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Effect of arbutin on the dipole potential and area per lipid of ester and ether phosphatidylcholine and phosphatidyl ethanolamine monolayers

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Abstract

The present results report for the first time a systematic study of the effect of arbutin on the dipole potential of lipid membranes. The dipole potential and the area per lipid were measured in monolayers of dimyristoylphosphatidylcholine (DMPC), 1,2-di-*O*-tetradecyl-*sn*-glycero-3-phosphocholine (dietherPC), dimyristoylphosphatidylethanolamine (DMPE) and 1,2-di-*O*-tetradecyl-*sn*-glycero-3-phosphoethanolamine (dietherPE), spread on aqueous solutions of different concentrations of arbutin. The decrease of the dipole potential of DMPC, both in condensed and expanded monolayers, is parallel to an increase in the area per lipid. In contrast, for dietherPC, the area per lipid is not affected, in spite of the fact that arbutin is also able to decrease the dipole potential in a less drastic extent. In the case of DMPE, the response is similar to that observed with dietherPC: the dipole potential decreases, while the area per lipid remains unchanged. However, when the carbonyl groups are absent in phosphatidylethanolamine derivatives such as the dietherPE, the dipole potential is not affected by arbutin, with a small decrease in the area. The effect of arbutin on the dipole potential differs from that of sucrose, trehalose and phloretin and is congruent with previous results obtained by FTIR on its interaction with the CO groups. Arbutin binding is interpreted in terms of the exposure to water of the phosphate and carbonyl groups at the membrane interface of the different monolayers.

Keywords: Dimyristoylphosphatidylcholine (DMPC); 1,2-di-*O*-tetradecyl-*sn*-glycero-3-phosphocholine (dietherPC); Dimyristoylphosphatidylethanolamine (DMPE); 1,2-di-*O*-tetradecyl-*sn*-glycero-3-phosphoethanolamine (dietherPE); Monolayer; Arbutin; Dipole potential; Surface pressure; Area per lipid

1. Introduction

The hydration of the lipids is attained by the interaction of water molecules with the polar head groups. These are composed by carbonyl and phosphate groups, which have been shown to be the hydration centers. Water polarized by these groups contributes to the dipole potential of the membrane interface [1]. The first layer of water polarized by the carbonyl and phosphate groups makes a major contribution to the dipole potential [2–4]. This potential has been related with the forces that oppose the membrane–membrane contact during adhesion processes [5,6].

FTIR experiments carried out in this laboratory have shown that arbutin, in a similar manner than other polyhydroxylated compounds such as trehalose, sucrose and phloretin, displaces the antisymmetric stretching of the phosphate groups to lower frequencies, in lipids dispersed in water [7]. This indicates the presence of strong hydrogen bonds between arbutin and the P=O group. In contrast, in the solid state this frequency increases [7,8].

Instead, a different pattern arose for the carbonyls. As previously reported, carbonyl groups present two populations: one hydrated and the other non-hydrated [9]. Trehalose displaces the frequency bands of both carbonyl populations to lower values, sucrose and phloretin do not affect them and arbutin increases the frequency of the hydrated population and decreases that of the dehydrated one [7,9–11]. Taking into account previous hypothesis of trehalose interaction with phospholipids, these results would be indicative that arbutin forms H-bonds with CO and PO groups, depending on the hydration state of the interface. In particular, arbutin displaces the frequency band of

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the non-hydrated carbonyl groups to lower frequencies indicating that this compound forms hydrogen bonds with them. In contrast, the hydrated population band frequency is displaced to higher frequencies indicating dehydration [7].

To understand these effects, it could be suggested that the binding of arbutin to the non-hydrated carbonyl groups would promote a reaccommodation of the interphase giving, as a result, a segregation of the hydrated carbonyl groups from the aqueous phase towards the lipid matrix. As the dipole potential of a lipid membrane is manifested at the water–hydrocarbon interface by the orientation of the phosphate-choline moiety [1], the carbonyl groups of the ester union and the water molecules polarized by them, the different interaction of those polyhydroxylated compounds with the PO and CO groups could change the dipole potential by different mechanisms.

Congruent with the FTIR results, trehalose, sucrose and phloretin affect the dipole potential in different extents. Trehalose and phloretin decreases it and sucrose produces a slight increase. However, in order to make a comparison of the structural changes at the interfacial groups with the different effects on the dipole potential, arbutin data have not been reported.

