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Penetration behaviour of alkylbetainate chlorides into lipid monolayers

F. Nsimba Zakanda[a,c,*], K. Nott[b], M. Paquot[a], G. Mvumbi Lelo[c], M. Deleu[a]

[a] Unité de Chimie Biologique Industrielle, Gembloux Agro-Bio Tech-Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium
[b] Unité de Chimie Générale et Organique, Gembloux Agro-Bio Tech-Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium
[c] Service de Biochimie, Département des Sciences de Base, Faculté de Médecine, Université de Kinshasa B.P.127 Kinshasa XI, R.D. Congo

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ABSTRACT

In this paper, the penetration behaviour of the alkylbetainate chloride surfactants (C_nBC, n = 10–16) into lipid monolayers of dipalmitoylphosphatidylserine (DPPS), dipalmitoylphosphatidic acid (DPPA), dipalmitoylphosphatidylethanolamine (DPPE), palmitoyoleoylphosphatidylcholine (POPC) and cholesterol (CHOL) is investigated using the Langmuir trough technique. The penetration of C_nBC is followed by measurement of the surface pressure increase ($\Delta\pi$) at a constant surface area after the injection of C_nBC into the aqueous phase, underneath the lipid monolayer previously spread at the air–water interface at 25 °C and at different initial surface pressures (π_i). The influence of both the lipid head group and the surfactant hydrocarbon chain length on the effectiveness of C_nBC penetration into these monolayers is discussed. The results have shown that C_nBC adsorb at the air–water interface giving evidence of their surface-active properties. The adsorption kinetics of C16BC into different lipid monolayers are lipid head charge and lipid head volume-dependent. The magnitude of the surface pressure increase ($\Delta\pi$) arises in the following order: DPPA > DPPS ≫ CHOL ≈ DPPE > POPC. C_nBC penetration into negatively-charged (DPPS and DPPA) monolayers does not seem to depend on surfactant alkyl-chain length compared to uncharged (CHOL) and zwitterionic (DPPE and POPC) monolayers for which $\Delta\pi$ increases with a larger alkyl-chain length. Electrostatic interactions are mainly involved in the affinity of C_nBC with monolayers but the hydrophobic effect plays also a role.

1. Introduction

Glycine betaine (N,N,N-trimethylglycine) also called betaine is present in various plants, animals and microorganisms [1]. It is an important naturally occurring compound that functions as compatible solutes or osmoprotectants [2]. Recently, a review of the synthesis methods and the potential applications of glycine betaine derivatives has been published [3]. Several surfactants including esters (alkylbetainates) are based on this molecule [4–9]. These compounds are generally referred to as "mild to the skin" [10,11] and are used as additives to other surfactants to improve their dermatological properties (e.g., betaines decrease the skin irritation of anionic compounds). From the point of view of practical applications, studies of the surface properties of alkylbetainates and their interaction with lipid membranes are of great importance.

Phospholipids and sterols are biological amphiphiles that are the building blocks of cell membranes. Because of the extremely complex nature of in vivo systems, lipid monolayers, bilayers, and liposomes are often used as models to investigate their biological counterparts [12]. Monolayer represents half of a lipid bilayer membrane [13,14]. It provides a simple model for evaluating membrane insertion of amphipathic compounds into the outer leaflet of a biological membrane [12,15]. Parameters such as the nature and the packing of the molecules spread to form the monolayer, the composition of the subphase (pH, ionic strength) and temperature can be varied in a controlled way and without limitation. Lipid monolayers are very useful to characterize drug–lipid or lipid–lipid interactions at a molecular level using the Langmuir trough technique [16]. The amphiphile is dissolved in the aqueous subphase and its interaction with the monolayer is monitored. These studies are interesting from the biological and biophysical standpoints as well as in industrial applications such as the formulation of pharmaceutical and cosmetic products [17,18].

