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Modulation of the adsorption of alkaline cations to phosphatidylglycerol by a dimannosyldiacylglycerol

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Interactions between alkaline cations and phosphatidylglycerol alone or mixed with dimannosyldiacylglycerol were studied by means of electrophoretic mobility and surface potential measurements. The data were interpreted using the Gouy-Chapman-Stern theory of the electric double layer. The mobilities were found to vary in the increasing order $Li^+ < Na^+ \approx K^+ < Cs^+$ with phosphatidylglycerol alone. As the molar ratio of dimannosyldiacylglycerol was increased, the mobility order was exactly reversed. Moreover, the presence of the glyceroglycolipid leads to a clear-cut discrimination between Na^+ and K^+. By fitting the zeta potentials obtained from the mobilities of phosphatidylglycerol dispersions, the following adsorption constants of the monovalent cations to the lipid were calculated (in M^{-1}): Li^+ 0.5, Na^+ 0.295, K^+ 0.295, Cs^+ 0.139. These adsorption constants were modulated by the presence of the dimannosyldiacylglycerol. For example, for a phosphatidylglycerol/dimannosyldiacylglycerol mixture in the 2:1 molar ratio as usually found in the membrane of a bacterium such as *Micrococcus luteus*, calculation gives the following adsorption constants (in M^{-1}): Li^+ 0.007, Na^+ 0.11, K^+ 0.682, Cs^+ 0.958. The combination of the zeta potentials obtained for lipid dispersions and surface potentials measured on lipid monolayers of the same composition showed that the interactions between the phospholipid and the glycolipid are mainly electrostatic in nature. This suggests strongly that the glycolipid might act as a modulator of the electrical properties of the bacterial membrane.

Introduction

The large diversity in glycolipid structure probably implies a variety of biological functions. Many attempts have been made to find out relationships between the structure of this class of lipids and some functional roles. Recently these two aspects have been reviewed for plant, mammalian and bacterial glycolipids [1,2]. The physical properties of these molecules have been extensively studied, in particular those galactolipids from plants [3] and glyceroglycolipids from *Acholeplasma laidlawii* membrane [4–8], with respect to both fatty-acyl chain composition and the structure of the polar head groups. The main result was that the diglucosyl- and monoglucosyldiacylglycerol from the mycoplasma membrane were found to exhibit a lamellar and non lamellar (cubic and reverse hexagonal-II) phases, respectively.

Concerning the interactions with the aqueous phase, the physical properties of glyceroglycolipids have been studied in monolayers [9,10]. The molecular packing of mono galactosyl- and di-

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galactosyldiacylglycerol appeared to be more expanded on ionic subphases than on pure water, but not affected by subphase composition.

In biological membranes, glyceroglycolipids exist together with other lipids among which phospholipids constitute the major part. This prompted us to study the phase properties of mixtures of glyceroglycolipids with phospholipids, in particular with phosphatidylglycerol, a widespread phospholipid in plants and bacteria. This allowed us to show that mixtures of the dimannosyldiacylglycerol, from the plasma membrane of the bacterium *Micrococcus luteus*, either with natural phosphatidylglycerol from the same source or with synthetic dipalmitoylphosphatidylglycerol, did not exhibit phase separation whatever the nature of the cations (monovalent or divalent) present in the aqueous phase [11]. Moreover, it was observed that the glyceroglycolipid restituted a fluid phase when mixed with dipalmitoylphosphatidylglycerol in the presence of divalent cations, suggesting that the mannose residues might modify the interactions between the cations and the phospholipid at the interface. In this respect, it has been shown, through electrophoretic mobility determinations [12], surface pressure [13] and surface potential [14] measurements in monolayers, that monovalent and divalent cations adsorb to phosphatidylglycerol.

In the present work, we investigated whether a glycolipid, such as the dimannosyldiacylglycerol from *M. luteus*, was able to modify the interactions of monovalent cations with phosphatidylglycerol. This study was carried out by a combination of surface potential measurements in monolayers and electrophoretic mobility experiments with liposomes. Data were interpreted using the electrical double-layer theory. They strongly suggest that the glycolipid modulates the adsorption of monovalent cations to phosphatidylglycerol.

