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Biochimica et Biophysica Acta 1193 (1994) 287–292

BBA Biochimica et Biophysica Acta

Lasalocid and biomimetic membranes: insertion in Langmuir films of lipids

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Received 28 October 1993; revised 11 April 1994

Abstract

The interfacial properties at the water surface of dipalmitoylphosphatidylcholine (DPPC) in monomolecular films containing various concentrations of lasalocid sodium salt (LAS) have been studied in the range from $r = 0.005$ to 0.1 (r = molar ratio LAS : DPPC). The data from which the results have been expressed were obtained from the following compression isotherms at constant temperature (22°C): of mixed films, of pure DPPC and of pure LAS. The incorporation of LAS resulted in pressure and concentration dependent molecular area increase, between 0 and 16 $mN\,m^{-1}$. The observed effect has been expressed as the area S_A^* occupied by each antibiotic molecule in the mixed films. The variations of S_A^* have been discussed at two levels of constant surface pressure, at 8 $mN\,m^{-1}$ which is situated in the phase transition region of the DPPC isotherm and at 4 $mN\,m^{-1}$ in the liquid expanded state. In both cases, the S_A^* values decreased as r increased. They have been related to the molar areas S_A of LAS, obtained from the compression isotherm of the pure antibiotic and expressed as S_A^*/S_A. This ratio was considerably greater than unity in the phase transition region within the studied concentration range and close to unity in the liquid expanded state. Using the method of Goodrich, the excess free energy of mixing G_{XS} has been calculated. The values were positive above $r = 0.01$ and they increased with increasing concentrations. The results indicate non miscibility and strong repulsion between the two kinds of molecules in the mixed films. Two different mecanisms of insertion have been suggested.

Key words: Langmuir film; Lipid/water interface; Cation carrier

1. Introduction

The antibiotic LAS belongs to a group of ionophores isolated from *Streptomyces*. These molecules act as cation carriers in cell membranes and lipid bilayers [1,2]. The principal application is the treatment of infections in veterinary medicine. Only recently it has been shown that LAS might be efficient against multi drug resistance (MDR) in cancer therapy [3].

The ionophore activity of such antibiotics depends on a liposoluble cation complex formation. The complexation takes place at the polar inside of the molecule, closed as a ring through hydrogen bonding between the end groups [1]. The complex is protonated in acidic solution and unprotonated when the environment is basic [4]. These two conformations are also likely formed in monolayers [5].

The driving force of the transport mechanism for this kind of ionophores is the concentration gradient between the two sides of the bilayer [6]. The ion exchange takes place at the membrane interface which is thus the site for the capture and release of the cation [7]. For this reason we have wanted to examine the interaction LAS–DPPC in monolayers which represent a model for one half of the bilayer. The compression isotherms of such films reveal interfacial phenomena.

In a former study we have observed molecular area increase in Langmuir films when LAS was added to DPPC for antibiotic concentrations close to $r = 0.01$ [8].

The purpose of the present investigation has been to examine the concentration dependence of the expansion effect on the lipid monolayer.

2. Materials and methods

The antibiotic LAS as sodium salt was purchased from Sigma Chimie and recrystallized from aqueous methanol and from a cyclohexane-acetone mixture,