The behaviour of the alkylbetainate chloride (C_nBC, n = 10–16) (Scheme 1) monolayers at the air–water interface at 20 °C has been previously investigated [19]. However, no information is available with regard to their penetration into lipid membranes. For this reason, the aim of this work is to study, using the Langmuir technique, the interaction of a homologous series of C_nBC with lipid monolayers spread on water at constant area and temperature. The chosen lipids have various polar head groups

* Corresponding author at: Unité de Chimie Biologique Industrielle, Gembloux Agro-Bio Tech-Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium. Tel.: +32 81 62 22 32; fax: +32 81 62 22 31.
E-mail addresses: nsimbazak@yahoo.fr, fnsimbazakanda@student.ulg.ac.be, fnsimba@unikin.cd (F. Nsimba Zakanda).

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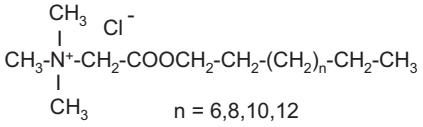


Scheme 1. Chemical structure of the alkylbetainate chlorides (C_nBC).

[dipalmitoylphosphatidylserine (DPPS), dipalmitoylphosphatidic acid (DPPA), dipalmitoylphosphatidylethanolamine (DPPE), palmitoyloleoylphosphatidylcholine (POPC) and cholesterol (CHOL)]. Their structures and intrinsic p*K*s are shown in Table 1. The influence of both the lipid head group and the surfactant hydrocarbon chain length on the effectiveness of C_nBC penetration into these monolayers is discussed.

2. Materials and methods

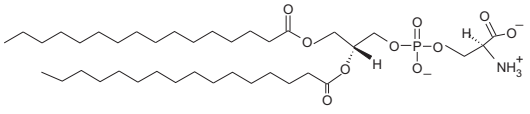
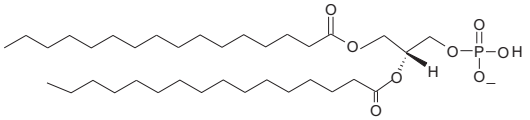
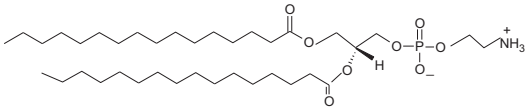
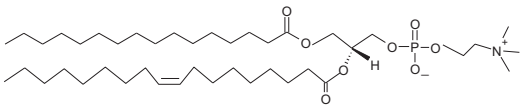
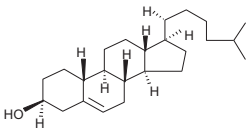
2.1. Materials

Alkylbetainate chlorides (C_nBC) were synthesized in two steps according to the method described in the literature [26]. Briefly, betainyl chloride was formed by adding thionyl chloride drop wise under constant stirring to a suspension of glycine betaine in dichloromethane at 60 °C. The reaction proceeded in a water bath, until the emission of sulfur dioxide ceased. The resulting product was washed with n-hexane. Primary alcohols (C_{10}–C_{16}) were acylated by the obtained betainyl chloride to produce alkylbetainate chlorides (C_nBC). Structures were confirmed by infrared spectroscopy and mass spectrometry. A typical IR spectrum of betainyl chloride displayed bands at 2960, 2870 cm^{-1} (C–H stretching), 2925, 2850 cm-1 (C–H stretching), 1800 cm^{-1} (C=O stretching), 675 cm^{-1} (C–Cl stretching). IR spectra of C_nBC displayed bands at 2918–2962 cm^{-1} (C–H, stretching), 1738–1751 cm^{-1} (C=O, stretching), 1402–1477 cm^{-1} (C–H, bending), 1190–1205 cm^{-1} (C–O, stretching). Electrospray ionization mass spectra displayed the signals of the $[M]^+$ ions at m/z = 258.1 (C_{10}BC), 286.3 (C_{12}BC), 314.3 (C_{14}BC) and 342.4 (C_{16}BC). Dipalmitoylphosphatidylserine (DPPS), dipalmitoyphosphatidic acid (DPPA), dipalmitoyphosphatidylethanolamine (DPPE), palmitoyloleoylphosphatidylcholine (POPC) and cholesterol (CHOL) were purchased from Advanti (99% purity). The solvents (chloroform and methanol) of analytical grade were obtained from Fluka. Reagents and solvents were used as received.

