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THE EFFECT OF SURFACE CHARGE DENSITY ON VALINOMYCIN-K^+ COMPLEX FORMATION IN MODEL MEMBRANES

J. CASPERS, M. LANDUYT-CAUFRIEZ, M. DELEERS and J.M. RUYSSCHAERT

Laboratoire de Chimie Physique des Macromolécules aux Interfaces, Faculté des Sciences, C.P. 206/2, Université Libre de Bruxelles, Bruxelles (Belgium)

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Summary

The model membrane approach was used to investigate the surface charge effect on the ion-antibiotic complexation process. Mixed monolayers of valinomycin and lipids were spread on subphases containing K^+ or Na^+. The surface charge density was modified by spreading ionizable valinomycin analogs on aqueous subphases of different pH or by changing the nature of the lipid (neutral, negatively charged) in the mixed film. Surface pressure and surface potential measurements demonstrated that a neutral lipid (phosphatidylcholine) or positively charged valinomycin analogs didn't enhance the antibiotic complexing capacity. However, a maximal complexation is reached for a critical lipid concentration in the valinomycin-phosphatidylserine mixed film. The role of the surface charge on the valinomycin complexing properties was examined in terms of the Gouy-Chapman theory. As a consequence of the negative charge of the lipid monolayer, the K^+ concentration near the surface is larger than the bulk concentration, by a Boltzmann factor. A good agreement was observed between the experimental results and the theoretical predictions. Conductance measurements of asymmetric bilayers containing a neutral lipid (egg lecithin) on one side and a negatively charged lipid (phosphatidylserine) on the other, confirm the role of the surface charge. Indeed, addition of K^+ to the neutral side of the bilayer containing valinomycin had no effect on the conductance whereas addition of K^+ to the charged side of the bilayer caused a 80-fold conductance increase.

Introduction

Several recent works have been devoted to the study of the changes in the cellular membrane permeability under the effect of various ionophores.

Respecting their mode of action, they can be divided into 2 main classes: the 'carriers' and the substances forming ion-conducting pores or channels. In the first case, a mobile carrier molecule binds the ion at one membrane solution interface, then migrates to the opposite interface and releases the ion into the aqueous phase. In the second process, several antibiotic molecules form an aggregate which bridges the membrane and may act as a pore.

Valinomycin has been shown to increase the K^+ permeability in natural and artificial lipid membranes and to act as a mobile carrier [1—4]. The influence of the surface charge of the membrane on its ion permeability has attracted the attention of several groups [5—10]. Attempts to demonstrate relationships between surface charge and membrane conductance have been made by means of experiments with black lipid bilayer membranes [5,6,9—11]. In the present study, the monolayer approach was chosen to investigate the surface charge effect on the ion-antibiotic complexation process.

The surface charge density was modified by spreading ionizable valinomycin analogs on aqueous subphases of different pH. The influence of the lipid was studied on lipid-valinomycin mixed films. An interpretation of the experimental results is given in terms of the Gouy-Chapman theory.

To complete the study, asymmetric lipid bilayers have been formed by apposition of two separate monolayers spread at the air-water interface. One monolayer was formed from negatively charged lipids and the other from neutral lipids [12,13]. The role of the surface charge asymmetry is investigated in terms of conductance change.

Materials and Methods

DL-α-Dipalmitoyl phosphatidylcholine, egg lecithin, glycerol monooleate and valinomycin were purchased from Sigma Chem. Co., and phosphatidylserine from Schwartz-Mann.

The antamanide sample was obtained as a gift from Dr. Birr, from the Max Planck Institute in Heidelberg. The valinomycin analogs in which a valyl residue is replaced by a lysyl or a glutamyl residue, have been generously supplied by Dr. V.T. Ivanov, from the Shemyakin Institute of Bioorganic Chemistry, U.S.S.R. Academy of Science, Moscow.

The substances dissolved in chloroform were spread at the air-water interface using a microsyringe 'Agla'. The ionic strength of the subphase was maintained at 10^{-2} or 10^{-1} M by addition of the calculated quantities of sodium or potassium chloride. The precision in the pH measurements is ±0.05 pH. The temperature was maintained at 23°C. The vibrating electrode technique was employed to measure the surface potential. The surface pressure isotherms were read on a Lauda Film Waage Balance. The accuracy is about ±0.05 dyne/cm for surface pressure measurements and ±5 mV for the surface potential measurements.

Asymmetric bilayers were formed by apposition of two separate monolayers spread at the air-water interface by the technique described by Montal and Muller [12,13]. The Teflon chamber to form the bilayer consisted of two compartments separated by a thin teflon partition containing a hole (0.25 mm^2) in its center. This teflon partition was 25 μm thick. The surface area of each com-

partment was 4 cm^2 and the volume 3 cm^3. Each compartment was equipped with Ag/AgCl electrodes connected with a Keithley Electrometer (Model 602). The complete system was enclosed in a Faraday cage. Membranes were formed by adding solutions simultaneously to both compartments and lifting the liquid until the hole was completely covered.

