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Biochimica et Biophysica Acta 1758 (2006) 1823–1829

BIOCHIMICA ET BIOPHYSICA ACTA

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FTIR analysis of the interaction of arbutin with dimyristoyl phosphatidylcholine in anhydrous and hydrated states

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Received 29 March 2006; received in revised form 1 June 2006; accepted 23 June 2006
Available online 20 July 2006

Abstract

In this paper, the interaction of arbutin with dimyristoylphosphatidylcholine bilayers was studied by FTIR spectrometry. The results show that arbutin interacts in different extents with the phosphate and carbonyl groups of membranes in the gel state, the liquid crystalline state or subjected to osmotic stress. The effect, in the presence of water, on the antisymmetric stretching of the phosphate groups is qualitatively similar to that found with other molecules composed by a glucose moiety such as trehalose and sucrose. However, significant differences were found between these compounds and arbutin in the carbonyl region. Arbutin displaces the PO_2^- antisymmetric stretching to lower frequencies in lipids dispersed in water. This indicates strong hydrogen bonding. In contrast, in the solid state, this frequency increases. The effect on the carbonyl groups varies depending on the hydration state of the bilayer, which is achieved by changing the phase state of the bilayer or by osmotic stress. The hydrocarbon region is not affected by arbutin in the excess of water. However, symmetric and antisymmetric stretching of CH_2 and CH_3 are strongly affected in the dry state.

Keywords: Arbutin; Dimyristoylphosphatidylcholine; Fourier Transform infrared spectroscopy; Carbonyl group; Phosphate group; Hydration

1. Introduction

Arbutin (4-hydroxyphenyl-beta-glucopyranoside) is a solute accumulated to high concentrations in drought and frost resistant plants. This hydroquinone derivative composed by glucose and a phenol moiety (Fig. 1) is isolated from the leaves of the bearberry shrub, cranberry, blueberry and most types of pears [1,2].

In model lipid systems, arbutin destabilizes phosphatidylcholine (PC) vesicles during drying and rehydration and stabilizes membranes containing nonbilayer-forming lipids during freezing. In liposomes containing the nonbilayer-forming lipids monogalactosyldiacylglycerol (MGDG) or phosphatidylethanolamine (PE), arbutin shows a protective function during drying, as measured by retention of carboxyfluorescein (CF) and extent of vesicle fusion [3,4].

Arbutin auto-fluorescence determinations suggest that arbutin interacts with hydrated membranes by insertion of the phenol moiety into the lipid bilayer. This interaction causes an increase in membrane leakage during air-drying by a mechanism other than vesicle–vesicle fusion [5].

The interaction between arbutin and lipid membranes and the resulting effects on membrane stability depend, in a complex manner, on the lipid composition. It has been suggested that the effect of arbutin is localized at the membrane interface because no effect in the hydrophobic core region was observed with steady-state anisotropy measurements with probes that localize at different positions in the membranes [5]. In addition, another indication that arbutin affects the membrane interfacial properties is the observation that it inhibits the phospholipase A_2 action in dry lipids but it does not in excess of water [6].

Thus, arbutin action seems to be related to the hydration state of the lipid membrane interface where carbonyl, phosphate and choline groups acquire different degrees of hydration [7,8].

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doi:10.1016/j.bbamem.2006.06.024

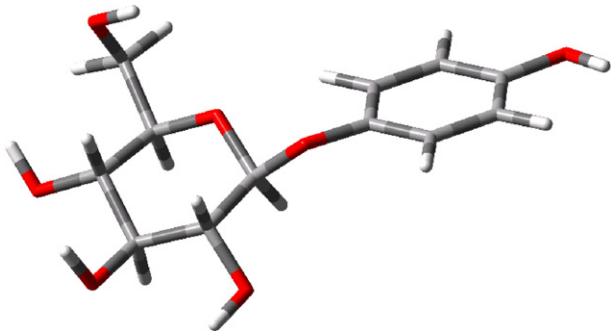

Fig. 1. Molecular structure of arbutin.

