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Methylation of ethanolamine groups in phosphoethanolamines is relevant for L-arginine insertion in lipid membranes

Ana Bouchet, Fabiana Lairion, Anibal Disalvo *

Laboratorio de Fisicoquímica de Membranas Lipídicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 2ªP (1113) Buenos Aires, Argentina

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ABSTRACT

The interaction of L-arginine with membranes composed by phospholipids with different degrees of methylation of the ethanolamine group was studied by means of surface and dipole potentials and surface pressure variations. The subsequent methylation of the amine head group appears to hinder the synergic response of the adsorption observed in phosphatidylethanolamine membranes. The kinetics of the binding process denotes that the methyl groups are relevant in regulating the specific interaction of the amino acid with the interface by hydrogen bonds. This response can be put in correlation with the function of signal transduction assigned previously to methyl lipids [F. Hirata and J. Axelrod, 1980] and appears to be relevant to understand the mechanism of insertion of arginine residues in peptides of biological interest.

1. Introduction

The insertion of peptides containing the basic amino acid L-arginine (Arg) in fully hydrated lipid membranes has been a matter of discussion since, in principle, a high energy cost would be needed to accommodate such a highly charged Arg side chain into the hydrocarbon core of the lipid membrane [2]. The fact that Arg is transported and accumulated in different types of cells, strongly suggests that, in spite of that restriction, this molecule is able to pass through cell or vesicle membranes which are usually composed by different mixtures of phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) [3–7]. It has been proposed that Arg insertion is favored by the stabilization of the guanidinium moiety in water pockets inside the membrane phase [8]. However, details of this specific interaction with different lipid membrane groups have not been reported.

Recently, it has been shown that Arg can only adsorb to PCs in the fluid phase, while it interacts with DMPE membranes even in the gel state. This last feature suggests that Arg perturbs the bilayer packing of PEs causing an increase in the chain mobility and area per molecule, as a consequence of a change in the H-bonding pattern [9]. The enhanced adsorption in gel PE in comparison to gel PC does not correlate with the lower hydration and higher lateral interaction of PEs in comparison to PCs (2 to 4 water molecules per lipid molecule for PEs [10–12] in comparison to 7–8 water molecules per lipid molecule for PCs in the same state). Clearly, other structural factors should be considered.

Electrophoresis mobility of liposomes showed that Arg adsorbs to PE membranes shifting the zeta potential to negative values indicating that the carboxylate group of the amino acid orients to the water phase [9,13]. The location of this group in an outer plane toward the aqueous phase in DMPE membranes suggests that guanidinium group sinks into the membrane phase interior (snorkeling). Simulation results have shown that the total number of H-bonds formed between Arg molecules and the solvent decreases while the number of H-bonds formed with the membrane increases as they get adsorbed onto the membrane surface [14]. As the positive moiety trimethyl ammonium of DMPC is projected into the water phase, the differences in the resulting surface arrangements would depend on the group linked to the phosphate of the phospholipids, such as choline or ethanolamine moieties. According to Cevc, the long range and medium intermolecular interactions are regulated by the interfacial properties, such as hydration and surface potential, independent of the acyl chain nature [15].

The different water reorganizations could differently affect the mechanism of adsorption and the kinetic patterns of Arg insertion in the two lipids. In particular, insertion of Arg molecules in the DMPE membranes would need to disrupt the existing hydrogen bonds network formed between the PE head groups. This may imply a strong interaction of Arg with the NH group of PE that would render the phosphates less bounded, enhancing the mobility of the phosphate and carbonyl groups and therefore modifying the reorganization of water molecules around the head groups [9]. This could give an indication of how water spaces could be created in

* Corresponding author. Tel.: +54 1149648249; fax: +54 1145083645.
E-mail address: eadisal@yahoo.com.ar (A. Disalvo).

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between the lipids increasing the mobility of the C-atom of the acyl chain near the head group which could favor the amino acid partition.

PCs and PEs are the two major constituents of mammalian plasma membranes. In some tissues PEs may be sequentially methylated by methyltransferases, yielding N-methyl-PEs, N,N-dimethyl-PEs, and, finally, PCs [16]. The partially methylated PE shows gel to liquid crystalline phase transition temperatures between those corresponding to PCs and PEs [17,18].

The number of methyl groups covalently bound to the amino moiety of these phospholipids seems to play an important role in determining domain shapes. Upon compression and expansion, bridges form between individual lipid domains. A so-called 'bridging' effect occurs only in those lipid monolayers containing a few methyl groups. Even N,N-dimethyl-DPPE monolayers show some degree of bridging at the expanding cycle, but this feature is absent in PC monolayers [19].