Arbutin (4-hydroxyphenyl-beta-glucopyranoside) has, as sucrose and trehalose, a glucose moiety (Fig. 1). Its effect on membrane properties is connected to the hydration of the lipid interface, since it can inhibit phospholipase A_2 hydrolysis in the absence of water [8,12], but it does not in the presence of excess water. In addition, the binding to the P=O and C=O groups is dependent on the hydration state of the membrane which is modified if the membranes are in the gel, liquid crystalline or anhydrous state [7].

It has been argued that it partitions into lipid membranes probably by the insertion of its phenol moiety [8]. In this sense, it would be structurally comparable to phloretin, also a polyphenol molecule.

In this paper, we report for the first time the effect of arbutin on the dipole potential of ester and ether derivatives of phosphatidylcholine and ethanolamine in expanded and condensed states, in correlation with changes in the area per lipid, as determined in monolayers of dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidyl ethanolamine (DMPE), ditetradecyl PC (dietherPC) and ditetradecyl PE (dietherPE). If the effect of arbutin depends on the interaction with carbonyls and/or phosphates, the exposure of the different phospholipid groups to the water phase could alter the access of the molecule and, in consequence, the effect on the dipole potential. This exposure could be modified by the hydrogen bonds between different phospholipids along the phosphate, carbonyl and ethanolamine groups.

2. Materials and methods

Dimyristoylphosphatidylcholine (DMPC), 1,2-di-*O*-tetradecyl-*sn*-glycero-3-phosphocholine (etherPC), dimyristoylphosphatidylethanolamine (DMPE) and 1,2-di-*O*-tetradecyl-*sn*-glycero-3-phosphoethanolamine (etherPE) 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 mixture as running solvent.

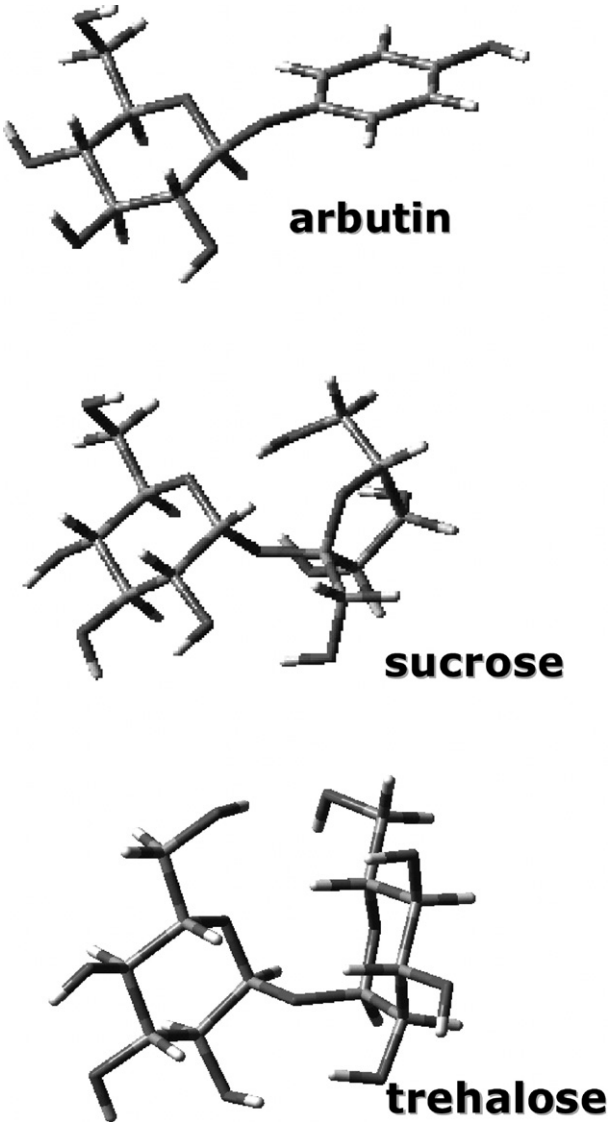

Fig. 1. Molecular structures of arbutin, trehalose and sucrose.

Arbutin (Arb) was obtained from Sigma and used as received after controlling the purity by UV analysis. Chloroform and KCl were analytical grade. All the aqueous solutions of different concentrations were prepared with ultra pure water of MilliQ quality (conductivity = 0.09 μS/cm). In all the solutions the resulting pH was 6.0 ± 0.2.