2.2. Penetration experiments

The experiments are conducted with an automated Langmuir system (KSV minitrough, KSV instruments Ltd., Helsinki, Finland) in which the mini-trough is filled with ~91 ml of milli-Q water. In a first set of experiments, each C_nBC is dissolved in water to obtain a concentration of 1.5 mM and is injected (without lipid monolayer) into the water subphase using a Hamilton syringe. The C_nBC final concentration in the subphase is 1.15 μM. It is assumed that alkylbetainate chlorides exist mainly as monomers within the water subphase) as this concentration is well below their measured cmc [8,9]. For the second set of experiments, the lipid monolayer (DPPS, DPPA, DPPE, POPC or CHOL) is prepared by carefully spreading droplets of the lipid solution (dissolved in chloroform/methanol 2:1 v/v) to obtain the desired initial surface pressure (π_i). When the monolayer surface pressure is stabilized at a π_i value, C_nBC is injected into the subphase to the same final concentration (1.15 μM). The surface pressure increase ($\Delta\pi$) induced by the C_nBC adsorption at the interface (without and with lipid monolayer) is measured with a Wilhelmy plate. The surface pressure is recorded until a plateau is reached, usually within 1 h. During the measurements the subphase is continuously gently stirred with a magnetic bar. The system is enclosed in a Plexiglas box in order to minimize water evaporation and to avoid contaminations. The subphase temperature is maintained constant at 25 ± 0.5 °C by circulating water through the base plate on which the mini-trough is mounted.

Table 1
Structures and intrinsic p*K*as of the lipids studied [20–25].

Lipid	Intrinsic p*K*a			2D structure
	Phosphate	Carboxyl	Amine	
1,2-Dipalmitoyl-sn-phosphatidylserine (DPPS)	2.1–2.2	2.1–4	9.7–9.9	
1,2-Dipalmitoyl-sn-phosphatidic acid (DPPA)	pKa_1: 3.5–4.0 pKa_2: 9.0–9.5	–	–	
1,2-Dipalmitoyl-sn-phosphatidylethanolamine (DPPE)	1.4–2.5	–	9.0–9.7	
1-Palmitoyl-2-oleoyl-sn-phosphatidylcholine (POPC)	1.4–2.0	–	1 0.5–11.5	
Cholesterol (CHOL)	–	–	–	

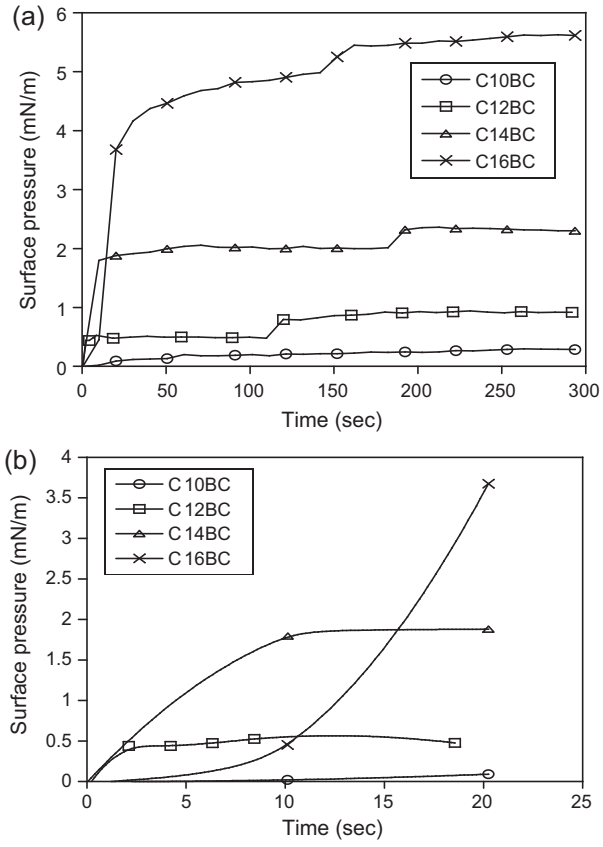


Fig. 1. Kinetics (a) and enlargement of the early stage (b) of the adsorption of alkylbetainate chlorides (C_nBC) at the air–water interface. C_nBC are injected at a concentration of 1.15 μM into the water subphase at 25 °C.