Results and Discussion

(1) Valinomycin monolayers

Fig. 1 shows the evolution of the surface potential ΔV obtained with a close-packed valinomycin monolayer spread on subphases containing KCl or NaCl increasing concentrations. Increasing NaCl concentrations do not modify significantly the ΔV value (500 mV). However, if valinomycin is spread on a support containing K^+, the surface potential reaches a value of 1010 mV for an ion concentration superior to 2.5 M. The minimum packing areas (A_0 value) obtained with subphase containing KCl or NaCl were in excellent agreement with the dimensions predicted from the space filling models [14,15]: 190 $Å^2$ when the subphase contains NaCl or a weak concentration of KCl and 160 $Å^2$ for a KCl concentration superior to 2.5 M. The ΔV value obtained for all NaCl concentrations is considered as that of the uncomplexed form (ΔV_f) and the value observed for a high K^+ concentration as that of the maximum complexation state (ΔV_c).

Under these conditions, the value of the association degree β can be determined from a relation similar to that used to determine the ionization degree of an acid or basic group at the air-water interface [15—19]:

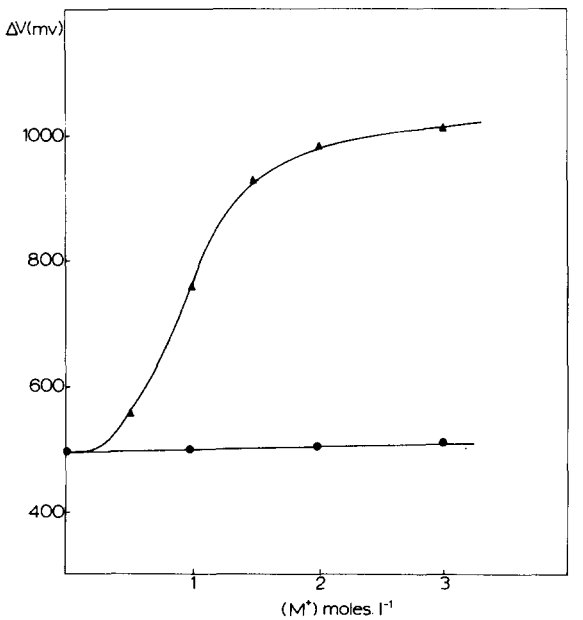

Fig. 1. Surface potential of valinomycin monolayer (measured at 28 dynes · cm^{-1}) as a function of the subphase ionic concentration. ▲, KCl; ●, NaCl; pH 5.5.

$$\beta = \frac{\Delta V - \Delta V_f}{\Delta V_c - \Delta V_f} \quad (1)$$

ΔV is the value of the surface potential measured for an intermediate ion concentration.

The equilibrium constant of the surface reaction:

Antibiotic + $M^+ \rightleftharpoons$ antibiotic-M^+

is [15, 17]:

$$K_c = \frac{\beta}{1-\beta} \cdot \frac{1}{[M^+]_s} = \frac{\Delta V - \Delta V_f}{\Delta V_c - \Delta V} \cdot \frac{1}{[M^+]_s} \quad (2)$$

$[M^+]_s$ is the surface concentration and is related to the bulk concentration $[M^+]$ by a Boltzmann factor:

$$[M^+]_s = [M^+]\, e^{-\epsilon\Psi/kT} \quad (3)$$

ϵ is the electronic charge, T the absolute temperature and k the Boltzmann constant. The electrostatic potential, ψ, can be determined by the Gouy-Chapman relation [20]. At 20°C, and for ψ expressed in mV:

$$\Psi = 50.4\ \mathrm{sh}^{-1}\ \frac{134\sigma}{c^{1/2}} \quad (4)$$

c, in M, is the monovalent electrolyte concentration in the subphase and σ is the surface charge density.

If N^+ is the number of molecules with a positive resultant charge, N the total number of spread molecules and A the area occupied per molecule, the surface charge density is:

$$\sigma = \frac{N^+}{NA} = \frac{\beta}{A}$$

From Eqns. 1—4, the evolution of β against the K^+ surface concentration is given in Fig. 2. The same figure shows also the results obtained with a monolayer of antamanide, an ionophore selective of Na^+ [17].

Comparison of the $\beta = f[M^+]_s$ functions for the two antibiotics shows a clear difference. For antamanide, Eqn. 2 gives a value of K_c (=10 M^{-1}) independent of the ionic strength. For valinomycin, however, a constant value of K_c cannot be determined by this method. This result confirms the observation of Kemp

TABLE I

SURFACE POTENTIAL (mV) OF THE VALINOMYCIN ANALOGS AS A FUNCTION OF THE pH AND THE NATURE OF THE CATION PRESENT IN THE SUBPHASE

Support		Valinomycin-Glu	Valinomycin-Lys
pH 2	0.1 M KCl	480	500
	0.1 M NaCl	460	500
pH 11.5	0.1 M KCl	450	430
	0.1 M NaCl	350	410

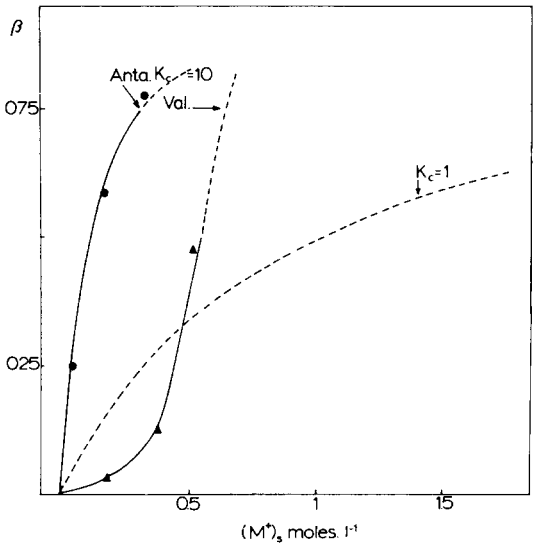

Fig. 2. Association degree β as a function of the surface ionic concentration $[M^+]_s$. ▲, valinomycin; ●, antamanide. The dotted line corresponds to a value of 1 M^{-1} for K_c.

and Wenner: the valinomycin-K^+ interaction in monolayers cannot be described by a simple association [14,15].

