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Interactions of ibuprofen with Langmuir monolayers of membrane lipids

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Abstract

DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) Langmuir monolayers were employed as model membranes for the studies of interactions with an amphiphilic drug — ibuprofen. The mode of interactions was found to be ibuprofen concentration dependent. The maximal value of compressibility constant, 270 mN/m is obtained for ibuprofen 10^{-5}–10^{-6} M solutions indicating that already in contact with such diluted drug solutions, the DPPC monolayer becomes more solid than in contact with pure water or saliva solutions, thus the organization of molecules is more compact or condensed. In contrary, the effect of concentrated ibuprofen solutions is opposite. Moreover, in the "diluted ibuprofen range" the molecules do not partition into the lipid layer since the limiting area for the lipid did not increase but in contrary was found to decrease upon contact with such solutions, and a disappearance of phase transition was observed. Contact with diluted solution of ibuprofen does not influence collapse pressure while making the condensed monolayer more solid.

In diluted solutions of *S*-ibuprofen (10^{-6}–10^{-5} M) the value of dipole moment is larger than for pure water or saliva subphases reflecting the organizing effect of ibuprofen. In concentrated ibuprofen solutions the dipole moment decreases which indicates partial compensation of charges upon incorporation of ibuprofen into the hydrophobic region of the lipid layer. Under these conditions, ibuprofen interferes with the cooperative interactions of the DPPC molecules in the layer and perturbs the structure of the monolayer.

Keywords: Langmuir monolayer; Ibuprofen; Lipid; Air–water interface

1. Introduction

Interactions of amphiphilic drugs with cell components involve changing the organization of the biological membrane. Binding to proteins is seen as the usual way of exerting their function [1] but the role of interactions with membrane lipids has been also repeatedly stressed [2–4]. Ibuprofen is a non-steroidal anti-inflammatory drug colorless, amphiphilic and relatively insoluble in water. Solubility increases from 0.078 g/dm^3 pH 4 to 291 g/dm^3 at pH 8, and pK_a value is 4.55 at room temperature [5] (Fig. 1).

Higher activity of the drug is exhibited by the *S*-configuration at the chiral center [6]. The *S*-enantiomer has the desired therapeutic effect while *R*-ibuprofen is inactive. Ibuprofen has been shown to partition into phosphatidylcholine liposomes [7] affect lipid melting temperature and change the enthalpy of the gel to liquid–crystalline phase transition in liposomes [4]. Ibuprofen was reported to interact in a concentration independent manner with monolayers of saturated phosphatidylcholines [4].

Lipid monolayers although much less complex than biological membranes have been shown to be excellent model interfaces to study the interactions with solution species [8–10]. Monolayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC are well characterized [11–16], and often used as models of outer membrane cell leaflet (Fig. 1). In the present study we describe the effect of ibuprofen (*S*-isomer and racemate) upon the organization of lipid membranes using as model system Langmuir monolayers of DPPC spread at the water–air and saliva-like Ringer solution–air interfaces. The monolayers are characterized by surface pressure (π) and electric surface potential (ΔV) measurements [17,18].

2. Experimental details

DPPC was purchased from Avanti Polar Lipids Inc., Ibuprofen racemate and *S*-ibuprofen were purchased from Sigma, chloroform from Aldrich. NaCl, KCl, and $CaCl_2$ were from

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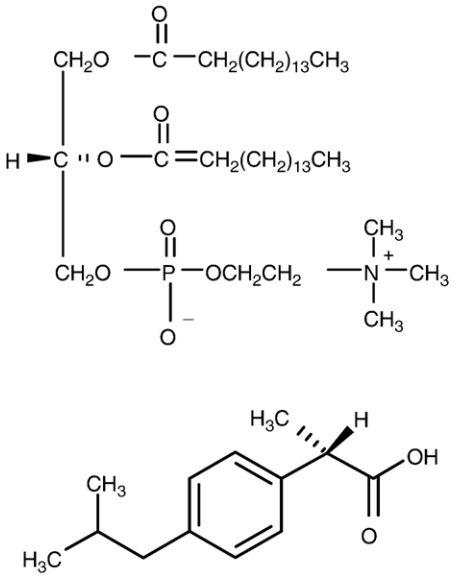

Fig. 1. Structures of ibuprofen and DPPC.

POCh Gliwice. Distilled water was passed through a Milli-Q® water purification system, (resistivity 18.2 MΩ̃. The subphase was either water or saliva-like Ringer solution: 0.15 M NaCl, 5.6 mM KCl and 0.7 mM $CaCl_2$. Temperature was constant 22±10 °C.