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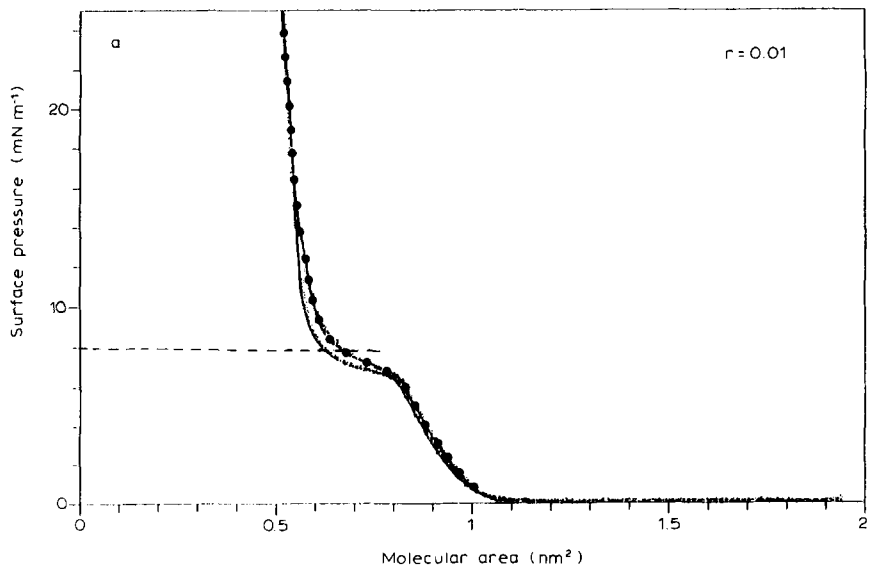
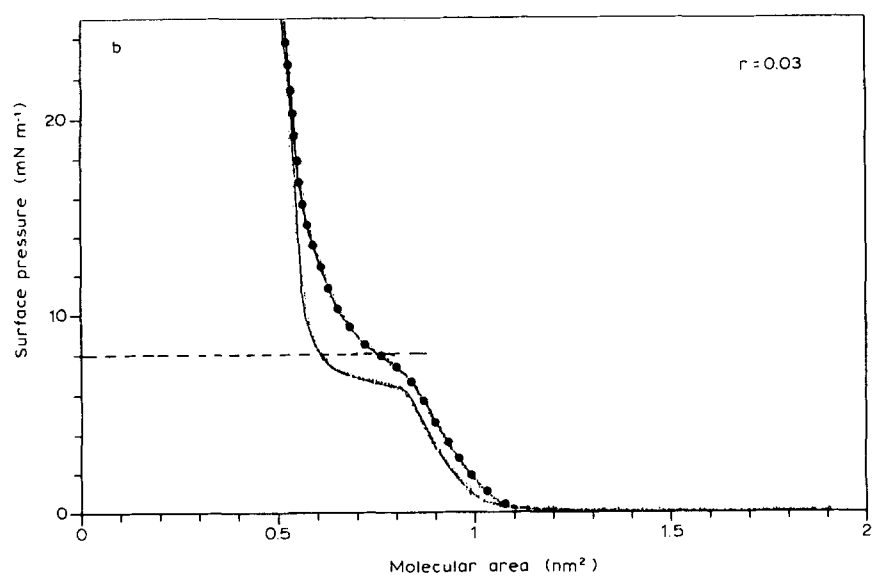
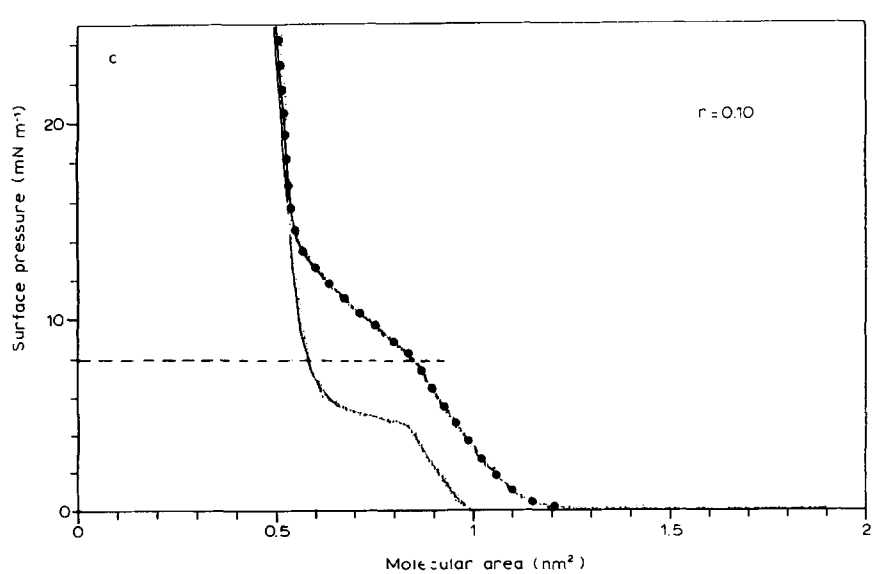
SSDI 0005-2736(94)00111-2


a
r = 0.01
Surface pressure (mN m⁻¹)
Molecular area (nm²)


b
r = 0.03
Surface pressure (mN m⁻¹)
Molecular area (nm²)


c
r = 0.10
Surface pressure (mN m⁻¹)
Molecular area (nm²)

m.p. 172°C (decomposition). The phospholipid DPPC was of the purest available quality from Sigma Chimie.

Stock solutions of the two compounds, $5 \cdot 10^{-4}$ M, were prepared in chloroform/hexane (2:3, v/v) and spread mixed or separately in the wanted molar ratios. The solvents were of analytical grade from Prolabo. They were distilled on molecular sieve for moisture removal.

The monolayers were spread on to a subphase of pure water treated on an Elgastat UHQ 2 system (resistivity 18 mΩ) from Labo Standa. All the experiments were done at pH 5.7 and at a temperature of 22°C.