The bridge effect is also seen in DPPE monolayer containing 5 mol% DPPC. The decreasing critical and onset temperature with increasing headgroup methylation implies that increasing methylation of the headgroup reduces the packing energy [19]. A single methylation of eggPE almost completely converts its hydration and bilayer repulsive properties to those of eggPC; little progression of hydration is seen with successive methylations [20].

Thus, headgroup methylation may play an important role in biological signal transduction [1,16,19]. These surface changes may affect surface forces involved in peptide or protein insertion such as dipole and surface potential.

How these surface properties affect the insertion of compounds of biological interest, like positive peptides (which mainly contain Arg), is unknown. For these reasons, the effect of L-arginine on lipid monolayers and bilayers was studied by varying the methylation of the ethanolamine groups with the purpose to determine the affinity of arginine for lipid membrane surfaces in which the phosphate group environment is modified by the lipid composition. Arg insertion was determined by measurements of the surface pressure, zeta potential and dipole potential of monolayers and bilayers composed by PC, N,N-dimethyl PE, N-monomethyl PE and PE at different Arg concentrations.

2. Materials and methods

2.1. Chemicals

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-sn-glycero- 3-phosphoethanolamine (DMPE), 1,2-dipalmitoyl-sn-glycero-3-phospho-ethanolamine (DPPE) and its derivates N-monomethylated (mmDPPE) and N,N-dimethylated (dmDPPE) were obtained from Avanti Polar Lipids, Inc (Alabaster, AL) and used as received. The purity of lipids was checked by thin layer chromatography using a chloroform:methanol:water (65:25:5) mixture as running solvent. L-arginine (Arg) was obtained from Sigma-Aldrich (Saint Louis, MO). Chloroform and KCl were of analytical grade. Water was MilliQ quality.

The pHs of the solutions used for zeta and dipole potentials measurements were adjusted to pH = 7 by titrating the L-arginine solution with HCl.

2.2. Liposome preparation

Multilamellar liposomes (MLVs) were prepared dispersing the lipids by vortexing in 1 mM KCl at temperatures higher than that of the phase transition, for 60 min. Large unilamellar vesicles were prepared by extruding the liposome dispersions through a polycarbonate membrane (pore size 100 nm) above the transition temperature of the lipids, and then, the samples were cooled down to the working temperature. LUVs of mmPE and dmPE of reproducible sizes were hard to obtain probably to the high temperature needed to extrude the lipids above the phase transition. This uncertainty led to compare MLV and LUVs adsorption in those samples at which reproducible vesicles could be obtained. As shown in Fig. 1 Supplementary the isotherms of both types of vesicles superposed and similar constant were obtained. Therefore, for practical reasons, and the good reproducibility, the complete study was done on MLVs.

2.3. Zeta potential

The zeta potentials (ζ) of DMPC, dmDPPE, mmDPPE and DMPE liposomes were determined in Zeta-Meter System 3.0 equipment, at 18 ± 2 °C. The voltage was fixed at 75 V. In this method, individual particles are visualized under the microscope and the mobility is determined automatically particle by particle. This requires large particles and high contrast. For this reason, multilamellar liposomes are used. The absorbance, mainly determined by the refractive index of the particles and the average size of the multilamellar dispersions are high enough for the four lipids used [21,22]. The total lipid concentration in all cases was 52 μM.

Once prepared, liposomes were cooled down to 18 °C and incubated at that temperature with different Arg concentrations (1–100 mM) for 1 h.

A total of 20 measurements were carried out focusing different particles for each sample. Data reported are the average of the measurements done for each condition with, at least, three different batches of liposomes.

The degree of coverage (θ) can be calculated by the relation between the zeta potential changes at each Arg concentration assayed. As the measures of zeta potential are done on individual particles in a relative way, the degree of coverage is independent of the number of particles and of the size of the particles provided these are large enough for the reasons given above and to avoid effects of curvature. An illustrative figure is included as Supplementary material (Fig. S1).

In this condition, the equilibrium between amino acid in solution and in the surface is

$$nA + S_e \leftrightarrows S_o$$

where S_e is the empty sites for adsorption of A and S_o represents those occupied by n molecules of A.

As the total sites, $S = S_e + S_o$, at equilibrium becomes $k_1\, A^n\, (S - S_o) = k_2\, S_o$, from where, when $n = 1$, the Langmuir binding isotherm can be written

$$\theta/(1-\theta) = k_2/k_1[A]$$

being $\theta = S_o/S$ is the mole fraction of occupied binding sites. Thus, if $S_o = 0$, $\theta = 0$ and if $S_o = S$, $\theta = 1$.