The difference between the values of the surface potential obtained for valinomycin and valinomycin-K^+ complex, supposes probably a modification in the molecular geometry during the complexation process [21]. The parameter β determined by relation 1 must thus be considered, in the case of this antibiotic, as an apparent 'parameter'.

(2) Valinomycin analog monolayers

To investigate the role of the charge density on the complexation process, the ionizable valinomycin analogs were spread at the air-water interface. The valinomycin analogs differ from the parent compound in the replacement of a L-valyl residue by an L-lysyl or an L-glutamyl residue [22]. If the pH of the subphase is maintained at pH 11.5 and at pH 2, respectively, for the two analogs, the free antibiotic forms are unionized. Fig. 3 shows the evolution of ΔV vs. K^+ concentration. Comparison of the isotherms obtained for the two valinomycin analogs (Fig. 3) with the valinomycin isotherm (Fig. 1) would suggest the non-cooperativity of the valinomycin analogs-K^+ complexation process. In the same concentration range, no significant change in the surface potential was observed on subphases containing NaCl. The specificity of these analogs for the K^+ is thus maintained.

A pH change from 11.5 to 2 for the lysyl analog induces a positive ionization of the lysyl residue. Table I indicates that this ionization increases the ΔV value.

The surface potential of a charged monolayer can be described by the equation [20,23]:

$$\Delta V = 12\ \Pi n\mu + \Psi \qquad (5)$$

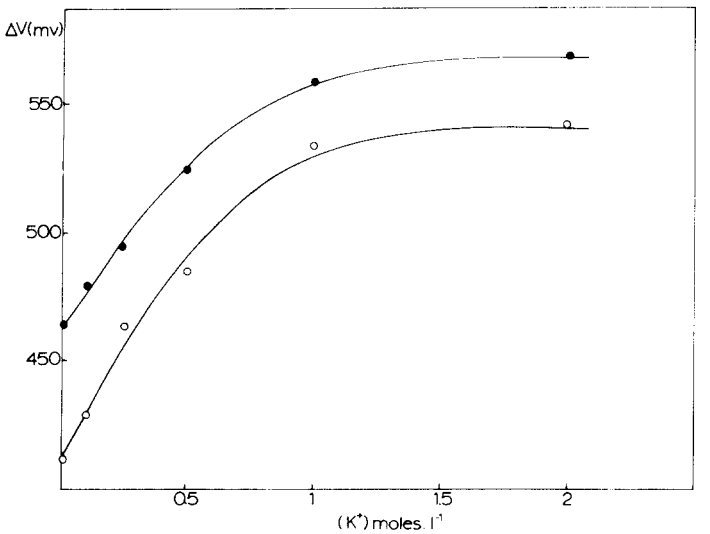

Fig. 3. Surface potential of valinomycin analogs (measured at 25 dynes · cm^{-1}) as a function of the subphase ionic concentration. ●, valinomycin-Glu at pH 2; ○, valinomycin-Lys at pH 11.5.

n is the number of molecules per $Å^2$ and μ, the contribution due to the vertical component of the total dipole moment. The positive electrostatic potential ψ is given in Eqn. 4. The increase of surface potential is clearly correlated to this positive electrostatic contribution. Moreover the effect is non specific and does not depend on the nature of the cation present in the aqueous phase (Table I). When the pH of the subphase changes from 2 to 11.5 for the other analog, the glutamyl residue is negatively ionized. The decrease of the surface potential is 110 mV when Na^+ is present in the subphase and only 30 mV in the presence of K^+.

With the K^+, the surface potential variation observed can be explained if we admit that a fraction of the spread molecules exist in the form of a neutral complex with this ion. However, the existence of a high proportion of the complexed form when K^+ concentration is 10^{-1} M, is improbable (Fig. 3). Thus, we have to admit that the ionization of the glutamyl group strongly modifies the K^+ surface concentration (Eqn. 3) in a favourable way to the reaction:

$$\text{Valinomycin-Glu}^- \rightarrow \text{Valinomycin-Glu}^-\text{-}K^+$$

With Na^+, the same increase in surface concentration exists but no 'charge compensation effect' is observed. In this case, the strong decrease of the ΔV value is due to the negative electrostatic potential contribution. If the surface antibiotic charge is positive (ionization of the lysyl residue), the formation of the complex with K^+ ion is obviously not obtained when the K^+ concentration is 10^{-1} M. We can even observe in this case that the formation of the complex remains unfavourable at much higher K^+ concentrations of the subphase. Indeed, at pH 2, when the KCl concentration passes from 10^{-1} to 2 M, the surface potential of the lysyl analog increases to 25 mV. This value represents only 23% of the increase obtained at pH 11.5 (Fig. 3) for a similar variation of the ionic strength of the support.

