THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

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Molecular Dynamics Study of the Interaction of Arginine with Phosphatidylcholine and Phosphatidylethanolamine Bilayers

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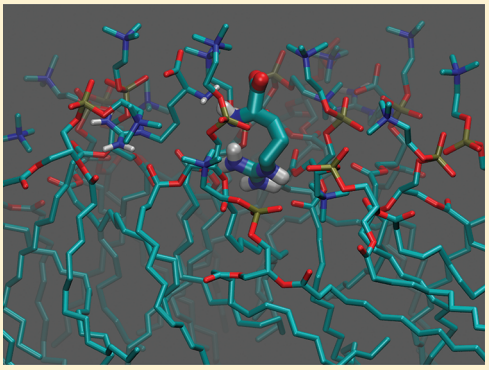
Supporting Information

ABSTRACT: In this work, the differential interaction of zwitterionic arginines with fully hydrated dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) bilayers was analyzed by molecular dynamics simulations. In both systems, arginine binds to lipids with the carboxylate moiety oriented toward the aqueous phase, in agreement with previous experimental determinations of ζ potential of DMPC and DMPE liposomes. The guanidinium groups are found at different depths within the bilayers indicating that some arginines are buried, especially in DMPE. We observe, in the DMPE system, that the strongest interaction occurs between the guanidinium group and the carbonyl oxygen of the lipid. In the case of DMPC membranes, the strongest interaction is found between the guanidinium groups of the arginines and the phosphate groups of the lipids. Unexpectedly, arginine zwitterions are stabilized through the creation of hydrogen bonds (HB), either with water or with polar groups of the lipids. The mechanisms of interaction seem to be different in both membranes. In DMPE bilayers, arginines insert by breaking the inner HB network of the polar head groups, consequently increasing the occupied area per lipid molecule. In the DMPC bilayers the arginines insert by replacing the already present water molecules within the membrane, without significant effects on the area per lipid.



INTRODUCTION

L-Arginine (Arg) is an important component of several peptides and proteins of biological relevance that interact with membranes.[1] The interaction of proteins and peptides with biological membranes is, in many cases, due to contacts between specific Arg residues and the lipid components of the membrane. For example, when crystallized, several Arg residues on the S4 helix of the potassium channel attach to the hydrophobic region of the lipid membrane.[2−4] The fact that Arg is transported and accumulated in different cell types, strongly suggests that this molecule would be able to penetrate cells or plasma membranes composed of different mixtures of lipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE).[5−9]

The insertion of peptides containing the basic amino acid Arg in fully hydrated lipid membranes has been a matter of discussion, since high energy costs are associated with the insertion of highly charged guanidinium groups into the hydrocarbon core of the lipid membrane.[10−12] One plausible mechanism to explain how Args insert into membranes is to consider that the insertion is favored by stabilization of the guanidinium moieties in water pockets inside the hydrophobic membrane phase.[13] This idea is at odds with the classical model of biological membranes, which treats the lipid bilayer as a homogeneous low dielectric slab, where the hydrocarbon and polar regions are separated by an ideal plane (the hydrocarbon-polar region interphase). Rather, the polar head groups at this interphase are imbibed in water including its hydration shells (occupying a finite region of c.a. One nm thick) that may play a substantial role in the kinetics and the thermodynamics of amino acid and peptide stabilization. Therefore, depending on the composition of the membrane (PEs with 2 to 4 water molecules per lipid molecule[14−16] and PCs with around 18 to 22 water molecules per lipid molecule, in the fluid phase[17]), the physicochemical characteristics of the interphase, according to the hydration level[16−19] and the differential topological feature derived from it, might affect the mode of insertion of arginine.

Previous experimental results have shown that the Arg monomer is adsorbed into PC and PE membranes increasing the negative zeta potential of liposomes in suspension.[20,21] This result was interpreted considering that the negative moiety of the amino acid, i. e. the carboxyl group, orients and projects to