The degree of coverage can be written in terms of fraction of area i.e. $\alpha_{occupied}/\alpha_{total}$. Moreover, as the total area is given by $n_L.a_L$ (where n_L is the number of lipids and a_L the area per lipid), $\alpha_o/\alpha_T = n_{Lo}.\ a_L/n_L\ aL = n_{Lo}/n_L$ provided the area per lipid is not altered in the process.

On the other hand, θ can be calculated from the zeta potential values for each Arg concentration as $\theta = \zeta_o - \zeta/\zeta_o - \zeta_{max}$, where ζ_o is the zeta potential of the liposome in the absence of Arg, ζ_{max} is the zeta potential value for liposomes saturated with Arg, and ζ is the zeta potential at any intermediate Arg concentration. Thus, when $\zeta = \zeta_o$, $\theta = 0$ and $\alpha_{occupied}/\alpha_{total} = 0$. When $\zeta = \zeta_{max}$, $\theta = 1$, and $\alpha_{occupied}/\alpha_{total} = 1$.

Therefore,

$$\theta = \frac{\Delta\zeta}{\Delta\zeta\,\mathrm{max.}} = \frac{(\mathrm{KC})^n}{1 + (\mathrm{KC})^n} \quad (1)$$

with $K = k_2/k_1$, the dissociation constant of the complex amino acid-site and n is the parameter of heterogeneity [23,24]. The latter

assumes that one amino acid binds to a cluster of n lipids to form a SA_n complex.

From Eq. (1), it is clear that when n = 1, the adsorption stoichiometry is 1:1 solute–site. When n < 1, as it is observed from the fitting, a molecule A can bind to more than one lipid. If the site is considered as the NH group in one phospholipid molecule, one Arg is binding to two NH groups, possibly of two different molecules.

2.3.1. Determination of dipole potential in monolayers

Dipole potential and surface pressures were determined in lipid monolayers spread on a clean air–aqueous solution interface containing different Arg concentrations. Data was collected when constant potential or pressures were reached and no further changes were observed with additions of lipids. In this condition, the lipids in the monolayer are in equilibrium with lipids forming liposomes in the subphase. A corresponding state between bilayer and monolayer is achieved since equilibrium is established by the transfer of lipid molecules to and from the monolayer and the outer bilayer of the vesicles [25–29].

Once the monolayer was formed and no further changes in surface pressure were observed by addition of lipids, the values of interfacial potential (V_{surf}) were determined through a circuit of high impedance, by means of an ionizing electrode on the monolayer and a reference electrode in the aqueous subphase (KCl 1 mM) using the following expression:

$$V_{surf} = V_{Ag/AgCl} - V_{grd} = V_{solution} - V_{grd} \quad (2)$$

where $V_{Ag/AgCl}$ is the potential of the reference electrode and V_{grd} the potential of the shield covering the ionizing electrode.

Temperature was set at the values indicated in each assay (mostly 18 and 28 °C) and measured with a calibrated thermocouple immersed in the subphase and maintained within ±0.5 °C.

The dipole potential of the monolayer (Ψ_D) was evaluated as

$$\Psi_D = V_{surf} - V_{lip} \quad (3)$$

where V_{surf} is the potential of the clean surface (without lipids) and V_{lip} the potential after the monolayer was formed.

Different values of Ψ_D were obtained for the clean surface of the amino acid solution assayed and with a monolayer of lipids, in the conditions described below.

2.4. Surface pressure measurements in monolayers

The formation of a lipid monolayer on the interface of solutions, with and without amino acid, was monitored by measurements of the surface pressure in a Kibron μtrough S equipment, at constant temperature (28 ± 0.5 °C) and area. The surface of an aqueous solution contained in a Teflon trough of fixed area was exhaustively cleaned. Then, a chloroform solution of the phospholipids was spread on the surface, up to reach a constant surface pressure for different Arg concentrations in the aqueous subphase. Results of surface pressure were expressed in mN/m.

2.5. Surface pressure changes induced by L-arginine adsorption

Different aliquots of a chloroform solution of phospholipids were spread on the clean surface to reach increasing surface pressures from 9 mN/m to that corresponding to a surface saturated by lipids. We have studied the effects of Arg on monolayers at different initial pressures in the range below and above that corresponding to bilayers at similar temperatures, c.a. 30 mN/m, according to the surface pressure–area isotherms of DMPC, DMPE and methylated PEs.