Material and Methods

Chemicals

Both phosphatidylglycerol (PG) and dimannosyldiacylglycerol (DMDG) were purified from the membrane of the strain of *Micrococcus luteus* (Institut Pasteur A 270) [15]. More than 75% of the acyl chains were composed of iso- and ante-iso methyl-branched myristic and palmitic acids. The lipids were pure as checked by thin-layer chromatography.

Salts were of analytical grade (Merck, Darmstadt, F.R.G.). Ultra-pure water from a Milli-Q apparatus (Millipore Corp., Bedford, MA) was used.

Monolayer experiments

Compression isotherms, surface pressure, π, and surface potential, ΔV, were measured with an experimental set-up described earlier [16], employing experimental procedures detailed elsewhere [13]. π was measured by means of a platinium plate connected to a torsion balance of our fabrication while ΔV was measured by means of two Americium electrodes. Throughout all experiments, reference surface potentials of aqueous subphases were around 10–20 mV. Film compression was reproducible at $\pm 5 \cdot 10^{-3}$ nm^2, whereas the reproducibility of ΔV determinations was at ± 3 mV. Lipids were spread in the form of chloroform/methanol (5 : 1, v/v) solutions. The subphase consisted of 1 mM NaCl or KCl at pH 6.4. Monolayer data presented are the average of two to three experiments carried out at 20 °C.

Electrophoretic mobility measurements

Multilamellar vesicles were prepared by the method of Bangham et al. [17]. Micrographs of these lipid dispersions were obtained by negative staining with phosphotungstate [18]. These pictures revealed that dispersions of PG alone and its mixtures with DMDG were mainly (88%) composed of vesicles with a mean radius of 0.08 ± 0.04 μm.

Electrophoretic measurements were carried out with a Mark II microelectrophoresis apparatus (Rank Broth., Bottisham, Cambridge, U.K.). Care was taken to focus at the stationary level [19]. Data were obtained by applying the electric field at constant current in the cylindrical cell, with a four electrode arrangement, two electrodes for applying the electric field and two electrodes for measuring the applied voltage. Particles were timed alternatively in each electric field direction by inversing the field polarity. Data presented are the average of at least 30 determinations in each direction.

Theory

The zeta potential was calculated using Henry's equation

$$\zeta = (3/2)\cdot\eta\cdot\mu/(\varepsilon_0\cdot\varepsilon_r\cdot f(\kappa a)) \quad (1)$$

with

$$\kappa = F(2I/\varepsilon_0\cdot\varepsilon_r\cdot RT)^{1/2} \quad (2)$$

In these equations, η is the viscosity of the aqueous phase, μ is the particle mobility, ε_0 and ε_r are the permittivity of free space and the aqueous phase, respectively, κ is the reciprocal Debye screening distance, I is the ionic strength, and a is the radius of the diffusing particle. F, R and T have their usual meaning. In our case and for a 0.1 M ionic concentration, κ takes the value of $1.052\cdot 10^9$ and a has a mean value of 0.08 μm. This gives a value for κa of 90 leading to $f(\kappa a) = 1.451$ [20].

The data were interpreted by means of the Gouy-Chapman-Stern theory for electric double layers. The charges located on negative lipids are spread uniformly in a plane which is considered at $x = 0$. The other assumptions are that ions are point charges and do not penetrate beyond the plane of negative lipid charges, the dielectric constant of the aqueous phase is equal to its bulk value up to the surface of the membrane and image charge effects can be ignored. These assumptions have been discussed earlier [21,22].

By combining the Poisson and the Boltzmann equations, the Gouy equation is obtained:

$$\sinh(e\Psi_o/(2kT)) = \sigma/(8N\varepsilon_0\varepsilon_r kT\cdot C)^{1/2} \quad (3)$$

in which Ψ_0 is the electrostatic potential at the surface of the membrane, σ is the surface charge density, C is the monovalent ion concentration, N is Avogadro's constant, ε_0 is the permittivity of free space, ε_r is the dielectric constant of the aqueous phase and e is the charge of the electron.