Received: October 6, 2011
Revised: March 23, 2012
Published: March 26, 2012

dx.doi.org/10.1021/jp2096357 | J. Phys. Chem. B 2012, 116, 4476−4483

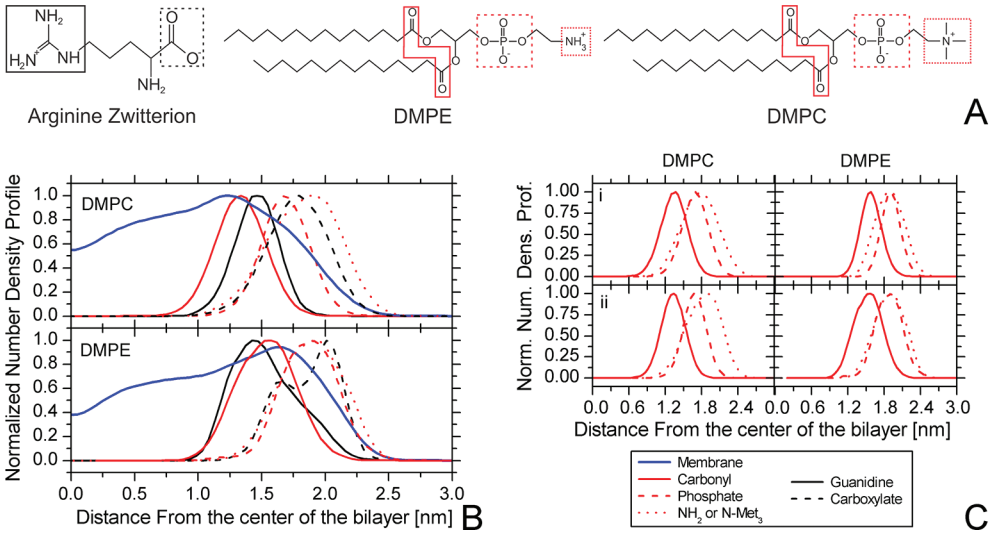


Figure 1. (A) Scheme of the lipids and arginine molecules in the zwitterionic state. The functional groups of each molecule use the same color coding and line styles in all figures. (B) Normalized number densities for all the components of the DMPC and DMPE membrane bilayer systems with zwitterionic arginine. (C) Normalized number densities of individual lipid functional groups. (i) Density profiles in control DMPC or DMPE membranes (devoid of arginine molecules). (ii) Density profiles in DMPC or DMPE membranes containing zwitterionic arginines.

the water phase.[20] In addition, the replacement of the carboxyl group by an hydrophobic residue such as a benzyl ether group charges the liposomes positively, indicating that the carboxylic group has more affinity for water.[20] Therefore, several theoretical studies have investigated the role of solvation in the process of insertion of amino acid side chains into membranes.[13,22,23] However, experimental results clearly indicate that Arg can adsorb into PCs only in the fluid phase while in the case of DMPE membranes it is adsorbed also in gel phase.[21] This peculiar feature of binding to PE in gel phase could involve subsequent structural changes coherent with a fluidification process triggered by specific interactions with H-bonding groups. Recently, it has been shown that the methylation of ethanolamine groups in PE hinders drastically the adsorption in the gel state.[24] Therefore, insertion in PE could be described by a binding followed by a partition, which affects the bilayer packing by an area increase, thickness decrease and/or changes on the water penetration.[20,21] Most likely, all these processes would have an effect in chain mobility.

Because Arg contains several potentially forming H-bond (HBs) groups (namely, guanidinium, α-amine and carboxylate groups, Figure 1A), it is important to determine which of those groups are specifically involved in the stabilization of Arg in PE bilayers.[25−27]

In this work, we therefore seek to analyze the mode of insertion of Arg monomers into dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) bilayers using molecular dynamics (MD) simulations, on the basis of interactions between lipid moieties (phosphates, amines), arginine residues (guanidinium, amines, carboxylate) and water molecules.

METHODOLOGY

To analyze the singular behavior of arginine in contact with different zwitterionic membranes, we constructed two different systems: one with DMPC and another with DMPE bilayers, both of them under conditions similar to previous experiments performed by us.[21] For each one of the phospholipid bilayers under study, we simulated a system with 10 molecules of arginine in the zwitterionic state, and one without arginines as a control. All the arginine molecules started with the same conformation, a stable zwitterionic state, which was obtained after a short 10 ns molecular dynamics simulation in water (Supporting Information Figure S1-A). The 10 arginine molecules were located at random position close to one membrane leaflet.

Arginine is found in the zwitterionic state in aqueous solution at pH's between 8.9 and 12.5 with a PI of 10.76. In the arginine zwitterion, the guanidinium group is positively charged, the carboxylate group is negatively charged and the functional α-amino group is neutral, rendering the molecule electrically neutral. Argninine zwitterions were simulated mimicking the experimental conditions used as a reference in this work.[21] Furthermore, ab initio calculations suggested a very basic character for neutral and zwitterionic states, with similar atomic charge distributions in both of them.[28] Therefore, the results obtained for the arginine zwitterion could be applied to alternative protonation states.

Four systems were studied in this work: two containing DMPC and DMPE membranes, each with 10 arginine molecules in their zwitterionic state, and two with DMPC and DMPE bilayers, without arginine molecules, used as controls. The membranes contained 128 lipid molecules (DMPC or DMPE) with 64 lipids in each leaflet, surrounded by about 6,000 water molecules. The control systems (without the arginine molecules) were constructed using an equilibrated DMPC membrane in water available from previous studies,[29] the DMPE membrane was created by replacing the carbon atoms of the trimethylammonium group of the PC lipids with hydrogens. Around 150 mM of KCl was added to each system and they were equilibrated for 10 ns, in order to allow the electrolytes to freely diffuse.