At each chosen surface pressure, an amino acid solution volume was injected in the subphase to reach a concentration of 21.4 mM and surface pressure changes were followed during time until a constant value was reached. The same procedure was followed for all monolayer compositions. Surface pressure and increases of surface pressure at constant surface area were automatically recorded. The spreading of the amino acid on a clean aqueous surface, as well as its injection into water, resulted in no changes of the surface pressure. Surface pressure values shown in the figures are the average of at least three measurements. The individual points were within 5% of the reported values.

The kinetic pattern of Arg insertion was evaluated in terms of the linearity of the variation of the surface pressure with the square root of the time (√sec). The experimental values were fitted with equation [4]:

$$\Delta\Pi = k \times t^n \quad (4)$$

to evaluate k and n [30], where k is a time dependent diffusion coefficient and n indicates the departure from a pure Fickean diffusion process.

3. Results

The variations of the degree of coverage measured by the relative values of zeta potential of individual DMPE, mmPE and dmPE liposomes with Arg concentration in the bulk solution show different patterns of adsorption (Fig. 1). The adsorption of L-Arg to these membranes deviate from a Langmuir type isotherm with the progressive demethylation as denoted by the n coefficient values lower than 1 (Table 1). As previously said, Arg doesn't adsorb on DMPC vesicles in gel state and therefore the zeta potential is not modified (data not shown). The adsorption on PCs is only observed in the fluid state and, in this case, it follows a Langmuir type isotherm (n = 1) [9]. The adsorption isotherm of Arg on DMPE at 18 °C, for which n = 0.74, suggests that the adsorption takes place in non-independent sites, probably producing surface rearrangements, in contrast to fluid DMPC for which n = 1.The deviation of the adsorption from a Langmuir type is relevant when the number of methylenes decreases(Table 1).

According to Eq. (1), adsorption can be ascribed to a Langmuirian process when n = 1. If this is the case, the degree of coverage is given by the fraction of lipids attached to Arg without changes in the area per lipid. However, this cannot be sustained when n is different

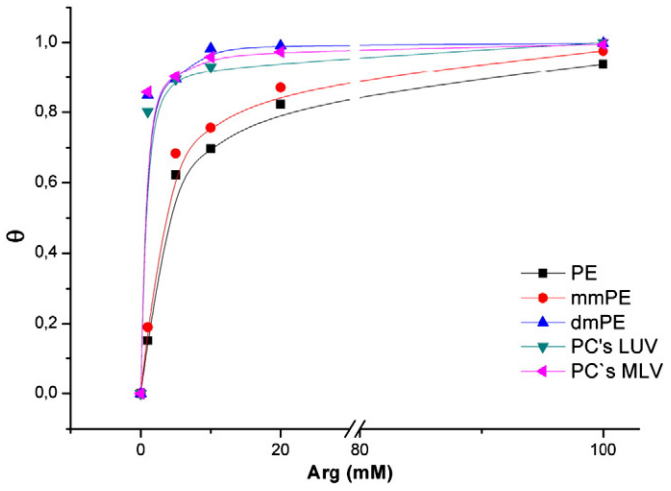

Fig. 1. Adsorption isotherm of Arg to DMPE, mmPE, dmPE and DMPC liposomes. The experimental data of zeta potential were fitted by Eq. (1) to obtain K and n values summarized in Table 1.

Table 1
Comparison of the surface potential, dipole potential and interaction parameters of PE and its methylated derivates with arginine.

Lipid	n[a]	Zeta potencial (mV)	Dipole potencial (mV)	Relative decrease in dipole potential (%)[b]	Affinity constant (K) M^{-1}[b]	k[c]	Surface pressure cut-off (mN/m)[d]
PC	1	-13.7 ± 2.1	529.8 ± 11	0	0.54×10^3	0.021	ND
dmPE	1	-29.9 ± 2.3	389.3 ± 15	28	0.18×10^3	0.027	ND
mmPE	0.9	-39.1 ± 3.2	395.2 ± 17	22	2.90×10^3	0.031	ND
PE	0.74	-45.3 ± 0.9	516.0 ± 22	12	2.00×10^3	0.085	50

[a] Obtained from the fitting of the curves of Fig. 1.
[b] Change in dipole potential produced by the presence of Arg 100 mM in the subphase at 20 °C.
[c] Obtained from the curves of ΔΠ vs √t fitting with the Eq. (4).
[d] Obtained from plots of Fig. 2.

from one. Therefore, the degree of coverage is a function of the change in the area per lipid ($f(a_L)$) induced by the amino acid, i.e.

$$\theta = n_{Lo}/n_L f(a_L).$$

The structural changes suggested by the non-Langmuirian type can be analyzed by considering the effects of Arg on the surface pressure of monolayers at initial surface pressures at, below and above to that corresponding to bilayers (c.a. 30 mN/m). Noticeable effects are observed when Arg interacts with DMPE monolayers at different initial surface pressures. The slope for DMPE is well defined with a clear cut-off at 50 mN/m (Fig. 2). In contrast, the subsequent methylation of the PE group produces a sharp decrease of the slope ΔΠ vs Π curves with respect to DMPE and no critical value can be determined when L-Arg was injected to the subphase of DMPC monolayer in the same range of surface pressure. Thus, surface pressure changes are produced by Arg insertion only when NH hydrogen bonding groups are available in the lipid.

