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The effect of compatible solute ectoines on the structural organization of lipid monolayer and bilayer membranes

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ABSTRACT

Compatible solutes are small organic osmolytes responsible for osmotic balance and at the same time compatible with the cellular metabolism. Here, we have investigated the effect of the compatible solutes, ectoine and hydroxyectoine, on the fluid–rigid domain structure of lipid monolayer and bilayer membranes. Mainly saturated dipalmitoyl-phosphatidylcholine membranes exhibiting a clear *le*/*lc* phase transition were used. Fluorescence microscopy showed that ectoines added to the aqueous subphase expand and fluidize the lipid monolayers especially at surface pressures below 30 mN/m. The domain structure at the *le*/*lc* phase transition is sensitively modified leading to smaller but more numerous domains in the presence of ectoines. Hydroxyectoine was more efficient than ectoine. These results are explained by the replacement theory assuming that the ectoines are likely to be expelled from the membrane surface thus favoring the hydration of the lipid membrane. This effect reduces the line tension, which is the interfacial energy at the domain edges leading to reduced domain sizes and increased number of rigid domains. Isotherms of negatively charged phosphatidylglycerol membranes show a similar expansion, while unsaturated lipids are less affected. Mixed phosphatidylcholine/phosphatidylglycerol membranes exhibit the same effect on the line tension increasing the tendency for a phase separation. This could be shown also in bilayer vesicles, where the compatible solutes have only a minor effect on the lipid main phase transition in pure DPPC membranes but reduce the extent of the pretransition. In mixed DPPC/DPPG bilayer membranes ectoines cause a phase separation leading to the enrichment of expanded DPPC domains. In conclusion, our study gives for the first time evidence that ectoines have an effect on lipid membranes increasing the hydration of the surface and thus increasing the mobility of the lipid head groups and fluidizing the lipid layer accordingly. This increased fluidity may be of advantage for cell membranes to withstand extreme conditions like temperature or osmotic pressure and might also accelerate cellular repair mechanisms.

1. Introduction

The ectoines (ectoine and hydroxyectoine) are zwitterionic, low-molecular weight and strong water binding organic molecules occurring in aerobic chemoheterotrophic and halophilic/halotolerant bacteria [1–4]. They belong to the class of most abundant compatible solutes and are synthesized and enriched within the cells during the environmental stress conditions viz., high temperature, freezing, extreme dryness and high salinity [5]. Interestingly, these solutes are biologically inert and do not interfere with the overall cellular functions even though they accumulate at high concentration in the cytoplasm, hence are named 'compatible solutes' (CSs) [3]. In addition to the function as osmoprotectants, CSs are distinguished as effective stabilizers of the biomolecules which include proteins, nucleic acids and biomembranes.

Several theories have been proposed to explain the protective mechanism of CSs, among which the 'preferential exclusion model' has gained most support [6–8]. It states that the CSs are strong water structure formers and are as such excluded from the immediate hydration shell of the biomolecules, for example, a protein, and preferentially hydrate the protein surface, consequently enhancing the stability of their native conformation by making denaturation thermodynamically less favorable. They do not directly interact with the protein surface, but slow down the diffusion of solvent molecules

Abbreviations: DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DPPG, 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol); DSC, differential scanning calorimetry; BODIPY-PC, 2-(4,4-Difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine; MLV, multilamellar vesicles; LUV, large unilamellar vesicles.

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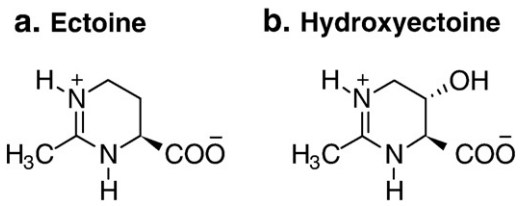

Fig. 1. Molecular chemical structure of a) ectoine and b) hydroxyectoine.

in the bulk phase by strongly interacting with water molecules [9], thereby restricting the structural alteration and enhancing their kinetic stability. Recently, ectoine preferential exclusion/interaction was shown using molecular dynamic simulations [10].

The literature survey shows that when compared to other biomolecules, protein–solute interaction *in vitro* has been extensively studied and a number of models have been proposed [6,11–13]. In addition, the effect of compatible solutes on nucleic acids and biomembranes has been studied lately. A comparative study of different compatible solutes was carried out on a model enzyme lactate dehydrogenase (LDH) and it was found that mannosylglycerate and hydroxyectoine were the best protectants [5,14]. Additionally, ectoine and hydroxyectoine have been shown to prevent protein aggregation, stabilize nucleic acids and protein structures and improve protein refolding. For more details readers are referred to a review [15], which describes the several applications of compatible solutes in different biological fields and further shows the effectiveness of ectoine and hydroxyectoine. From various studies, ectoines (especially hydroxyectoine) have been shown to be very potent stabilizers of biomolecules. Hydroxyectoine, a hydroxyl derivative of ectoine, with its –OH group can partly replace the water molecules lost from the hydration shell and further stabilize the biomolecules. Here both ectoine and hydroxyectoine have been used to investigate the effect on lipid systems.

Over the decades, the study of cellular membrane has led to the use of various model systems. The model membrane constitutes a simplistic system to study physical properties of plasma membranes. The multilamellar or unilamellar liposomes have been extensively used as a model to study the lipid–protein interaction as well as solute interaction with bilayer membrane systems [16–18]. In addition, lipid monolayers at the air–water interface have been used, which provide several advantages over liposomes and offer an excellent membrane model system [19,20]. Main advantage of using monolayer systems is that the lipid composition can be varied and regulated independent of lipid lateral packing density and the lipid surface area exposed to the medium is known. It can be used to study two-dimensional and surface phenomena, such as adsorption, surface activity, wetting, ordering and phase transition [20]. Importantly, lipid monolayers represent models for biological membranes and biologically important interfaces, such as the pulmonary surfactant monolayer in the lungs. In model lipid membranes, especially monolayers, large domains can be observed by epi-fluorescence microscopy [21] and atomic force microscopy (AFM) [22]. These domains appear as a liquid ordered (*Lo*) phase, coexisting with a liquid disordered (*Ld*) phase. The shape and size of lipid domains vary with lipid composition and other physical parameters and mainly exhibit circular shape over time. Studies indicate the presence of interfacial energy, also known as 'line tension', at the phase interfaces which is a key factor in determining domain size [23,24]. The distribution of domain size and shape mainly depends on the competition between line tension and long-range electrostatic repulsion. The line tension tends to increase domain size in order to minimize the total boundary length and entropy and favors compact, often circular domain shape, whereas, electrostatic dipolar repulsion accounts for other shapes [25,26].

