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# Interaction of Phenylalanine with DPPC Model Membranes: More Than a Hydrophobic Interaction

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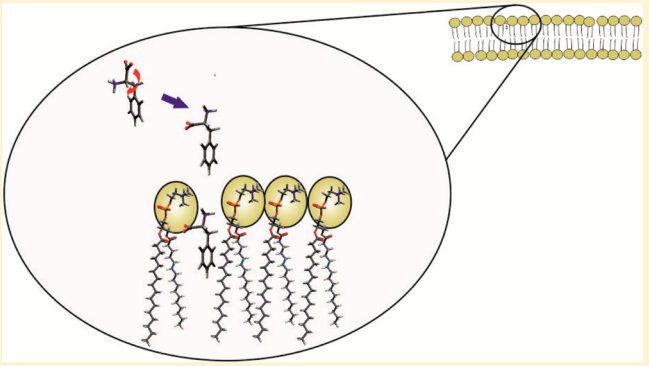
**ABSTRACT:** The negative free energy previously reported is explained by the stabilization of a PC-Phe (phosphocholine-phenylalanine) complex in the presence of water shown by the decrease in the symmetric stretching frequency of the phosphate group of the lipid ($PO_2^-$). An entropic contribution due to the disruption of the water network around the phenyl and in the membrane defect may be invoked. The dipole potential decrease is explained by the orientation of the carboxylate opposing to the CO of the lipids with oxygen moiety toward the low hydrated hydrocarbon core. The symmetric bending frequency of $NH_3^+$ group of Phe, decreases in 5.2 $cm^{-1}$ in relation to water congruent with zeta potential shift to positive values. The Phe to DPPC dissociation constant is $K_d$ = 2.23 ± 0.09 mM, from which the free energy change is about −4.54 kcal/mol at 25 °C. This may be due to hydrophobic contributions and two hydrogen bonds.



## INTRODUCTION

Phenylalanine (Phe) plays an important role in different physiological processes such as antimicrobial activity of certain peptides as clavanin, where its presence provides the peptide sufficient hydrophobicity, affinity, and conformational flexibility, necessary for its action.[1] Also, it is known that in pathological concentrations, phenylalanine self-assembly into amyloid fibrils.[2] According to White et al. the free energy of partition of this amino acid is around −1.4 to −1.8 kcal/mol both in interface scale and in octanol scale, indicating that the process is favored energetically. This behavior is considered to be a consequence of the presence of the highly hydrophobic phenyl group.[3]

In aqueous solution, Phe has a very high hydrophobicity that could influence the hydrogen bond network and its environment.[4] In addition, it is known that Phe can form a cation or an anion in aqueous solutions showing an amphoteric character.[5] This amino acid also presents a third form as a zwitterion.[6] It was reported by Kaczor et al. that L-Phe presents six conformers; two of these differing only in the arrangement of the amino acid group relative to the phenyl ring. They achieve the lowest energy by displaying a strong stabilizing intramolecular hydrogen bond of the O−H···N type and the carboxylic group O=C−O−H.[7] Whether these configurations may have consequences in the binding of Phe to lipid membranes and how water may affect it is uncertain. Recent results have shown that Phe interacts in lipid monolayers near the collapse pressure and with lipid bilayers subjected to a high hypertonic stress.[8] Thus, packing and hydration appears to affect Phe insertion. In the first case, it is expected that lateral pressure would promote packing imperfections due to mismatch of lipid head groups. This may cause a redistribution of water or a concomitant partial dehydration affecting affinity of the amino acid by the interphase. Vice versa, water extrusion by the hypertonic media increases the packing promoting similar defects by changes in curvature and/or dehydration, at least in certain regions of the bilayer. The partial exposure of hydrophobic regions may give the necessary surface hydrophobicity, which is expected to influence molecular arrangements of the phenyl group in phospholipids bilayers and monolayers.[9] The hydrophobic defects may include water arrangements to favor hydrophobic interaction.[10] Thus, the different states of hydration may affect the Phe insertion.[11,4]

Previous work has demonstrated that defects are related with the relative populations of hydrated and non-hydrated carbonyl groups of the phospholipids. Since they seem to involve water exposure of hydrophobic regions, the nature of the interactions of Phe is a point of immediate interest, in particular, if structural packing defects where Phe is intended to insert are water accessible in the gel state. In this context, in order to give an insight on the molecular interaction of Phe with different regions of DPPC molecules the effect on Phe on membranes groups in a bilayer at different degrees of hydration was studied by means of ATR/FTIR spectroscopy.