The insertion of Arg in the lipid interface can be also visualized by the relative decrease in dipole potential, performed in independent measures. Except for PC, the relative dipole potential decrease is lower with the depletion of the methyl groups, denoting a different final dipole arrangement of the interface with the insertion of Arg according to the methyl group number.

The sequence found for the adsorption process is congruent with the kinetics of Arg insertion in interfaces with different degrees of methylation. The relative increase of the surface pressure vs time curves showed that the insertion rate and the final equilibrium of surface pressure decrease in the order phosphatidylethanolamine > monomethyl phosphatidylethanolamine = dimethyl phosphatidylethanolamine ≅ -phosphatidylcholine (Fig. 3).

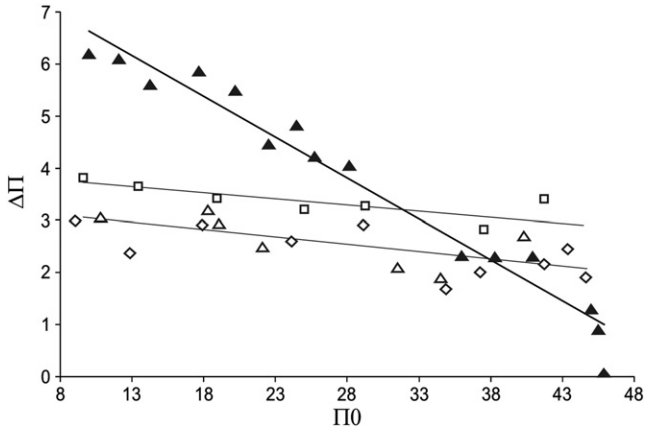

Fig. 2. Surface pressures changes (ΔΠ) of DMPC(◊), dmPE(Δ), mmPE (□) and DMPE (▲) monolayers at different initial pressures (Π_0) induced by the injection of arginine to achieve a concentration of 21.4 mM in the subphase.

A similar kinetic behavior of Arg insertion to that found in lipids with different degrees of methylation was found in lipid monolayers in which different ratios of PE and PC were mixed (Fig. 4). We determined that the presence of a minimum percentage of PC in PE monolayers decreases the arginine interaction to values comparative to PC, as observed when PE was partially methylated.

In Table 1, the values of k corresponding to the fitting of Eq. (4) are shown.

The kinetic data of surface pressure plotted as a function of the square root of time denotes that the n values are equal to 0.54, 0.53 and 0.47 for dmPE, mmPE and DMPE, respectively. Comparable values were obtained in mixtures of PC and PE.

4. Discussion

Since the penetration and adsorption of proteins into different types of membranes has been explained in terms of the partition of different amino acids, it seems reasonable to study the influence of specific residues, such as L-Arg, present in different proteins and peptides interacting with membranes.

It has been suggested that the guanidinium group is essential for arginine-rich basic peptides translocation [31]. Thus, a possible site of insertion can be the negative electrostatic charge around the phosphate groups.

The difference in the structural changes produced by Arg on lipid surfaces with different degrees of methylation is summarized in Table 1. The affinity constant increases with the negative zeta potential from PC to PE being the non-Langmuirian character of the adsorption more pronounced. Considering that Arg adsorption is promoted when the lipids are in the fluid state, in DMPC membranes, as it was demonstrated previously [9], it is reasonable to think that adsorption would be favored by the phosphate exposure to the aqueous phase. In this regard, some considerations about the origin of the zeta potential value are pertinent.

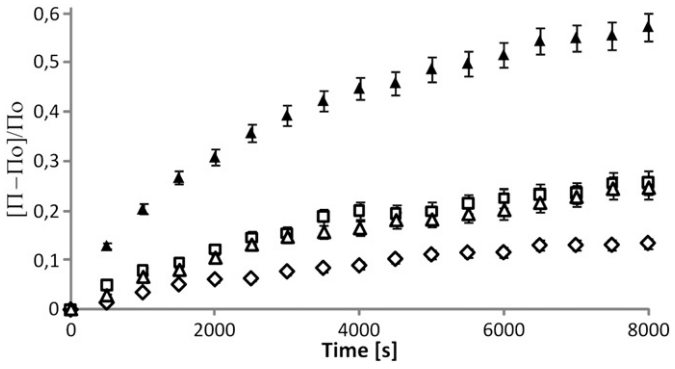

Fig. 3. Relative increase in the surface pressure ($\Pi - \Pi_0/\Pi_0$) as a function of time of gel DMPE (▲), gel DMPC (◊), gel mm-DPPE (□), gel dm-DPPE (Δ) monolayers by the injection of arginine to achieve a concentration of 21,4 mM in the subphase. At an initial surface pressure $\Pi_0 = 10$ mN/m.

