of solvent system) or a phosphatidylcholine-valinomycin mixture (weight ratios: 40:1, 30:1, 20:1 and 10:1) and were stored at 4°C for less than a week.

The solvents were of chromatographic standard grade: chloroform and butanol were from Aldrich (Milwaukee, WI, USA) and hexadecane was from Fluka (Neu-Ulm, Germany).

1, 0.1, 0.01, 0.001 and 0.0001 M potassium chloride solutions were used as electrolytes for the experiment. Potassium chloride was analytical grade and was roasted prior to use at 400°C for 4 h to remove traces of organic material. Water purified by Milli-Qll (18.2 M, Millipore, USA) was used in all solutions and all cleaning procedures.

All experiments were performed at room temperature 20 ± 1°C.

Methods

Preparation of the bilayer membranes

Bilayer membranes were obtained as bubbles at the Teflon cap constituting a measuring vessel component. The use of hexadecane as the solvent allows one to obtain membranes of thickness and capacity values similar to those of membranes formed from monolayers (Benz et al. 1975). A small quantity of butanol has a negligible effect on the impedance parameters of the bilayers created: however, it considerably accelerates the formation of the membranes. The formation of the bilayers was monitored visually and electrically by measuring the membrane capacitance at low frequency (1 Hz). The capacity of the membranes increased with time after the formation of bilayers until a steady-state value was reached some 10–20 min later. The measurements were begun only after the low frequency capacitance was stable; increasing by less than 1% per hour. When the capacitance had stabilized it was assumed that diffusion of solvent out of the bilayers was complete. The area of the bilayers was determined with a microscope with a built-in micrometer scale in the lens and was between 4×10^{-2}–8×10^{-2} cm^2 (the values were given for the bilayers area with subtracted margin).

Impedance analysis

Electrochemical impedance spectroscopy was performed with an ac impedance system (EG&G, Princeton Applied Research, Model 388) that included a personal computer, a two-phase lock-in amplifier (Model 5208) and a potentiostat/galvanostat (Model 273), in which a four-electrode input was applied within the pre-amplifier. The electrochemical cell contained two identical reversible silver–silver chloride electrodes and two identical current platinum electrodes, and was described exactly by Naumowicz et al. (2003, 2005), Naumowicz and Figaszewski (2003).The use of the four-electrode system in the studies of electric phenomena occurring in membranes makes it possible to considerably reduce the errors caused by electrode and electrolyte impedance (Kalinowski and Figaszewski 1995). A 4-mV amplitude sine-wave signal perturbation was applied in the 0.1–10,000 Hz frequency range. Data analysis was performed by means of software using a nonlinear least square fit (Equivcrt.Pas) elaborated by Boukamp (1988).

Results and discussion

The effect of valinomycin on the electric properties of the phosphatidylcholine lipid membrane was examined

in the presence of different amounts of the ion carrier using electrochemical impedance spectroscopy. The impedance technique was used to characterize the membrane features as this method has been shown to be able to accurately measure the membrane capacitance and resistance on bilayer lipid membranes. The experimental impedance values presented here refer to the bilayer surface area unit. Impedance measurements of the lipid membrane were carried out in our study with unmodified membranes and with membranes modified by four different valinomycin concentrations and five different KCl concentrations. Except for the Z values, all recorded impedance spectra are characterized by common general features and the same dynamic behaviour. For this reason, the data for one KCl concentration and for one valinomycin concentration, respectively, are shown in the paper.

Every point of Fig. 1 represents a mean of six independent membrane measurements. Very simple impedance diagrams were obtained in the absence of the ion carrier; they had the form of capacitive semicircles in the entire analyzed frequency range; it was the evidence of the lipid bilayer being a dielectric layer with leakage (Fig. 1a). The possibility of misinterpretation of the recorded data is reduced by the simplicity of the equivalent circuit used for data analysis (inset in Fig. 1a). R_0 is the electrolyte solution resistance; it was assumed to be ohmic in nature and expected not to perturb the membrane properties. R_m represents the resistance of the membrane, and C_m is the membrane capacitance. Resistance and capacity of the PC membranes change insignificantly with increasing electrolyte concentration in the studied KCl concentration range. Based on the equivalent circuit mentioned above, the nonlinear least squares analysis was used to simulate the impedance plots; then the values of R_m and C_m were extracted from the fit. The NLLQ fit is represented by the solid line in Fig. 1a and is in good agreement with the data obtained.