The arginine molecules were placed at random positions in the aqueous face near to one of the two membrane leaflets into the systems after 10 ns of equilibration with the electrolytes (Supporting Information Figure S1-A and B). To keep the amino acids in contact with only one leaflet, they were restricted to move within 10 Å of one membrane leaflet

dx.doi.org/10.1021/jp2096357 | J. Phys. Chem. B 2012, 116, 4476−4483

following the same procedure described previously for the simulation of bilayer exposed to asymmetric concentrations of electrolytes.[29] Finally, 100 ns of MD simulation were performed on each system.

All MD simulations were performed using the Gromacs package version 3.3.3.[30−32] A direct cutoff for nonbonded interactions of 1 nm, and particle mesh Ewald for long-range electrostatics were applied. Berendsen baths[33] were used to couple the simulation boxes with a semi-isotropic pressure of 1 atm and a temperature of 323 K for the DMPC bilayer and 333 K for the DMPE bilayer in order to keep the bilayers in the liquid-crystalline phase.[34] Phospholipids, water/ions and arginines were coupled to separate Berendsen thermostats with a relaxation time of 0.1 ps. All bond lengths of lipid and Arg molecules were constrained using the LINCS algorithm[35] whereas the SETTLE algorithm[36] was used for water molecules. The united-atoms force field of Berger et al.[37] was applied for the phospholipids and the OPLS all-atom force field for the arginines.[38] All the systems were solvated with SPC water molecules.[39] KCl ions were modeled using the default parameters from the OPLS force field.[38] The time step in all the simulations was set to 2 fs.

Trajectories were analyzed using the standard tools from the Gromacs package.[40] The number densities profile, the water orientation and the number of hydrogen bonds were calculated and averaged over the last 20 ns of simulation, while the rest of the analysis were computed over the complete trajectory (100 ns). The number of hydrogen bonds was calculated based on an angle Acceptor−Donor-Hydrogen cutoff of 30 degrees and a distance donor−acceptor cutoff of 3.5 Å

RESULTS AND DISCUSSION

Density Profiles of Lipids and Arginines. The normalized number densities for all the components in PC and PE membranes with Arg are shown in Figure 1B. The effect that arginines have on the lipidic molecular structure is highlighted in a comparative manner in Figure 1C.

Our results reveal different mechanisms for arginine stabilization in both types of membranes systems, Arg-DMPC and Arg-DMPE. In DMPC, the guanidinium group lies between the carbonyl and the phosphate groups, while in DMPE bilayers it is found beyond plane formed by the lipid's carbonyl groups (Figure 1B) closer to the center of the membrane. On the other hand, the carboxylate group of Arg is localized between the lipid's trimethylammonium and phosphate groups in DMPC and in an outer plane toward the water phase in DMPE (black dashed line in Figure 1B).

The carbonyl−phosphate (CO−PO) distances observed in the arginine containing systems are similar to the distances observed in both control systems (Figure 1C). In DMPC, the insertion of Arg displaces the choline groups to the water phase, while in DMPE, NH_3 group remains in the same plane as the PO group. Additionally, in PE, the position of the PO group is more diffused in the presence of arginine, characterized by a wider distribution (Figure 1C).

The deep insertion of guanidinium groups into DMPE membranes and the outer plane localization of carboxylate groups show a tendency to orient arginines normal to the membrane plane or alternatively a thinning of the bilayer by local structural deformation. However, the presence of arginine in DMPE bilayers does not modify the relative position of the phosphate and ammonium groups weakening the hypothesis of membrane deformation. In contrast, although the phosphate group of DMPC bilayers with Arg remains at approximately the same position as in the control system, the position of the trimethylammonium group is slightly shifted toward the aqueous solution leaving the carboxylate of the amino acid in an inner plane with respect to the aqueous phase (Figure 1C).

Experimentally, adsorption of Arg to DMPC and DMPE liposomes produces a shift to negative values in ζ potential measurements.[20,21] There are two possible explanations for this observation: (I) If Arg is neutral at pH 10 in solution, when adsorbed, the negative charge could arise from the deprotonation of some Arg molecules (some Arg would have a charge of −1 and others would remain neutral, since adsorbed Args may display altered pKas). (II) If Arg would be in solution in a cationic form, the shift to negative values in zeta potential measurements could be the consequence of the adsorption of counterions on the exposed groups, mainly polarizable anions such as chlorides. These two points are antagonist in nature, since in the first case, the negative end of arginine dipoles point to the aqueous phase, whereas the second case presents a scenario where the guanidinium groups are the ones facing the water phase.