In the present study we report the interaction of ectoine and hydroxyectoine with the lipid molecules which constitute the biomembranes. Here, a different approach has been used to study the effect of ectoines on the phase behavior of lipids. The Langmuir film balance and video-enhanced epi-fluorescence microscopy were used to analyze the phase transition of lipids and domain formation including the domain shape and size. Subsequently, the effect of the solute on aforementioned line tension which defines the shape and size of lipid domains will be discussed. In addition, the result obtained from differential scanning calorimetry (DSC) to study the effect of ectoines on the phase transition temperature of multilamellar vesicles is presented.

2. Materials and methods

2.1. Materials

The lipids used in this study, namely 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (POPG) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). 2-(4,4-Difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine (BODIPY-PC) was obtained from Molecular Probes (Eugene, OR). Ectoine ((S)-2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) and Hydroxyectoine ((4S,5S)-

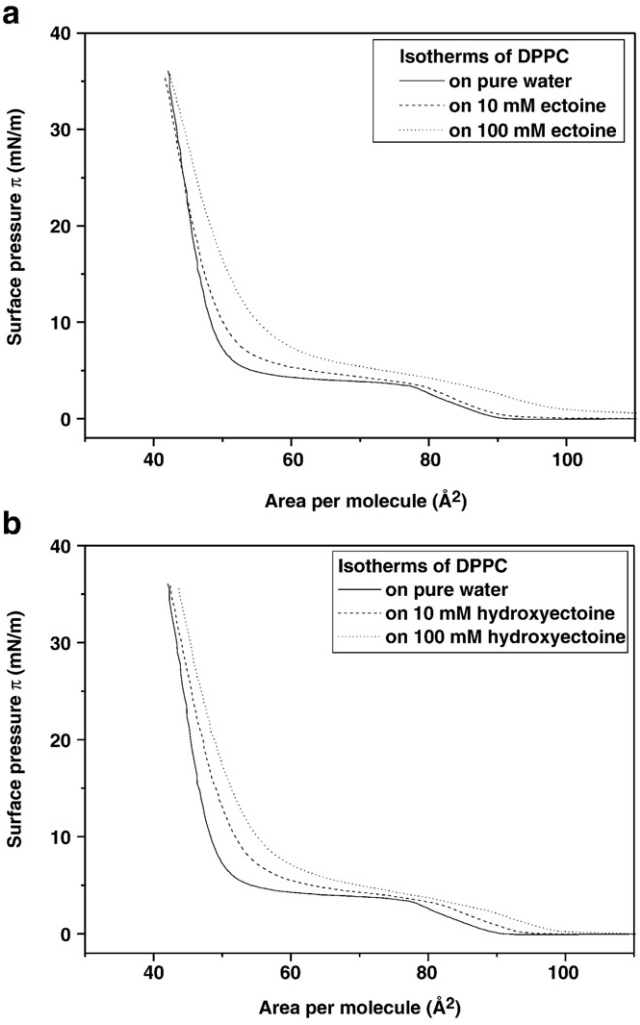

Fig. 2. Surface pressure–area (π–A) isotherms of monolayer consisting of DPPC on aqueous subphases containing a) ectoine and b) hydroxyectoine in 10 and 100 mM concentration. All the measurements were performed at 20 °C.

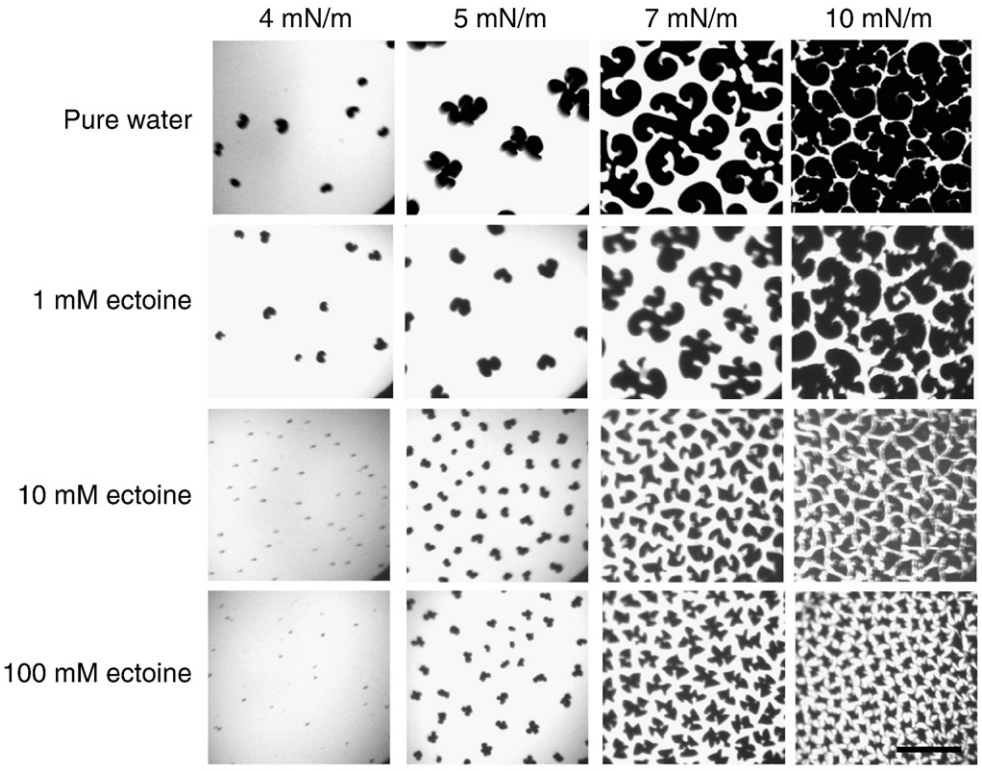

Fig. 3. Video-enhanced fluorescence microscopic images of DPPC monolayers on subphases containing different concentration of ectoine, measurements performed at 20 °C. Images taken at different surface pressures are shown. All samples were doped with 0.5 mol% of fluorescent dye BODIPY-PC. The scale bar is 50 μm.

2-methyl-5-hydroxy-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) were obtained from Bitop AG, Witten, Germany. All lipids were used without further purification. Chloroform and methanol were high pressure liquid chromatography grade and purchased from Roth (Karlsruhe, Germany). Water was purified and deionized by a multicartridge system (MilliPore, Billerica, MA) and had a resistivity >18 MΩ m.