The frequency response was different when valinomycin was present in the membrane (Fig. 1b). The impedance spectra of the bilayer modified with valinomycin contained a capacity semicircle and, in addition, a low-frequency semicircle related to the potassium ion transport in the area close to the membrane surface. The existence of two semicircles shows the presence of an additional impedance branch connected with the kinetic of passage of the potassium ions across interface membrane/electrolyte solution. This branch is in series with the impedance of the pure membrane. The equivalent circuit used to describe the transport of ions through the bilayer is depicted in Fig. 1b. This circuit takes into account the impedance components of the membrane

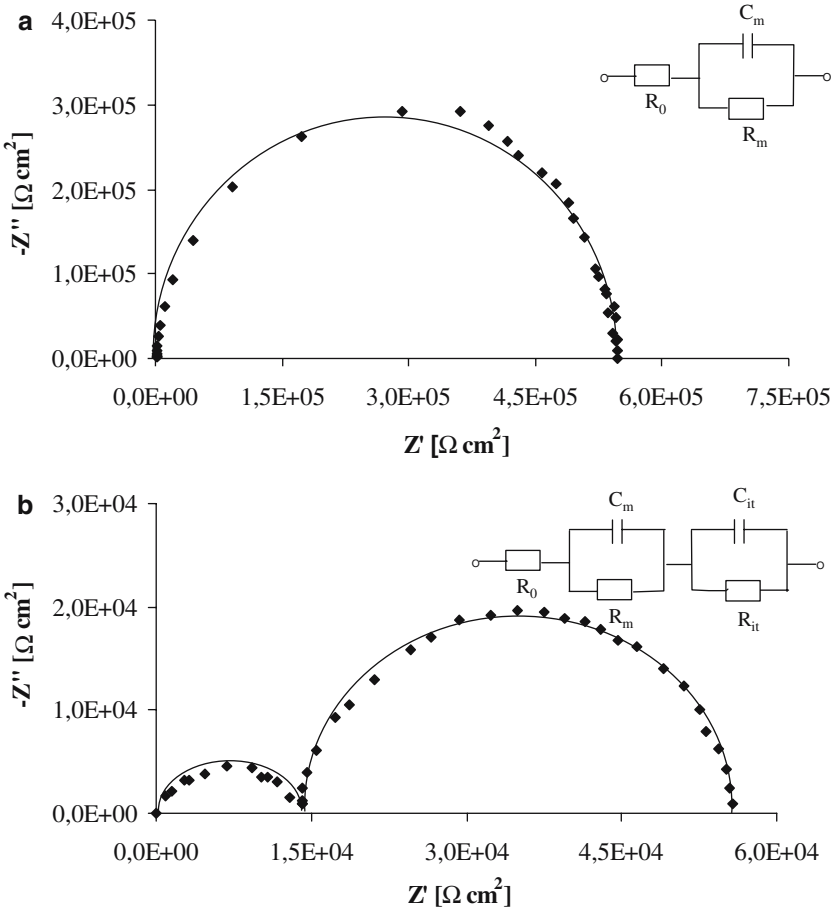

Fig. 1 Impedance spectra recorded for 0.001 M KCl: **a** a bilayer formed by phosphatidylcholine only, **b** a phosphatidylcholine bilayer modified with valinomycin (total valinomycin surface concentration is equal 1.24×10^{-13} mol cm^{-2}). The equivalents circuits used for impedance data analysis are shown in the *inset*. The *solid lines* represent the results of the fitting procedure

and the impedance representing the situation at the membrane interface. The membrane impedance is composed of the electric capacity of the membrane C_m, and of the electric resistance of the charged complex transport inside the membrane R_m. Capacity and resistance of the transfer across interface membrane/electrolyte solution are denoted by C and R_{it}, respectively (*it* stands for transfer across interface). Based on this equivalent circuit, the nonlinear least squares analysis was used to simulate the plots; then the values of the impedance parameters were extracted from the fit (the NLLQ fit is represented by the solid lines in Fig.1b).