The Boltzmann equation relates the concentration at the interface with the bulk concentration:

$$C_0 = C_b\cdot\exp(-e\Psi_o/(kT)) \quad (4)$$

Adsorption of the monovalent cations to the surface of the membrane is described by use of a Langmuir adsorption isotherm which assumes a stoichiometric 1 : 1 association between the lipid and the cation:

$$PG^- + Na^+ \leftrightarrows PG\text{-}Na \quad (5)$$

with the adsorption constant:

$$K = [PG\text{-}Na]/[PG^-][Na^+] \quad (6)$$

The surface charge density is expressed by:

$$\sigma = \sigma_{max}/(1 + KC_0) \quad (7)$$

where σ_{max} is the maximum surface charge density on the membrane (one electronic charge/phospholipid).

The zeta potential is calculated by considering the variation of the electrostatic potential with the distance x from the interface:

$$\Psi(x) = (2kT/e)\ln((1+\alpha\cdot\exp(-\kappa x))/(1-\alpha\cdot\exp(-\kappa x))) \quad (8)$$

where

$$\alpha = (\exp(e\Psi_o/2kT)-1)/(\exp(e\Psi_o/2kT)+1) \quad (9)$$

Combination of these equations allows one to compare the experimental zeta potentials with theoretical ones and therefore to calculate an adsorption constant for the cation to the lipid. These calculations were carried out with the aid of a microcomputer (Wang 2200VP) and using a modified version of an algorithm kindly provided by S. McLaughlin.

Results

Monolayer experiments

Surface pressure, π, and surface potential, ΔV, were continuously recorded with film compression for phosphatidylglycerol, DMDG and their mixtures on 1 mM NaCl and KCl aqueous subphases.

The isotherms were analyzed by plotting the mean molecular area versus the glycolipid molar fraction for a surface pressure π of 30 mN/m. The results are reported in Fig. 1A. In this type of diagram, any straight line is to be considered to represent either ideal mixing or phase separation, while any deviation from linearity indicates inter-

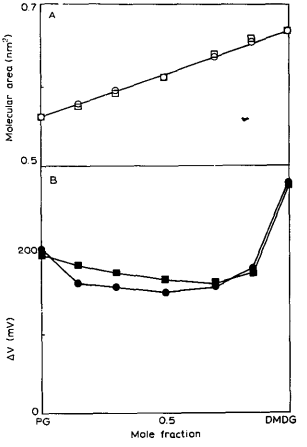

Fig. 1. Mean area per molecule (A) and surface potential (B) versus mole fraction for dimannosyldiacylglycerol (DMDG) mixed with phosphatidylglycerol (PG), in the presence of 1 mM Na^+ (○, ●) and 1 mM K^+ (□, ■). The reference surface pressure was 30 $mN \cdot m^{-1}$.

molecular interactions between the two components. As can be seen, and both in the presence of Na^+ and K^+, no deviation from linearity was observed at the surface pressure of 30 mN/m. A small and negative deviation was detected at lower surface pressures ($\pi = 20$ and 10 mN/m, data not shown). It has been previously shown that the two lipids exhibit high miscibility properties. No phase separation was detected in either monolayers or liposomes, whatever the nature of the cations (monovalents or divalents) present in the aqueous phase [11]. Therefore, the diagram shown in Fig. 1A accounts for a mixed lipid phase in which intermolecular interactions, cannot be detected and do not alter lipid molecular packing.

The same type of diagrams were constructed from the surface potential data obtained for the same surface pressure of 30 mN/m. As shown in Fig. 1B, large negative deviations from linearity were observed. It is more pronounced in the presence of Na^+ than in the presence of K^+ up to a glycolipid molar fraction of 0.7. Above this, the film mixture behaves the same on both cations. For PG alone, ΔV was around 180 mV for both cations, in agreement with previous determinations [14]. A higher value of 280 mV was found for the glycolipid alone. This surface potential value, as well as the surface pressure, was insensitive to the nature and the concentration (tested up to 100 mM) of the cations present in the subphase. The effects of salts on glycolipid monolayers has been studied in the case of simple glycolipids such as stearyl glycoside or cerebrosides [23]. For these molecules, and for high ion concentrations (0.5 M), film expansion was observed which depended on the nature of the cations present in the subphase. In our case, interactions between cations and the glycolipid mannose residues at the interface cannot be detected and do not perturb the film organization.