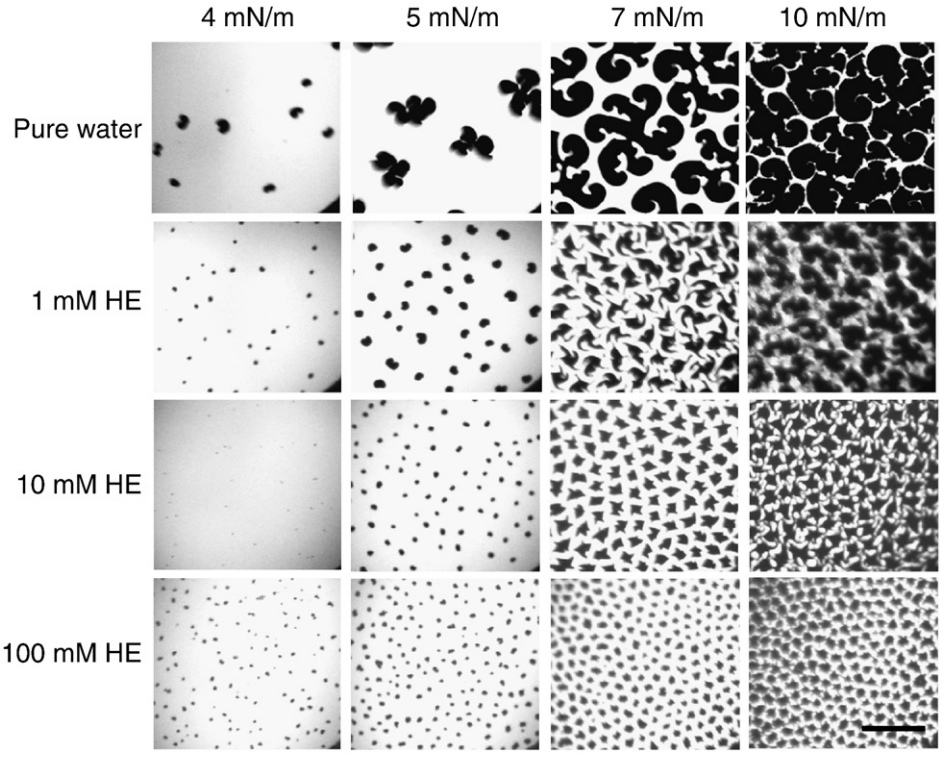

Fig. 4. Video-enhanced fluorescence microscopic images of DPPC monolayers on subphases containing different concentration of hydroxyectoine (HE), measurements performed at 20 °C. Images taken at different surface pressures are shown. All samples were doped with 0.5 mol% of fluorescent dye BODIPY-PC. The scale bar is 50 μm.

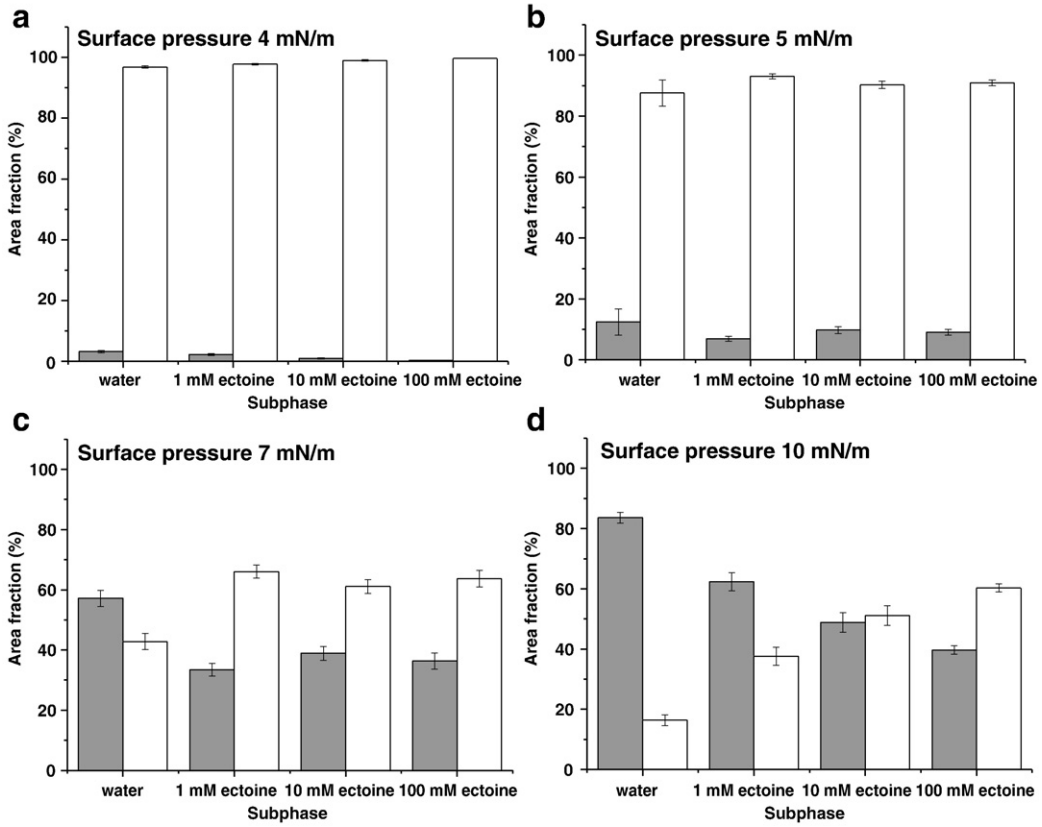

Fig. 5. Analysis of the area fraction of rigid and fluid domains in DPPC monolayers spread on ectoine containing aqueous subphases at surface pressures a) 4 mN/m b) 5 mN/m c) 7 mN/m and d) 10 mN/m. The gray columns represent dark (rigid) areas and white columns represent bright (fluid) areas.