The isotherms were measured with a Krüss film balance using a pendulum 100 and they were recorded with an IBM-AT computer.

The films were spread with a Hamilton CR 200 syringe (50 μl corresponding to 1 nm^2 per molecule at 150 cm^2). The compressions were performed at a speed of 0,15 nm^2 min^{-1}.

3. Results

The contact between LAS and DPPC can be achieved in different ways.

(I) The film is spread from a solution containing the two compounds.

(II) After spreading a film of pure DPPC, one drop of LAS stock solution is added to the film.

(III) After spreading a film of pure DPPC, a drop of LAS stock solution is injected into the subphase at the bottom of the trough.

We have plotted the compression isotherms surface pressure– molecular area of the mixtures at different values of r from films prepared as mentioned above.

Spreading procedure (I)

Compression isotherms of mixed films from LAS and DPPC together with the isotherm of pure DPPC have been plotted under identical conditions for the following concentrations: $r = 0.005$, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.1. Examples are shown in Fig. 1(a), (b), and (c) for $r = 0.01$, 0.03 and 0.1, respectively. As seen from these curves, the presence of LAS results in concentration dependent modifications of the physical state in the monolayer.

The compression isotherm for the mixed film of LAS-DPPC is situated above the isotherm of DPPC for all the studied concentrations which means that for a given pressure, the molecular areas become larger. This area increase is pressure dependent. A consequence is, that the first order phase transition between liquid expanded LE and liquid condensed LC states, discussed in detail by Pallas and Pethica [9], takes place at surface pressures, higher for the mixed films than for the pure lipid, but the starting point of the phase transition tends to move to lower molecular areas as the LAS concentration increases. For these reasons, the films of pure DPPC and those of DPPC containing LAS are not always in the same physical state at the same surface pressure. This difference becomes significant for the highest r values of our experiments.

We have chosen to express the observed effect quantitatively as the contribution of each antibiotic molecule S_A^* to the area in the mixed film. The films of the mixture have been compared with the film of pure lipid at a given pressure where the films undergo the same physical strain. We have, nevertheless, selected surface pressures where the two films are in the same physical state. The area S_A^* occupied by the antibiotic molecule in the film can be expressed as

$$S_A^* = S_{LA}\left(1 + \frac{N_L}{N_A}\right) - \frac{S_L N_L}{N_A} = S_{LA}\left(1 + \frac{1}{r}\right) - \frac{S_L}{r}$$

where N_L and N_A are the numbers of moles of lipid and antibiotic, respectively in the spreading mixture, S_L and S_{LA} the molecular areas of lipid and lipid-antibiotic mixture (mean value), respectively at a fixed value of the surface pressure, and $r = N_A/N_L$.

We have, on one hand, expressed the values of S_A^* at 8 mN m^{-1} since the molecular area expansion is the most important at surface pressures around this value. The variations of S_A^* with the LAS concentration are plotted in Fig. 2 from $r = 0.005$ to $r = 0.05$, concentrations at which the two films are in the transition state. From $r = 0.06$ and above, the mixed films have not yet reached the transition state at this surface pressure.

In Fig. 2, linear decrease of S_A^* is seen as the concentation increases (calculated correlation coefficient 0.99). The values of S_A^* have been related to the molecular area S_A of pure LAS at 8 mN m^{-1}, obtained from compression isotherms in a previous study [5] and inserted in Fig. 2. The value of S_A at 8 mN m^{-1} has been estimated to 0.9 nm^2. The relationship has been expressed as the ratio S_A^*/S_A shown in Table 1. As seen from the table, the ratio diminishes when r increases, but values greater than unity remain all through the studied concentration range.

Fig. 1. Compression isotherms of DPPC, continuous line, and of mixed films of DPPC + LAS, line with full circles, at different molar ratios: (a), (b), (c). Subphase: pure water, temperature: 22°C, pH 5.7. Dotted horizontal lines: indicate the surface pressure at which the S_L and S_{LA} values have been determined.