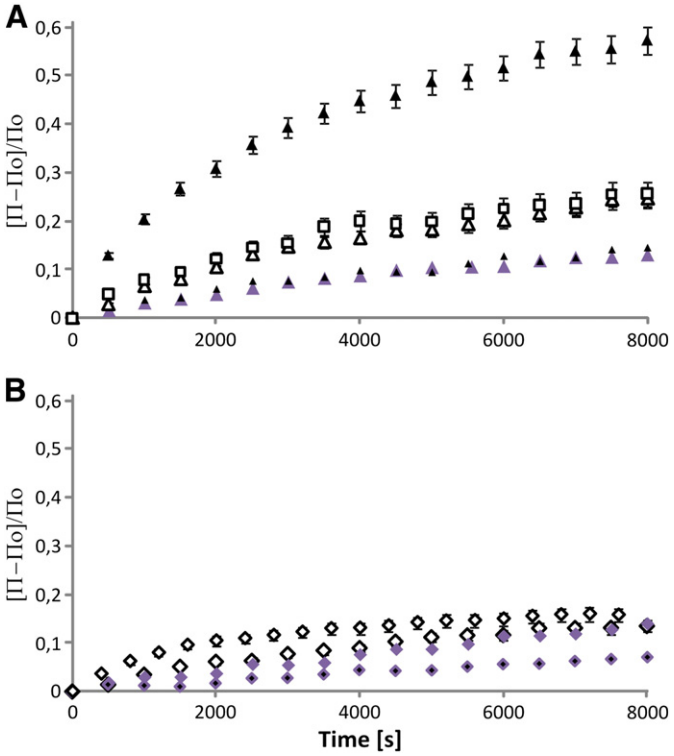

Fig. 4. Relative increase in the surface pressure ($\Pi-\Pi_0/\Pi_0$) as a function of time by the injection of arginine to achieve a concentration of 21.4 mM in the subphase at 18 °C for monolayers of PE:PC mixtures (A) and PC:PE mixtures (B).A — DMPE:DMPC 1:0.1 (▲) at $\Pi_0 = 10$ mN/m and DMPE:DMPC 1:0.1 at $\Pi_0 = 30$mN/m (▲)DMPE (▲), mm-DPPE (□), dm-DPPE (Δ),B — DMPC:DMPE 1:0.1 (◆) at $\Pi_0 = 10$ mN/m and DMPC:DMPE 1:0,1 (◆) a $\Pi_0 = 30$ mN/m.DMPC (◊) $\Pi_0 = 10$ mN/m, DMPC (◊) $\Pi_0 = 30$ mN/m.

The membrane surface potential cannot be measured directly. However, net charges at the surface particle affect distribution of ions in the interfacial regions resulting in the formation of an electrical double layer. The liquid layer surrounding the particle exists as two parts; an inner region, called the Stern layer, where the ions are strongly bound and an outer diffuse region, where they are less firmly attached. Within the diffuse layer there is a motional boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to the electric field), ions within the boundary move with it, but any ions beyond the boundary do not travel with the particle. This boundary is called the surface of hydrodynamic shear or slipping plane. The potential that exists at this boundary is known as the Zeta potential. If no net charges are present, surface charge would be zero. This is what would be expected for zwitterionic lipid particles, either multilamellar or large unilamellar. However this is not the case even at low ionic strength for PC, PE and methylated PEs. One possibility, discussed in literature, was to consider that the positive end (choline or ethanolamine groups) is displaced toward an inner membrane plane leaving the negative end (the phosphate group) more exposed to water. However, for the external electrical field, the separation of the two opposite charges in the membrane surface is negligible.

Thus, it is more reasonable to think that the measured zeta potential cannot be ascribed only to the attached surface charges, in which case the surface potential of zwitteionic ions should be zero, but rather to the specific adsorption of polarizable anions to the surface in the Stern or inner Helmholtz layer. Mobility determination requires, at least, low ionic concentration and in this condition, anions, mainly chlorides adsorb to the surface. The more polarizable the anions are the more negative is the zeta potential [34].

