Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Monolayers as Membrane Models. A Study of Antibiotic Action on Lipids

A. Hochapfel [a], H. Hasmonay [a], M. Jaffrain [a] & P. Peretti [a]
[a] Laboratoire de Physique et Biophysique des Milieux Mal Ordonnés, Université Paris V, 45 Rue des Saints-Pères, 75270, Paris Cedex 06, France

Version of record first published: 24 Sep 2006.

To cite this article: A. Hochapfel , H. Hasmonay , M. Jaffrain & P. Peretti (1992): Monolayers as Membrane Models. A Study of Antibiotic Action on Lipids, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 215:1, 221-228

To link to this article: http://dx.doi.org/10.1080/10587259208038528

Mol. Cryst. Liq. Cryst. 1992, Vol. 215, pp. 221–228
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in the United States of America

MONOLAYERS AS MEMBRANE MODELS. A STUDY OF ANTIBIOTIC ACTION ON LIPIDS.

A. HOCHAPFEL, H. HASMONAY, M. JAFFRAIN, P. PERETTI
Laboratoire de Physique et Biophysique des Milieux Mal Ordonnés, Université Paris V, 45 Rue des Saints-Pères, 75270 Paris Cedex 06, France.

(*Received March 11, 1991*)

Abstract
Langmuir films of the lipids dipalmitoylphosphatidylcholine, docosanoic acid and octadecanol, in presence of two antibiotic ionophores, monensin sodium salt and lasalocid sodium salt have been studied. An important area increase as a consequence of antibiotic addition has been observed in the π-A isotherms of the phospholipid. The effect has been considered as a result of ionic cavity formation accompanied by rearrangements in the lipid hydrocarbon chains. A smaller area increase of monolayers from the single chain lipids has been observed only when monensin sodium salt was added. Monolayer studies of monensin sodium salt allowed molecular area estimations of the pure antibiotic in the film.

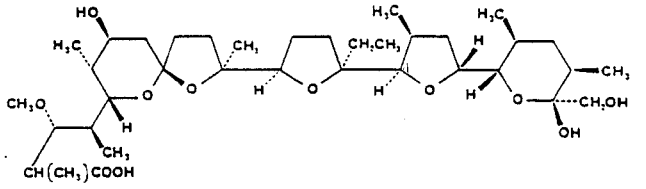
Keywords: monolayer, dipalmitoylphosphatidylcholine, monensin, docosanoic acid, lasalacid, octadecanol

INTRODUCTION

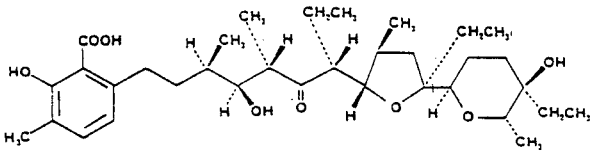
Monensin (MON) and Lasalocid (LAS) are two antibiotic ionophores isolated from *streptomyces* . Their principal utilization in therapy is at present as anticoccidal agent in poultry and also as growth stimulants in cattle feeding.

Due to their properties as ionophores, these compounds have become especially interesting in biochemistry for the investigation of membrane transport and trans membrane cation gradients [1, 2, 3, 4, 5].

The two compounds, monocarboxylic molecules with a long chain of cyclic ethers, belong to the Nigerecin group of antibiotics :



MONENSIN



LASALOCID

MON and LAS have been studied in bilayer systems such as BLM and vesicles from phospholipids and composite lipids where they are reported to be situated in the membrane interior in a cyclic configuration leading to the formation of ionic cavities. The ether and polar groups are directed towards the cavity inside where they complex with ions which are allowed to pass across the lipid barrier. The non polar side of the molecules is directed towards the hydrocarbon chains of the bilayer [6].

The antibiotic activity of these compounds is supposed to originate from the ion carrier properties [3] and it is interesting to compare the two molecules MON and LAS because they have individual ion selectivity [2].

The membrane is reported to swell as a consequence of ionic cavity formation. The purpose of our study has been to investigate this effect on Langmuir films of lipids containing the antibiotics, spread on an aqueous subphase. Such monolayers represent one half of the biomembrane and the isotherms provide quantitative information on molecular areas and their changes at the interface and also on phase transitions in the system.