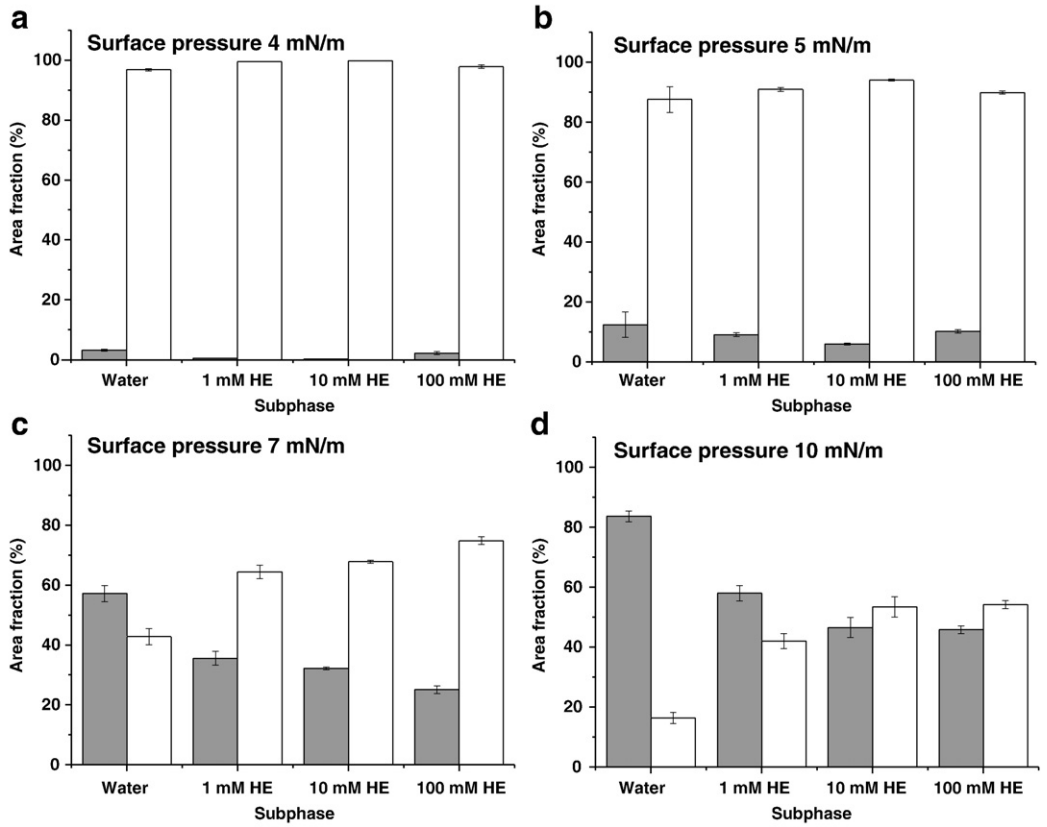

Fig. 6. Analysis of the area fraction of rigid and fluid domains in DPPC monolayers spread on subphases containing hydroxyectoine (HE) at surface pressures a) 4 mN/m b) 5 mN/m c) 7 mN/m and d) 10 mN/m. The grey columns represent dark (rigid) areas and white columns represent bright (fluid) areas.

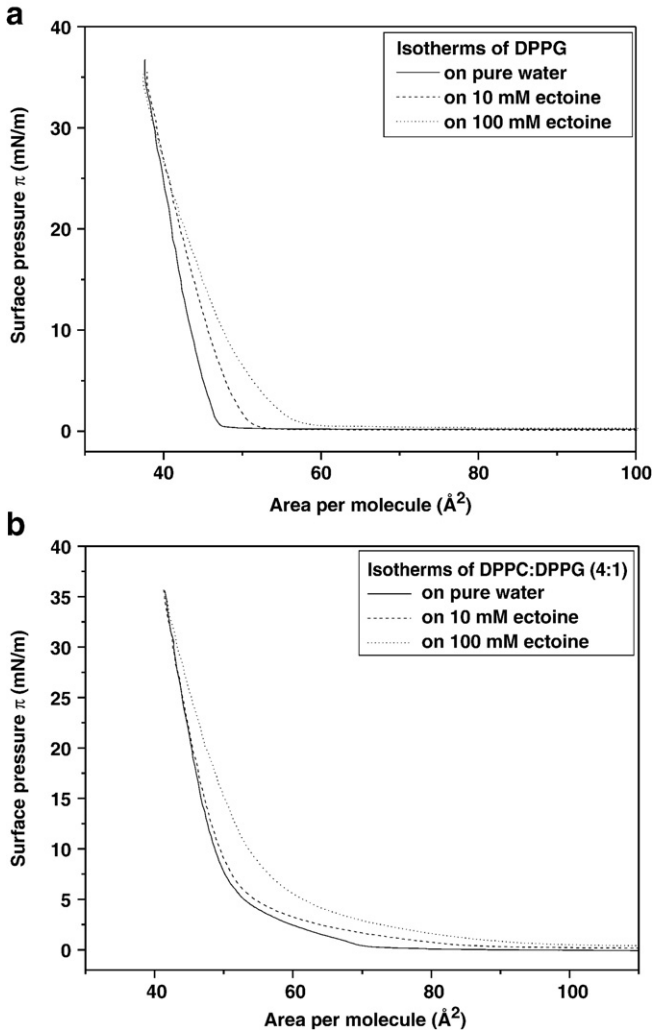

Fig. 7. Surface pressure–area (π–A) isotherms of monolayer consisting of a) DPPG and b) DPPC:DPPG on subphases containing 10 mM and 100 mM ectoine. All the measurements were performed at 20 °C.

The buffer solution used to hydrate lipid films consisted of N-2-(hydroxyethyl)piperazine-N′-2-ethansulfonic acid (HEPES) from Merck (Darmstadt, Germany). Lipids were dissolved in chloroform/methanol solution (1:1, v/v). The preparation of vesicles was carried out with a Liposofast-miniextruder from Avestin (Ottawa, Canada).

2.2. Vesicle preparation

The lipids DPPC or DPPC/DPPG mixture with a molar ratio of 4:1 was dissolved in chloroform/methanol (1:1, v/v) and dried under a stream of nitrogen at 50 °C. Traces of solvents were removed by keeping under vacuum at 50 °C overnight. The lipid films were hydrated by adding a buffer containing 1 mM HEPES, pH 7.0. The vesicle suspension (2 mg/ml) was kept for 30 min at 50 °C in a water bath and was vortexed for 30 s at regular intervals to get multilamellar vesicles (MLVs). The resulting multilamellar vesicles (MLVs) were converted into large unilamellar vesicles (LUVs) at 50 °C by membrane extrusion using liposofast containing a polycarbonate membrane with a pore diameter of 100 nm.

2.3. Surface pressure–area isotherm

All the film balance experiments were performed on an analytical Wilhelmy film balance (Riegler and Kirstein, Mainz, Germany) with an operational area of 144 cm^2. All surface pressure–area measurements were performed on pure water or varying concentrations of ectoine or hydroxyectoine, at 20 °C. The spread monolayers were composed of DPPC, DPPG, POPC, POPG or DPPC/DPPG (4:1, mol ratio) prepared in a chloroform/methanol solution (1:1, v/v) and spread onto the subphase. After an equilibration time of 10–15 min for the solvent to evaporate, the monolayers were compressed at a rate of 2.9 cm^2/min.

2.4. Fluorescence light microscopy

Domain structures of lipid samples doped with 0.5 mol% BODIPY-PC were visualized by means of a video-enhanced epi-fluorescence microscope (Olympus STM5-MJS, Olympus, Hamburg, Germany) equipped with an xy-stage and connected to a CCD camera (Hamamatsu, Herrsching, Germany). The images were captured at desired surface pressures by stopping the barrier. All the measurements were performed on a subphase containing pure water or varying concentrations of ectoine or hydroxyectoine, at 20 °C. The total area and number of the non-fluorescent domains in fluorescence images of the monolayers were analyzed using the program ImageJ, developed at the U.S. National Institutes of Health (http://rsbweb.nih.gov/ij/index.html). Domain area and number analysis were performed at least with four pictures taken in independent experiments.

2.5. Differential scanning calorimeter (DSC)

Calorimetric experiments were performed using PC controlled Setaram micro DSC III differential scanning calorimeter connected to a programmable water bath. One of the two chambers was filled with the sample; the other was filled with the corresponding buffer solution. The volume of the vesicle suspension was 800 µL with lipid concentrations up to 2 mg/mL. The buffer system was 1 mM HEPES (pH 7.0) containing varying concentration (1, 10, and 100 mM) of ectoine or hydroxyectoine. A scan rate of 30 °C/h was used in the temperature range between 10 and 60 °C. Phase transition temperatures were taken at the maximum of the transition peak.