3. Results

3.1. Alkylbetainate chlorides (C_nBC) adsorption at the air–water interface

Fig. 1a–b illustrates the time course for the adsorption of C_nBC (at a concentration of 1.15 μM in the subphase) to the air–water interface. An increase in the surface pressure is detectable instantaneously, attaining a first plateau. After a time between 50 and 200 s, a second fast and small increase in surface pressure is observed reaching a second plateau (Fig. 1a). The pressure increase is indicative of C_nBC surface activities. As the alkyl chain length increases the final surface pressure increases. $C_{16}BC$ shows a maximal surface pressure much higher than the three others. At equilibrium, the C_nBC monolayers exhibit surface pressures of 0.4, 0.9, 2.2 and 5.7 mN/m for $C_{10}BC$, $C_{12}BC$, $C_{14}BC$ and $C_{16}BC$, respectively. Fig. 1b shows that the adsorption rate during the early stage is influenced by the alkyl-chain hydrophobic character. It is high and asymptotical for $C_{12}BC$ and $C_{14}BC$, low and linear for $C_{10}BC$ and low and sigmoidal for $C_{16}BC$ during the early stage of adsorption.

3.2. Hexadecylbetainate chloride (C16BC) penetration into lipid monolayers: effect of different lipid polar heads

In water (pH 5.7), the head group of DPPS has two negative charges and one positive charge, the one of DPPA has one negative charge, the ones of DPPE and POPC have one positive charge and one negative charge while the one of CHOL is uncharged.

The interaction of $C_{16}BC$ with lipids is analyzed by measuring the surfactant-induced surface pressure increase on lipid monolayers preformed at the air–water interface at 30 mN/m. At such a surface pressure the properties of a lipid monolayer (the packing of molecules) correspond to those of most of natural or artificial lipid bilayers [27]. This surface pressure can thus be suggested to be relevant for biological purposes [28,29].

The $C_{16}BC$ (at a concentration of 1.15 μM) is injected into the water subphase under the preformed lipid monolayers maintained at a constant area. Addition of C16BC in the subphase underneath all the lipid monolayers induces a significant increase of surface pressure, whatever the nature of the lipid (Fig. 2a). It indicates that the alkylbetainate is able to adsorb and penetrate into the lipid monolayer. This penetration is nevertheless very low into the zwitterionic POPC monolayer.

The penetration rate of $C_{16}BC$ into the lipid monolayer (first region of the kinetic plots) is influenced by the charge and the steric hindrance of the lipid head group. It decreases in the following order according to the slope of the linear part of the curve: DPPA > CHOL > DPPS > DPPE > POPC (Fig. 2b).

Surface pressure profile versus time after $C_{16}BC$ injection is asymptotical for DPPA and CHOL, sigmoidal for DPPS and DPPE and almost linear for POPC (Fig. 2a). The maximal surface pressure increase reached at the plateau follows the order: DPPA > DPPS > DPPE ≈ CHOL > POPC.

To investigate whether the $C_{16}BC$, the most surface-active C_nBC in this study (Fig. 1) is able to interact with membranes with physiological lipid packing densities, lipid monolayers formed by anionic DPPS, uncharged CHOL and zwitterionic POPC are chosen to examine the role of the polar head charge on the interaction of $C_{16}BC$ with lipids. The penetrating ability of $C_{16}BC$ into lipid monolayers is investigated at four initial surface pressures (5, 10, 20 and 30 mN/m).