The experimental values of the R_m^{-1}, C_m, R_{it}, and C_{it} parameters are presented in Figs. 2 and Fig. 3 as functions of potassium ion concentration in the solution and of total valinomycin surface concentration in the membrane. The total carrier surface concentration in the individual forming solution was calculated using Eq. 9. Taking into account the partition coefficient of the carrier as equal to 9.3×10^4 (Benz et al. 1973), the following total valinomycin surface concentrations were obtained: 1.24×10^{-13}, 1.64×10^{-13}, 2.42×10^{-13} and 4.63×10^{-13} mol cm^{-2}. For the sake of clarity, experimental errors have been omitted in Figs. 2a and 3a (deviations amounted to 15% of mean resistance values and the divergence of results increased with the increasing amount of the carrier which made the membrane unstable). The C_{it} and R_{it} values were determined for 0.0001, 0.001, and 0.01 M KCl because the formation of the second semicircle was observed in this electrolyte concentration range only; at frequencies lower than 398 Hz.

It can be concluded that an increase in potassium ion concentration at constant valinomycin surface concentration provokes a marked decrease in R_m (Fig. 2a) and R_{it} (Fig. 3a) as well as a marked increase in C_{it} (Fig. 3b). The dependence of C_m on KCl concentration was not taken into account in Fig. 2b because no clear variation was observed of the C_m values with electrolyte concentration [more or less constant membrane capacity value was also observed by other authors who studied the effect of valinomycin on potassium ion transport across lipid bilayers (Naumann et al. 2003)]. Both resistance values were also observed to decrease with increasing carrier concentration at constant K^+ ion concentration (increasing membrane conductivity), whereas the capacity of transfers across interface and membrane capacitance values were observed to increase. Increase in the membrane capacity can be explained by increasing valinomycin concentrations in the membrane, which results in increasing the electric permittivity of the lipid bilayer.

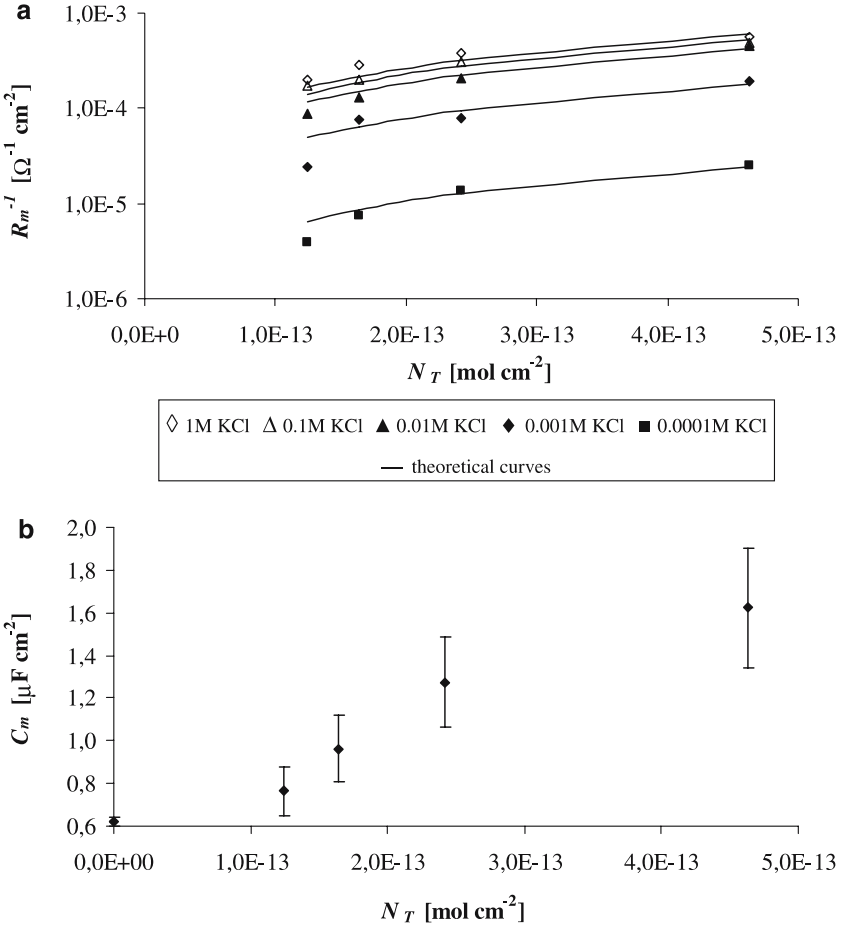

Fig. 2 The dependence of the conductance of the membrane (**a**) and the capacitance of the membrane (**b**) upon total valinomycin surface concentration at various electrolyte concentrations. The experimental values are marked by *points* and the theoretical values by *solid lines*