3. Results

The effect of the organic osmolytes ectoine and hydroxyectoine (Fig. 1), also named compatible solutes, on the phase behavior of artificial lipid monolayer and bilayer membranes has been investigated. Lipid layers composed of zwitterionic dipalmitoyl-phosphatidylcholine (DPPC), negatively charged dipalmitoyl-phosphatidylglycerol (DPPG), in one component membranes as well as mixed 4:1 molar ratio DPPC/DPPG membranes have been investigated. Unsaturated lipids palmitoyl-oleyl phosphatidylcholine (POPC) and the corresponding phosphatidylglycerol (POPG) have been considered as well. Lipid monolayers have been investigated by the film balance technique monitoring both, the area pressure isotherm as well as the corresponding fluorescence microscopic pictures of the domain structures. Differential scanning calorimetry (DSC) was used to study the lipid bilayers with respect to their phase transition temperature.

3.1. Isotherms monitored by film balance measurements and video-enhanced fluorescence microscopy

The influence of ectoine and hydroxyectoine on the isotherms of DPPC monolayers is presented in Fig. 2. The isotherm on an aqueous subphase in the absence of compatible solutes as well as on a subphase containing 10 or 100 mM ectoine (Fig. 2a) or correspondingly the hydroxyectoine (Fig. 2b) was taken. On pure water the well known *le–lc* phase transition at 5 mN/m of DPPC is clearly visible with an almost constant pressure during compression of the film between areas of 78–50 $Å^2$. Isotherms taken on ectoine containing subphases

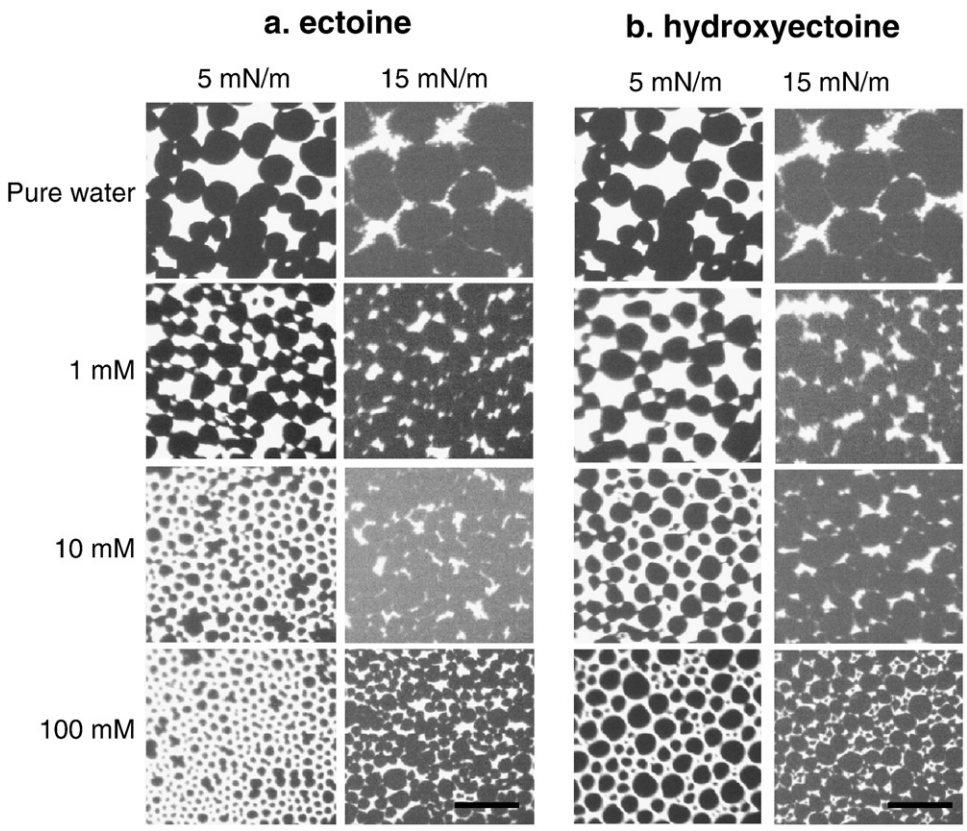

Fig. 8. Video-enhanced fluorescence microscopic images of DPPC:DPPG (molar ratio 4:1) monolayers doped with 0.5 mol% of fluorescent dye BODIPY-PC. The subphase contains water or increasing amounts of a) ectoine and b) hydroxyectoine (HE). Images taken at surface pressures, 5 mN/m and 15 mN/m are shown. The scale bar is 50 μm.

are shifted to higher area values and the *le–lc* phase transition is broadened. At high pressures (>35 mN/m) the isotherms merge thus clearly showing that the ectoine does not influence the lipid structural organization at higher surface pressures. A similar behavior is shown by hydroxyectoine. However, the effect is more pronounced compared to ectoine and expansion is also seen at higher pressures (35 mN/m) but only on 100 mM hydroxyectoine.

The fluorescence pictures of the monolayers doped with BODIPY-PC, a fluorescent dye that is preferentially soluble in the fluid *le* phase are shown in Figs. 3 and 4. Dark domains represent the rigidified *lc* phase, light areas the more fluid *le* phase. Lipid films on pure water exhibit the well known kidney shaped *lc* domains in the phase transition region. The extent of the *lc* phase increases with increasing surface pressure (decreasing area). At 4 mN/m only a few small almost unstructured domains are visible. The characteristic kidney shape starts to form at 5 mN/m and is best visible at 7 mN/m as a left handed clearly developed nanostructure which is then linked together at higher pressure at the end of the phase transition. Ectoine leads to an almost complete disappearance of these preformed structures at 4 mN/m. At 5 mN/m the well formed kidney structures loose their shape and the domain size shrinks considerably. At 5 mN/m and 7 mN/m the well developed domain structure is lost continuously with increasing ectoine concentration. This effect is even more enhanced on a subphase containing hydroxyectoine (Fig. 4). A quantitative evaluation of the microscopic pictures is given in Figs. 5 and 6. Fig. 5a–d shows the relative distribution of lipids within the fluid condensed (dark) and fluid expanded (bright) regions. It is clearly visible that at low pressure (4 mN/m) the bright (fluid) domains dominate with about 90% and neither ectoine nor hydroxyectoine has a considerable effect on the area fraction. At 7 mN/m the area fraction is close to 50% but increases drastically with higher concentration to about 70% of fluid fraction. The same is true for 10 mN/m where the fluid area distribution is roughly 20% on water but increases to 55–60% in the presence of ectoine or hydroxyectoine. In parallel the number of rigid domains (data not shown) increases on ectoine containing subphases reaching values of 50 at 4 mN/m and 70 at 7 mN/m on a 120×120 μm² area in case of 100 mM ectoine. The corresponding values for hydroxyectoine are 150 and 210 domains per unit area. Pictures at 10 mN/m could not be evaluated due to the merging domains. This result clearly shows that ectoine and hydroxyectoine (having much stronger effect) both decrease the domain line tension in DPPC monolayers.