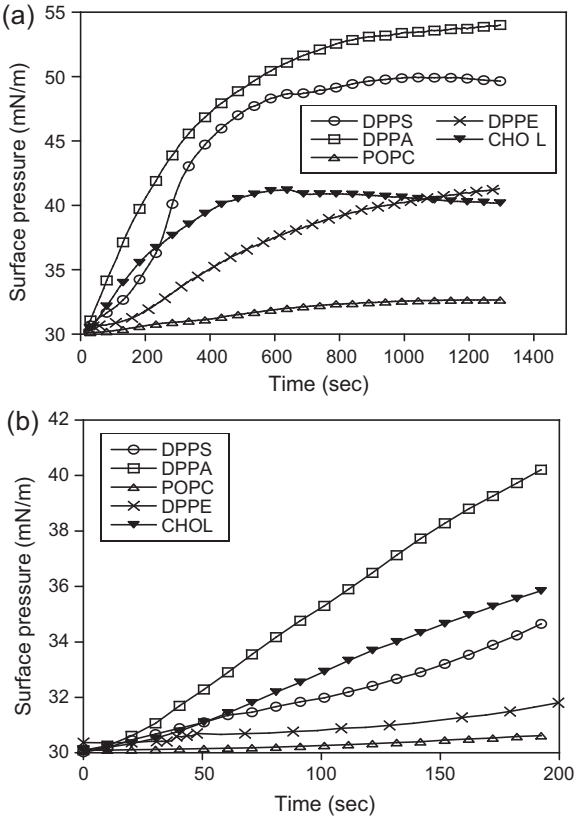


Figure 2. Kinetics (a) and enlargement of the early stage (b) of the hexadecylbetainate chloride ($C_{16}BC$) adsorption into different lipid monolayers initially compressed at 30 mN/m. The concentration of $C_{16}BC$ in the water subphase (pH 5.7) is 1.15 μM, and the subphase temperature is 25 °C. The time of injection correspond to $t = 0$.

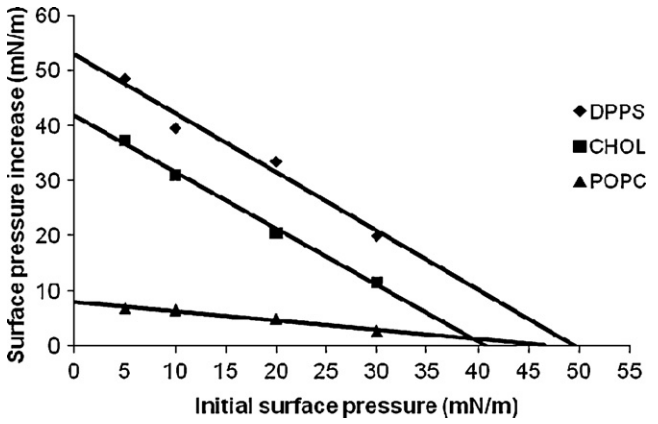


Fig. 3. Ability of hexadecylbetainate chloride (C_{16}BC) to penetrate into lipid monolayers with different polar heads. Maximal surface pressure increase as a function of the initial surface pressure (π_i) of the monolayer. The concentration of C_{16}BC in the water subphase (pH 5.7) is 1.15 μM, and the subphase temperature is 25 °C.

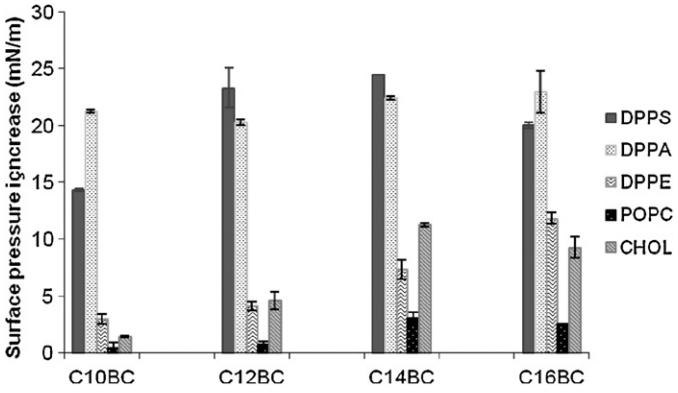


Fig. 4. Surface pressure increase ($\Delta\pi$) induced by the insertion of C_nBC (final concentration in the subphase of 1.15 μM) into lipid monolayers with different polar heads at initial surface pressure of 30 mN/m on water (pH 5.7) at 25 °C.