Zeta potential measurements

Because of electrolytic phenomena and in particular when working around pH 7, changes in the pH of the aqueous phase are observed. These pH changes depend on the number of electrophoretic determinations, i.e. on the time the electric current is going through the sample. Therefore, the aqueous phase of the lipid suspension has to be buffered, preferentially using anions and cations which do not adsorb to the lipids in order not to alter the zeta potential.

Control experiments were carried out with Tris and Mops, already used by others [12], and with phosphate as buffer. Typically, for a set of 40 electrophoretic measurements, which is a minimum for statistical analysis of the data, the pH was found to vary from pH 7.7 to 6.9 in the presence of Tris and Mops. Furthermore, dependence of electrophoretic mobility on pH was observed over this pH range, suggesting pH-dependent adsorption of these two molecules to the lipid vesicles. This is in agreement with a previous report which showed adsorption of Tris to PG monolayers in a pH-dependent manner. The maximum adsorption was found around pH 7.5 [13]. In contrast, an anion like phosphate does not adsorb

TABLE I

ELECTROPHORETIC MOBILITIES μ AND CORRESPONDING ZETA POTENTIALS ζ OBTAINED FOR DISPERSIONS OF PHOSPHATIDYLGLYCEROL AND ITS MIXTURES WITH DIMANNOSYLDIACYLGLYCEROL IN THE PRESENCE OF ALKALINE CATIONS

μ is expressed in 10^{-8} $m^2 \cdot s^{-1} \cdot V^{-1}$ and ζ is expressed in mV.

	Li^+ (0.1 M)		Na^+ (0.1 M)		K^+ (0.1 M)		Cs^+ (0.1 M)	
	μ	ζ	μ	ζ	μ	ζ	μ	ζ
PG	−4.8±0.8	−71±11	−5 ±1	−77±15	−5 ±1	−77±15	−5.5±0.9	−82±11
PG/DMDG mixtures								
8.5:1.5			−4.9±0.9	−74±13	−3.8 ±0.6	−57± 9		
2:1	−5.8±0.5	−86± 8	−5.2±0.4	−77± 6	−4.2 ±0.6	−63± 9	−4.0±0.4	−59± 7
1:1	−5.5±0.6	−81± 8	−4.2±0.4	−63±11	−3.39±0.4	−50± 6	−3.2±0.5	−47± 7
3:7	−4.2±0.5	−63± 7	−3.7±0.3	−53± 4	−3.3 ±0.3	−48± 4	−3.43±0.3	−51± 5
1.5:8.5			−2.3±0.8	−34±12	−2.3 ±0.3	−34± 5		

to anionic lipid surfaces. With this buffer, the pH was also found to vary from 7.6 to 6.8 after 40 electrophoretic measurements, but the electrophoretic mobilities measured for PG and PG/DMDG vesicles remained unchanged over this pH range. In the following, all experiments were carried out with phosphate as buffer and the pH was taken as the average of the pH values measured at the beginning and at the end of a set of experiments.

Electrophoretic mobilities were determined on vesicles of PG alone or mixed with DMDG in various molar ratios, in the presence of monovalent cations at a concentration of 0.1 M in the water phase. As can be seen in Table I, a rather large dispersion of electrophoretic data was observed. This was not due to defocussing the microscope from the stationary level. The symmetry of the particle mobility rate was checked with respect to the sign of the electric field. In an attempt to reduce this dispersion, at least 60 to 80 determinations were carried out for each lipid sample. The standard deviation for the corresponding set of data was only slightly reduced. The differences between the various average mobility values are small and for many of them, an overlap between extreme values was still observed. So, a statistical analysis of the electrophoretic data was carried out, consisting of a t-test comparison of the various populations two by two, with an error risk of 5%. The differences between mean mobility values which are reported in Table I can be considered as statistically significant. In the following, we will only retain these average values without considering their dispersion.

The electrophoretic mobilities shown in Table I exhibit dependence on both the nature of the monovalent cations present in the subphase and on the vesicle composition. For PG alone, they increase in the order $Li^+ < Na^+ = K^+ < Cs^+$ at previously shown [12], while for PG/DMDG mixtures, they decrease in the order $Li^+ > Na^+ > K^+ > Cs^+$. For each cation, the electrophoretic mobility tended to decrease with increasing glycolipid mole fraction.