Pure DPPG monolayers are also expanded in the presence of ectoine or hydroxyectoine and the effect is similar for both solutes, hence, only the isotherm on ectoine is shown (Fig. 7a). Fluorescence pictures could not be taken here because the DPPG does not undergo the *le–lc* phase transition. Mixtures of DPPC/DPPG (4:1 molar ratio) are presented in Fig. 7b. On a pure water phase a slight remainder of the *le–lc* phase transition originating from the DPPC component is still visible which disappears on compatible solute containing subphases leading again to a strong expansion with increasing solute concentration for both, ectoine (Fig. 7b) as well as hydroxyectoine (data not shown). Again the curves merge at higher pressure. For the mixed monolayers we found a clear microscopic structure (Fig. 8a and b). In the absence of solutes the domains are round with a diameter of about 25 μm^2 at 5 mN/m. Both ectoine and hydroxyectoine reduce the domain size drastically at 5 mN/m and the number of rigid domains increases (Fig. 9). At higher pressures no clear geometrically differentiated domains are visible any more but a clear fluidization is observed by an increased relative light/dark area ratio. Due to the merging domain boundaries these pictures could not be analyzed quantitatively.

Unsaturated lipids (POPC and POPG) were investigated as well (data not shown). In both cases only minor effects of the solutes could be observed for ectoine and hydroxyectoine.

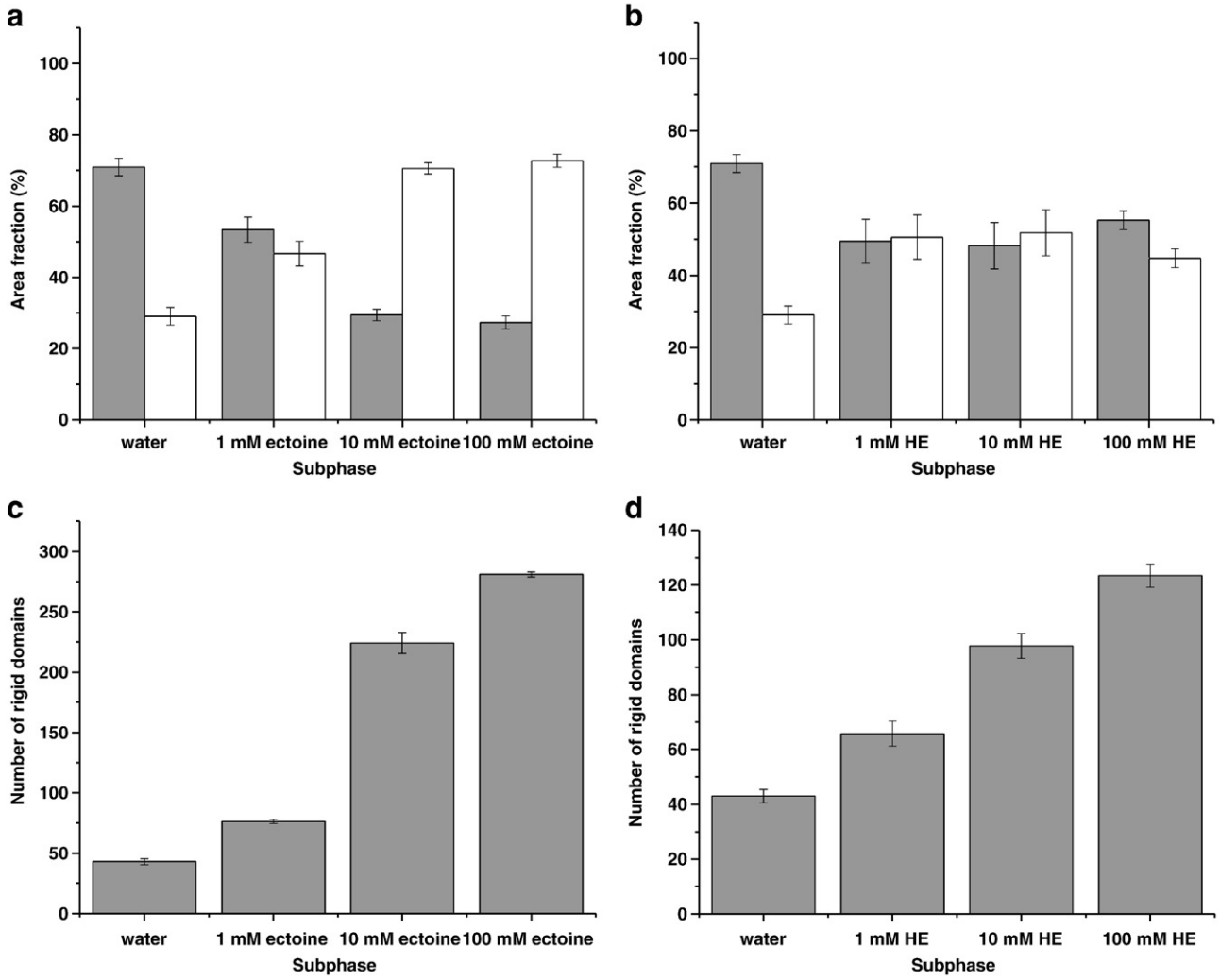

Fig. 9. Analysis of the area fraction of rigid and fluid domains in DPPC:DPPG (molar ratio 4:1) monolayers spread and compressed to surface pressure 5 mN/m on subphases containing different concentrations of a) ectoine and b) hydroxyectoine (HE). The corresponding numbers of domains in $120 \times 120\ \mu m^2$ area are given in figure c) ectoine and d) hydroxyectoine. The grey columns represent dark (rigid) areas and white columns represent bright (fluid) areas.

3.2. Calorimetric investigation of lipid bilayers

Multilamellar DPPC as well as DPPC/DPPG lipid vesicles has been investigated by differential scanning calorimetry (DSC) in the absence and presence of ectoine or hydroxyectoine (Fig. 10a and b). Pure DPPG does not form reproducible multilayers and is thus not included. In DPPC MLVs the pre- as well as main transition is clearly visible. The main transition is not affected, while the pretransition is only slightly influenced by the ectoine. Hydroxyectoine causes a slight upward shift and a broadening of the main phase transition and the pretransition is slightly broadened with mixed DPPC/DPPG MLVs which we observe as a clear tendency for a phase separation (Fig. 11a and b). Ectoine in the subphase leads to a sharpening of the main transition as well as the reappearance of the pretransition. On a hydroxyectoine containing subphase we observe a clear separation of the main transition peak into two giving clear evidence for a phase separation leading to more DPPG depleted DPPC domains (shoulder marked by arrow) accompanied by the formation of DPPG enriched domains (upward shifted main transition marked by arrow head). Similar results were obtained with large unilamellar vesicles (results not shown).