2.1. Determination of the dipole potential in monolayers

Dipole potential (Ψ_D) was determined in monolayers formed on an air–water interface by spreading chloroform solutions of the different lipids over an aqueous subphase (KCl 1 mM) with or without Arb, as described before [6,13].

The values of the interfacial potential were determined through a circuit of high impedance, connecting an ionizing electrode above the monolayer and a reference electrode in the aqueous subphase, using the following expression:

$$V_{surf} = V_{Ag/AgCl} - V_{grd}$$

where V_{surf} is the potential of the clean aqueous surface, measured as the potential difference between an Ag/AgCl reference electrode, immersed in the solution underneath the surface ($V_{Ag/AgCl}$) and the grid displaced c.a. 2 mm above the surface (V_{grd}). This grid is the sensor of the ionizing electrode that

emits alpha particles in order to achieve the electrical connection across the air. The dipole potential of the monolayer (Ψ_D) was evaluated as:

$$\Psi_D = V_{lip} - V_{surf}$$

where V_{surf} is the potential of the clean surface (without lipids) described above and V_{lip} the potential measured with the same set-up after the lipid monolayer was formed on the air–water interphase. The values of monolayers potentials were taken within an experimental error of ±20 mV. 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.

Different values of Ψ_D were obtained between the V_{surf} for the clean surface of arbutin solutions and V_{lip} obtained with monolayers of lipids formed on each solution. These differences are reported for the arbutin concentration assayed.

2.2. Formation of lipid monolayers. Measure of the surface pressure and area per lipid calculation

The formation of monolayers on the air–water interface of aqueous solutions with and without arbutin was monitored by measurements of the surface pressure of the different lipid monolayers in a Kibron μtrough S equipment, at constant area and temperature.

Aliquots of a chloroform solution of lipids were spread on a clean surface of water or aqueous solutions with 100 mM arbutin (highest concentration tested) and left to reach constant surface pressures, until no changes were observed with further additions of lipids (saturation). Results of surface pressure were expressed in mN/m.

All dipole potential measurements were determined as described above when the surface pressure of the monolayers reached that saturation point (see Fig. 2). In this condition, it has been demonstrated that lipids in excess form aggregates in the subphase and that the thermodynamic and interfacial properties are comparable with those of a bilayer [6,13,14].

With this procedure, the lipids are stabilized spontaneously according to the aqueous solution properties and temperature, without forcing the lipids by the lateral pressure, as it would be the case in the determinations of the dipole potential along a surface pressure/area curve. The compression or expansion of the monolayer, at constant amount of lipids in the surface, may change the conformation of the lipids at the interface, thus affecting the dipole potential.

The areas per lipid were calculated from curves of monolayer surface pressure vs. nmoles of lipid added to a trough of known constant area (Fig. 2). As observed, the titration of the surface with increasing aliquots of a chloroform lipid solution reaches a saturation. The plateau of saturation was the best straight line obtained with, at least, three points. The mean value of this line with its corresponding standard deviation is depicted in the figure.

The area was calculated from the nmoles corresponding to the first saturation point in this line, for the different monolayers. The criteria to take this first point was to compare the standard deviation of this point with that of the plateau. When the difference of this point was higher than the standard deviation of the saturation line, it was not considered as first point of saturation.

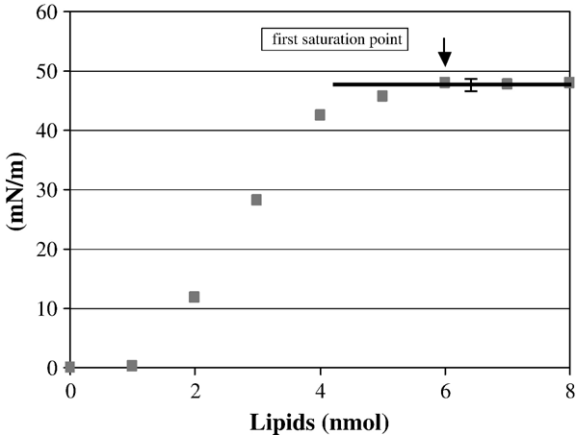

Fig. 2. Graphical determination of the area per lipid from the saturation curve of surface pressure, at constant area and temperature.