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## METHODS

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar lipids and L-phenylalanine (Phe) from Sigma-Aldrich and used without further purification. Chloroform was of analytical grade. Fresh stock solution of Phe was prepared immediately in order to avoid the fiber formation.[12] MiliQ water, obtained in an Osmoion 10.2 equipment, was used to prepare all aqueous solutions at a final pH of 5.0. In this condition the zwitterion specie was the predominant chemical form.[6,13] The lipid films were prepared from a 4 mM DPPC stock solution in chloroform[14] and resuspended with a 29 mM Phe aqueous solution to a final lipid concentration of 20 mM. That is, the final Phe/PC ratio was 1.45. The MLV's suspensions were frozen and thawed five times in order to favor the mixture of Phe with the lipids. All the spectra were recorded at this ratio for different degrees of hydration.

The ATR-FTIR spectra of L-Phe aqueous solution was recorded during water evaporation on ZnSe crystal (45°) at a temperature of 25 °C, a relative humidity of 40% and pH 5.0. Spectra of L-Phe aqueous solutions were measured between 650 and 4000 $cm^{-1}$ on a Thermo Scientific 6700 spectrometer. The spectrometer was connected to a system of circulation of dry air in order to avoid the interference of water vapor and carbon dioxide. All experiments were repeated at least three times and were analyzed with a Microcal Origin program (version 8.5). On ZnSe crystal 50 $\mu$L of the lipid sample with Phe was placed. The degree of hydration is monitored through the evolution of the water band of the lipid sample following the relation between the intensity of the water band and the asymmetric vibration of the methylene group. In cases where absorption bands appeared to be a summation of components, a combination of Fourier deconvolution and curve-fitting procedures was used to obtain estimates of the position of the component bands and to reconstruct the contours of the original band envelope.

The zeta potentials of DPPC MLV's were determined in Zeta-Meter System 3.0 equipment. All measurements were done at 22 ± 2 °C and pH 5.0. 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. The total lipid concentration in all cases was 0.15 mM and the Phe concentration was increased from 0 up to 11 mM. A total of 20 measurements were carried out focusing different particles for each sample. Data reported are the average of measurements done for each condition with, at least, three different batches of liposomes.[15]

## RESULTS

In the presence of Phe, the symmetric stretching frequency of the $PO_2^-$ groups decreases with the increase of hydration (Figure 1). This effect of water on the symmetric phosphate groups is not observed, as already informed, when pure lipids are hydrated.[16] The inspection of Figure 1 indicates that, in the absence of water, the sym $PO_2^-$ frequency is higher in the presence of Phe. At high hydration the $PO_2^-$ frequency becomes lower than that corresponding to pure lipids, indicating that, in the presence of water, Phe has a favorable interaction. No changes have been observed in the asymmetric $PO_2^-$ frequency and in the hydrophobic acyl chains at least in these experimental conditions.

The results in Figure 2 show the effect of water on Phe incorporated to bilayers, according to the procedure described in the experimental section. The wavenumber associated with the C−C stretching band of the aromatic ring increases with the

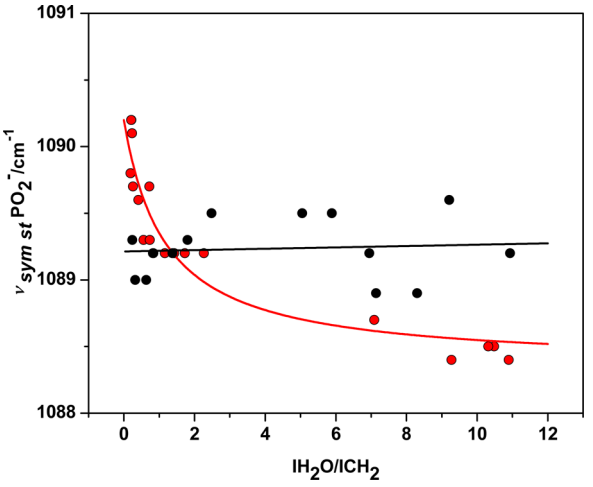


**Figure 1.** ATR-FTIR frequency of sym vibration of $PO_2^-$ as a function of the hydration degree. Red dots represent Phe:DPPC experimental points and the black solid line is the fitting curve. Black dots correspond to DPPC experimental points and gray solid line corresponds to the fitting curve.