4. Discussion

The action of protective solutes is a major factor in maintaining the integrity of cells during stress which could be extreme environmental conditions such as high salt, low or high temperatures or desiccation. Ectoines are synthesized by aerobic, chemoheterotrophic and halophilic organisms to guarantee their survival under these extreme conditions. The major task of ectoines is to protect the biopolymers which are proteins, nucleic acids and lipids against dehydration accompanied with conformational changes with the consequence of the loss of the biological activity. The influence of ectoines has been extensively investigated mainly on proteins and also on DNA [27] and their action is explained by the "preferential exclusion model" [7,10,28] where the solutes are excluded from the macromolecular surface. This is thought to slow down the water diffusion from, e.g., protein surfaces with the consequence to stabilize their hydration shell leading to a conformational stability which stabilizes the protein structure, e.g., against heat or salt. This effect is driven by hydrogen bond formation of the ectoine in water. The effect of compatible solutes on lipid membranes has also been investigated in the past [29,30] but mainly focussing on alcohols including sugars, glycerol and amino acids [18] coming to the same conclusion that the preferential exclusion of the solute from the membrane–solvent interface is the key factor for the favorable *in vivo* effect.

The same explanation holds for the effect of ectoines which would mean that the hydrated state is favored by ectoine. A protective effect of ectoines against cell damage has been shown [28] in erythrocytes which becomes more resistant against lysis caused by SDS treatment

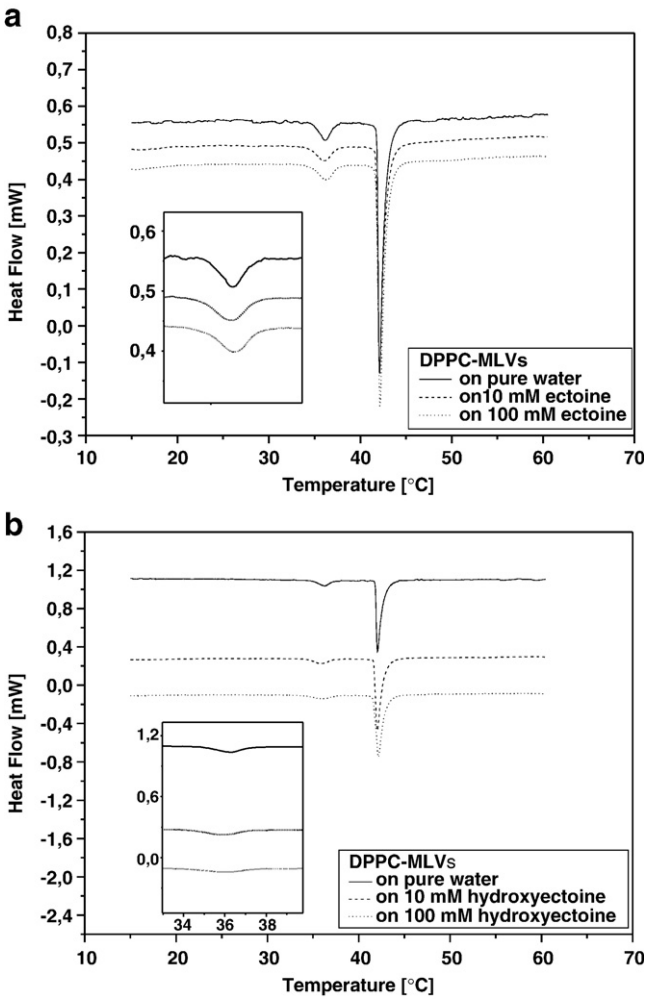

Fig. 10. Differential scanning calorimetry scans of DPPC multilamellar vesicles in 10 mM and 100 mM concentration of a) ectoine and b) hydroxyectoine containing aqueous dispersions. Pretransitions are enlarged in the insets.

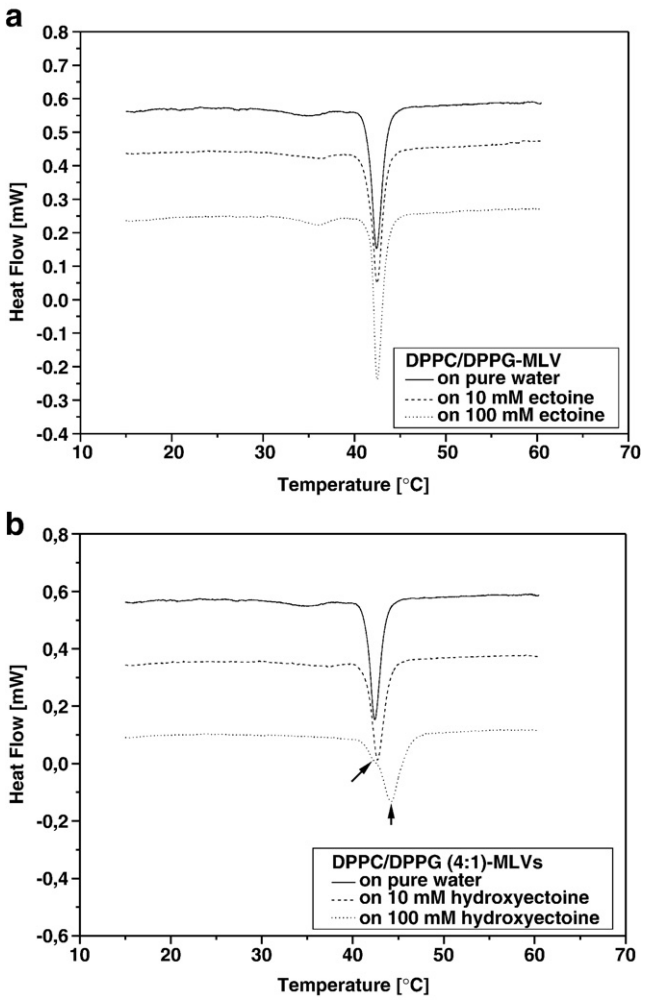

Fig. 11. Differential scanning calorimetry scans of DPPC:DPPG (molar ratio 4:1) multilamellar vesicles in 10 mM and 100 mM concentration of a) ectoine and b) hydroxyectoine containing aqueous dispersions. The arrows in panel b point to the separated main transition exhibiting the lower phase transition of DPPC enriched (arrow) and the higher phase transition of DPPG enriched narrow head) domains.

if they are pretreated by ectoines. To further elucidate this membrane effect we investigated lipid monolayers and lipid bilayers concentrating on domain structures. These lateral compositions (sometime called rafts) are of special interest since they are involved in cellular signaling processes. Fluid phase coexistence in lipid membranes is characterized by the formation of a liquid ordered phase (liquid condensed, *lc* phase in lipid monolayers) and a liquid disordered phase (liquid expanded, *le* phase in lipid monolayers). The *lc* phase exhibits a higher degree of ordering and packing preventing water to penetrate the hydrophobic core.