Thus, values of zeta potentials do not correspond to the Coulombic but rather to the non-Coulombic surface potential, which affects ion distribution near bilayer and thus affects vesicular drag in an external electrical field.

In consequence, the changes in the zeta potential produced by the presence of Arg reflected in a shift to negative values of the zeta potential can be interpreted as an effect on the distribution of ions at the slipping plane produced by the presence of the amino acid. Therefore, Arg interaction cannot be considered only as driven by electrostatic forces but also by polarization forces. As observed in Fig. 1 (supplemental) no significant differences are observed between MLVs and LUVS, denoting that no significant topological changes are present in both types of particles.

At 18 °C, Arg adsorbs to DMPE liposomes following a non-Langmuir isotherm with an affinity binding constant $K = 2\times10^3\ M^{-1}$ and $n = 0.74$. This means that the adsorption takes place by promoting surface rearrangements involving area and surface pressure changes.

Isothermal titration calorimetry has shown that the enthalpic change for the Arg adsorption to DMPE is −24 kJ/mol, compared to −7 kJ/mol in DMPC, both in the gel state, which is congruent with the increased adsorption on PE (to be published). This result suggests that Arg adsorption on PE is energetically high enough to promote structural rearrangements at the interphase. Arginine molecules could be stabilized in PE membranes by the formation of strong H-bonds with the ammonium, PO or CO of the lipid group.

Arg binds to the NH free groups rendering the phosphate groups less bounded allowing the entrance of water with a concomitant increase in surface pressure as shown in Fig. 3.

The variation of the slope of the $\Delta\Pi/\Pi$ curve, noticeable only in DMPE, determines a clear cut off value that is not observed when a single methylation is present. Thus, methylation hinders the strong interaction with the NH groups. According to Yu and Hui, DMPE, mmPE, and dmPE are in the solid condensed state in the range of surface pressures corresponding to the initial pressure range [19], (Fig. 2). Thus, all lipids are in the gel state and differences in the Arg interaction cannot be ascribed to the phase state but to differences in the surface arrangements for each lipid. In this regard, it was previously observed that the area per lipid for PE increases in around 8% in presence of Arg, while no change was observed in PC membranes in liquid condensed state. This supports the idea that the degree of coverage is a function of the change in the area per lipid ($f(a_L)$) induced by the amino acid as $\theta = n_{Lo}/n_L.f(a_L)$.

Possibly, the concomitant hydration of the phosphocholine head group is favored by the voluminous choline groups in its adjacencies when the membrane is in the fluid state.

These conclusions are supported by recent Molecular Dynamics results denoting that Arg forms a higher number of H-bonds with PE membrane than with PC, indicating a deeper minimum of energy for the stabilization of the amino acid in PE membranes, which is again in the same direction of the high enthalpic change pointed above [14].

It has been argued that headgroup methylation may play an important role in biological signal transduction [16]. Increasing headgroup methylation exhibits marked changes in the shape of solid domains, in monolayers [19]. In this regard, the number of methyl groups at the amino moiety of the phospholipids is supposed to play an important role in determining the domain shapes. According to the present results, these domain properties may be connected with the number of groups with the ability to form hydrogen bonds.

The so-called 'bridging' effect occurs only in those lipid monolayers containing a few methyl groups. Even dmDPPE monolayers show some degree of bridging at the expanding cycle, but this feature is absent in DPPC monolayers. The bridge effect is also seen in DPPE monolayer containing 5 mol% DPPC. The consequence of PE methylation on melting volume apparently reflects the fact that the ability to form hydrogen bond is lost after the addition of the third methyl group [32]. This topological property affects the partition of the amino acid: the higher the bridging, the higher is the Arg interaction.

The sequence of insertion, according to the affinity constant values, decreases in the order DMPE ≅ N-methyl-DPPE > N, N-dimethyl-DPPE > DMPC. Except for PC, the increase in affinity is comparable to that found for the decrease in the melting volume [32].

It is interesting to notice that the sequence found for the affinity constant obtained by liposome mobility is similar to that found in the Arg insertion in monolayers at surface pressures below 30 mN/m, (Fig. 2). In this condition, monolayers are in the lower middle of the isotherm branch towards the liquid expanded region. However, above 30 mN/m, the monolayer approaches the collapse pressure. It is likely that, at high pressures, topological rearrangements do not allow to obtain a congruent sequence.

A more precise correlation can be made when the magnitude of the surface pressure at equilibrium is compared with the decrease in the zeta potential from PE to PC (Table 1). The zeta potential of liposomes increases to negative values along de-methylation from PC to PE. Gel DMPC zeta potential is not affected by Arg.