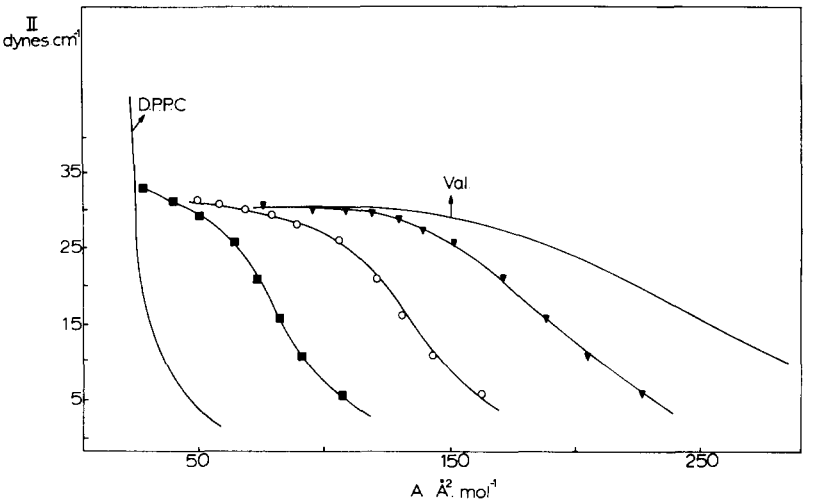

Fig. 4. Surface pressure-mean molecular area curves for mixed monolayers of phosphatidylcholine (DPPC) and valinomycin, on 10^{-1} M KCl; pH 5.5. ▲, $X_V = 0.75$; ○, $X_V = 0.50$; ■, $X_V = 0.25$.

These results prove the qualitative influence of the charge on the formation of the valinomycin-K^+ complex at the interface.

In the membrane, the surface charge density will depend on the nature of the hydrophilic part of the lipid. To investigate the influence of this charge density of the complexation process, valinomycin-lipid mixed films were formed in the air-water interface.

(3) Mixed films of valinomycin and phospholipids

Figs. 4 and 5 show the surface pressure evolution of the mixed films as a

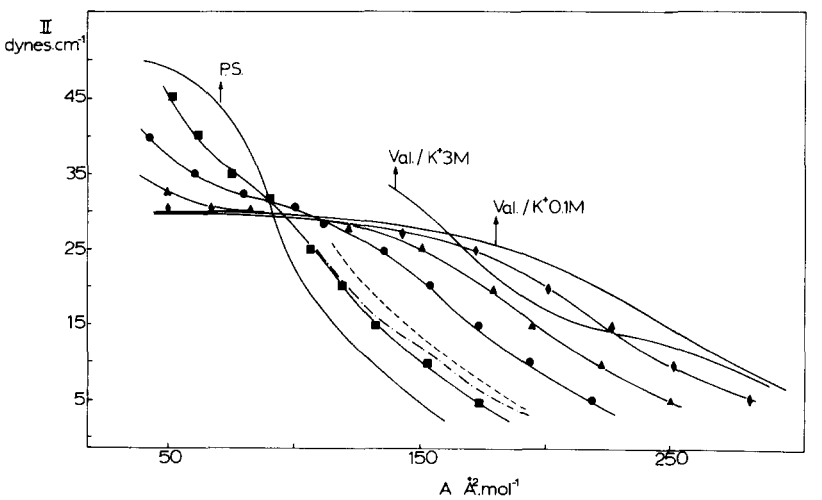

Fig. 5. Surface pressure-mean molecular area curves for mixed monolayers of phosphatidylserine and valinomycin, on 10^{-1} M KCl; pH 5.5. ◆, $X_V = 0.85$; ▲, $X_V = 0.65$; ●, $X_V = 0.45$; ■, $X_V = 0.20$. Experimental values are in agreement with theoretical predictions, except if $X_V = 0.20$. - - - - -, calculated curve for $X_V = 0.20$ (free form of valinomycin); · · · · · ·, calculated curve for $X_V = 0.20$ (complexed form of valinomycin).

function of the mean molecular area on various subphases in which the ionic strength is maintained at 0.1 M NaCl or KCl. For this value of K^+ concentration, the valinomycin spread alone exists at the interface in the free form (Fig. 1). If the mixed monolayer behaves ideally [14,24], the average molecular area A_{pv} in the two-component films can be written:

$$A_{pv} = A_p X_p + A_v X_v \tag{6}$$

A_p and A_v are the molecular area in the two single-component films at the same surface pressure, X_p and X_v are their molar fraction in the mixture. The agreement obtained between the experimental and the calculated curves in the case of the mixtures valinomycin-dipalmitoyl phosphatidylcholine (Fig. 4) demonstrates the absence of interaction between the two film components and confirms the results obtained by other authors [14]. We find a rather similar situation if we replace the neutral phosphatidylcholine, with the phosphatidylserine negatively charged at the pH of the experience on a 10^{-1} M NaCl subphase.