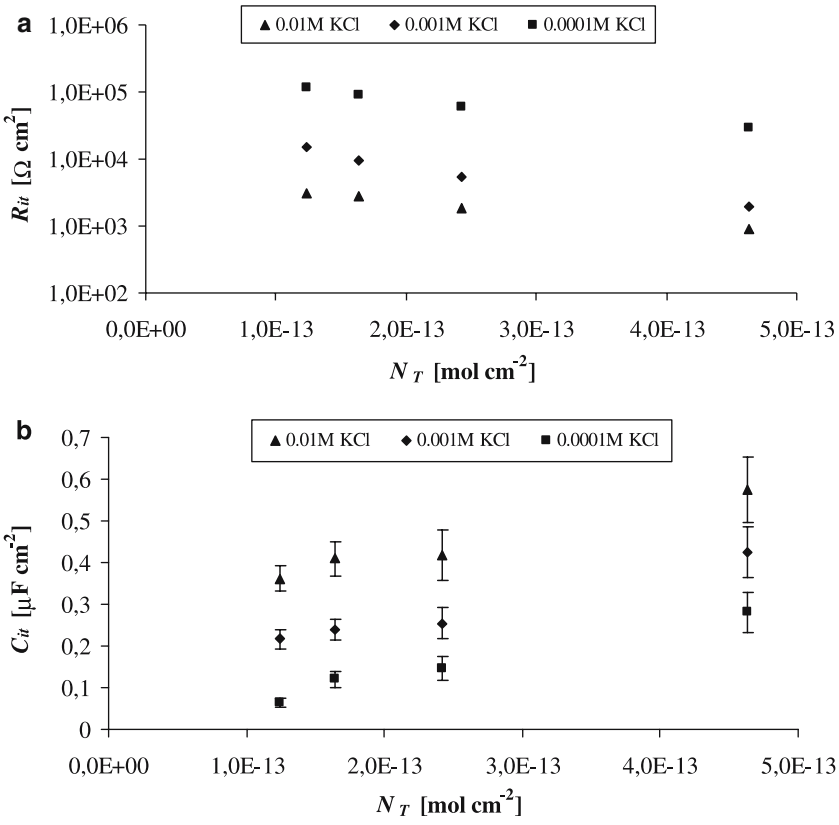

Fig. 3 The dependence of the resistance of the transfer across interface (**a**) and the capacitance of the transfer across interface (**b**) upon total valinomycin surface concentration at various electrolyte concentrations

As previously mentioned, the effect of a carrier on the conductivity of the lipid bilayer may be expressed by Eq. 11. This Eq. 11 can be written in the form: $y = ax$, where $y = R_m^{-1}$, $x = N_T$ and $a = S/d^2 \cdot \mu_{MS} \cdot F \cdot (K_h \cdot a_M)/(1 + K_h \cdot a_M)$. The a coefficient determined by the linear regression was applied to present the agreement of the Eq. 11 data (solid lines) with the experimental data (points) in Fig. 2a. It can be seen that the agreement between the theoretical and experimental values is good, which verifies the correctness of equations presented in this article. When comparing proportionality of the membrane conductance with the total valinomycin surface concentration, as presented in Fig. 2a, it is obvious that a single valinomycin molecule is the smallest transporting unit. From the linearity of this dependence in a broad concentration range, it is also evident that the molecule participates as a carrier and not a channel (Lauger 1972). Conductance is proportional to the potassium ion concentration logarithm, if the K^+ concentration is lower than 0.1 M (not shown here). This fact, together with the Fig. 2a data, suggests that the 1:1 valinomycin-K^+ complex is the carrier of charge in the membrane. Deviations from linearity observed at greater potassium ion concentrations suggest that the increase in conductance is slower than the increase in potassium ion concentration. Such a saturation is characteristic of a carrier system at high concentration of transported individuals where a great part of the carrier appears in the form of a complex. It is in agreement with the earlier results obtained for classical BLM systems (Lauger 1972).