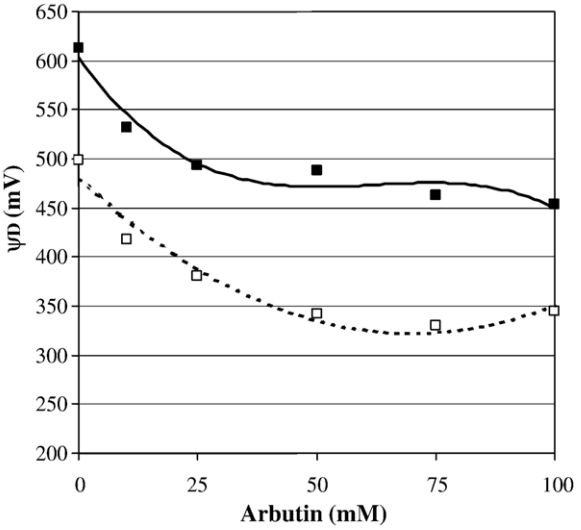

Fig. 3. Effect of the arbutin concentration in the subphase of DMPC monolayers at 18 °C (■) and 28 °C (□).

Considering that each aliquot of chloroform solution corresponds to 1 nmol of lipids, each determination is affected by an error of ±0.5 nmol, that gives a corresponding variation in the area for each lipid.

3. Results and discussion

The dipole potential of DMPC monolayers is decreased by the increase of arbutin concentration in the subphase, both at 18 and 28 °C (T_t DMPC ≅ 24 °C). The curve for data at 28 °C (liquid expanded state) displays ca. 100 mV below that corresponding to data taken at 18 °C (condensed state) in the whole range of concentrations assayed (Fig. 3), in accordance with the differences in packing between those states.

The maximum effect on decreasing the dipole potential is achieved for Arb at about 75 mM. In these conditions, Arb decreases in 170 mV the dipole potential of liquid expanded monolayers and 150 mV for the condensed state (Fig. 4). The similar difference implies that arbutin affects, in the same way the condensed and the expanded monolayers. In other words, the lipid phase state does not affect the arbutin effect on the dipole potential.

At Arb 75 mM, a decrease is also observed in monolayers of dietherPC (T_t ≅ 27 °C), spread at 18 or at 28 °C. However, in this case, the dipole potential decreased only 50 mV at the same surface pressure, in contrast to the 170 mV observed in liquid expanded monolayers of DMPC (Fig. 4). The difference in dipole potential between pure DMPC and dietherPC indicates that the presence of carbonyl groups contributes to the dipole potential as previously shown [2,3]. Nevertheless the dipole potential can also be affected by arbutin in the absence of this group.

The decrease in dipole potential of DMPC, in the expanded (28 °C) and condensed (18 °C) phase, is in accordance with the increase in the area per lipid, denoting that arbutin intercalates as a spacer independently of the lipid phase state

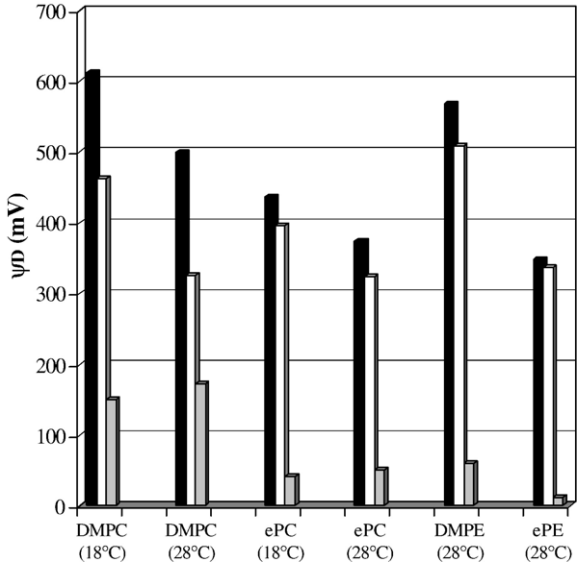

Fig. 4. Dipole potentials and decrease of the dipole potential in DMPC, dietherPC, DMPE and dietherPE at 18 and 28 °C. Black bars: pure lipids, White bars: lipid spread on 75 mM arbutin at the indicated temperatures, gray bars: difference between the dipole potential without and with arbutin.