In previous reports, arbutin has been shown to depress the gel to liquid crystalline phase transition temperature of dry phospholipids, as measured by differential scanning calorimetry, with a pattern similar to that seen in phospholipids dried with trehalose [5]. Unlike trehalose, however, arbutin does not protect dry liposomes from leaking their contents. These results were obtained with lipid membranes dried in the presence of trehalose and different ratios of arbutin. However, no report comparing the arbutin action with that of trehalose in dry membranes and in the excess of water is available.

Thus, it is of interest to analyze the insertion of arbutin in lipids in different phase and hydration states by studying the specific interaction with carbonyl and phosphate groups. For this purpose, a detailed analysis by FTIR has been done on the gel and the fluid state of aqueous suspension and in dry samples of DMPC at different arbutin–lipid ratios.

As shown in Fig. 1, this molecule is composed by one glucose moiety. Therefore, it will be important, in the context of water replacement by H bonding compounds, to analyze how arbutin interacts with lipids in comparison to other well-known compounds containing glucose such as trehalose and sucrose [7].

The present results provide a new insight on the relevance of the hydration level on the molecular insertion of arbutin in lipid membranes showing a qualitative difference with trehalose and sucrose on the effect of carbonyl groups.

2. Materials and methods

2.1. Lipid sample preparation

Dimyristoylphosphatidylcholine (DMPC) was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and used without further purification. Arbutin was purchased from Sigma-Aldrich, Inc.

Lipids in chloroform solution were dried to form a film under a stream of nitrogen. Lipids were rehydrated in deuterated water or in solutions of different concentrations of arbutin in D_2O (10, 50 and 100 mM) heating above the transition temperature and gently agitation during 15 min to produce multilamellar vesicles. The final lipid concentration was 40 mg/ml.

To study the action of arbutin on the external face, the liposomes were prepared by rehydrating the lipid film in 0.5 ml of D_2O. Then, liposomes were centrifuged during 15 min at 2000 rpm. The pellet was dispersed twice with D_2O solutions of arbutin at different concentrations.

MLV samples with and without arbutin inside and outside were left, at room temperature, during 1 h before measurements.

The same procedure was followed to prepare samples in the dehydrated state. The liposomes obtained were lyophilized and used to prepare KBr pellets for FTIR spectra.

2.2. FTIR measurements

FTIR measurements were carried out in a Perkin Elmer 1600 spectrophotometer, provided with a DTGS detector .The resolution of the equipment was 2 cm^{-1}.

The interaction of arbutin with the phospholipid head groups in lipids dispersed in D_2O was studied employing a cell with AgCl windows. For each condition, spectra were taken for DMPC bilayers in the gel (18 °C) and in the liquid crystalline state (25 °C).

After a total of 1024 scans, spectra were analyzed using the mathematical software provided by Perkin Elmer for FTIR equipment. Mean values of the main bands in each condition were obtained from a total of three different batches of samples. The anhydrous samples were prepared by lyophilization and the infrared spectra of solid samples were obtained in KBr pellet after 64 scans at room temperature [8].

D_2O was used as a solvent to disperse the lipids in order to visualize the carbonyl region [9]. Spectra of pure D_2O were subtracted in all analyzed samples to avoid solvent interference with the phosphate group. In the subtracted spectra, the D_2O bands are absent and that corresponding to the antisymmetric stretching of the phosphate group are centered in the region observed in solid DMPC.

The contours of C═O stretching bands were obtained by Fourier Self Deconvolution using band width parameters between 18 and 20 cm^{-1} and a band narrowing factor of 2 as defined by the mathematical software GRAMS/32 Spectral Notebase. Deconvolution was used to obtain the peak frequencies of the component bands reported for the two populations of carbonyls: the non-hydrated (centered at 1742.5 cm^{-1}) and hydrated (centered at 1724 cm^{-1}) populations in the gel state, and the non-hydrated (1737 cm^{-1}) and hydrated (1722 cm^{-1}) populations in the fluid state [9,10,14]. The shifts of these two populations were studied as a function of arbutin concentration in the gel, liquid crystalline and solid states.

DMPC/arbutin spectra were obtained from the spectra of DMPC dispersed in a D_2O arbutin solution to which the spectrum of pure arbutin was subtracted.