The results of zeta potential and kinetics of insertion suggest that the interaction and final stabilization of a guanidinium-phosphate complex are modulated by the steric hindrance due to the presence of bulky methylene groups in the phosphate adjacencies.

The relative increase in surface pressure induced by Arg decreases with the methylation of the ethanolamine group, to a minimum close to the experimental error, independent of the initial surface pressure. As a comparison the results obtained with mixture 1:10 PC:PE and 1:10 PE:PC are included. This is a very important and interesting result, since it puts into relevance the cooperativity of the surface groups of adjacent molecules in the membrane arrangement. This is also sustained by the n coefficient lower than 1 suggesting a 2:1 lipid–Arg stoichiometry in the non Langmuirian process.

Inspections of the changes in PC and PE mixtures, induced by the amino acid in the sub phase, are shown in Fig. 5.

The ΔΠ vs time curves showed that the insertion rate and the final equilibrium surface pressure decreased in the order phosphatidylethanolamine > monomethyl phosphatidylethanolamine ≈ dimethyl phosphatidylethanolamine ≈ idem phosphatidylcholine, similar to those for the affinity constant and the surface pressure increases shown in Figs. 1 and 2.

The diffusion regime is mostly linear with the square root of time, denoting a purely diffusion process in mmPE, dmPE and PC. In contrast, in the case of PE, the kinetics could be described by the so called anomalous subdiffusion [33]. In this case, the diffusion can be described by

$$<r^2> = 4D\ t^n.$$

This equation can be written as

$$<r^2> = 4D\ t^{(n-1)}t \cong 4\check{D}_{(t)}t$$

with $\check{D}_{(t)}$ is a time dependent diffusion coefficient. When diffusion follows this equation it can be concluded that the matrix is not laterally homogeneous and that it contains obstacles of some kind. Although diffusion experiments cannot predict the nature of obstacles, the comparison of methylated and nonmethylated lipids suggests that this may be of kinetic nature, which microscopically can be ascribed to the formation of H bonds in the overall process of insertion.

Thus, as the n coefficient is below 0.5 within the experimental error, diffusion is coupled to structural rearrangements of the matrix in which the solute (in our case Arg) diffuses in.

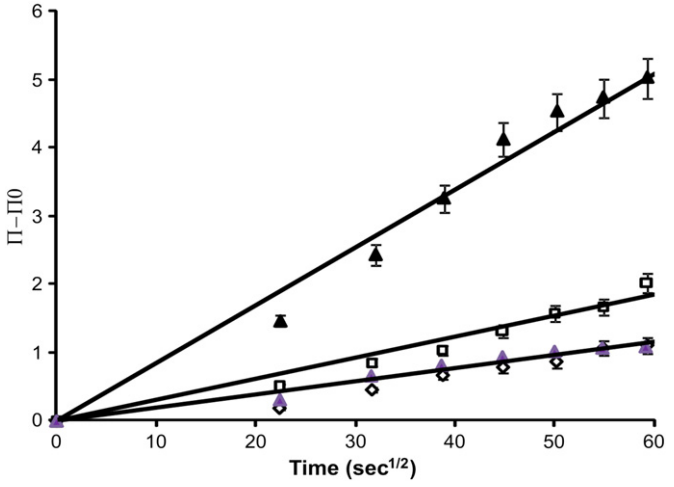

Fig. 5. Variation of surface pressure after injection of Arg on monolayers with DMPE (▲), DMPC (◊), mmDPPE (□) and DMPE: DMPC 1:0,1 (▲) as a function of time$^{1/2}$. Initial surface pressure for all the curves was 10 mN/m.Regression coefficients (▲) 0.981, (□) 0.984, (◊), (▲) 0.98.

5. Conclusions

- The effect of Arg on PE monolayer's surface pressure becomes more relevant in the absence of methyl groups on the ethanolamine moiety.
- A single methylation changes abruptly the ΔΠ/Π curves making it independent of the initial pressure.
- The response of mmPE and dmPE to Arg insertion falls in between PE and PC because headgroup methylation has significant different packing effects in bilayer membranes. This agrees with previous headgroup hydration measurements and the formation of domain shapes [18].
- In PCs there is no possibility to form hydrogen bonds with the amine group. Thus, neither the zeta potential nor the dipole potential is changed by arginine in DMPC in the gel state.
- The more negative zeta potential the greater the ability of the NH_2 to form hydrogen bonds.
- The surface pressure changes seem to be a function of the ability of the NH_2 to complete the H bonds. An incomplete net of hydrogen bonds between the Arg and the NH_2 does not produce a change in the surface tension.

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