However the agreement between experimental and calculated values is not observed with the phosphatidylserine-valinomycin system spread on a 10^{-1} M KCl subphase, if a critical value ($X_v \approx 0.20$) is reached. A better agreement is observed if the area occupied per valinomycin molecule (Eqn. 6) is assimilated to the area of the complexed form (valinomycin isotherm on a 3 M KCl subphase), in a well defined interval of surface pressure (Fig. 5). This result seems to prove that the antibiotic passes from the free to the complexed form if the phosphatidylserine molar fraction reaches a critical value.

In order to test this hypothesis, the different mixed films were studied by surface potential.

Fig. 6 shows the evolution of the surface potential of the valinomycin-

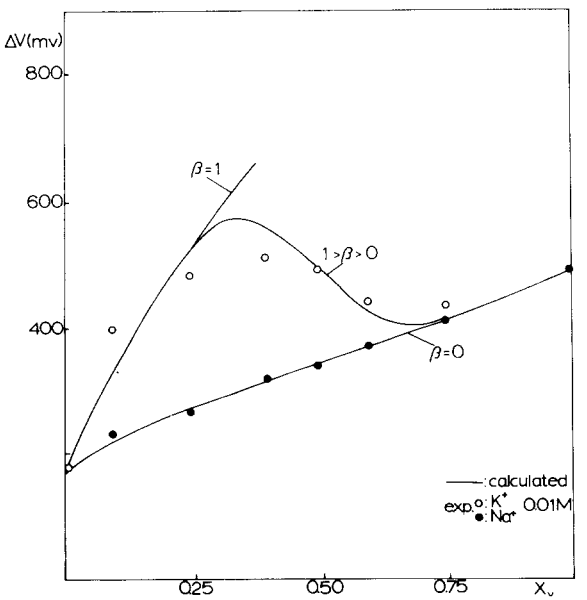

Fig. 6. Comparison of the calculated and experimental values of the surface potential ΔV of mixtures valinomycin-phosphatidylserine on 10^{-2} M KCl.

phosphatidylserine mixed films (close packed films) as a function of the antibiotic molar fraction. The $\Delta V = f(X_v)$ curves show, at low KCl concentration of the subphase (10^{-2} M) a maximum near $X_v \approx 0.35$. This effect is not observed in presence of NaCl.

To give a quantitative explanation of the shape of the $\Delta V = f(X_v)$ curve as a function of the nature of the cation present in the subphase, it was essential to define the various contributions to the surface potential of the mixed films. The surface potential of a miscible phosphatidylserine-valinomycin film might be described by a single expression:

$$\Delta V_{pv} = 12\ \Pi(n_p\mu_p + n_v\mu_v) + \Psi_{pv} \tag{7}$$

n_p and n_v are the number of molecules of each component per Å^2, μ_p and μ_v are the vertical dipolar contribution of the two components (in mD) in the mixture.

For N_p phospholipid molecules and N_v valinomycin molecules, the total area occupied by the mixed film is: $N_pA_p + N_vA_v$ and it can be written for the dipolar term of the mixture:

$$\Delta V_{pv}^{dip} = \frac{12\ \Pi}{A_{pv}}(\mu_pX_p + \mu_vX_v) \tag{8}$$

A_{pv} (in Å^2) is defined in Eqn. 6. The determination of this term supposes the numerical evaluation of the dipolar moment value of the two components alone.

For the valinomycin monolayer, if no electrostatic contribution is taken into account, the surface potential can be simply described as a function of the vertical component of the dipolar moment:

$$\Delta V_v = 12\ \Pi n\mu_v = 12\ \Pi\frac{\mu_v}{A_v}$$

From Eqn. 1, it follows that:

$$\Delta V_v = 12\ \Pi\frac{\mu_c}{A_c}\beta + \frac{12\ \Pi\mu_f}{A_f}(1-\beta)$$

μ_c and μ_f are respectively the vertical contributions of the dipolar moments of the complexed and free forms of the antibiotic, A_c and A_f are the areas occupied by each of the two forms. In the present case, one can define an apparent dipolar moment:

$$\mu_v = [\mu_c\beta + \mu_f(1-\beta)] = \frac{\Delta V_vA_v}{12\ \Pi} \tag{9}$$

with $160\text{Å}^2 < A_v < 190\text{Å}^2$

Eqn. 9 should permit to calculate the dipolar moment, μ_v, of the valinomycin as a function of the association degree β. An electrostatic contribution due to the formation of a positively charged valinomycin-K^+ complex at the interface should modify these calculated values. According to Eqn. 5, the surface potential should include an additional term.

However, for low values of β ($\beta < 0.5$) and of the KCl concentration of the aqueous phase, ψ can be calculated from the Gouy-Chapman theory (Eqn. 4).

This calculated value represents less than 2,5% of the ΔV value. On the other hand, if β is high ($\beta > 0{,}5$), the high KCl concentration of the subphase should also tend to minimize the importance of the electrostatic correction to the dipolar term. In conclusion, Eqn. 9 appears as a correct approximation to evaluate the valinomycin dipolar moment. We obtain for the free form of the antibiotic ($\beta = 0$), 2.5 Debye and for the complexed form ($\beta = 1$), 4.3 Debye.