Although the contribution of ions at the electrical double layer cannot be disregarded, our molecular dynamics simulations clearly show that the guanidinium groups are deeply inserted into the membrane phase and the carboxylate groups pointing toward the aqueous phase, sustaining the first interpretation of the ζ potential results.[20]

We dissected the density profiles of each arginine chemical group (guanidinium and carboxylate) in DMPC and DMPE to analyze the internal composition of the average density profiles (see Figure 2). The bimodal distribution observed for the

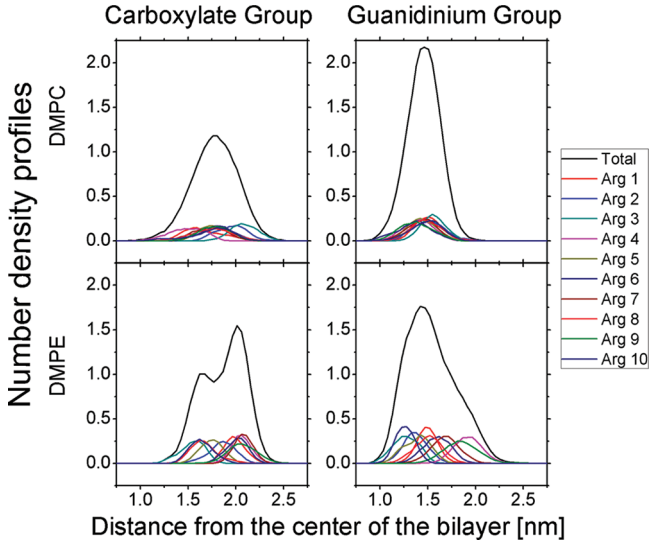


Figure 2. Number density profiles of the arginine functional groups (carboxylate and guanidinium groups) in DMPC or DMPE bilayers, in units of $1/nm^3$. The total, as well as the individual traces, of each Arg molecule is shown.

carboxylate group in DMPE membranes suggests that arginine molecules are buried preferentially at two different depths within the bilayer (in the Z direction). Nonetheless, a different behavior is observed in the case of DMPC bilayers (Figure 1B and 2), where both groups are homogeneously distributed along the Z axis. The density profiles in Figure 2 also show broader distributions in DMPE compared to DMPC, indicating

dx.doi.org/10.1021/jp2096357 | J. Phys. Chem. B 2012, 116, 4476−4483

that the molecules are more dispersed along the Z axis in DMPE (Figure 2).

In summary, arginine molecules insert normal to the membrane surface in both types of lipid membranes, with the guanidinium pointing inward and the carboxylate facing the aqueous phase (Supporting Information Figure S1−C). This particular orientation of the arginines is in very good agreement to the previously proposed explanation for the experimentally observed negative charging of liposome surfaces upon addition of arginine.[20]

The dipole moment of the Arg molecule is defined as the vector formed between the center of mass (COM) of the negatively charged carboxylate group and the positively charged guanidinium group. The modulus of this vector would be proportional to the value of the dipole moment.[41] Figure 3

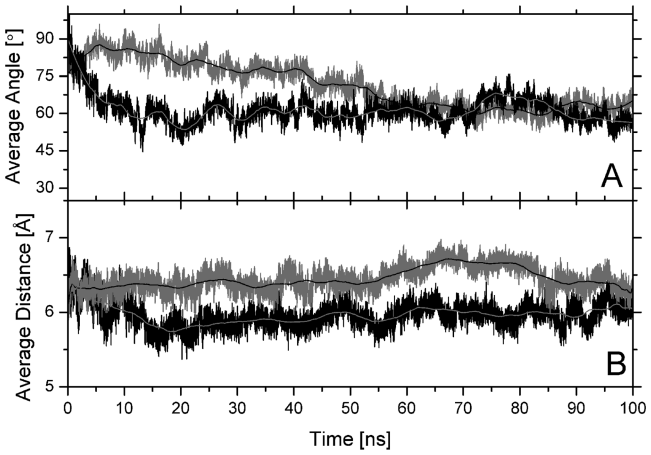


Figure 3. Time evolution of arginine dipoles' orientation and modulus. (A) Time evolution of the angle formed between the bilayer normal and the arginine dipole. (B) Time evolution of the distance between the guanidinium and carboxylate groups (proportional to the value of the arginine dipole). Color code: black for DMPC and gray for DMPE bilayers.