Earlier studies on antibiotic penetration into lipid monolayers show considerable pressure i.e. area increase under certain conditions [7, 8]. The drugs were in such case polyenic ionophores [9].

Our study is concerned with both MON and LAS as Na-salts (hereafter referred to as MON-Na and LAS-Na) and their effect on monolayers of different lipids; one phospholipid, dipalmitoylphosphatidylcholine (DPPC) and two single chain lipids, docosanoic acid (C22Ac) and octadecanol (C18OH).

EXPERIMENTAL PART

The antibiotics used in this study were: MON-Na (90 - 95%) and LAS-Na (97%) from Sigma. The amphiphiles were the following: DPPC (>99% by TLC) from Sigma, C22Ac from Aldrich recrystallized in acetone (mp. 80 -82°C) and C18OH from Merck recrystallized in acetone (mp. 59°C).

The solvents were Prolabo analytical grade. Chloroform and hexane were distilled on molecular sieve 4 Å for moisture removal.

Stock solutions of type 1 for the phospholipid experiments, 5.10^{-4} M in chloroform/hexane 2:3, were prepared for MON-Na, LAS-Na and DPPC. The DPPC/antibiotic solutions in molar ratios of 240:1, 120:1 and 60:1 were mixed just prior to use. The type 1 stock solution was also used for the films of the pure antibiotic.

Stock solutions of type 2 for the single chain lipid experiments, $2.49.10^{-3}$ M in ethanol/hexane 1:9, were prepared for MON-Na, LAS-Na, C22Ac and C18OH. The lipid/antibiotic solutions in the molar ratio 120:1 were mixed just prior to use.

The films were spread on a subphase of water purified in a Bioblock four cartridge system ORC-U3-R3-M3.

The isotherms were plotted on a Krüss film balance and 50 µl of the different stock solutions were spread with a Hamilton CR-200 syringe. The volumes agreed with 100 $Å^2$ molecule^{-1} for type 1 and 20 $Å^2$ molecule^{-1} for type 2 at 150 cm^2 within the balance frame. The 15 cm width piston advanced at a speed of 1.5 cm.min^{-1} during all the runs. The working temperature was 22°C.

RESULTS AND DISCUSSION

The π-A isotherms of DPPC and mixtures with MON-Na and LAS-Na spread on an aqueous subphase are shown in Figure 1. Three lipid/antibiotic molar ratios have been studied, 240:1, 120:1 and 60:1. These concentrations were chosen since Raman studies in our laboratory had already revealed strong carbon chain perturbation in the lamellar phase of DPPC when the antibiotics were added in the 120:1 molar ratio[10].

As seen from the curves, area expansion takes place above 6 mN.m^{-1}. The curves tend to meet above 15 mN.m^{-1}. The effect clearly increases with increasing amount of the antibiotics.

The break on the DPPC isotherm, characterizing a phase transition, disappears as the antibiotic concentration is raised. The effects from the two antibiotics are similar but slightly superior from MON-Na.

It is interesting that the monolayer expansion takes place in a part of the DPPC isotherm considered as liquid crystalline [11]. Moreover, some authors report that the chain packing at 12.5 mN.m^{-1} is similar to the packing in bilayers [12].

The π–A curves for the single chain lipids C22Ac and C18OH together with their mixtures with MON-Na and LAS-Na spread on water are shown in Figures 2 and 3 respectively. One molar ratio, 120:1 has been studied.

An area increase due to antibiotic addition is observed in the liquid-expanded part of the isotherms below 10 mN.m^{-1}. Above this pressure, the curves tend to overlap. As seen, the effect from MON-Na is here much higher than from LAS-Na.

In order to express the influence of the antibiotics on the lipid monolayers, we have determined the area S_A occupied by each molecule of MON-Na according to the molecular areas from the Figures 1,2 and 3. The results are shown in Table I. We have selected the pressure levels at 8 mN.m^{-1} for DPPC and 4 mN.m^{-1} for C22Ac and