To evaluate the cation-to-lipid adsorption constants, the experimental zeta potentials obtained from the above electrophoretic mobilities (Table I) were compared to calculated values (see Theory, in Materials and Methods). In these calculations, a distance of 0.2 nm of the plane of shear from the interface plane was used, a reasonable value as discussed elsewhere [12], and the molecular area of the PG in bilayers was assumed to be 0.63 nm^2. The calculated values of electrostatic potentials and adsorption constants are given in table II. For Li^+, mobility data fitted well with a distance x less than 0.2 nm, depending on the PG to DMDG molar ratio.

The adsorption constants obtained by fitting zeta potentials for PG alone vary like the lyotropic serie $Li^+ > Na^+ = K^+ > Cs^+$, in accord with published results [12,24]. The reversed order $Li^+ < Na^+ < K^+ < Cs^+$ was observed upon addition of DMDG to PG, up to a 1:1 molar ratio. For higher DMDG concentrations, the same tendency

was observed, at least with Li^+, Na^+ and K^+. It is worth noting that similar constants of 0.3 M^{-1} were measured for the adsorption of Na^+ and K^+ to PG and that addition of the glycolipid to the phospholipid bought about a clear cut discrimination between the two cations.

Surface potential and zeta potential combinations

It should be remembered that the surface potential ΔV which is measured is the sum of two terms, an electrostatic one Ψ_o, and a dipolar one, ΔV_p:

$$\Delta V = \Psi_o + \Delta V_p$$

The dipolar term, which is also referred to as the polarization potential, is proportional to the vertical component of the overall dipole moment of the film-forming molecules. This also includes the dipoles of the orientated water molecules which constitute the hydration shell of the lipids at the interface.

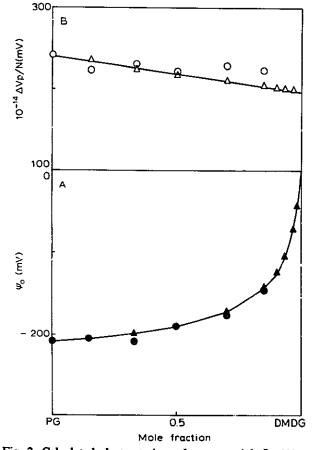

Fig. 2. Calculated electrostatic surface potential Ψ_o (A) and dipolar potential (B) for phosphatidylglycerol (PG) and its mixtures with dimannosyldiacylglycerol (DMDG) in monolayers, as function of the glycolipid mole fraction. Ψ_o was calculated using either the adsorption constant $K = 0.295\ M^{-1}$ found for Na^+ interacting with PG alone (▲) or the values determined for the corresponding PG/DMDG mixtures (●) with respect to the measured electrophoretic mobilities (Table II). The dipolar potentials (△, ○) were obtained by substracting the above calculated Ψ_o values (▲, ●) from the corresponding surface potential data (●) of Fig. 1.

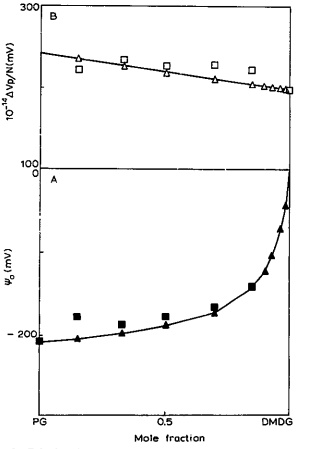

Fig. 3. Calculated electrostatic surface potential Ψ_o (A) and dipolar potential (B) for phosphatidylglycerol (PG) and its mixtures with dimannosyldiacylglycerol (DMDG) in monolayers, as function of the glycolipid mole fraction. Ψ_o was calculated using either the adsorption constant $K = 0.295\ M^{-1}$ found for K^+ interacting with PG along (▲) or the values determined for the corresponding PG/DMDG mixtures (■) with respect to the measured electrophoretic mobilities (Table II). The dipolar potentials (△, □) were obtained by substracting the above calculated Ψ_o values (▲, ■) from the corresponding surface potential data (■) of Fig. 1.