shows the angle between the arginine's dipole and the bilayer normal (proxy for the orientation of arginine molecules) and the distance between these two groups which is proportional to the value of the dipole moment, both measured along the simulated trajectories. The results display a similar orientation of arginine dipoles in both types of membranes, with an average angle of around 60° (Figure 3A). The distance between the carboxylate and guanidinium groups' COMs is in average 0.5 Å larger in PE than in PC membranes (Figure 3B). This is consistent with a deeper insertion of the guanidinium group and an outer location of the carboxylate group in PE membranes. Additionally, it comes out from Figure 3 that equilibration times in both membranes are different. In the case of DMPC, the system equilibrates at about 20 ns, whereas in DMPE the equilibrium is reached at nearly 70 ns. This may be due to breakage of the hydrogen bond network formed between lipid heads[42−44] after arginine insertion in DMPE membranes. Reasonably, the narrow density profiles observed for the phosphate and carbonyl groups in control DMPE (Figure 1C) could be the result of movement restrictions via formation of intermolecular hydrogen bond networks with lipid molecules.[42−44] In the presence of Arg in DMPE membrane, the width of the peaks for the density profile of both chemical groups increases, probably due to the perturbation of the intermolecular hydrogen bond networks, but it is not the case in DMPC bilayers, where the density profiles remain unmodified (Figure 1C). Therefore, in DMPE membranes, arginine adsorption disrupts the existing hydrogen bond networks formed between the PE head groups, enhancing the mobility of the phosphate and carbonyl groups. The increased mobility of phosphate groups is in accordance with the previously reported increase in surface area on DMPE monolayers,[21] with the resulting reorganization of the water molecules surrounding the head groups. This point is analyzed in the next section.

Further scrutiny of the results shown in Figure 3 corroborates that, although similarly oriented, arginine molecules are found in more extended conformation in DMPE membrane. The required energy to force an extended conformation of an Arg molecule in the DMPE interphase is probably compensated by a larger number of hydrogen bond interactions formed between the amino acid and the PE lipids, as we demonstrate in the "hydrogen bond formation" section.

Water Molecules in PC and PE Membranes: Ordering and Change in the Amount of Water Due to the Presence of Arginine. A characteristic difference between PE and PC membranes is their hydration level. It is known that PCs are more hydrated than PE membranes.[45,46] Therefore, it was worthy to analyze how the hydration level affects the process of insertion and how it may be modified upon addition of arginine molecules in each system. We therefore calculated solvent density profiles as well as the individual orientation of the water molecules for both PE and PC lipid systems. We also report the solvation profiles of the amino acid.

The orientation of the water molecules was evaluated as the average Cosine of the angle (α) between the dipole of the water molecules and the Z axis of the system. In the bulk, the water molecules do not have a preferential orientation. Hence, it is expected that $\langle \cos \alpha \rangle = 0$, where α is the angle between the water dipole and a given orientation. However, if the orientation of the water molecules is calculated in the presence of a membrane, $\langle \cos \alpha \rangle$ is negative as a consequence of the formation of hydrogen bonds between the oxygen of the glycero-phosphatidyl groups and the hydrogen of the water molecules. The ordering effect vanishes and $\langle \cos \alpha \rangle$ tends to zero as the distance from the membrane increases, displaying a well-defined hydration zone in the area among the CO and PO groups. This behavior was generally found in all the studied systems (Figure 4A), except that in PE where the curve is more negative and narrow than in PC, centered close to the CO, probably due to the existence of the known HBs in PE. Moreover, the dipole potential in PE is higher than in PC membranes, consistent with tighter and more organized water and lipid dipole arrangements.[47] This could be ascribed to the different topological features of both membranes.

The difference between the density profile of water for both systems with and without arginines is shown in Figure 4B. The difference $\Delta\delta_{wat} = \delta_{wat\text{-}arg} - \delta_{wat\text{-}noarg}$ (where $\delta_{wat\text{-}arg}$ is the number density profile of the system with arginine molecules and $\delta_{wat\text{-}noarg}$ is the number density profile of the control system) becomes positive if the addition of arginine molecules increases the density of water molecules on a given slice in the Z direction, it becomes negative if the density of water molecules decreases and is zero if the density remains unchanged. As seen in the figure, the presence of arginines increases the density of water molecules in the PE bilayers up to a distance of 1 Å from the carbonyl oxygen. PC bilayers, on

dx.doi.org/10.1021/jp2096357 | J. Phys. Chem. B 2012, 116, 4476−4483

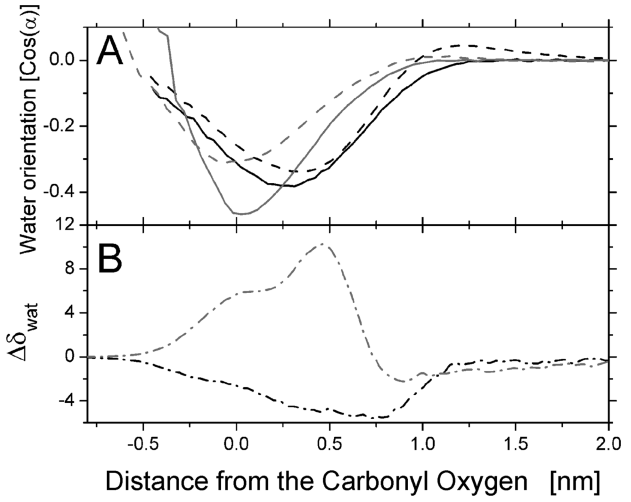


Figure 4. (A) Average orientation of water molecules with respect to the bilayer normal for the PC and PE bilayers. Control systems (without arginines) are shown in solid lines while the systems with the arginines molecules are shown in dashed lines (color code: black for DMPC and gray for DMPE). (B) Difference curves of $\Delta\delta$**wat** ($\Delta\delta$**wat** = δ**wat-arg** − δ**wat-noarg**) for the DMPC systems in black and DMPE systems in gray. $\Delta\delta$**wat** means the difference density profile curve of water molecules, δ**wat-arg** is the water density profile for the system with arginine molecules and δ**wat-noarg** is the water density profile for the system without arginine molecules. In both figures, for comparison, the systems were centered at the position of the carbonyl oxygen atoms since these atoms are located at the interphase between the hydrophobic and hydrophilic regions of the lipids.