The heterogeneous equilibrium constant can be calculated from Eq. 11. The slope of the straight line in the equation (now denoted by p) may be presented in the following way:

$$p = \frac{S}{d^2} \cdot \mu_{MS} \cdot F \cdot \frac{K_h \cdot a_M}{1 + K_h \cdot a_M} \tag{18}$$

The above equation can be modified to the following form, which is more suitable for calculation:

$$\frac{p}{a_M} = \frac{S}{d^2} \cdot \mu_{MS} \cdot F \cdot K_h - K_h \cdot p \tag{19}$$

This is an equation of the type $y = ax + b$, where $y = p/a_M$, $x = p$, $a = K_h$ and $b = S/d^2 \cdot \mu_{MS} \cdot F \cdot K_h$.

Equation 19 is presented in Fig. 4. The heterogeneous equilibrium constant of the 1:1 valinomycin-K^+ ion complex formation calculated from the a parameter amounts to about 0.44×10^3 dm^3 mol^{-1}. The value given for the heterogeneous equilibrium constant of the valinomycin-Rb^+ complex formation works out to be 0.4 dm^3 mol^{-1}, there is only one value accessible in literature for K_h (Benz et al. 1973). The correctness of our obtained value was checked by the determination of the percent of complexed valinomycin, which can be readily determined if the total valinomycin surface concentration and surface concentration of the complex are known. It amounts to 4.2% for 0.0001 M KCl, 30.6% for 0.001 M KCl, 79.9% for 0.01 M KCl, 97.1% for 0.1 M KCl, and 99.6% for 1 M KCl (percent of complexed valinomycin, taking into account the literature

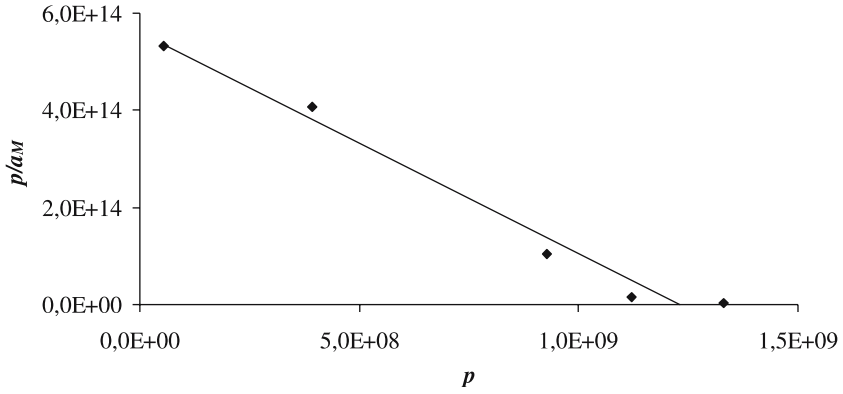

Fig. 4 The plot representing Eq. 19 for calculation of the heterogeneous equilibrium constant, where $p = S/d^2 \cdot \mu_{MS} \cdot F \cdot (K \cdot a_M)/(1 + K \cdot a_M)$

value of the heterogeneous equilibrium constant (Benz et al. 1973), is equal to 0% for 0.0001 M KCl, 0% for 0.001 M KCl, 0.4% for 0.01 M KCl, 3.2% for 0.1 M KCl, and 19.8% for 1 M KCl).

Knowledge of the stability constant of the 1:1 valinomycin-K^+ ion complex permitted us to determine the theoretical membrane conductance values as a function of potassium ion concentration at a given valinomycin concentration and to compare them with the experimental data. In order to obtain theoretical conductance values, calculations were based on Eq. 11 as denoted from the equation of the type $y = ax$, where $y = R_m^{-1}$, $x = (K_h \cdot a_M)/(1 + K_h \cdot a_M)$ and $a = S/d^2 \cdot \mu_{MS} \cdot F \cdot N_T$. This calculation was illustrated graphically in Fig. 5. As can be seen in Fig. 5, the theoretical and experimental results agree. Very good agreement between theoretical lines and experimental points can be observed in the high valinomycin concentration range in the bilayer. Small divergences were observed at small carrier concentrations in the bilayer. A low valinomycin-K^+ ion complex concentration in the bilayer can cause experimental errors resulting in differences of experimental and theoretical conductance values.