3. Results

In Fig. 2, the FTIR spectrum region, showing the phosphate bands, of pure DMPC and DMPC in the presence of arbutin is shown.

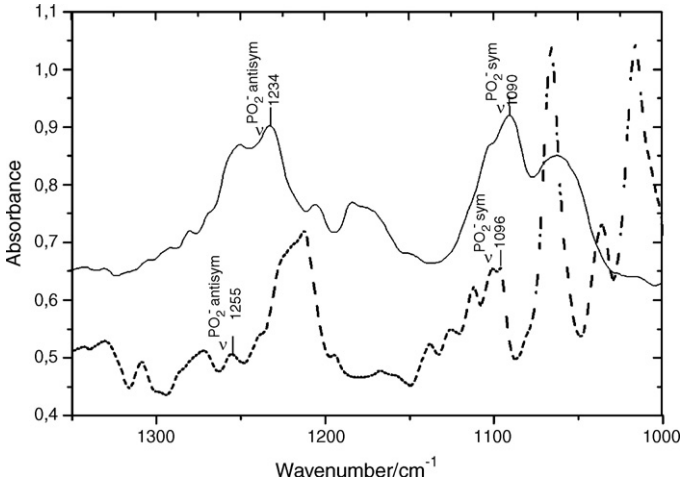

Fig. 2. FTIR spectra in the region of phosphate groups of DMPC. A—DMPC pure (full line); B—DMPC with 100 mM arbutin (dashed line).

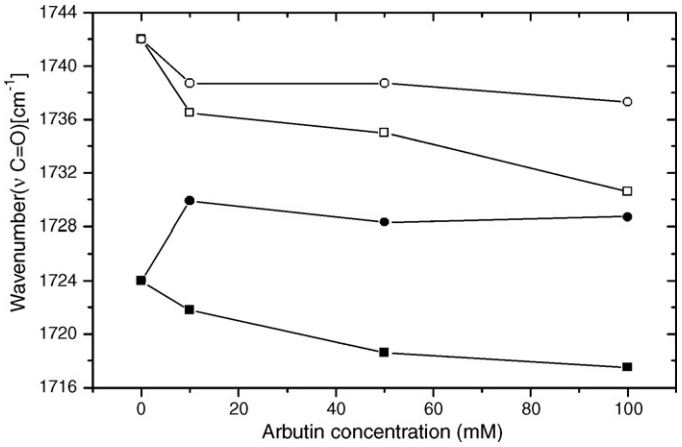

Fig. 3. Frequency shifts of the carbonyl groups in DMPC bilayers in the gel state dispersed in different arbutin solution as a function of arbutin/lipid ratio at 18 °C. Non-hydrated population: arbutin in the outer (□) and in the outer and inner (○) liposome solution. Hydrated population: arbutin in the outer (■) and in the outer and inner (●) liposome solution.

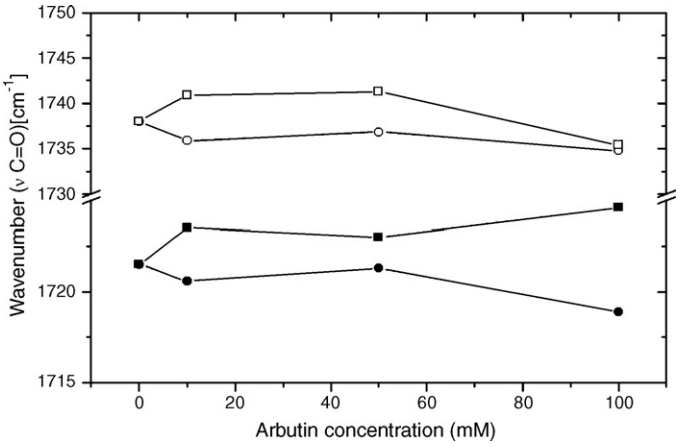

Fig. 5. Frequency shifts of the carbonyl groups in DMPC bilayers in the fluid state dispersed in different arbutin solution as a function of arbutin/lipid ratio at 25 °C. Non-hydrated population: arbutin in the outer (□) and in the outer and inner (○) liposome solution. Hydrated population: arbutin in the outer (■) and in the outer and inner (●) liposome solution.