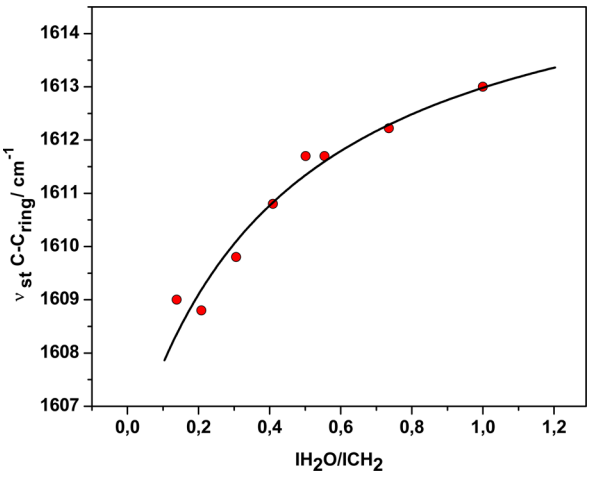


**Figure 2.** ATR-FTIR frequency of C−C vibration of aromatic ring as a function of the hydration degree. Red dots represent Phe:DPPC experimental points and black solid line is the fitting curve.

increase of water. Further inspection of the data denotes that the phenyl ring C−C St of Phe in water decreases from 1611.6 ± 0.2 to 1609.8 ± 0.8 $cm^{-1}$ in the absence of water, which would indicate that the possible interaction between Phe and DPPC is mediated by water exclusion.

A similar behavior is observed when the asym St $CO_2^-$ of Phe is analyzed in the presence of lipids, that is, the frequency shifts from 1583.8 ± 0.5 $cm^{-1}$ in the dry state to 1590.6 ± 0.9 $cm^{-1}$ at high hydration ($\Delta\nu$ = 6.8 $cm^{-1}$) (Figure 3).

In Figure 4A and B two independent experiments are compared. In part A, it is clearly observed that the symmetric bending frequency of $NH_3^+$ group of Phe, decreases in 3.3 $cm^{-1}$ in relation to the $I_{H2O}/I_{CH2}$.[17]

In addition, in Table 1, the frequency of symmetric $NH_3^+$ decreases 5.2 $cm^{-1}$ when Phe in water (3rd column) interacts with hydrated phospholipids (5th column). In part B, the zeta potential of the liposomes becomes positive with the binding of Phe. Taken together, the exposure of the positive group is congruent with the hydration denoted by the frequency decreases. A similar behavior was observed by analyzing the


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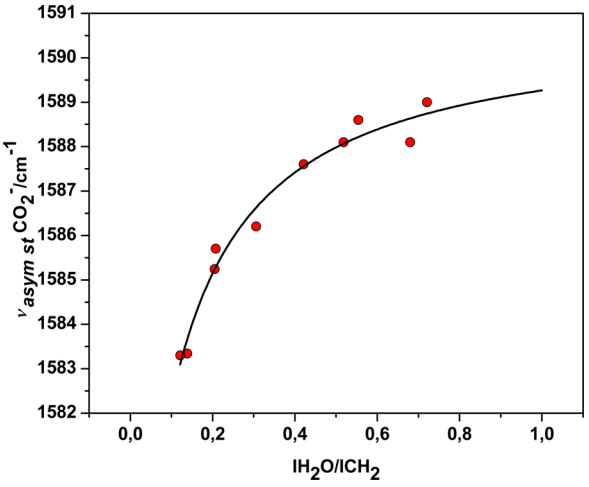


**Figure 3.** ATR-FTIR frequency of asym stretching vibration of $CO_2^-$ as a function of the hydration degree. Red dots represent Phe:DPPC experimental points and the black solid line is the fitting curve.