Surface pressure vs. molecular area isotherms were recorded using the KSV LB trough 5000 equipped with hydrophobic barrier. The experiment was controlled with software version KSV 5000. A Wilhelmy balance was used as a surface pressure sensor. Surface potential and surface pressure were recorded simultaneously as a function of molecular area. The accuracy of: measurements of area per molecule was 0.004 nm^2, of surface pressure was 1 mN m^{-1} and of surface potential was 10 mV.

The spreading solutions were prepared by dissolving DPPC samples in chloroform with a typical concentration 1±0.1 mg/ml. 40–50 μl of these solutions were spread on the air–water interface. After spreading the solution is left for 15 min for solvent evaporation. Compression is done with a barrier speed of 7.5 cm^2/min.

3. Results and discussion

Small additions of *S*-ibuprofen to both subphases lead to slight decrease of area per molecule in the monolayer, A_0. Addition of ibuprofen directly to the lipid sample in chloroform lead to the same observation (Fig. 2) This means that in the dilute ibuprofen concentration region it does not partition into the layer but interacts with it improving the organization of the molecules in the layer.

At *S*-ibuprofen concentrations in the subphase, C_{IBU} higher than 10^{-5} M the drug partitions to the layer as seen by the increase of surface pressure already at the initial trough area (Fig. 3A). The same behavior is noted for the saliva-like subphase (Fig. 3B). This indicates that ibuprofen interacts with the molecules in the layer. In the absence of lipids, ibuprofen does not form a Langmuir monolayer itself, however, increase of surface pressure to 1 mN/m can be detected on a subphase containing *S*-ibuprofen.

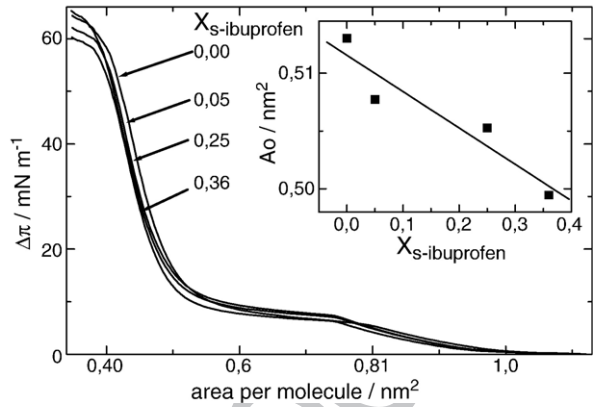

Fig. 2. Langmuir isotherms for DPPC and in ibuprofen–DPPC mixtures on water subphase. Mole fraction of ibuprofen in the mixture, $X_{S\text{-ibuprofen}}$. Inset A_0 for DPPC in ibuprofen–DPPC mixtures.

Above concentration 1.5×10^{-5} M ibuprofen in the subphase, the phase transition between liquid expanded, and liquid condensed phases disappears and surface pressure increases

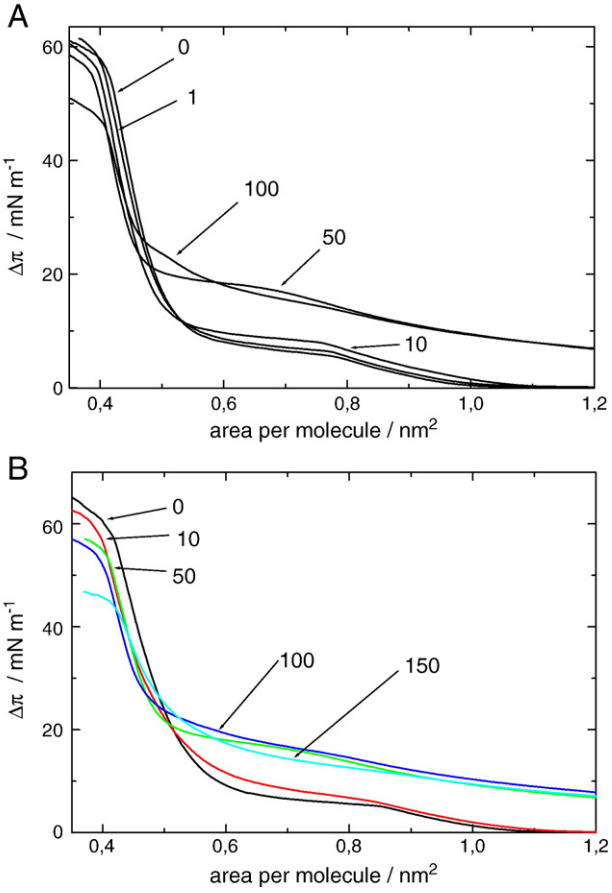

Fig. 3. DPPC isotherms on water (A) and Ringer solution (B) subphases in the presence of small concentration of *S*-ibuprofen (numbers denote concentration in μM).