The assigned band positions are plotted as a function of arbutin concentration inside and outside and only outside in the gel state (18 °C) and the liquid crystalline state (25 °C).

Arbutin inside and outside of DMPC liposomes at 18 °C decreases the frequency of the non-hydrated population (P_1) from about 1742.0 cm^{-1} in pure DMPC [8] to about 1737.3 cm^{-1} in the maximum concentration assayed (100 mM) (Fig. 3). In the same figure, data corresponding to this band when arbutin is only outside are also shown. In this case, the effect of arbutin on the decrease of non-hydrated population band is more pronounced than that observed when arbutin is outside and inside.

The effect of arbutin on the hydrated carbonyl band is markedly dependant on whether arbutin is inside and outside or only outside (Fig. 3). In the first case, the carbonyl band is displaced to higher frequencies. In contrast, arbutin outside produces a downward shift about 7 cm^{-1}.

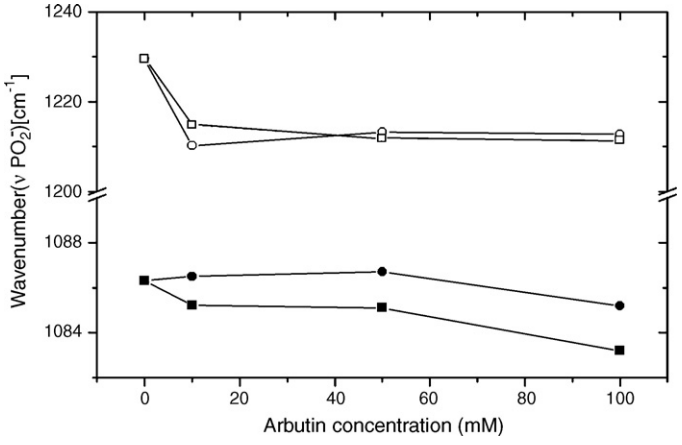

Fig. 4. Frequency shifts of PO_2^- stretching mode as a function of arbutin/lipid ratio of DMPC in the gel state at 18 °C. Antisymmetric stretching mode: arbutin in the outer (□) and in the outer and inner (○) liposome solution. Symmetric stretching mode: arbutin in the outer (■) and in the outer and inner (●) liposome solution.

In fully hydrated phosphatidylcholine in the gel state, the characteristic phosphate group vibrational band assigned to the PO_2^- anti symmetric stretching mode (ν_a PO_2^- as) is centered at 1229.5 cm^{-1} and the PO_2^- symmetric stretching mode (ν_b PO_2^- s) at 1085.0 cm^{-1}. In Fig. 4, it is observed that the PO_2^- antisymmetric mode is affected in equal extents by arbutin, inside and outside or only outside, with an important decrease in the frequency of around 15 cm^{-1} in the gel phase. However, the PO_2^- symmetric stretching band is only affected when arbutin is outside, showing a slight decrease.

The effects of arbutin on the carbonyl modes in DMPC liposomes in the liquid-crystalline state (25 °C) are less noticeable than that observed in the gel state (Fig. 5).

Non-hydrated carbonyl stretching band shows a similar trend than that observed in the gel phase when arbutin is inside and outside, although much less pronounced. The frequency is slightly shifted within the experimental error when it is only outside.

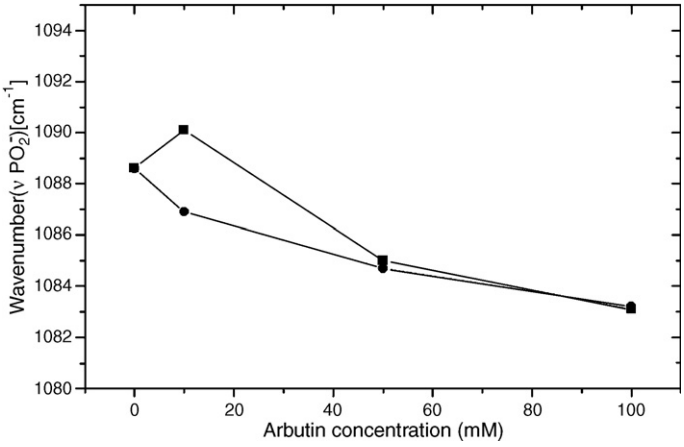

Fig. 6. Frequency shifts of the PO_2^- symmetric stretching in DMPC bilayers dispersed in different arbutin solution as a function of arbutin/lipid ratio at 25 °C. Arbutin in the outer (■) and in the outer and inner (●) liposome solution.