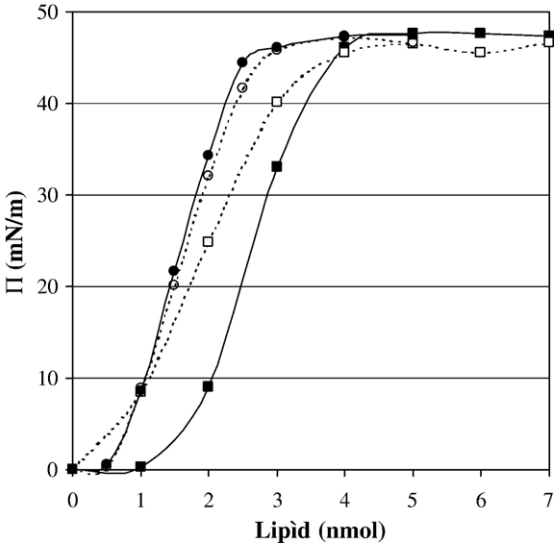

Fig. 6. Variation of the surface pressure with the addition of DMPC (□) and etherPC (○) to the air aqueous solution interface of water (filled points) and 100 mM arbutin (empty points) at 28 °C.

(Figs. 5, 6 and Table 1). The spacer effect is slightly smaller in the condensed than in the liquid expanded monolayers. In accordance, the decrease in dipole potential induced by a similar concentration of arbutin is smaller in condensed than in expanded monolayers.

The decrease observed in monolayers of dietherPC spread at 18 or at 28 °C is produced without affecting the area per lipid, which remains unchanged. Therefore, it may be inferred that the area changes mentioned above are related with the presence of carbonyl in the lipid interphase. In addition, these results suggest that arbutin affects the reorganization of dipoles around other groups besides carbonyls and by a mechanism other than as a spacer effect.

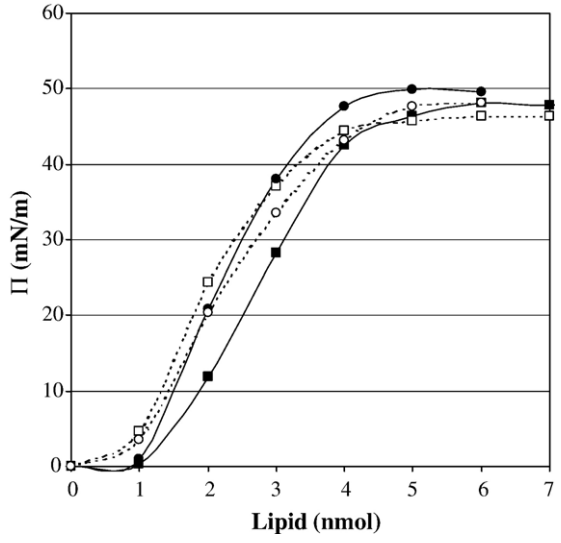

Fig. 5. Variation of the surface pressure with the addition of DMPC (■) and etherPC (●) to the air aqueous solution interface of water (filled points) and 100 mM arbutin (empty points) at 18 °C.

As reported previously, another important constitutive group contributing to the dipole potential is the phosphate [15]. Therefore, a possible explanation for this mechanism could be water partially displaced from the phosphate groups projected into the water phase, as in the case of trehalose [10]. In this regard, it has been reported that, due to the spatial stabilization of the glycerol backbone parallel to the membrane plane, the phosphate group is more exposed to water in the alkyl derivative than in the acyl ones, in which the glycerol would be laying normal to the membrane surface [16]. This is congruent with the increase in area per lipid observed in dietherPC, in comparison to the DMPC (Table 1). Thus, it is reasonable that arbutin may interact with the phosphates of the dietherPC without an expanding effect. However, no evidence of a net displacement of water has been obtained from measurements of water activity (a_w) of liposomes of dietherPC which decreased only about 2% in the presence of Arb 100 mM.

Taking into account these considerations, in order to investigate the influence of phosphates on the arbutin effect, the dipole potential was measured on DMPE ($T_t \cong 49.5$ °C) and dietherPC ($T_t \cong 56$ °C) monolayers. Thus, at 28 °C, both monolayers are in the condensed state, at the saturation surface pressure. Therefore, these data can be compared with those obtained at 18 °C with DMPC and dietherPE which, at that temperature are in the same state.