In order to determine the k_D value and to propose a correct equivalent circuit to reproduce the electric properties of the phosphatidylcholine membrane modified with valinomycin, the experimental data were substituted both for Eqs. 14, 15 and 16, 17. The results were found to agree at low frequencies i.e,. smaller than 5.906 Hz (data not shown). This fact was decisive in that the transfer across interface parameters could be related to the semicircle occurring in the impedance spectrum at low frequencies. The mean k_D value determined from Eqs. 14 and 15 amounts to $(18 \pm 7) \times 10^4$ s^{-1}. With the dissociation rate constant of the complex being known, the association rate constant was thus calculated from the $k_R = K_h \times k_D$ relationship. The resulting value was $(8 \pm 3) \times 10^8$ M^{-1} s^{-1}. Very different association and dissociation rate constant values of the complex could be found in the literature. The k_D values presented a range from 21 ± 5 s^{-1} (from the studies of carrier behaviour in semi-polar solvents system) to 2×10^4 s^{-1} (the case phosphatidylinositol membranes) and to 2×10^5 s^{-1} (the case of monooleic membranes). The k_R was found to range from 5×10^4 M^{-1} s^{-1} (the case phosphatidylinositol membranes) to 2×10^5 M^{-1} s^{-1} (the case of monooleic membranes); even a larger value, by four orders of magnitude, was given in the case of valinomycin and potassium ion association in methanol (Benz and Lauger 1976; Lauger 1980; Naumann et al. 2003; Haynes 1972;

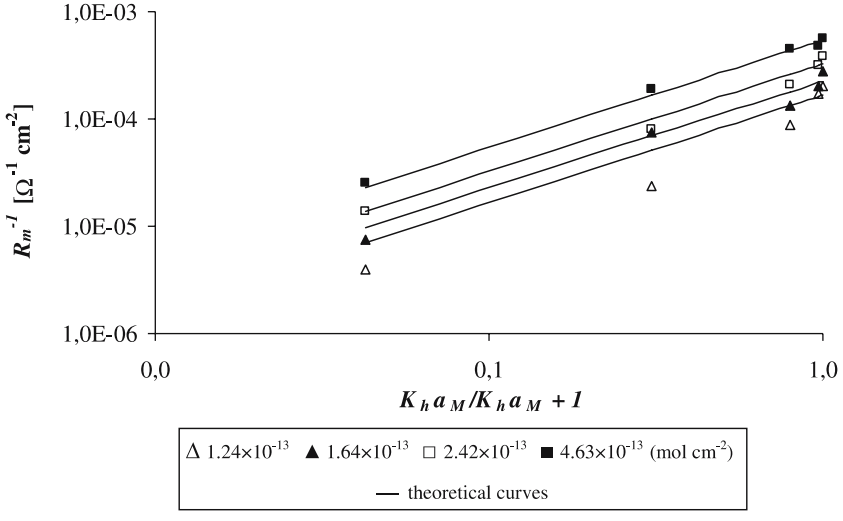

Fig. 5 The variation of membrane conductance as a function of potassium ion concentration at various valinomycin surface concentrations. The experimental values are marked by *points* and the theoretical values by *solid lines*

Grell et al. 1972). The rate constant values can depend on membrane structure and fluidity. Evidence can be found in the literature for a great effect of lipids within the membrane (the hydrocarbon chain length, the number of double bonds, the resulting functional group charge) upon the ion transport intermediated by valinomycin (Benz et al. 1973; Benz et al. 1977; Benz and Cros 1978).

Conclusions

Application of impedance spectroscopy to the study of the electrochemical behaviour of lipid bilayers allows one to provide a quantitative description of 1:1 valinomycin-K^+ ion complex. Basing on derived mathematical equations, a new method for the calculation of the heterogeneous equilibrium constant of the complex, association rate constant of the complex and dissociation rate constant of the complex was proposed.

Data presented in this work, received from mathematical derivation and confirmed experimentally are of great importance for interpretation of phenomena occurring in lipid monolayers and bilayers. In our opinion, these results can help in a better understanding of biological membranes and in their biophysical studies. A new, simple and very interesting method proposed by us can be used with success for the determination of the parameters used to describe any 1:1 carrier-monovalent ion complex.

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