When the hydrated carbonyl band is analyzed, it is observed that, in contrast to the trend observed in the gel phase, arbutin inside and outside decreases the frequency and when it is only outside it increases it (Fig. 5).

The PO_2^- antisymmetric stretching vibration band is, in both cases, slightly affected, within the experimental error, either by arbutin inside and outside or only outside (data not shown). However, the PO_2^- symmetric stretching vibration band is shifted by 6 cm^{-1} downward at 100 mM arbutin (Fig. 6).

The CH_2 and CH_3 symmetric and antisymmetric stretching vibration bands were not affected by arbutin at the two temperatures and conditions assayed (data not shown).

In the absence of water, the components of the C═O stretching vibration band obtained after deconvolution are shifted to lower frequencies by arbutin (Fig. 7A). In the same condition, arbutin produces a shift to higher values of the PO_2^- antisymmetric and symmetric stretching modes (Fig. 7B).

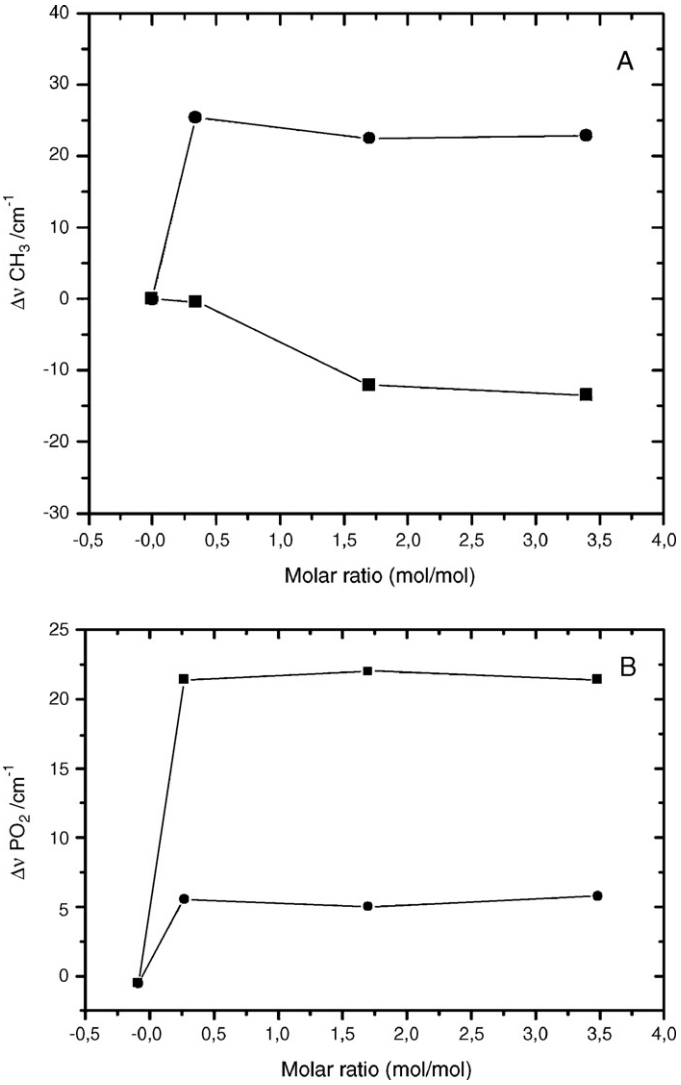

Fig. 7. Effect of arbutin on DMPC in anhydrous state. A—frequency shift of the components of the carbonyl band obtained by deconvolution. B—frequency shift for the PO_2^- antisymmetric stretching (■), and PO_2^- symmetric stretching (●).