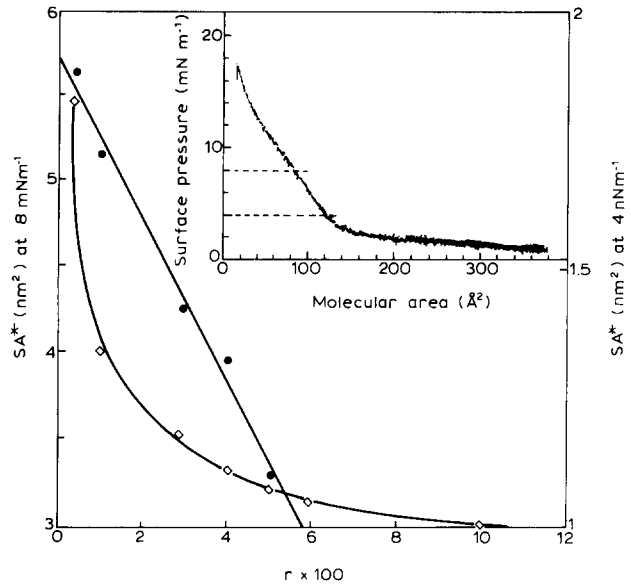


Fig. 2. Variation of the molecular area occupied by LAS (S_A^*) in the film of LAS + DPPC with varying molar ratios (r); ● at 8 mN m^{-1}, ◇ at 4 mN m^{-1}. Subphase: pure water, temperature: 22°C, pH 5.7. Inset: compression isotherm of LAS. Subphase: same conditions. Dotted horizontal lines: indicate the surface pressures at which the S_A values have been determined.

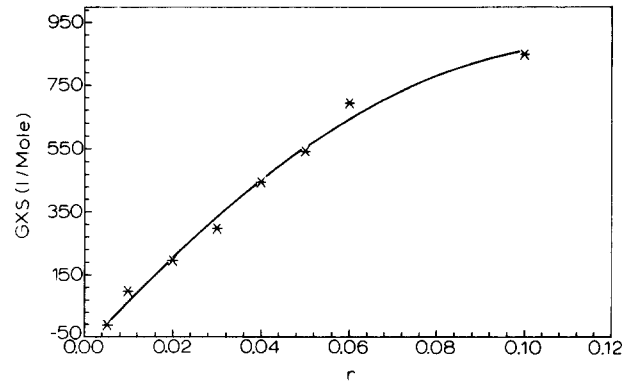


Fig. 3. Variation of the free energy of mixing (G_{XS}) in the films of LAS + DPPC with varying molar ratios (r). Subphase: pure water, temperature: 22°C, pH 5.7.

On the other hand, we have expressed S_A^* at 4 mN m^{-1} in the concentration range from $r = 0.005$ to 0.1. At this surface pressure, the films are all in the LE state. The experimental values are shown in Fig. 2 from which a tendency is seen of regular decrease of S_A^* as the antibiotic concentration increases. The S_A value, estimated to 1.2 nm^2 from the LAS compression isotherm in Fig. 2, results in S_A^*/S_A ratios close to unity as seen in Table 1.

Another way of expressing the data from the compression isotherms is to use the method proposed by Goodrich [10] for the calculation of the excess free energy of mixing G_{XS} which is given by the relation:

$$G_{XS} = N\left[\int_{\pi_0}^{\pi_1} S_{LA}\, d\pi - \frac{N_L}{N_L + N_A}\int_{\pi_0}^{\pi_1} S_L\, d\pi - \frac{N_A}{N_L + N_A}\int_{\pi_0}^{\pi_1} S_A\, d\pi\right]$$

where N is the Avogadro number.

In this study, the integrals have been evaluated graphically within the pressure interval from $\pi_0 = 0$ at 1.5 nm^2 to $\pi_1 = 16$ mN m^{-1} representing the interval where the difference between the compression isotherms of pure DPPC and of DPPC + LAS is significant.

The graph plotted in Fig. 3 gives the variations of G_{XS} as a function of r.

The figure shows that G_{XS} is nearly always positive, except for the lowest r values where the area differences measured from the isotherms are small and thus subject to greater experimental error.

When we operated as indicated in (II) at a concentration of $r = 0.05$, we have only been able to observe a slight difference between the compression isotherm of pure DPPC and of the mixed film.

Under the experimental conditions (III), at $r = 0.05$, we could observe no modification of the DPPC isotherm after injecting the LAS solution.

4. Discussion

The values $S_A^*/S_A > 1$ for the experimental conditions (I) in the transition region at 8 mN m^{-1}, must signify that repulsive forces are acting between molecules of DPPC and LAS. The existence of repulsive forces is furthermore evidenced from the positive values of G_{XS} and also from the results of the experiments (II) and (III).

These results concur with poor miscibility between the LAS and DPPC molecules.