As known, PEs present a strong head–head interaction due to the formation of a direct hydrogen bond between the phosphate and the ethanolamines of adjacent molecules [17,18]. Therefore, the phosphate groups in PE will be less exposed to the aqueous phase than in PC. It must be noticed in the curves of Fig. 7 and Table 1 that the surface pressure of PE is lower than those of PC in the same conditions, denoting a lower hydration of the lipids at the interface. A lower surface pressure means a

Table 1
Effect of 100 mM arbutin on the dipole potential, area per lipid and surface pressure of different monolayers

	Water				Arbutin			
	nmol Lipids	Dipole pot. (mV)	Π saturation (mN/m)	A^2/molecule	nmol Lipids	Dipole pot. (mV)	Π saturation (mN/m)	A^2/molecule
DMPC (18 °C)	6	612.6 (±9.9)	48	56.3 (±4.7)	5	462.5 (±33.1)	46	67.5 (±6.8)
DMPC (28 °C)	5	499.1 (±13.0)	47.5	67.5 (±6.8)	4	326.3 (±40.9)	46	84.4 (±10.6)
etherPC (18 °C)	5	437 (±23.6)	49.5	67.7 (±6.8)	5	395.6 (±10.3)	48	67.7 (±6.8)
etherPC (28 °C)	4	373.3 (±10.7)	47.5	84.7 (±10.6)	4	323.6 (±8.0)	47	84.7 (±10.6)
DMPE (28 °C)	6	568.6 (±13.7)	45	56.1 (±4.7)	6	509.9 (±38.0)	40.5	56.1 (±4.7)
etherPE (28 °C)	6	348.7 (±12.2)	44.5	56.3 (±4.7)	7	337.4 (±19.8)	39	48.2 (±3.4)

lower effect of the lipid on the water surface tension. That is, water network is less perturbed because of a lower hydration of the lipids. Thus, the exposure of phosphate to water is much lower in PE than in PC, as indicated by the 4 water molecules per lipid, as reported previously [19].

Data in Fig. 4 and Table 1 shows that arbutin decreases the dipole potential of DMPE in 60 mV, a value similar to that found with dietherPC at 18 °C, without affecting the area per lipid (Fig. 7). In contrast to PCs, arbutin does not affect the dipole potential nor the area per lipid, when carbonyl is absent, in dietherPE (Figs. 4, 7 and Table 1), i.e., in this type of interphase, arbutin has no constitutive group of the membrane to interact with.

The observation that arbutin has a slightly higher effect on DMPE than on dietherPC on the dipole potential would be due to the possibility that the carbonyls are partially accessible to arbutin, in spite of the packing of the phosphates and ethanolamines, or that phosphates are still partially exposed to water. If this last possibility would be true, arbutin should also decrease the dipole potential in dietherPE, because it has a similar packing than the ester form. As this is not observed experimentally, it may be concluded that arbutin interacts with CO groups in DMPE.

Thus, we conclude that the dipole potential decrease is due to the interaction of arbutin with carbonyls and phosphates, in an independent way.

This conclusion is in accordance with published FTIR results, showing that arbutin, in the presence of water, decreases the antisymmetric stretching mode of the phosphate groups and the frequency of the dehydrated population of carbonyls and increases that of the hydrated one [7]. These results would be indicative that arbutin forms H-bonds with CO and PO groups.

In the light of these results, the interaction of arbutin with the anhydrous population of carbonyls (which would be those oriented parallel to the plane of the membrane) was observed by the displacement to lower frequencies. This interaction would explain the area increase by insertion of the molecule in the plane of the membrane. This area expansion would promote a reorientation of the hydrated ones, formerly normal to the membrane, towards the hydrocarbon core, causing dehydration as derived by the frequency shift to higher values. Therefore, there would be less dipoles oriented to the water phase, thus explaining the observed decrease of the dipole potential concomitant with the spacer effect.

In the case of the dipole potential decrease observed for the dietherPC, without a concomitant spacer effect, arbutin–phosphate interaction would take place without water displacement, since water activity is not considerably altered. This is also in accordance with FTIR results. In excess of water, arbutin displaces the frequency of the antisymmetric stretching to lower values. However, in dehydrated samples arbutin displaces the same phosphate band to higher values, which suggests that arbutin cannot interact directly with the phosphate when it is dehydrated [7]. It is derived from these results that, in the absence of carbonyls and of area changes, the dipole potential decrease would be a consequence of the interaction of arbutin with the hydrated phosphates, probably opposing its own dipole to the constitutive ones.