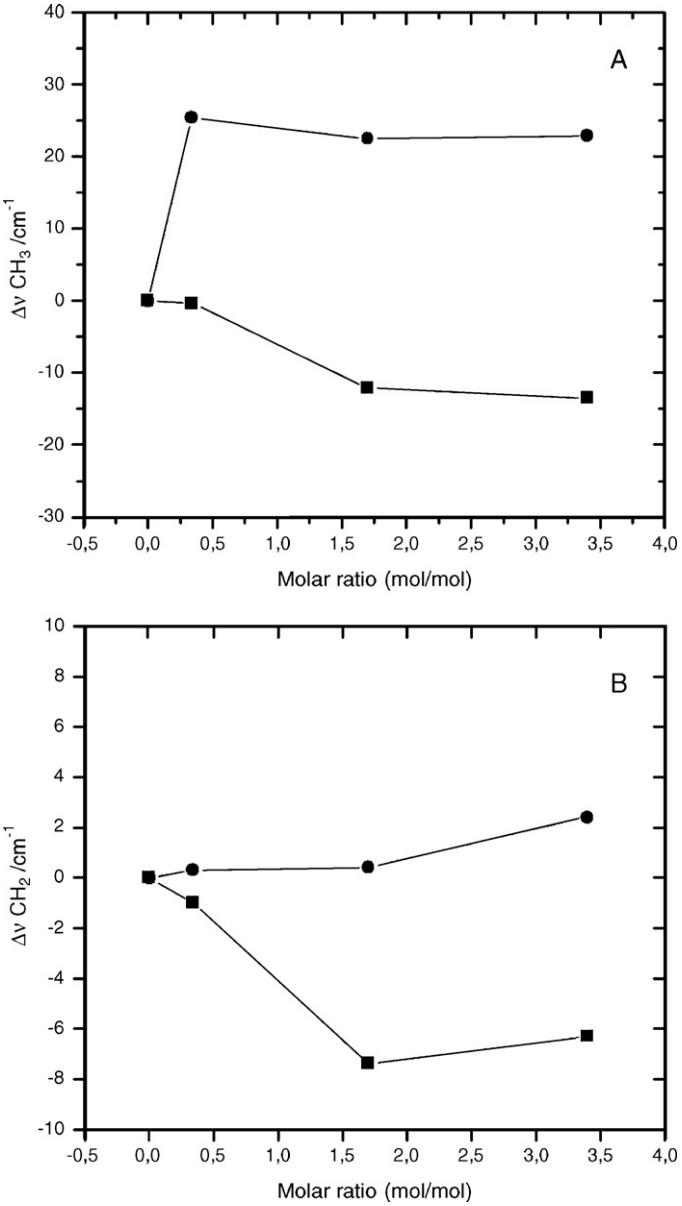

Fig. 8. Effect of arbutin on DMPC in anhydrous state. A—frequency shift for the CH_3 antisymmetric (■) and symmetric (●) stretching modes. B—frequency shift for the CH_2 antisymmetric (■) and symmetric (●) stretching modes.

In Fig. 8A, it is observed that the CH_3 symmetric stretching band is displaced to higher frequencies and the antisymmetric to lower ones.

In addition, while the CH_2 symmetric stretching band is not affected, the antisymmetric one is displaced to lower values (Fig. 8B).

4. Discussion

The effect of arbutin differs significantly when it interacts with DMPC in the gel (18 °C) and the liquid crystalline state (25 °C) in the presence of water or in the anhydrous state.

Quite different responses are also found when arbutin is only outside of gel and fluid DMPC liposomes with respect to those

in which arbutin in evenly distributed between the inner and the outer media.

The main difference observed in the action of arbutin on lipids in the excess of water in comparison to lipids in the anhydrous state is that, in the first case, the PO_2^- antisymmetric stretching band is displaced to lower values while in solid it is shifted to higher ones (Figs. 4 and 7B). This qualitatively different behavior indicates that arbutin would be able to form hydrogen bonds with the phosphate group when they are hydrated, but it does not when they are dehydrated. Previous results reported by Crowe were indicative of this behavior, although the assays were done in the presence of trehalose [5].

The decrease of the PO_2^- symmetric stretching mode at 25 °C is also indicative of the formation of H-bonds in the presence of water.