N−H stretching of the Phe; i.e., the N−H stretching frequency increases in the presence of lipids from 3115.5 ± 0.2 to 3123.2 ± 0.6 $cm^{-1}$ ($\Delta\nu$ = 7.7 $cm^{-1}$). However, this analysis could be done only at low hydration degrees due to the overlapping of this mode frequency with the −OH stretching of water.

## DISCUSSION

The interactions between phosphates with the lattice in the dry state are disrupted or weakened by the Phe insertion in the bilayer. The incorporation of water decreases the sym $PO_2^-$ frequency giving support to the proposal that the phosphate is isolated in the PC-Phe dry lattice.

The negative free energy previously reported by White and Wimley is explained by the stabilization of a PC-Phe complex in the presence of water.[3,18] However, this free energy decrease may have enthalpic and entropic contributions.

When Phe is in pure water, the phenyl groups are surrounded by water in which dipoles may interact with the $\pi$ orbitals by dispersion forces. The increase in the frequency associated with the C−C stretching band (Figure 2) would denote that this interaction is disrupted when inserted in the membrane, that is, a weaker interaction would take place. In order to favor the process an entropic contribution due to the disruption of the water network around the phenyl and in the membrane defect may be invoked. This insertion would promote $PO_2^-$ spaces available in the surface for water as shown by the frequency decrease detailed in Figure 1.

The significant increase of asym st $CO_2^-$ frequency of Phe might be explained considering that the dimer is displaced to monomer and that the carboxylate group becomes hidden from water when hydration increases.[19] This unexpected result is congruent with the shift to positive zeta potential values (Figure 4B) and with previous observations in which the dipole potential of lipid monolayers decreases with Phe insertion.[20,21] If it is accepted that the dipole potential is due to the carbonyl groups of the phospholipid normal to the membrane plane with the oxygen toward the water phase, making the membrane interior positive, the orientation of the carboxylate of Phe would be opposing to the CO of the lipids with oxygen moiety toward the low hydrated hydrocarbon core.

Control experiments followed the profile of the carbonyl groups indicating that in the present experimental conditions, the

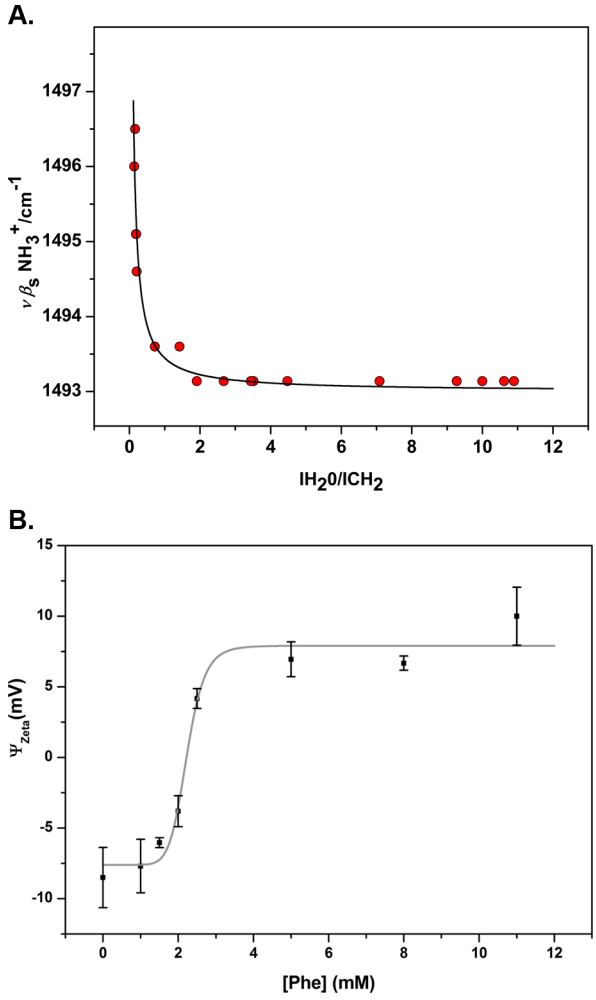


**Figure 4.** (A) ATR-FTIR frequency of sym $\beta$ vibration of N−H as a function of the hydration degree. Red dots represent Phe:DPPC experimental points and the black solid line is the fitting curve. (B) Zeta potential of DPPC liposomes as a function of [Phe]. The line indicates the best fit of the experimental data, according the equation