Arbutin may have access to the phosphate groups in dietherPC, but in the dietherPE the different packing achieved

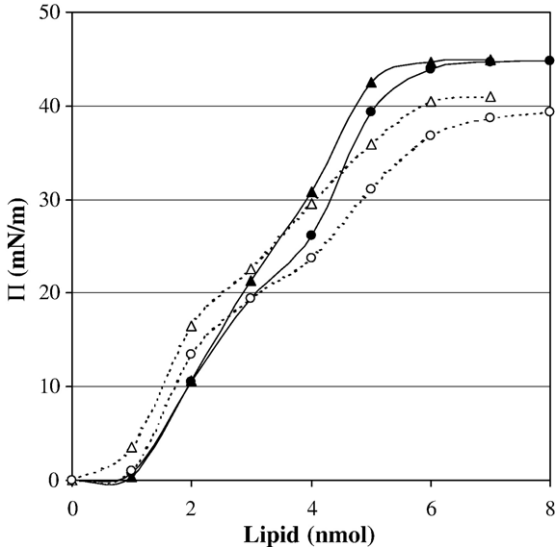

Fig. 7. Variation of the surface pressure with the addition of DMPE (△) and etherPE (○) to the air aqueous solution interface of water (filled points) and 100 mM arbutin (empty points) at 28 °C.

as a consequence of the strong PE–PE interaction, excludes arbutin. The slight effect on DMPE would suggest that this effect is due to a partial access to the carbonyls. In the absence of carbonyl the packing of the phosphates is even higher and arbutin does not affect the dipole potential of PE.

These results are very different to those found with trehalose and phloretin [4,10]. These polyhydroxylated compounds decrease the dipole potential, due to a strong interaction with the PO groups and/or with carbonyls. When FTIR experiments were made with hydrated lipids, the displacement to lower values in the presence of trehalose was ascribed to the formation of hydrogen bonds between PO and Tre with a water replacement (10).

Trehalose decreases the dipole potential parallel to an increase in the area per lipid, due to a specific interaction with carbonyls ([3,10], Lairion et al. (2006)) to be published]. In this condition, there is a decrease in the frequency value of the phosphate groups and both populations of carbonyls. In contrast to arbutin, trehalose also decreases the PO antisymmetric frequency in the anhydrous state thus confirming the water replacement hypothesis. From this, it is deduced that, in the presence of water, trehalose forms stronger H bonds with phosphate than with water itself [4,9].

The effectiveness of phloretin to decrease the dipole potential of monolayers in the fluid state is also related to an increase in the area, as a consequence of a direct interaction with the phosphate groups, and with a relatively significant water displacement [12]. FTIR experiments have shown a shift of the antisymmetric stretching of the phosphate groups to lower frequencies and no effect on the frequency bands of both populations of carbonyls.

The comparison of the present results with those reported for trehalose and phloretin are indicative that the decrease of the dipole potential by arbutin follows a different mechanism. It seems that it interacts with PO and CO groups but without a net water displacement. The involvement of the phosphate groups as playing a role in the magnitude of the dipole potential has been reported recently in relation of the interaction of trehalose, sucrose and phloretin (4,10.15). In addition, metal cations affecting the hydration sphere of the phosphate groups also seems to affect the dipole potential [20].

The contribution of carbonyls had been previously known (2.3). However, in this paper it is clearly stated that the organization of the carbonyl dipoles depends on the type of polyhydroxylated compound interacting with the interphase.

As we discussed previously, the interaction of arbutin with the anhydrous population of carbonyls would probably promote a reorientation of the hydrated ones, towards the hydrocarbon core (dehydration) and there would be an interaction with the hydrated phosphates (without water displacement), so that there is not a net water displacement as a consequence of arbutin interaction with lipids.

We cannot disregard the possibility that the decrease in the dipole potential is due to the insertion of its own dipole in the membrane, opposing that originated by the constitutive dipoles, CO and PO. This is based in the observation that the decrease in dipole potential produced by trehalose is, at similar concentrations, lower than that produced by arbutin, in spite of the fact that trehalose displaces water and increases the area in a larger extent.

These results suggest a dynamic response of the interfacial hydration, achieved by fluctuating distribution of groups that polarize water.

Acknowledgments

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