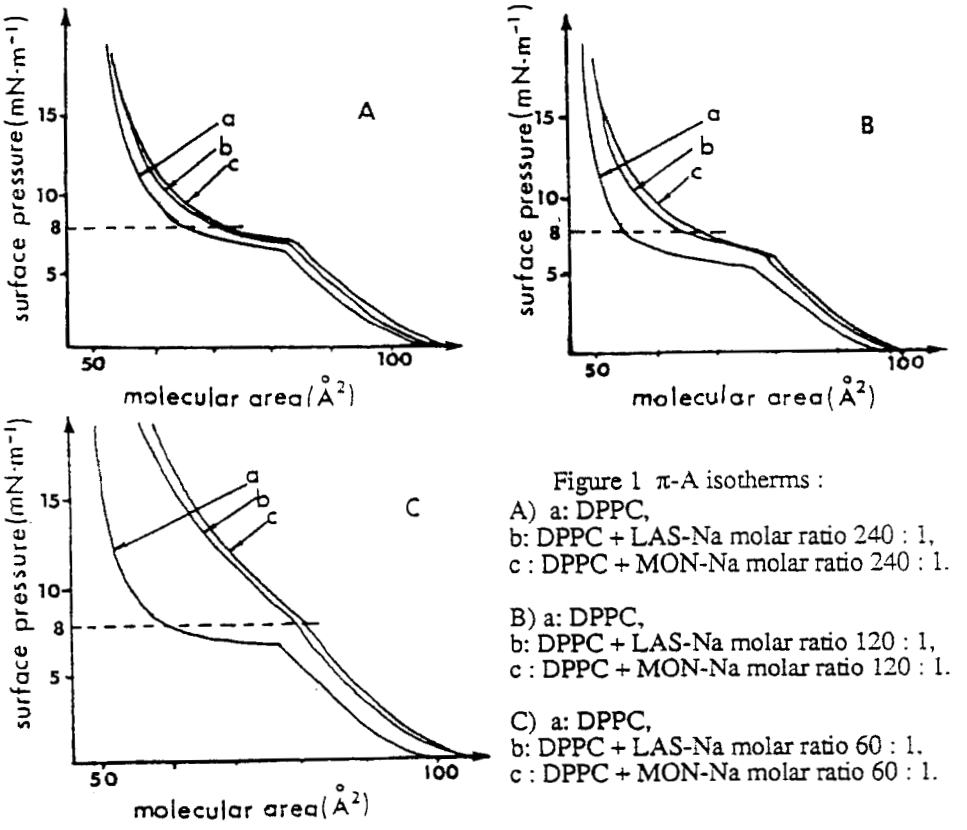


Figure 1 π-A isotherms :
A) a: DPPC,
b: DPPC + LAS-Na molar ratio 240 : 1,
c : DPPC + MON-Na molar ratio 240 : 1.

B) a: DPPC,
b: DPPC + LAS-Na molar ratio 120 : 1,
c : DPPC + MON-Na molar ratio 120 : 1.

C) a: DPPC,
b: DPPC + LAS-Na molar ratio 60 : 1.
c : DPPC + MON-Na molar ratio 60 : 1.

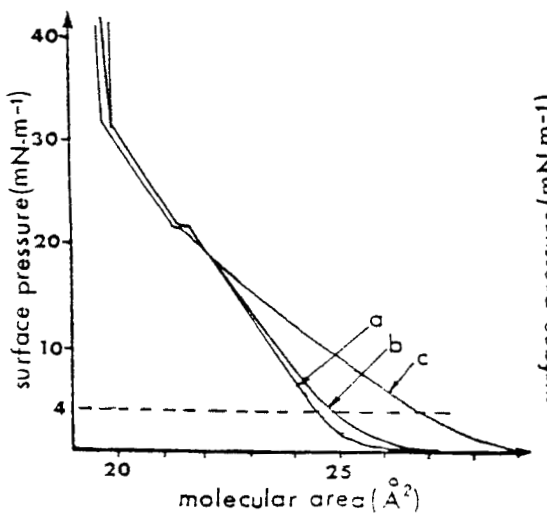


Figure 2 π-A isotherms a: C22Ac.
b: C22Ac + LAS-Na molar ratio 120 : 1
c : C22Ac + MON-Na molar ratio 120 : 1

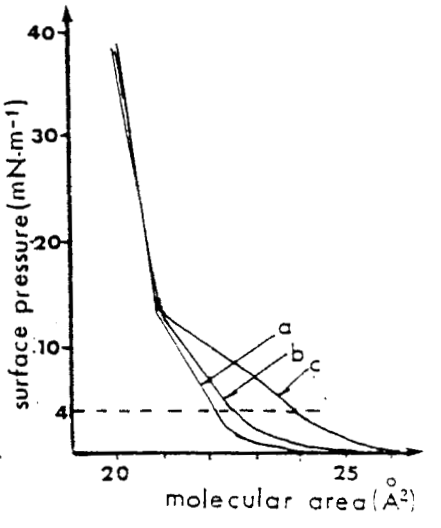


Figure 3 π-A isotherms a: C18OH,
b: C18OH + LAS-Na molar ratio 120 :1
c : C18OH + MON-Na molar ratio 120:1