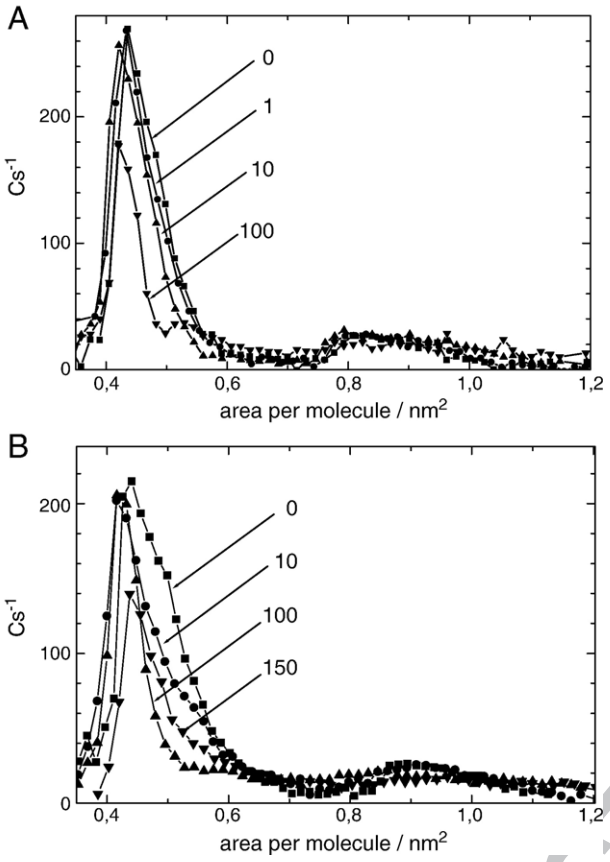

Fig. 4. Changes of compressibility modulus C_s^{-1} along the isotherm for increasing concentration of ibuprofen in water (A), and saliva (B) subphase, C_{IBU} — numbers denote concentration in μM.

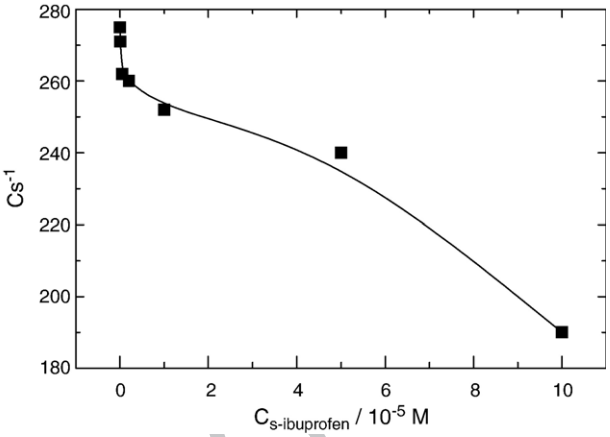

Fig. 5. The dependence of compressibility modulus C_s^{-1} on concentration of ibuprofen in water subphase.

smoothly starting from the highest area per molecule (Fig. 3). Collapse pressure first slowly decreases under influence of highly diluted *S*-ibuprofen solutions while at higher concentrations than 10^{-5} M, a sharp decrease in collapse pressure with increasing concentration is observed until the solubility limit of ibuprofen is attained. The solubility of ibuprofen in saliva-like solution, pH 6 is 0.01 M. Thus, collapse pressure dependence may be used to determine *S*-ibuprofen concentration in the range 2×10^{-5}–2×10^{-4} M. Fig. 4 presents the changes of the compression modulus, C_s^{-1} with changing area per molecule for selected concentrations of ibuprofen in water and Ringer (saliva-like) solution.

The compression modulus, (reciprocal of compressibility), is defined as [19–24]:

$$C_s^{-1} = -A(d\pi/dA),$$

where A is area per molecule, and π is surface pressure. C_s^{-1} equal to 50–100 mN/m denotes a liquid expanded film. The maximal value of compressibility modulus is high, 270 mN/m and is obtained for *S*-ibuprofen 10^{-5}–10^{-6} M solutions indicating that in contact with diluted ibuprofen solutions the monolayer is more solid than on saliva itself (Table 1, Fig. 5).