The limiting surface pressure or exclusion pressure [30,31] is determined by extrapolating the regression of the plot to the *x* axis (Fig. 3). The limiting surface pressure is defined as the maximum initial surface pressure above which the penetration of C_{16}BC into the monolayer no longer occurs. This parameter is a measure of the membrane-penetrating power of the surfactant [32].

The maximum surface pressure increase ($\Delta\pi$) is plotted as a function of the initial surface pressure (π_i). In all cases, $\Delta\pi$ decreases linearly with increasing π_i (Fig. 3). C_{16}BC penetrates much more readily into DPPS and CHOL monolayers than into POPC monolayer under similar surface pressures. The exclusion surface pressures obtained are 49.8, 40.7 and 47.1 mN/m for DPPS, CHOL and POPC monolayers, respectively.

Moreover, the ordinates at the origin for DPPS and CHOL linear regression are much higher than the surface pressure increase obtained after the C_{16}BC adsorption in the absence of lipid monolayer. DPPS and CHOL seem thus to exert an attractive effect on C_{16}BC adsorption while the penetration of C_{16}BC into POPC monolayer is only governed by C_{16}BC interfacial properties.

3.3. *Alkylbetainate chlorides (C_nBC) interaction with lipid monolayers: effect of the betainate alkyl-chain length*

Pure lipid monolayers of DPPS, DPPA, DPPE, POPC and CHOL at an initial surface pressure of 30 mN/m are formed to study the effect of the alkyl-chain length on the C_nBC adsorption and penetration process. The maximal surface pressure increase ($\Delta\pi$) for each system is shown at Fig. 4. The C_nBC insertion into negatively-charged DPPS and DPPA monolayers is almost not alkyl chain length-dependent except for C_{10}BC which is less inserted into DPPS monolayers. However, for zwitterionic DPPE and POPC and uncharged CHOL monolayers, the insertion of C_nBC tends to increase with the alkyl chain-length (Fig. 4).

4. Discussion

Alkylbetainate chloride surfactants (C_nBC, n = 10–16) are water-soluble surfactants in accordance with their HLB values (>22) according to the Davies system which ranks surfactants by their effective polarity and is not limited to non-ionic surfactants such as the Griffin index [33]. The interfacial behaviour of C_nBC (n = 10–16) monolayers at the air–water interface at 20 °C has been investigated in our previous work [19]. The results have shown that the C_nBC with a hydrocarbon chain length ≥14 are able to form stable monolayers at an air–water interface.

In the present work, the adsorption kinetics at an air–water interface and the ability of alkylbetainate chlorides (C_nBC) to penetrate into lipid monolayers with different polar heads are studied.

C_nBC adsorb at the air–water interface giving evidence of their surface-active properties. The increase of surface pressure after the first equilibrium suggests rearrangement of the surfactant monomers at the interface from an undefined orientation of the alkyl-chains at the water surface to an upright orientation of the molecules when additional molecules reach the surface. The maximal surface pressure induced by the adsorption increases with the alkyl-chain length of the surfactants. This is due to the increase of hydrophobic interactions between the alkyl-chains at the air–water interface allowing a higher interfacial concentration to be reached as commonly observed for other surfactants [34]. The rate of adsorption at the early stage increases as the alkyl chain-length increases from C_{10}BC to C_{14}BC and decreases for C_{16}BC. This suggests that the adsorption energetic barrier induced by the molecular film at the air–water interface is higher for C_{16}BC than for C_{12}BC and C_{14}BC. The longer the hydrophobic alkyl chain of non-adsorbed molecules, the higher the energy they need to pass the hydrophilic regions [34].