For the phosphatidylserine spread alone, the surface potential is given by:

$$\Delta V_p = \frac{12\,\Pi\mu_p}{A_p} + \Psi_p$$

At the pH of our experiments, the phosphatidylserine film is completely ionized [25]. The calculated values of ψ_p (Eqn. 4) are -131 mV for $c = 10^{-1}$ M and -189 mV for $c = 10^{-2}$ M. The measured values of the surface potential, ΔV_p, are respectively 230 and 180 mV. From the ΔV_p and ψ_p values, we can determine the dipolar moment μ_p of the ionized phospholipid. The very similar values obtained on the two subphases (594 and 607 mD) are compatible with the hypothesis usually admitted: there is a very light influence of the salt concentration in the subphase on the dipolar contribution to the surface potential [23].

The values of μ_p and μ_v are now calculated. It is essential to evaluate the electrostatic contribution ψ_{pv} in the mixture. For a negatively charged film, the surface charge density, σ, will be equal to N^-/NA where N^- is the number of charged molecules, N is the total number of molecules and NA is the total film area. In the case of a one component system, completely ionized, $N^-/N = 1$ and $\sigma = 1/A$ where A is the area occupied per spread molecule. If the film is now partially ionized, the ionization degree of the film is defined by $\alpha = N^-/N$, and $\sigma = \alpha/A$. Finally, in the case of a film with two components, one negatively charged and the other positively it follows that:

$$\sigma = \left| \frac{N_p^- - N_v^+}{N_p A_p + N_v A_v} \right|$$

And, if the two components are completely ionized:

$$\sigma = \left| \frac{X_p - X_v}{A_{pv}} \right|$$

A_{pv} is defined by Eqn. 6.

If $N_p^- < N_p$ and $N_v^+ < N_v$, $N_p^-/N_p = \alpha$ and $N_v^+/N_v = \beta$; Eqn. 4 gives:

$$\psi_{pv} = 50.4 \text{ sh}^{-1} \frac{134 |X_p \alpha - X_v \beta|}{A_{pv} c^{1/2}} \tag{10}$$

Because the phosphatidylserine is totally ionized, α is equal to 1. From Eqns. 7, 8 and 10, one can calculate, for all compositions of the valinomycin-phosphatidylserine mixtures, the ΔV value for the two extreme situations: absence of complexation ($\beta = 0$) and optimal complexation ($\beta = 1$).

Table II allows to compare the calculated results and the experimental values obtained on the two subphases (KCl and NaCl).

A reasonable agreement is obtained between the experimental results on NaCl subphases (10^{-2} M) and the ΔV calculated values for $\beta = 0$. Because of the

TABLE II

SURFACE POTENTIAL CALCULATED (FOR $\beta = 0$ AND $\beta = 1$) AND MEASURED (ON 10^{-2} M KCl OR NaCl SUBPHASE) OF MIXED FILMS VALINOMYCIN-PHOSPHATIDYLSERINE

X_v	ΔV calculated (mV)		ΔV measured (mV)	
	$\beta = 0$	$\beta = 1$	Na^+	K^+
0	—	—	180	180
0.10	228	341	240	405
0.25	282	527	275	490
0.40	323	689	330	520
0.50	349	830	350	500
0.60	374	956	380	450
0.75	412	1052	420	450
1	—	—	500	500

absence of specificity of valinomycin for Na^+, this result demonstrates the validity of the calculation used to determine the resultant surface potential.

If KCl is present in the aqueous phase (10^{-2} M), reasonable agreement between experimental and calculated ΔV values for $\beta = 1$ is observed only for low valinomycin concentrations ($X_v < 0{,}25$). If one increases the proportion of valinomycin in the mixed films, the ΔV experimental values show a maximum, then decrease and reach the values obtained on NaCl. A decrease of the molar fraction of the negatively charged phospholipid induces a decrease of the complexed fraction of valinomycin. No similar curves were observed in presence of a neutral lipid (egg lecithin). Fig. 1 indicates the absence of valinomycin-K^+ complex for KCl concentrations in the range 10^{-2}—10^{-1} M. The effect observed in presence of phosphatidylserine must be attributed to the charge density associated with the lipid film. The surface charge density will increase the K^+ surface concentration and allows the complexation process. At low phosphatidylserine surface concentration, the ion distribution is much less modified. We are passing from a situation characterized by a high K^+ surface concentration ($X_p \approx 1$) to a situation in which the K^+ surface concentration and bulk concentration are similar ($X_p << 1$).

The possibility to determine the surface concentration $[K^+]_s$ in presence of the mixed films should allow to calculate a $\Delta V_{pv} = f(X_v)$ function in agreement with the experimental results. The electrostatic contribution, ψ_{pv}, for $0 < \beta < 1$, can be determined by a graphical method illustrated in Fig. 7. An arbitrary series of values between 0 and 1, for different mixtures of valinomycin-phosphatidylserine are given to β. The ψ_{pv} values are calculated from Eqn. 10 and plotted on the ordinate in the diagram $\psi_{pv} = f(X_v)$. Each curve corresponds to a defined value of β. The value of $[K^+]_s$, for each ψ_{pv} value is then determined by Eqn. 3 and plotted on the second axis of the ordinates. From Fig. 2, by interpolation on the diagram, the β values which correspond to those different K^+ surface concentrations can be found. The graph obtained by joining these points gives us the evolution of the $\psi_{pv} = f(X_v)$ function.