The monolayer of *S*-isomer is rather stabilized or organized by diluted ibuprofen solutions as opposed to the effect of more concentrated solutions called further on "concentrated range". Moreover, in the "diluted ibuprofen range" ibuprofen molecules do not partition to the lipid layer (since at the dilute ibuprofen range the limiting area for the lipid did not increase but contrary was found to decrease), delay the appearance of phase transition and do not influence collapse pressure while making the condensed monolayer more solid (Fig. 6).

Decompression of the monolayer after compression in the presence of 0, 10^{-6} and 10^{-4} M ibuprofen does not give identical results. At high concentrations of *S*-ibuprofen upon multiple compression–decompression cycles the isotherm is shifted towards smaller areas.

Fig. 7 shows the increasing surface potential at already large areas per molecule reflecting the "organizing" role of diluted

t1.1 Table 1
t1.2 Characteristic parameters of the Langmuir DPPC monolayers on diluted ibuprofen subphases

t1.3 Subphase	C_s^{-1} max mN/m	A_0 nm^2 /molec	A_{coll} [a] nm^2/molec	π_{coll}, mN/m	μ D
t1.4 Water	269±20 [b]	0.516±0.005	0.415±0.004	57±1	0.65±0.2
t1.5 Saliva	218±20	0.545±0.005	0.415±0.004	57±1	0.78±0.2
t1.6 Water+10^{-6} M *S*-ibuprofen	268±20	0.499±0.005	0.398±0.004	57±1	0.7±0.2
t1.7 Water+10^{-6} M racemate–ibuprofen (diluted solution effect)	238±20	0.503±0.005	0.393±0.004	58±1	0.9±0.2
t1.8 Water+3×10^{-5} M *S*-ibuprofen	252±20	0.499±0.005	0.400±0.004	56±1	1.5±0.2
t1.9 Water+3×10^{-5} M ibuprofen racemate	240±20	0.500±0.005	0.395±0.004	57±1	0.9±0.2

t1.10 [a] area per molecule corresponding to collapse pressure.
t1.11 [b] average values of 5 to 8 experiments±standard deviation.

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concentrations of ibuprofen. This confirms interactions of as low as 10^{-6} M *S*-ibuprofen with the lipid molecules already at the areas per molecule corresponding to highly diluted layer. A semi-quantitative analysis of the surface potential isotherm was made using the Helmholtz equation [19,24]:

$$\mu = \varepsilon\varepsilon_0 \Delta V A,$$

where μ is the vertical component of the dipole moment, $\varepsilon_0 = 8.8542 \times 10^{-12}$ Fm, A is the area per molecule and ε and ε_0 are the permittivities of the monolayer and of vacuum, respectively. The maximal apparent dipole moment $\mu_A = \mu/\varepsilon$ is calculated and depicted in Table 1. Highest value of dipole moment is obtained for 3×10^{-5} M *S*-ibuprofen. At higher concentration it decreases again showing partial compensation of charges upon incorporation of *S*-ibuprofen into the hydrophobic region of the lipid layer. Under these conditions ibuprofen interferes with the cooperative interactions of the DPPC molecules in the layer.

If racemate is used instead of *S*-ibuprofen the "dilute range effect" is not so clearly observed. The characteristic parameters for the monolayers on the subphases studied are compared in Table 1. Same behaviour of ibuprofen is seen in case of lipid–cholesterol Langmuir monolayers as shown in Fig 7. Collapse pressure value may be used to follow the concentration of the drug since it decreases proportionally upon adding ibuprofen into the solution.