TABLE II

ADSORPTION CONSTANTS K (IN M^{-1}) AND ELECTROSTATIC POTENTIALS Ψ_0 (IN mV) CALCULATED FROM ELECTROPHORETIC MOBILITIES SHOWN IN TABLE I FOR DISPERSIONS OF PHOSPHATIDYLGLYCEROL AND ITS MIXTURES WITH DIMANNOSYLDIACYLGLYCEROL IN THE PRESENCE OF ALKALINE CATIONS

	Li^+ (0.1 M)		Na^+ (0.1 M)		K^+ (0.1 M)		Cs^+ (0.1 M)	
	K	Ψ_0	K	Ψ_0	K	Ψ_0	K	Ψ_0
PG	0.5	−86.62	0.295	−94.12	0.295	−94.12	0.139	−102.95
PG/DMDG mixtures								
8.5:1.5			0.296	−89.37	1.752	−65.82		
2:1	$7.7 \cdot 10^{-3}$	−110.56	0.110	−94.69	0.682	−74.05	0.958	−69.69
1:1	$d = 1.55$ Å							
	$9.08 \cdot 10^{-3}$	−93.69	0.314	−73.79	0.702	−57.9		
3:7	$d = 1.55$ Å							
	$6 \cdot 10^{-3}$	−69.63	0.145	−62.94	0.43	−55.88	0.294	−58.71
1.5:8.5			0.206	−38.47	0.233	−38.15		

The dipolar part of the surface potential can be estimated if the electrostatic part is obtained independently. This can be done using the electrostatic theory, as described in Material and Methods. Calculations of Ψ_0 were carried out for Na^+, and K^+, taking into account the charge surface dilution brought about by addition of DMDG to PG and the ionic conditions used for the monolayer experiments. In a first step, calculations were carried out with the adsorption constants determined for PG along. In a second step, Ψ_0 was calculated taking into account the various cation adsorption constants determined for the different PG/DMDG mixtures studied. Results are shown in Fig. 2 for Na^+ and in Fig. 3 for K^+.

In Figs. 2A and 3A, the full lines show the changes in electrostatic potential Ψ_0 which are expected to occur when diluting PG with DMDG and assuming that the adsorption constants of the two cations to PG remain unchanged. Substracting the calculated Ψ_0 values for PG alone (195 mV) from the experimental surface potential values (200 mV, Fig. 1B) yields the polarization potentials of this lipid in the presence of the two cations. These polarization potentials are expressed in terms of $\Delta V_0/N$, in order to correct for the differences in molecular area which exist between PG and DMDG [25]. From these corrected values and that obtained from the surface potential measured for DMDG alone (Fig. 1) and which is dipolar in nature, it is possible to construct the polarization potential diagrams shown in Figs. 2B and 3B. In these diagrams, the straight lines account for an ideal mixing of the two lipids, i.e., no change in the cations to PG adsorption constants and no detectable dipolar interactions between PG and DMDG.

In the second step, Ψ_0 was calculated for the various PG/DMDG mixtures taking into account the relevant cation-to-lipid adsorption constants determined through electrophoretic mobility experiments (Table II). Then, they were substracted from the surface potential data of Fig. 1B to obtain the desired polarization potentials. Calculated Ψ_0 values and corresponding corrected polarization potentials are shown in Figs. 2B and 3B for Na^+ and K^+, respectively. They have to be compared with the full lines presented in these figures. In terms of polarization potentials, the calculated values are distributed evenly around the straight line of reference for both cations (Figs. 2B and 3B). In terms of electrical potential, a marked deviation from the theoretical full line is observed with K^+. This suggests that in PG/DMDG mixtures, changes in surface potential which result from the interactions between the two lipids are not dipolar but mainly electrostatic in nature.

Discussion

The above electrophoretic mobility data and their interpretation using the Goy-Chapman-Stern theory confirm the adsorption of cations to PG

previously described [12,14]. An adsorption constant of about 0.3 M^{-1} was found for Na^+, in reasonable agreement with the value of 0.6 M^{-1} obtained by Eisenberg et al. [12] using the same technique but with Mops as buffer. A value in the range 0.1–0.4 M^{-1} was also reported for the same couple, through pH-titration of the lipid in monolayers (14).