the other hand, have a slight decrease in the number of observed water molecules at the same distance. Farther away (at a distance greater than 1.2 Å from the carbonyl oxygen), no significant changes are observed in any of the considered membranes. This emerges as a clear distinction between arginine modes of insertion in both membrane systems. The same behavior is observed in Supporting Information Figure S2, with the number density profile in the presence and absence of arginines (Supporting Information Figure S2) showing greater number of water molecules at the interphase of the PC than the PE bilayers, with or without arginines. The difference is maintained up to a distance of 0.5 nm from the carbonyl oxygen. The difference is observed even though the change in the amount of water molecules decreases in PC and increases in PE membrane upon the addition of Arg.

In combination with the increase in phosphate mobility, these results strongly suggest that the mechanism of arginine insertion in PE membranes involves the access of water molecules to the bilayer matrix. This is in complete agreement with previous experimental results of the generalized polarization (GP) values of Laurdan.[21,48,49] Under similar conditions, arginines reduce the GP values of Laurdan only in the case of PE membranes, implying that the interaction of Arg with PE promotes a different reorganization of the water molecules. In Figure 4, it is observed that the number of water molecules increases in PE upon addition of arginine molecules, but maintaining a greater disorder than the observed in PC membranes, also in agreement with experimental results.[21]

In addition, we followed the number of water molecules around the amino acids, by measuring the cumulative number of water molecules around each individual arginine functional group (Supporting Information Figure S3). We see that the first water molecule, associated to carboxilate groups, is in closer contact in PC membrane than in PE, whereas the water molecules associated to guanidinium groups remain in closer proximity in PE over PC membrane, in agreement with the results discussed above.

Hydrogen Bond Formation. The Figure 5 shows the number of HBs between: (i) Arg and lipids (Figure 5A), ii) Lipids and solvent (Figure 5B) and iii) Arg and solvent (Figure 5C). Furthermore, it is worth to mention that for Figures 5A and C the HBs are presented per arginine molecule but for Figure 5B they are reported per lipid molecule.

Hydrogen Bonds Formed between Arginine and Lipid Molecules. Figure 5A shows the total number of HBs per arginine molecule as well as the breakdown of the number of HBs with the individual functional groups of arginines and lipids. According to the figure, there is an increase in the total number of Hbs per arginine molecule in DMPE when compared to DMPC membranes and it shows the ability of argininès carboxylate to form HB with the lipid ammonium groups of DMPE (second panel). The later interaction further stabilizes the arginine molecules in DMPE compared to DMPC membranes, providing a potential explanation to the observed extended conformation of the molecule in PE, as previously mentioned

If we focus on the guanidinium groups of the amino acids, we notice different modes of interaction with the functional groups of both membranes. In DMPE, guanidinium groups form higher number of HBs with carbonyl oxygen, whereas a greater number of HBs is achieved with DMPC's phosphate groups. This is in agreement with Arg molecules penetrating deeper into DMPE membranes (previously discussed in Figures. 1 and 2). Finally, formation of HBs between the α-amino group and the lipid phosphate and carbonyl groups are not significant. Therefore, the strong interaction of Arg with the amine groups of PE leaves the phosphate groups less bounded, explaining the higher mobility of the phosphate groups in PE membranes evidenced also by a wider density profile (Figure 1). In addition, this would denote the possibility that more water spaces could be created between the lipids, resulting in an increased mobility of the carbon atoms of the acyl chain near the headgroup. This correlates with the reported decrease in the transition temperature of DMPE, as measured with fluorescence anisotropy and generalized polarization.[21]

Hydrogen Bonds Formed between Lipid and Water Molecules. DMPC membranes show a larger number of interactions with water than DMPE, both in the presence and absence of arginines.[14] We see that the total number of HBs formed between lipid and water molecules slightly decreases upon adsorption of arginines on DMPC, whereas no significant changes are observed for the case of DMPE membranes (Figure 5B). The breakdown of the interactions shows a similar picture for the carbonyl and phosphate groups of the lipid molecules. The reduction in the number of HBs in DMPC upon addition of arginines can be explained by the fact that Arg molecules have to compete to remove solvent molecules during the partitioning process. Additionally, in DMPE membrane, the lipid's ammonium groups are also able to forms Hbs with the solvent.