In the dry (or poorly hydrated) state, the PO_2^- antisymmetric and symmetric stretching modes are displaced to higher frequencies (Fig. 7B). The shift to higher frequencies is indicative of the strengthening of the PO bond and it can be related to the increase in the packing of the phosphate groups. The changes in the CH_2 and CH_3 antisymmetric and symmetric stretching modes are also indicative of an increase in the packing of the lipids in the dry state induced by arbutin. This effect is not observed in the gel and the fluid state (Fig. 8).

Another observation in the dry state is that the components of the carbonyl stretching band decreases with arbutin (Fig. 7A). This behavior is similar to that found in suspensions for the non-hydrated population when arbutin is inside and outside or only outside (Fig. 3).

In the dry (or poorly hydrated) state, the absence of water makes plausible that the decrease in the frequency of the carbonyl groups could be explained taking into account the H-bonds established between the C═O and OH groups.

The decrease in frequencies in lipids suspended in aqueous solution denotes the formation of H-bond of the non-hydrated carbonyl groups. At least two possibilities can explain this observation. One is that carbonyl groups bind to arbutin, the other that the carbonyl groups become more exposed to water.

It is interesting to note that the hydrated population in lipids dispersed in water shows an upward shift in frequency when arbutin is inside and outside. This would suggests that the hydrated population is loosing water due to arbutin.

In contrast, when arbutin is only outside, the decrease in the frequency of the hydrated population suggests a displacement of water molecules by arbutin, thus forming stronger H-bonds.

The effects of arbutin on the carbonyl groups in the dry state, on the non-hydrated population in lipid suspensions and on the hydrated population when arbutin is only outside suggest that arbutin binds to the CO when they are dehydrated. When arbutin is only outside, the osmotic stress produced by the different concentration outside and inside would cause the dehydration of the CO groups where arbutin can consequently bind.

When arbutin is inside and outside, the carbonyl groups would stabilize in a position with a low exposure to the water phase.

In the fluid state the change induced by arbutin are much more attenuated. This may be due to the fact that in the fluid state the polar groups are more hydrated, which makes more difficult the displacement of water for arbutin insertion. The above results suggest that arbutin would induce two effects: the dehydration of the carbonyl population and the formation of H-bonds with those groups.

Table 1
Comparison of the effect of arbutin on the PO_2^- and C═O vibrational bands at 18 °C with sucrose and trehalose

	DMPC	Trehalose	Sucrose	Arbutin
PO_2^- antisym/cm^{-1}	1229.5	1196.7 (−32.8)	1202.8 (−26.7)	1213.2 (−16.3)
PO_2^- sym/cm^{-1}	1086.3	1087.3 (1.0)	1087.9 (1.6)	1086.7 (0.4)
C═O non-hydrated/cm^{-1}	1740.9	1735.7 (−6.3)	1739.8 (−2.1)	1738.7 (−3.3)
C═O hydrated/cm^{-1}	1724.0	1718.0 (−6.0)	1723.0 (−1.0)	1728.3 (4.3)

Values in parenthesis correspond to the difference in frequency with respect to pure DMPC at the same temperature. Concentrations were fixed in all case at 50 mM.

Previous FTIR studies have shown that trehalose and sucrose [7] produce a decrease in the PO_2^- stretching bands, which has been interpreted as a result of the replacement of water at the membrane interface [8,9]. It is interesting to take into account that the three compounds have a glucose moiety in its structure [11].

The effects on the carbonyl and phosphate groups are shown in Table 1. At 50 mM, the PO_2^- antisymmetric stretching frequency follows the sequence trehalose <sucrose <arbutin. However, the PO_2^- symmetric stretching band does not show an important change.

The compounds displace the frequencies of the C═O non-hydrated population to lower values with respect to pure DMPC (negative values in brackets). The magnitude of the decrease is in the order: trehalose>arbutin>sucrose. In contrast, the hydrated population frequency is decreased by trehalose, not affected by sucrose and increased by arbutin.

Taken together, it can be inferred that the interaction with the phosphate group is qualitatively similar in the three compounds since all of them decrease the antisymmetric frequency. This can be related to the presence of the glucose moiety in the three molecules. However, the selective interaction of those molecules with lipid bilayers can be related to the carbonyl group exposure and distribution.