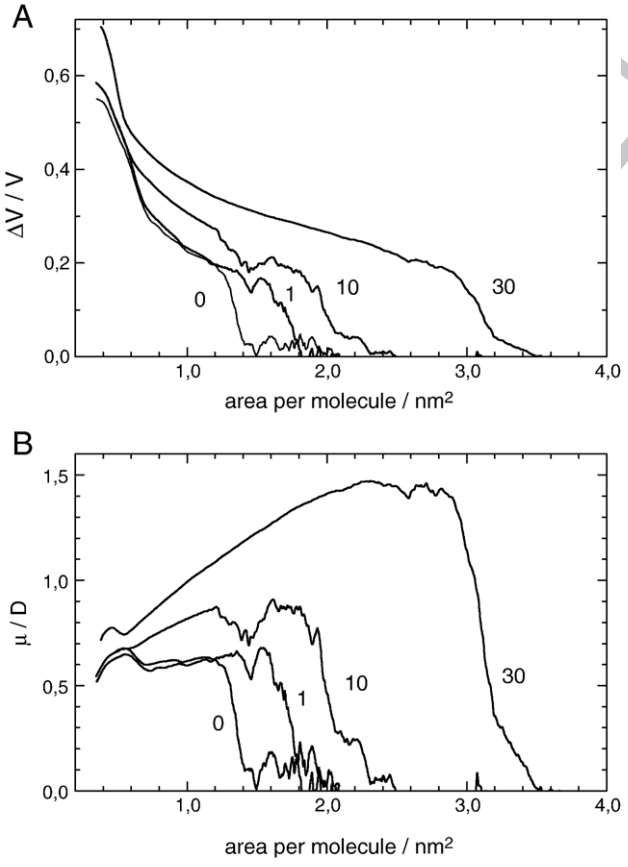

Fig. 6. Surface potential (A) and apparent dipole moment (B) vs. area per molecule isotherms for DPPC on diluted *S*-ibuprofen subphases (numbers denote concentration in μM).

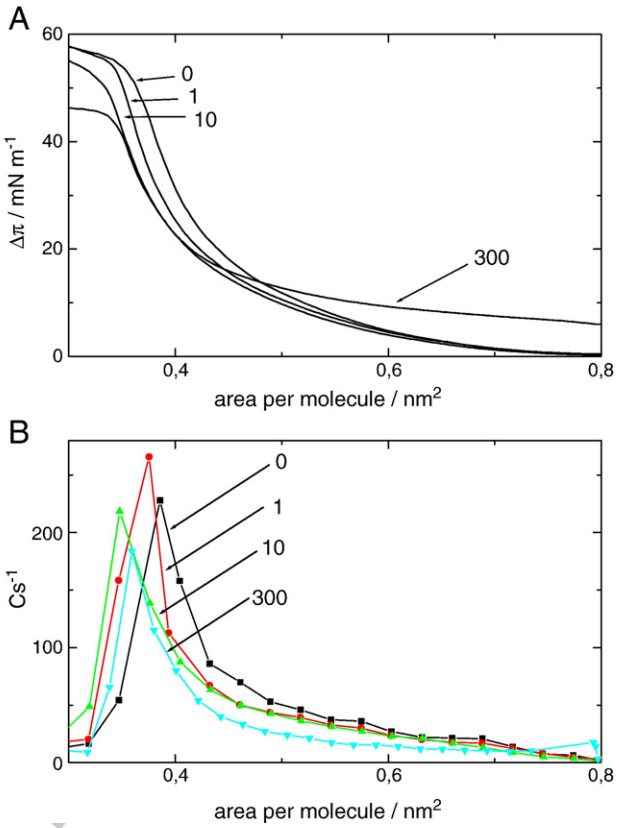

Fig. 7. DPPC–cholesterol isotherms (A) apparent compressibility modulus C_s^{-1} (B) on Ringer solution subphases in the presence of ibuprofen racemate; C_{IBU}: (numbers denote concentration in μM).

4. Conclusion

Studies of liposomes by Fatouros and Antimisiaris [25] have demonstrated the ability of membrane incorporated amphiphilic drugs to enhance membrane integrity and stabilise liposomes. Ibuprofen incorporation improves the stability of DPPC: cholesterol liposomes [7], which is explained as due to interdigitating between the lipid chains of the bilayer and increasing rigidity of the bilayer.

The studies performed here show that *S*-ibuprofen at concentrations lower than 10^{-5} M exerts similar influence on the lipid monolayer to that of anesthetics which influence ordering of membranes by accumulation at the interfacial region of domains of gel and fluid lipid [26]. Above 3×10^{-5} M the analysis of isotherm indicates that the structure of the DPPC monolayer changes in contact with the drug since its molecules partition into the monolayer and affect the typical phase transitions observed for the lipid. The tight packing of the monolayer is perturbed and fluidizing influence is observed. When ibuprofen is present in the subphase the organization of molecules into a liquid monolayer starts already at larger areas per molecule. This is evidenced by the earlier onset of the increase of surface potential and pressure. The layer becomes

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also more liquid (see compression modulus) than in the absence of the drug. Electrochemical results reported recently confirm are in agreement with these observations [27]. Similar effect has been shown recently for lipopeptide drugs — fengycin [10] and surfactin [28]. The ibuprofen concentration dependent interaction with lipid component of membranes should be of importance when the membrane functions in the presence of various concentration of this drug are considered.

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