The exclusion pressure values (Fig. 3) higher than the estimated lateral pressure for biological membranes suggest that C_{16}BC is able to insert into lipid bilayer in vivo. However, insertion of C_{16}BC into a lipid monolayer is greatly dependent on the polar head and the structure of the lipid. According to the maximum surface pressure increase induced by C_{16}BC insertion observed in Fig. 2a and Fig. 3, the affinity of C_{16}BC is greater for negatively-charged lipids like DPPS and DPPA. It suggests the existence of electrostatic interactions between the positive charge of C_{16}BC and the negative charges of the lipids. Among the negatively-charged lipids, DPPA is slightly more favorable for the insertion of C_{16}BC. The existence of charge-charge repulsion between neighboring DPPA head groups [35,36] can increase the effective head group size making them more accessible for the insertion of cationic C_{16}BC as suggested by Gambinossi et al. for the tetracycline [37]. This can also explain the higher rate of penetration observed for DPPA (Fig. 2b).

The affinity of C_{16}BC is also higher for monolayers composed by lipids with a low steric hindrance head group like CHOL and DPPE in comparison to POPC monolayer (Figs. 2 and 3). A compensation of the head–tail size mismatch is likely to play a major role in determining the level of C_{16}BC insertion into CHOL monolayer. Indeed, the sterol rings of CHOL can fit nicely in the C_nBC alkyl-chain region and its hydroxyl group helps to anchor it at the C_nBC head–alkyl-chain interface. CHOL can be thought as an intercalator in the C_nBC monolayer, functioning as a spacer to compensate the head–tail size mismatch and increase the packing density of the

alkyl-chain region (Figs. 2–4) as it was suggested by Ishitsuka et al. [38] for POPC. The bulky methyl groups and the positive charge of POPC head group may prevent C_{16}BC molecules to insert into POPC monolayer to a greater extend.

The favored insertion of C_{16}BC into the zwitterionic DPPE monolayer in comparison with the zwitterionic POPC is unexpected since it is known that the packing of DPPE is higher than that of POPC due to the formation of hydrogen bonds between the DPPE neighbor molecules. DPPE molecules leading to a lower hydration degree and to a much lower area per lipid for PE [39,40]. In addition, POPC is in an expanded state even at 30 mN/m [32] and corresponds thus to an elastic monolayer more susceptible to be penetrated by an amphiphatic compound. The fact that DPPE has strong tendency to be organized in structures with a high intrinsic negative surface curvature when constrained within a monolayer, lowers the lateral pressure in the interface [41]. This can create insertion sites for amphipatic components [42]. On the other hand, the steric hindrance is less important for C_nBC molecules to insert into DPPE monolayer than into POPC monolayer with bulky methyl groups in the head groups as in C_{16}BC molecules. Bouchet et al. [43] have found the same result for the insertion of Arginine into DMPE and DMPC monolayers. The higher magnitude of $\Delta\pi$ with the increase of C_nBC alkyl chain-length in the case of neutral lipid monolayers indicates that hydrophobic interactions are also involved in the insertion of C_nBC into a lipid monolayer.

5. Conclusion

Alkylbetainate chlorides (C_nBC, $n = 10–16$) are able to adsorb at the air–water interface. Insertion of C_nBC into lipid monolayers is greatly dependent on the nature of the lipid. The negatively-charged monolayers are more favorable to the penetration of alkylbetainate chlorides than the uncharged and the zwitterionic monolayers. The penetration ability of C_{16}BC into DPPS, CHOL and POPC is above the threshold lateral pressure of native biological membranes. The penetration of C_nBC is almost alkyl-chain length-independent into the negatively-charged DPPS and DPPA monolayers for an alkyl-chain length ≥ 12. In the case of neutral monolayers, however, the value of $\Delta\pi$ increases as the surfactant alkyl-chain length increases. The surface pressure change is strongly affected by the monolayer forming lipid type and is in the order of DPPA > DPPS $\gg$ CHOL $\approx$ DPPE > POPC. These results indicate that as well as the net charge of the monolayer surface, the size of the head group is another important factor that must be considered in the penetration process of C_nBC. Electrostatic interactions are mainly involved in the affinity of C_nBC with monolayers but the hydrophobic effect play also a role when electrostatic interactions cannot occur.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.colsurfb.2011.03.039.

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