In other words, the phosphate is a site for H-bonding compounds that cannot distinguish between arbutin, sucrose or trehalose. In contrast, carbonyl groups appear to play an important role in the specific interaction of H-bond compounds with lipids.

It is well established that there is a “bound” hydration shell surrounding the head group of membrane phospholipids that plays an important role in bilayer stability [12,13]. This hydration shell may change from seven water molecules per lipid to eighteen water molecules per lipid at the gel–liquid crystalline transition.

The C═O stretching band of phospholipids in aqueous dispersions displays an asymmetry which changed when the lipid passed from the gel into the liquid crystalline phase [14].

This means that the exposure of the carbonyl groups to the water phase is different for each phase state.

Thus, arbutin interaction may be affected by the different carbonyl arrangements present at different hydration levels. That would be the reason for which the effect of arbutin is more pronounced in gel state than in fluid state.

In this regard, it is important to notice that in the gel and fluid states, the arbutin effect differs when it is outside and inside or only outside. In this last case, the effect of arbutin could produce an osmotic stress due to the difference of concentration of arbutin between the inner and the outer solution. Hence, the hypertonic shock would be causing water rearrangements affecting arbutin insertion. This is congruent with previous observations in DMPC liposomes in the gel state [15–17].

Various direct interlipid hydrogen bonding possibilities exist between the phosphate or carbonyl oxygens of adjacent phospholipids. In consequence, the configuration of the sites to which water binds to the phospholipids in bilayers should be described as different arrays of hydrogen bonds at the carbonyl region [18–20].

Early studies distinguished tightly bound and weakly associated water molecules [20–22]. Indeed the first layer of water makes a major contribution to the dipole potential, as influenced by the carbonyl and phosphate groups [23–25]. Preliminary results in this laboratory demonstrated that arbutin decreases the dipole potential of DMPC monolayers and in alkyl derivates, which can be related to the interaction of arbutin with PO and CO groups (Lairion and Disalvo, to be published).

5. Conclusions

The interaction of arbutin with the phospholipid head groups is different in the absence or in the presence of water.

In the absence of water, the increase of frequency of the PO_2^- group can be ascribed to restrictions in the vibration mode probably due to packing in the phosphate region. The increase in packing in the hydrocarbon chain is also increased by arbutin in anhydrous lipids.

In the presence of water, the hydrocarbon region is not affected by arbutin as shown by the CH_2 and CH_3 stretching modes of vibration of the acyl chains, in all the experimental conditions assayed. The effect appears confined at the membrane interface. The decrease in the PO_2^- antisymmetric stretching mode appears similar to that observed with trehalose and sucrose. This behavior might be due to the interaction of the three compounds through the glucose moiety. This possibility should be verified by theoretical calculations and further experiments.

A similar response in the PO_2^- antisymmetric stretching mode was found when arbutin is inside and outside or only outside of lipid membranes.

In contrast, the interaction pattern with carbonyl group differs from the sugars and is strongly dependant on whether the membrane is in the gel or the liquid crystalline states, and whether arbutin is evenly or unevenly distributed between the inner and outer media.

In all the cases assayed, arbutin interacts with the non-hydrated population of the carbonyl groups, as inferred from the shift to lower frequency. Arbutin outside can cause an osmotic hydration shock, enhancing the interaction with the hydrated population. The interaction with the C═O and PO_2^- groups decreases in fluid membranes as a consequence of the hydration in comparison with the gel state. In conclusion the arbutin insertion in the membrane is dependent of the hydration state of the interfacial groups.

Acknowledgements

This work was supported with funds from Agencia Nacional de Promoción Científica y Tecnológica, (PICT 0324), CONICET (PIP 5476 and PIP 6457), CIUNT (Universidad Nacional de Tucumán), and UBACyT (047).

EAD and ABA are members of the Research Career of CONICET (National Research Council, Argentina). MAF is recipient of a fellowship from the same institution.

The authors are grateful to Dra A, Gomez Zavaglia for the critical reading of the manuscript and to Lic M.L. Roldan and Lic A. Ledesma for assistance with the software programs for the preparation of the figures.

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