Hydrogen Bonds Formed between Arginine and Water Molecules. The total number of HBs formed between Arg and solvent molecules is higher in the case of DMPC membranes (Figure 5C). The rest of the panels show the breakdown of the interactions for the different amino acid chemical groups, where

dx.doi.org/10.1021/jp2096357 | J. Phys. Chem. B 2012, 116, 4476–4483

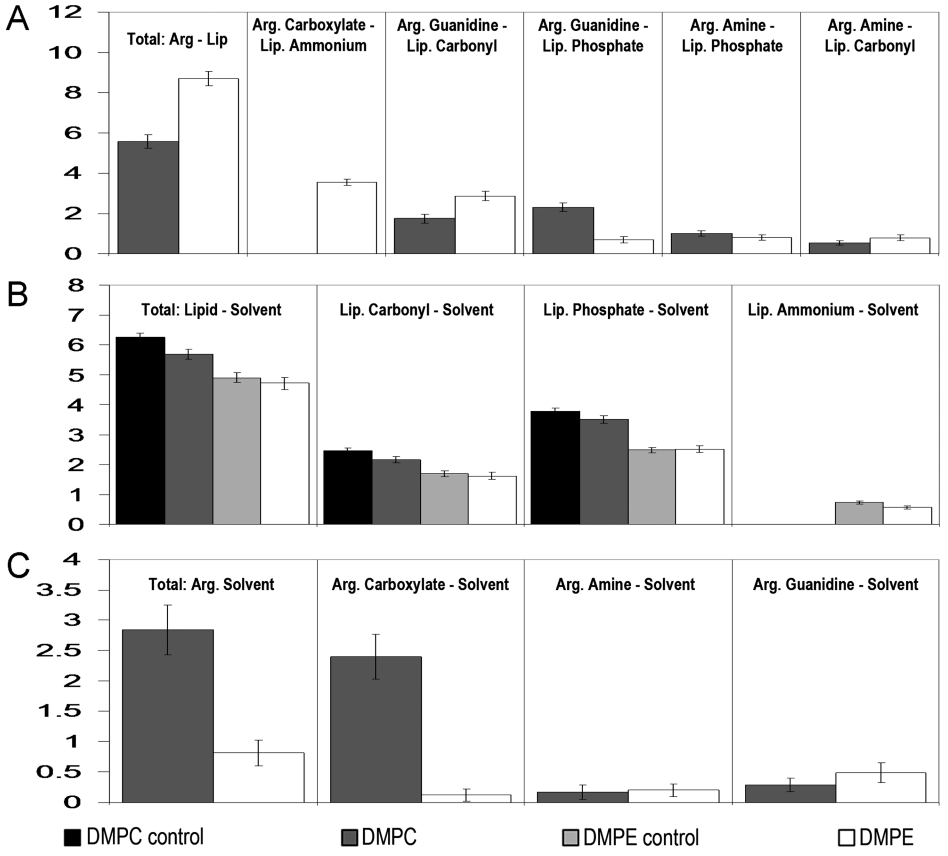


Figure 5. (A) Number of hydrogen bonds per arginine molecule formed between lipids and the amino acid molecules. (B) Number of hydrogen bonds per lipid molecule formed between lipids and the solvent molecules (for the lipids functional groups, the phosphate and the carbonyl oxygen groups contain the corresponding ester oxygen atoms that are also able to form Hbs). (C) Number of hydrogen bonds per arginine molecule formed between amino acid and the solvent molecules.

we observe that the carboxylate groups remain much more hydrated in DMPC membranes. Instead, in DMPE the carboxylate groups of the amino acid remain less hydrated since they are involved in intermolecular HBs with the lipid ammonium groups (as it was shown previously Figure 5A).

The total number of HBs formed between arginines and solvent molecules decreases during the course of the simulation, producing desolvation of arginines as the HBs are exchanged with the lipids in the process of adsorption into the membrane surface (Figure. 6A). The preference of Arg to form HBs with PE instead of water was thermodynamically tested, showing strong enthalpy changes upon addition of arginine molecules to PE lipids, measured by isothermal titration calorimetry (data not shown).

Hydrogen Bonds Formed between Lipid Molecules. In Figure 6B, we observe that the control DMPE system evolves a larger amount of HBs compared to the arginine containing DMPE system, suggesting that the interphase of DMPE bilayers is stabilized by inter/intra molecular HBs. The number of HBs in DMPE membranes with arginines decreases, implying exchange of intermolecular lipid–lipid to lipid-arginine HBs. The latter interaction is confirmed in Figure 6A by the observation that Arg molecules dehydrate during the binding process.