The dipolar contribution to the surface potential is calculated from Eqn. 8. The valinomycin dipolar moment is obtained, for all β values, from Eqn. 9. Eqn. 7 allows to calculate the resultant surface potential. Fig. 6 allows the com-

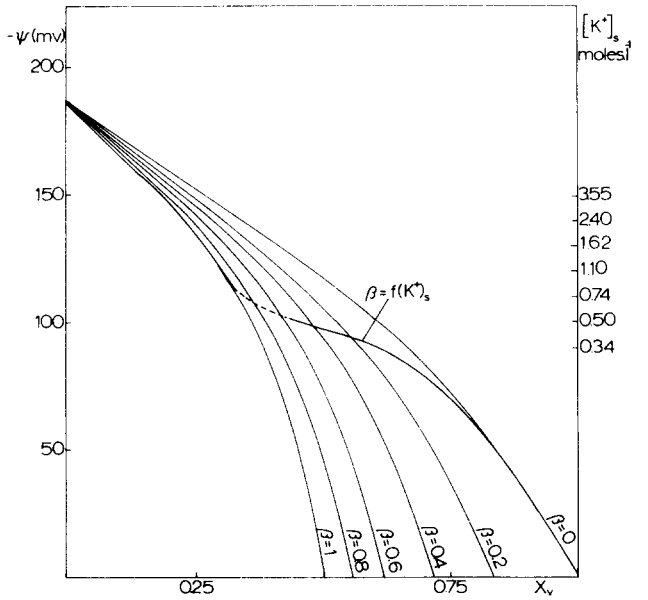

Fig. 7. Graphical determination of the electrostatic potential ψ of mixtures valinomycin-phosphatidylserine on 10^{-2} M (see text).

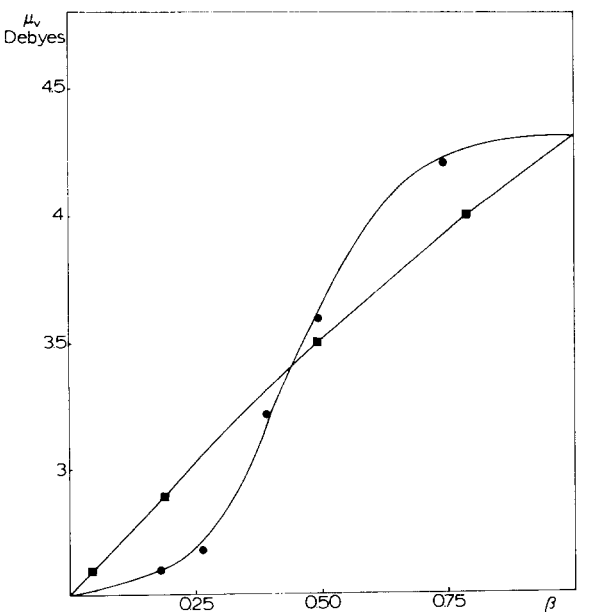

Fig. 8. Comparison of two evolutions of the valinomycin dipolar moment with the association degree β on 10^{-2} M KCl. ■, calculated from Eqn. 9; ●, calculated from the experimental results with the hypothesis $K_c = 1\ M^{-1}$.

parison of the theoretical curves and the experimental points.

The divergence observed if $X_v < 0.25$ can be due to an overevaluation of the ψ_{pv} term. But in the range $0.25 < X_v < 0.75$, the discrepancy between the experimental and calculated ΔV values is a consequence of the assimilation to the association degree of the apparent parameter determined by Eqn. 1. For $K_c = 1$ (mentioned by other authors [26–28] at the lipid-water interface), Fig. 2 shows the evolution of β as a function of $[K^+]_s$, according to Eqn. 2. From Fig. 7, ψ_{pv} is recalculated for each mixture composition. The difference between $\Delta V_{experimental}$ (Fig. 6) and this ψ_{pv} calculated value gives the dipolar contribution. From Eqn. 8, we calculated the dipolar moment of valinomycin alone.

Fig. 8 illustrates the evolution of the valinomycin dipolar moment as a function of β compared with the linear function resulting from Eqn. 9. The sigmoidal relationship may be due to the conformational change undergone by the valinomycin during the complexion process at the interface and can explain the divergences observed between the experimental and calculated values of ΔV_{pv}, in the Fig. 6.

In conclusion, a good agreement is observed between the experimental results and the theoretical predictions. This monolayer approach agrees with the basic results obtained by McLaughlin and Eisenberg on lipid bilayers [29]. Clearly, the lipid charge modulates the valinomycin-K^+ complexation process, as a consequence of a modification of the K^+ concentration in the layer adjacent to the surface.

(4) Asymmetric bilayers

Measurements on black lipid membranes showed that the membrane conductance was influenced by the ionization of the lipid [5,6]. However, these studies did not distinguish definitively the electrostatic and hydrophobic contributions to the conductance because the nature of the lipid hydrocarbon chain was different for charged and neutral lipids. For these reasons, asymmetric bilayers were formed, in the present work, using the Montal's technique [12,13,30]. Monolayers were spread on the surface of the electrolyte solution. One monolayer was formed from a glycerolmonooleate-egg lecithin mixture

TABLE III

CONDUCTANCE OF ASYMMETRIC BILAYERS (IN $\Omega^{-1} \cdot cm^{-2}$) IN THE PRESENCE OF VALINOMYCIN

Neutral layer : glycerolmonooleate/egg lecithin (20 : 80), Charged layer : glycerolmonooleate/phosphatidylserine (20 : 80). Conductance was measured in an interval of applied voltage between −60 mV and +60 mV. The negative side corresponds to the high K^+ concentration compartment (0.1 M KCl + 0.005 M NaCl) and the positive side to the high Na^+ concentration compartment (0.1 M NaCl + 0.005 M KCl).