C18OH since the major area expansions are situated around these values. If S_{L+A} is the mean molecular area of lipid + antibiotic, S_L the molecular area of the lipid, N_L the number of lipid molecules and N_A the number of antibiotic molecules, we have :

$$S_L N_L + S_A N_A = S_{L+A}(N_L + N_A) \text{ then } S_A = S_{L+A}\left(\frac{N_L}{N_A} + 1\right) - S_L \frac{N_L}{N_A}$$

TABLE I Molecular areas for DPPC, C22Ac, C18OH, mean molecular areas for the mixture with MON-Na and occupied area by each MON-Na molecule in the films. The values are obtained from Figures 1,2 and 3.

lipid	π(mN.m^{-1})	S_{L+A}(Å^2)	S_L(Å^2)	N_L/N_A	S_A(Å^2)
DPPC	8	71	66	240	1271
	8	65	55	120	1265
	8	80	60	60	1280
C22Ac	4	26.6	24.5	120	279
C18OH	4	23.9	22.4	120	204

As seen from Table I, the values of "occupied area" S_A by each MON-Na molecule in the DPPC layer is practically the same for the ratios 240:1, 120:1 and 60:1. This means that the area expansion must strictly be due to the antibiotic molecules since it is proportional to the concentration.

Table I shows that the expansion in the case of the simple lipids, C22Ac and C18OH, represents a much smaller value of the "occupied area" , 279 and 204 Å^2 respectively, than the values 1265-1280 Å^2 for DPPC.

We wish to recall that the lipid isotherms are very sensitive to minor exterior factors, the more so in the case of phospholipids, and this leads to dispersion of the π-A curves. Therefore, the given results have been confirmed in a great number of experiments. The lipid + antibiotic curves have been compared to the curves for the pure lipids using the same stock solutions, plotting the curves within a short time and under conditions as identical as possible.

In order to draw other conclusions from these experiments, an estimation of the antibiotic molecular areas alone are necessary. A value as great as 1271 Å^2 is unlikely considering the molecular formula, moreover at maximum surface concentration of MON in monolayers the molecular area has been reported [5] as being 417 Å^2.

We have made another approach to this question in monolayer studies of MON-Na. These molecules actually spread out in a compressible film. This might be a

consequence of an amphiphile behavior on the water surface. Monolayer studies of other pure antibiotic ionophores are also found in the literature [8,13].

The π-A curves of MON-Na are shown in Figure 4. They have been obtained under exactly the same conditions as the curves of the lipid mixtures. We have not examined any other behavior of these films at present, because our purpose has been to find area values at 4 and 8 mN.m-1 for the antibiotic alone in order to compare them with the "occupied areas" in Table I.

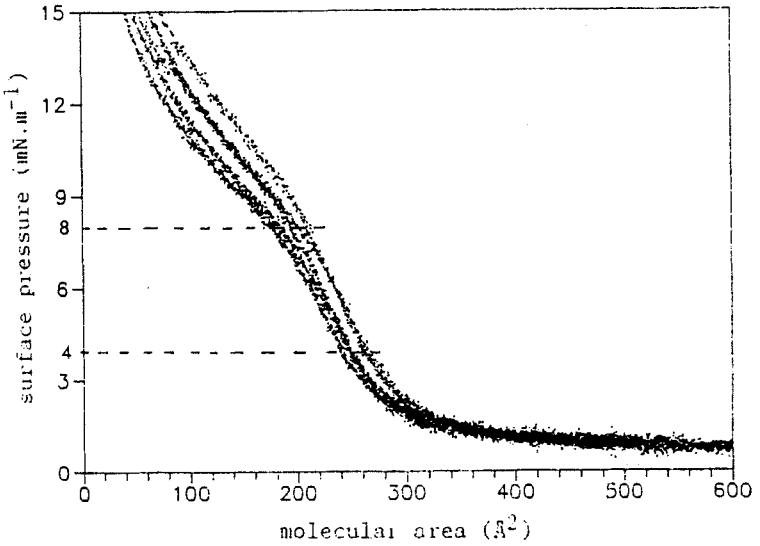


Figure 4 : π-A isotherms for MON-Na at different spreading areas.