In accord with previous observations [12], the measured electrophoretic mobilities vary according to the increasing sequence $Li^+ < Na^+ = K^+ < Cs^+$, which corresponds to a decreasing cation-to-lipid adsorption constant (Table II). The same decreasing order was found for Li^+, Na^+ and Cs^+ adsorbing to PG monolayers [14].

Interestingly, addition of the glycolipid to the phospholipid completely reverses the sequence. For the 2:1 PG/DMDG molar ratio, as usually found in the membrane of the bacterium *M. luteus* [15], adsorption constants extend over a large scale, from $7.7 \cdot 10^{-1}$ M^{-1} for Li^+ up to 0.96 for Cs^+, with a marked discrimination between Na^+ and K^+ (Table II). A clear-cut discrimination between these two ions is also observed in mixed monolayers of the two lipids in terms of surface potential (Fig. 1B) but not in terms of lipid molecular packing (Fig. 1A). Interpretation of these surface potential data using the Gouy-Chapman-Stern theory and on account of the cation adsorption constants determined by the electrophoretic experiments indicate that mixing of the two lipids does not significantly alter their polarization potential or at least in a way which cannot be detected (Figs. 2B and 3B). In addition to the fact that mixing of the two lipids occurs without significant change in their molecular packing (Fig. 1A), this suggests that the consequences of the interactions between PG and DMDG are electrostatic in nature. They lead to a surface charge density dilution accompanied by changes in the cation-to-lipid adsorption constants.

A straightforward interpretation of such results remains difficult to achieve. It has been recently suggested that the changes in electrophoretic mobilities can be accounted for by changes in the water polarization at the interface [26]. Such a contribution cannot be rule out. Nevertheless, it cannot explain the observed differences between the monovalent cations.

When describing cation interactions with anionic groups at the interface, there are two physically distincts models of ion binding to consider: site-specific and ion-atmosphere binding [27]. From binding constant studies of alkaline cations to polyelectrolyte phosphates [28–30], DNA [31–33] and nucleotides [34], giving the relative order $Li^+ > Na^+ > K^+ > Cs^+$, parallel to the increasing ionic radius sequence [35,36], it has been concluded that cations could interact with the phosphate group through their hydration shell [28,29]. This conclusion could apply to PG alone since the same sequence of cation-to-lipid adsorption constants is observed.

From another point of view, it is known that interactions of cations with an organized interface strongly depends on their respective hydration number and on the water structure near the surface [37–45]. Alkaline cations in water are solvated [35,46–51] but, although Li^+ and Na^+ are usually strongly solvated by highly structured water, K^+ and Cs^+ are less solvated and their hydration shell is more fluid. Therefore, the reversed sequence $Li^+ < Na^+ < K^+ < Cs^+$, which is observed for PG/DMDG mixtures suggests that the cations would interact with the phospholipid through their hydration shell. The size of the hydration shell of PG/DMDG mixtures is not known but presumably it depends on the organization of the polar head groups at the interface. The non-acylated glycerol moiety of PG has been shown to extend parallel to the interface plane [52]. The orientation of the dimannosyl residue of DMDG is still unknown. Nevertheless, through NMR experiment, it has been shown that the mannosyl residue of a monomannosyldiacylglycerol extends away from the bilayer surface [53]. The presence of a second mannose residue more than likely imposes a new distribution of water at the interface and it is suggested that changes in lipid hydration as well as changes in cation hydration, could be involved in the way alkaline cations interact with PG and its mixtures with DMDG.

The functional role of glyceroglycolipids in biological membranes is far from being well understood [2]. These molecules are very often found to be associated with PG, in plants and bacteria [54,55]. In the case of the bacterium *M. luteus*, it has recently been shown that PG and DMDG

interact together within the bacterial membrane [56]. This is in agreement with our previous observation that these two lipids form highly miscible phases. It was shown also that the glycolipid can restitute membrane fluidity when PG is complexed by divalent cations. The data presented in this communication strongly suggest that the glycolipid could also play a role in modulating the electrical properties of the membrane.

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