System				Valinomycin in the membrane phase		
				10^{-5} M	10^{-4} M	10^{-3} M
0.005 M Na^+ 0.1 M K^+	neutral layer	charged layer	Na^+ 0.1 M K^+ 0.005 M	$1.1 \cdot 10^{-6}$	$1.1 \cdot 10^{-6}$	$1 \cdot 10^{-6}$
0.005 M Na^+ 0.1 M K^+	charged layer	neutral layer	Na^+ 0.1 M K^+ 0.005 M	$2.2 \cdot 10^{-6}$	$2.7 \cdot 10^{-5}$	$8.2 \cdot 10^{-5}$

(20 : 80), the other from a glycerolmonooleate-phosphatidylserine mixture (20 : 80). The aqueous phase in a compartment consisted of 10^{-1} M KCl + 0.005 M NaCl at pH 7.3 and of 10^{-1} M NaCl + 0.005 M KCl at the same pH in the other compartment.

If K^+ (0.1 M) was present in the compartment adjacent to the neutral side of the bilayer, conductance did not depend on the valinomycin concentration in the membrane phase (Table III). The K^+ surface concentration gradient between the 2 sides of the bilayer could be qualitatively described by: $[K^+_{surface}]_{chargedside} \gg 0.05$ M and $[K^+_{surface}]_{neutralside} = 0.1$ M and an increase of the valinomycin concentration in the membrane phase does not modify the transport process. However a drastic conductance increase was observed if the K^+ was present in the compartment adjacent to the charged side of the bilayer. The K^+ surface concentration gradient would be higher ($[K^+_{surface}]_{chargedside} \gg 0.1$ M and $[K^+_{surface}]_{neutralside} = 0.005$ M) and an increase of valinomycin concentration from 10^{-5} to 10^{-4} M gives approximately a ten-fold increase in conductance. A modification of the membrane/valinomycin concentration from 10^{-4} M to 10^{-3} M, however, induces only a 3-fold charge in conductance indicating the begin of a saturation process.

Finally, the conductances obtained, for a 10^{-3} M valinomycin concentration in the lipid phase, are 80-fold higher if K^+ is present in the compartment adjacent to the charged side than if K^+ is present in the compartment adjacent to the neutral side. Again, the accumulation of negative surface charges on one side of the bilayer, induced an increase of the K^+ surface concentration and enhanced the complexation process.

Conclusions

In alcoholic solutions, the stability constant of a valinomycin-K^+ complex decreases from 10^5 to 10^2 M^{-1} as the water content is increased to 60% (in mol) [21,22]. This solvent dependence of the complexing selectivity can explain the high K^+ concentration necessary to the valinomycin complex formation at the air-water interface. In the present paper, we present evidence of the influence of the lipidic environment on the interfacial complexation process.

$$\underset{\text{(monolayer)}}{\text{Valinomycin}} + K^+_{(\text{adjacent to monolayer})} \rightleftharpoons \underset{\text{(monolayer)}}{\text{Valinomycin} - K^+}$$

The arrangement of the lipid monolayer with polar head groups oriented at the interface means that an electrostatic field extends out into the surrounding water. This field is relatively small for zwitterionic phospholipids (phosphatidylcholine) but is appreciable for a lipid carrying a net negative charge (phosphatidylserine). The effect of the electrostatic field will be to attract positively charged ions into the vicinity of the interface between the lipid layer and the aqueous solution. Surface pressure and surface potential measurements demonstrated that neutral lipid (phosphatidylcholine) or positively charged valinomycin analogs did not enhance the antibiotic complexing capacity. However, a maximal complexation is reached for a critical phosphatidylserine concentration in a valinomycin-phosphatidylserine mixed monolayer.

The role of the surface charge on the valinomycin complexing properties was examined in terms of the Gouy-Chapman theory. As consequence of the negative charge of the lipid monolayer, the K^+ concentration near the surface is larger than the bulk concentration by a Boltzmann factor. A good agreement was observed between the experimental results and the theoretical predictions.

Conductance measurements of asymmetric bilayers containing a neutral lipid (egg lecithin) on one side and a negatively charged lipid (phosphatidylserine) on the other, confirm the role of the surface charge: addition of K^+ to the neutral side of the bilayer containing valinomycin had no effect on the conductance whereas addition of K^+ to the charged side of the bilayer caused a 80-fold conductance increase. Such model membranes are of interest because similar lipid asymmetry exists in biological membranes. Indeed, it was proposed that, in membranes of human red blood cells, phosphatidylethanolamine and phosphatidylserine are mainly in the cytoplasmic monolayer of the bilayer and that phosphatidylcholine and sphyngomyelin are in the external monolayer [31]. Our results suggest that accumulation of negatively charged lipids on one side of the bilayer would enhance drastically the recognition process between a membrane site and specific charged molecules.

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