Area Per Lipid. The evolution of the area per lipid along the trajectory for the systems with and without arginines is shown in Figure 7. This figure illustrates that the area per lipid does not significantly change upon insertion of arginine molecules into the DMPC bilayer. The final area per molecule

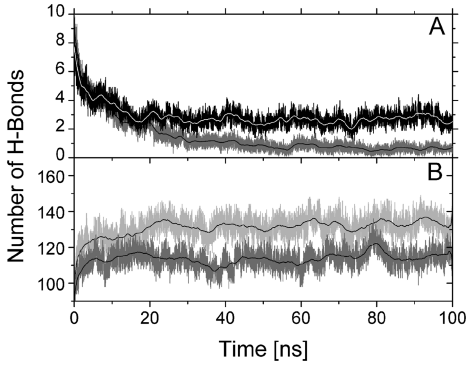


Figure 6. (A) Time evolution of hydrogen bonds formed between arginine and solvent molecules (DMPC and DMPE are shown in black and gray respectively). (B) Time evolution of the lipid–lipid hydrogen bonds in control DMPE membranes (without arginines) and DMPE membranes with zwitterionic arginines (control DMPE is shown in light gray and arginine containing DMPE in dark gray).

in PC is higher than in PE membranes, (65 Å^2 for PC and 52 Å^2 for PE), in agreement with previously reported areas.[15,42,50]

As we mentioned, arginines replace the water molecules located at the interphase of PC membranes and their adsorption do not require an area increase to attain the interaction. In contrast, the addition of arginines to DMPE bilayers increases the area per lipid, acting as a spacing molecule. The phenomenon can be explained by disruption of the HB network because of insertion of arginine molecules

dx.doi.org/10.1021/jp2096357 | J. Phys. Chem. B 2012, 116, 4476–4483

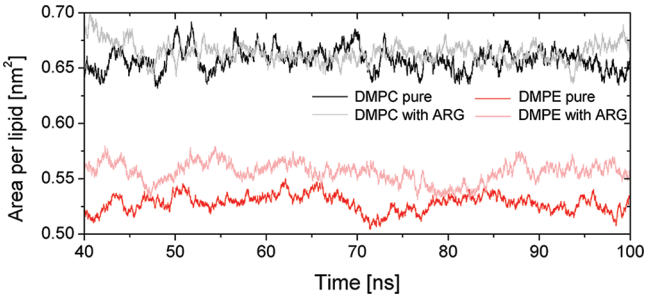


Figure 7. Time evolution of the per lipid area in both DMPC and DMPE systems. Only the last 60 ns are shown since the system is already equilibrated.

between lipids, with consequent entrance of water, both affecting lipid packing.

CONCLUSIONS

The present results indicate that the mechanism for Arg insertion into DMPE lipid bilayers proceeds through formation of HBs between carboxylate-NH_3 and guanidinium-carbonyl groups. In this process, the area is enlarged and the phosphates increase their mobility in total agreement with previous experimental findings (Bouchet et. al).[21] Formation of HBs between DMPE lipids and Arg carboxylate or guanidinium groups seems to energetically compensate the breakage of the lipid membrane intermolecular HB network. In this manner, the inserted arginines work as spacers in the membrane interphase that creates solvation pockets. Importantly, arginine molecules are further stabilized in PE membranes by the formation of HBs between the arginine's carboxylates and the lipid NH_3 groups in PE membranes, which is absent in PC systems. In this regard, NH_3 groups appear to be essential, explaining the reason why Args can even insert in PE membranes in their gel state.

In PC membranes instead, Arg is partitioned between the water solution and the lipid bilayers, without significant changes in the lipid area. In this case, the guanidinium groups interact with phosphates displacing at most two water molecules per Arginine. Previous theoretical works have shown that arginine is able to interact with a PC membrane because of the solvation of its guanidine group.[13,23,51] Our results additionally show that Arg may enter solvated into the membrane in preformed sites (water pockets) between the phosphates in PCs. This may explain why Arg partition in DMPC is only observed in the fluid state of the membrane.

In conclusion, the results of this work fit with the proposal that highly charged amino acid can partition into lipid membranes by two mechanisms: in fully hydrated membranes, such as fluid PC, by partitioning into the water in the membrane. In less hydrated membranes, such as PEs, by the formation of strong HBs with specific groups that may drive to the creation of a water pocket.

These two different mechanisms should be taken into account to explain insertion of Arg-containing peptides in complex membranes composed of two of the most important lipids in natural membranes, such as PEs and PCs.

ASSOCIATED CONTENT

Supporting Information

Figure S1: Initial and final configuration of the system with DMPC and 10 arginine molecules. Figure S2: Number density profile for the water molecules in all simulated systems. Figure S3: Solvation profile analysis. This information is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by ANII (Agencia Nacional de Investigación e Innovación). Programa de Apoyo Sectorial a la Estrategia Nacional de Innovación, INNOVA URUGUAY (Agreement No. DCI - ALA/2007/19.040 between Uruguay and the European Comisión). We thank Mario Pujato for proofreading the manuscript.

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