$$\frac{\Psi_z - \Psi_{zo}}{\Psi_z^{max} - \Psi_{zo}} = \frac{[Phe]^n}{[Phe]^n + K_d}$$

where $\Psi_z$ corresponds to the change of zeta potential, $\Psi_{zo}$ is the zeta potential in the absence of Phe, $\Psi_z^{max}$ is the zeta potential at the higher [Phe], $K_d$ is the Phe dissociation constant to the DPPC liposomes, and $n$ is the cooperative coefficient. A coefficient different from 1 corresponds to non-Langmuir isotherms in which adsorption sites are not independent.[14]

membrane is in the gel state as was previously reported and that Phe does not alter such a profile.[22,23]

The dissociation constant of Phe to DPPC calculated by fitting the curve of Figure 4B is $K_d$ = 2.23 ± 0.09 mM. From this value a free energy change of about −4.54 kcal/mol at 25 °C can be obtained. This negative value is higher than that reported by White and Wimley,[3] which was ascribed to the hydrophobic interaction of Phe with the lipid bilayer. The additional −2.56 kcal/mol is roughly equivalent to the energy of two hydrogen bonds.

The inspection of Figure 4B indicates that the adsorption of Phe does not follow a behavior corresponding to independent sites. Instead, the sigmoid profile suggests a strong cooperative process that would promote changes in the packing of the


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**Table 1. Wavenumbers for Principal Groups of Phe at Different Conditions of Hydration with and without DPPC**

| functional group | dry Phe $\nu$/cm$^{-1}$ | hydrated Phe $\nu$/cm$^{-1}$ | dry Phe with DPPC $\nu$/cm$^{-1}$ | hydrated Phe with DPPC $\nu$/cm$^{-1}$ |
|---|---|---|---|---|
| C−C arom ring | 1605.5 ± 0.4 | 1612.0 ± 0.4 | 1609.8 ± 0.8 | 1611.6 ± 0.2 |
| asym St $CO_2^-$ | 1580.0 ± 1 | 1589.4 ± 0.5 | 1583.8m ± 0.5 | 1590.6 ± 0.9 |
| $\beta$ sym $NH_3^+$ | 1496.5 ± 0.7 | 1498.4 ± 0.3 | 1496.5 ± 0.5 | 1493.2 ± 0.8 |

bilayer.[15] This is congruent with the surface pressure increase in DPPC monolayers reported previously.[9]

The insertion of the phenyl groups, normal to the membrane surface, illustrates on the hydrophobic interaction with the membrane surface. In Figure 5, it is schematically represented the

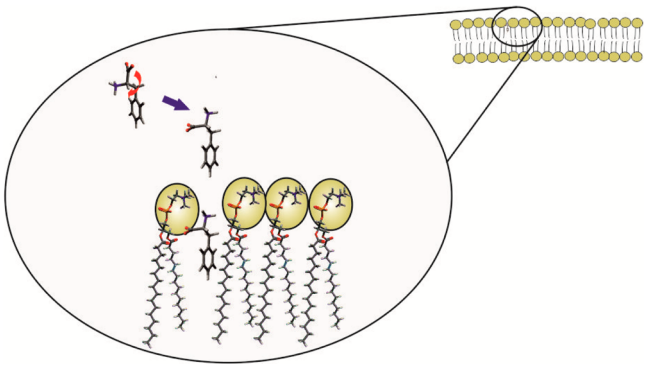

**Figure 5.** Schematic representation of the Phe insertion in the lipid interphase. Red arrow represents the rotation of the carboxylate group as a previous step to the insertion of Phe into the membrane.

rotation of the carboxylate group (see red arrow) as a previous step to the insertion of Phe into the membrane. This would give an average dipole orienting the oxygen groups to the membrane interior and $NH_3^+$ facing the water phase, congruent with dipole potential decrease and positive values of zeta potential.

## CONCLUSIONS

It may be concluded that although the phenyl group may interact hydrophobically with the membrane, the dipole interaction and the hydrogen bonding of the N−H with the water phase also contribute to PC-Phe stabilization.

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### Notes

The authors declare no competing financial interest.

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