Under the usual experimental conditions, 50 µl of stock solution, a pressure appeared at large molecular areas. This must mean that the molecules still interact and are not freely spread out.

For this reason, we have plotted curves for smaller volumes of the stock solution 1 (see Experimental Part) : 18, 16, 14, 12 and 10 µl. These experiments allowed us to evaluate the minimum molecular area of about 450 Å^2 at a pressure near zero. This is the area above which the molecules must be spread out in order to form a monolayer.

The molecular areas from the experimental curves in Figure 4 meeting this requirement (18-10µl) gave the following values : 180-220 Å^2 at 8 mN. m^{-1} and 240-270 Å^2 at 4 mN.m^{-1}.

If these areas are representatives of the molecular areas in the lipid/antibiotic monolayers, an area of at least one thousand Å^2 is left in the films from the DPPC

mixture. This effect may have the same origin as the observed swelling of membranes. The dimensions exeed the values of 7 to 10.5 Å reported for aqueous pores formed by other antibiotic ionophores in bilayers[14]. These results might indicate that the lipid molecules also participate in the ionic cavity constitution and that their aliphatic chains are tilted out of the vertical position with respect to the interface. This might be consistant with the Raman studies [10].

The observed area increase in the case of the simple lipids C22Ac and C18OH is no greater than the expected contribution from MON-Na in Figure 4. This increase might only indicate that the antibiotic is situated in the monolayer and it does not reflect either ionic cavity formation or increased disorder in the hydrocarbon chains.

CONCLUSION

The present study has evidenced area expansion in monolayers of the phospholipid DPPC in presence of antibiotic ionophores. This might be, as in bilayer systems, a consequence of ionic cavity formation. The importance of this effect also seems to indicate simultaneous rearrangements in the lipid hydrocarbon chains. The area increase from MON-Na was slightly superior to the increase from LAS-Na.

Monolayer studies of the single chain lipids, C22Ac and C18OH, disclosed a much smaller area increase from MON-Na and insignificant effects from LAS-Na. The two antibiotics must therefore be implanted differently in these films.

The results have shown that the effect of the two antibiotics in the lipid monolayers does not indicate a general behavior.

REFERENCES

1. H. H. Mollenhauer, D. J. Morré and L. D. Rowe, Biochim. Biophys. Acta, 1013, 225 (1990).
2. Y. N. Antonenko and L. S. Yaguzhinsky, Biochim. Biophys. Acta, 938, 125 (1988).
3. F. G. Riddell, S. Arumugam and B. G. Cox, Biochim. Biophys. Acta, 944, 279 (1988).
4. E. Sada, S. Katoh, M. Terashima and M. Yamamoto, Biotechnol. Bioengin., 29, 652 (1987).
5. R. Sandeaux, J. Sandeaux, C. Gavach and B. Brun, Biochim. Biophys Acta, 684, 127 (1982).
6. F. M. Menger, Bol. Soc. Chil.Quim.,35, 33 (1990).
7. L. L. M. Van Deenen and R. A. Demel, Biochim. Biophys. Acta, 94, 314 (1965).

8. R. A. Demel, L. L. M. Van Deenen and S. C. Kinsky, J. Biol.Chem., 240, 2749 1965).
9. S. C. Kinsky, Ann. Rev. Pharmacol., 10, 119 (1970).
10. M. Picquart, J.Rzeszotarska, M. Jaffrain and P. Peretti, Bioraman 90 Reims (France) 1990.
11. D. Papahadjopoulos, in G.B. Ansell, J.N. Hawthorne and R.M.C. Dawson (Eds.), Form and Functions of Phospholipids, Elsevier Sci. Pub. Comp., Amsterdam, London, New York, 1973 p.147.
12. O. Albrecht, H. Gruler and E. Sackmann, J. de Phys., 39, 301 (1978).
13. H. E. Ries Jr., Langmuir, 6, 883 (1990).
14. T. E. Andreoli, V. W. Dennis and A. M. Weigl, J. Gen. Physiol., 53, 133 (1969).