The increasing S_A^*/S_A ratio with diminishing r values as shown from experimental procedure (I), could

Table 1
The values S_A^*/S_A; S_A^*: area occupied by the LAS molecule in the mixed film LAS + DPPC; S_A: molecular area of LAS in the monolayer of the pure compound) for various values of r (r: mol LAS/mol DPPC) on an aqueous subphase, temperature 22°C, pH 5.7

$r = N_A/N_L$	0.005	0.01	0.03	0.04	0.05	0.06	0.1
S_A^*/S_A at 8 mN m^{-1}	6.2	5.7	4.7	4.4	3.7		
S_A^*/S_A at 4 mN m^{-1}	1.5	1.1	1.0	0.9	0.9	0.9	0.8

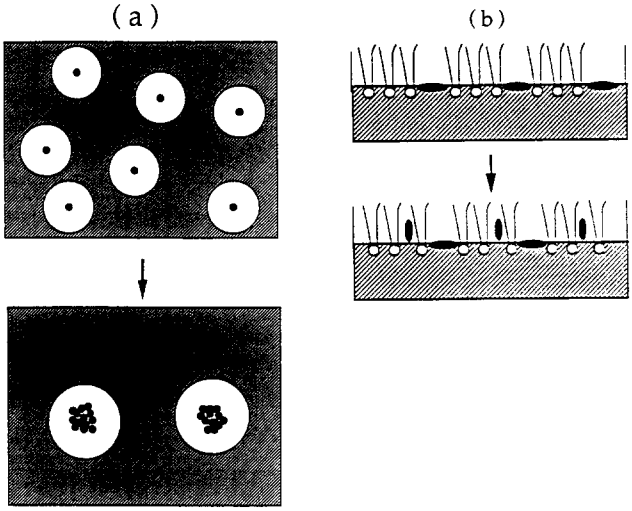


Fig. 4. (a) Illustration of one possible mechanism of LAS insertion in the monolayer of DPPC. At low molecular ratios: repulsion effects from isolated LAS molecules. At higher concentrations: repulsion effects from aggregates. (b) Illustration of another possible mechanism of the LAS effect on the lipid monolayer. At low concentrations: positioning of the macrocyclic ring complex flat at the interfeace until saturation. At higher concentrations: the LAS molecules start to enter into the lipid bilayer and they reorientate.

be explained through the fact that single LAS molecules are dispersed in the lipid layer at low concentrations and that they tend to aggregate as the concentration is increased. These situations are shown in Fig. 4 (a).

The values of S_A^*/S_A close to unity at 4 $mN\,m^{-1}$, might signify that the molecules are too far apart to interact significantly at this surface pressure. The decreasing tendency could, however, be due to aggregation of the antibiotic at the interface.

Although the LE state might represent the fluid state of the membrane, our experiments show that the LAS molecules have the most significant effect on the lipid membrane in the phase transition region of the film.

The literature contains information on monolayer packing conditions representing the bilayer packing in fully hydrated smectic mesophases of phosphatidylcholines where equivalent surface pressures of 20 to 25 $mN\,m^{-1}$ are reported [11,12] and for other biological lipid systems even higher values are given [13,14]. Our experiments show that at about 16 $mN\,m^{-1}$, the compression isotherm of the mixed film superimposes the lipid isotherm and this might indicate that the antibiotic molecules are then expelled from the monolayer. Below this surface pressure, the molecules contribute to the formation of a film more rigid than the film of the pure phospholipid.

The result of procedure (II) might signify that there is interaction between the monolayer of DPPC and one large aggregate of LAS.

It seems likely that procedure (III) leads to precipitation of LAS in the water subphase, since the compound is nearly insoluble in water, and that none of these molecules are getting into contact with the lipid film.

Another interpretation of the variations in the S_A^*/S_A ratio with the LAS concentration, can be suggested. The highest observed values of S_A^* in the low concentration region might be consistant with the fact that the molecular ring of LAS occupies a flat position at the interface of the mixed film in the way which they also likely do in monolayers of pure LAS at high molecular areas [5,15]. The molecules might penetrate into the region of the lipid chains and reorientate after a stage of saturation at the interface as indicated in Fig. 4 (b). Results from microcalorimetric measurments recently performed and not yet published, seem to indicate that the LAS molecules might affect the lipid bilayer interior only above concentrations $r = 0.04$. This might be due to implantation of the antibiotic molecules at the interface followed by insertion into the hydrophobic region.

The main result of our investigation is the observation of concentration dependent repulsive effects between LAS and DPPC molecules at the interface. In bilayers and membranes, the LAS molecule achieves the ion transport acting as a shuttle which moves rapidly from one side of the bilayer to the other. We suggest that the repulsive forces might influence the transport phenomena in opening up the membrane and thus favor the passage of the cation complex.

Acknowledgment

This work was supported by DRET (DGA).

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