Line tension and domain boundary fluctuations may be a key regulator and the driving force for domain coarsening [31]. García-Sáez et al. [26] have shown that the interfacial energy at the domain edge is the major parameter that determines not only the membrane lateral organization but also the distribution of domain size. Although they investigated temperature dependent effects we concentrated on ectoines as line active species. García-Sáez et al., found that the hydrophobic mismatch between the *le* and the *lc* phase lipids causes an increased line tension leading to bigger domains to minimize the interfacial length [26]. Baumgart et al. showed that line tension drives the shape of domains in vesicles [32].

We now showed that the formation of ectoine–water complexes and thus the cosmotropic effect of ectoine on the water structure shown before can stabilize lipid monolayers and bilayers as well. Those artificial lipid layers however are considered to be excellent models for cell membranes. As shown in Fig. 12a, a lipid monolayer in water is stabilized by hydrophobic interactions of the apolar lipid tail and hydrophilic interactions of the polar lipid head groups to water. In an ectoine solution the hydrophilic interactions are increased by the ectoine water complexes as shown in Fig. 12b which results in an increase of the mobility of lipids and thus the fluidity of the lipid monolayers. The higher fluidity of lipids is advantageous for cell membranes for signaling processes. Also repair mechanisms can be accelerated.

The effect of ectoine on fluidity of lipid monolayers was shown by film balance measurements using lipid monolayers. By increasing the surface pressure on a DPPC lipid monolayer in water the formation of rigid well-shaped *lc* domains can be observed at higher pressure. These rigid domains are much smaller and more numerous in ectoine solutions. As the concentration of ectoine increases, the size of the rigid domain decreases. Even at concentration down to 1 mM an effect can be observed which is pretty low compared to other work on proteins considering concentrations up to 2.4 M [26].

In summary, we can state that the phase equilibrium at the *le*/*lc* phase transition in DPPC membranes is especially sensitive for ectoine present in the aqueous subphase. The unsaturated POPC (or POPG) monolayer does not exhibit such a phase transition. Due to the unsaturated character of one of the chains, the monolayer is already in an expanded state and is

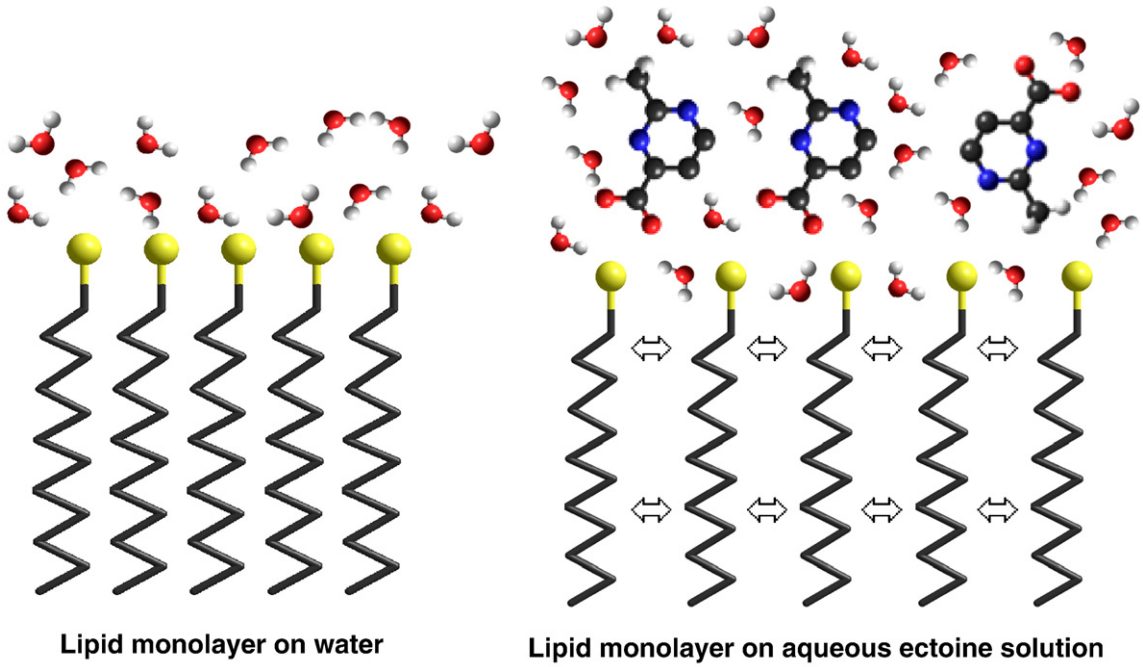

Fig. 12. Schematic image of the exclusion model for compatible solutes at membrane surfaces leading to an expansion and thus fluidization of the lipids. Organized water clusters at the membrane surface are formed around the solutes thus increasing the water activity at the membrane surface.

thus not much affected by ectoines. Negatively charged and rigid DPPG monolayers, although they do not undergo a pressure dependent phase transition are also sensitive against ectoines exhibiting a strong expansion. In mixed DPPC/DPPG membranes we obtained clear evidence that the fluidization of DPPC is the driving force to increase the tendency for a phase separation. In addition to the monolayer experiments we were able to show that in multilamellar vesicles made of DPPC and DPPG in a 4:1 molar ratio the thermotropic phase transition is separated into two distinguishable peaks clearly demonstrating that fluidized DPPC is separated from the DPPG enriched areas which melt at higher temperature. DPPC bilayers are only slightly affected which fits to our observation, that in monolayers at pressure above 30 mN/m the effect of ectoine is minor. This is convincing since a lateral pressure of about 30 mN/m is assumed for lipid bilayers. Rudolph and Goins [18] investigated DPPC multilayer vesicles under the influence of the amino acid proline and in agreement with our results observed a slight broadening of the pretransition and slight upward shift of the main transition but at concentrations of up to 3 M [26].

Comparing the effects of ectoine and hydroxyectoine reveals small but significant differences. Hydroxyectoine seems to be slightly more effective than ectoine due to the presence of an OH group resulting in an additional potency to form hydrogen bridges with the water molecules, further a strong interaction with the lipid head group might be expected. Although the major effect on lipid domain structure for both solutes seems to be the exclusion from the membrane surface.

In conclusion, we can state that ectoines act as membrane domain modifiers and thus possible lipid raft regulators. The harmonization of hydrophobic mismatches within a membrane may lead to a considerable elastic membrane stabilization smoothing out the interface and thus minimizing the sum of elastic and hydrophobic energy of the interface.

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