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Chlorpromazine associates with phosphatidylserines to cause an increase in the lipid's own interfacial molecular area — role of the fatty acyl composition

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Abstract

Partition coefficients of the drug chlorpromazine were determined for five different molecular species of diacylglycerophosphatidylserine in a monolayer kept at constant surface pressure (20 mN/m). Two models of adsorption of chlorpromazine in phosphatidylserine monolayers were compared. The first model correlated the amount of inserted drug molecules with the induced increase in area. The second model introduced the effect of drug adsorption on the lipid's own area by comparing the effect of increasing temperature on the lipid's own interfacial area. From the second model, the extrapolated work of insertion of one drug molecule per lipid molecule in a monolayer kept at 20 mN/m was correlated to the partition of the drug in liposomes. The work of insertion of chlorpromazine was insignificant in the unsaturated dioleoylphosphatidylserine and was maximum in the saturated distearoylphosphatidylserine monolayers. The presence of one double bond in the acyl chains dramatically reduces the work of insertion of chlorpromazine between lipid molecules and also reduces the effect chlorpromazine induces on the lipids own interfacial area in monolayers.

Keywords: Phosphatidylserine; Chlorpromazine; Molecular species; Monolayer; Adsorption; Partition coefficient

1. Introduction

The packing of biological membranes is determined by both the headgroups and the type of acyl chains of the glycerophospholipids. The activity of membrane-bound proteins (receptors, ion channels, enzymes) are often very dependent on the class of phospholipid, the particular acyls and the physical state of the phospholipid. Thus, C_{55}-isoprenoid alcohol kinase from *Staphylococcus aureus* requires glycerophospholipids for activity and gives far higher activity with phosphatidylcholines (PC) than phosphatidylethanolamine (PE) or phosphatidylserines (PS), and maximal activity with PC is obtained with short chain (e.g.

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octanoyl) acyls while hexadecanoyl- and octadecanoyl-containing PC gave no activation [1]. Furthermore, the activity of PLA_2 is highly dependent on the structure of its membrane substrate [2–4], and subtle variations in the acyl composition substantially affects the physical states and thereby the activity of cytochrome b_5 reconstituted in PC liposomes [5]. The physical features of liposomes with different degrees of acyl unsaturation also influences protein kinase C [4] in a way that argues against specific lipid–protein interactions [6]. Anionic glycerophospholipids determine the topology, and hence activity, of membrane proteins in general [7], and these phospholipids alter the structure of human recombinant prion protein which is associated with membranes in living cells [8]. Recently it was found that when the fluidity of membranes composed of egg PC and dioleoyl phosphatidic acid was varied by changing the cholesterol content in the membranes, the conformation of the nicotinic acetylcholine receptor was varied in a way that suggested alterations in the receptor's activity from inactive through active to desensitised [9].

The observations discussed above strongly suggest that the activity of membrane-bound enzymes, receptors and ion channels may be influenced by the lipid composition of the membranes. Therefore, one should expect that perturbation of the lipid organisation by amphiphilic molecules should influence the structure/activity of membrane-bound proteins without direct interaction between the protein and the amphiphile. Membrane perturbation with CPZ and other amphiphilic, psychotropic drugs have indeed been shown to induce a host of genes in both bacteria and mammalian cells [10]. Thus, it is possible that chlorpromazine's (CPZ) claimed effect on the D_2-receptor is partially due to perturbation by CPZ of the membrane that contains the receptor. The study of interaction of CPZ with pure lipid membranes is therefore of interest to study the action of CPZ in terms of lipid composition.

In a previous study, we have shown that CPZ induces a strong effect on monolayers of acidic phospholipids as compared to monolayers of zwitterionic lipids [11]. The present work further investigates the interaction of CPZ with monolayers and liposomes of different diacylglycerophosphatidylserine species by focusing on the drug-induced increase of the lipid molecular interfacial area. These measurements allow us to correlate the induced expansion of the monolayer with the partition coefficient of the drug in liposomes via the work of insertion of the drug in the monolayer.

2. Materials and methods

2.1. Chemicals

Dipalmitoylphosphatidylserine (DPPS), distearoylphosphatidylserine (DSPS), 1-stearoyl,2-oleoylphosphatidylserine (SOPS), dioleoylphosphatidylserine (DOPS), 1-palmitoyl,2-oleoylphosphatidylserine (POPS) were purchased from Avanti Polar Lipid (Alabaster, AL). The lipids were dissolved at 1 mg/ml in chloroform (> 99%) obtained from Merck (Whitehouse Station, NJ). Chlorpromazine hydrochloride was from Sigma (St. Louis, MO). The 5 mM phosphate buffer, pH 7.4, was prepared with NaH_2PO_4 (> 99%) and Na_2HPO_4 (> 99.5%) from Merck. KCl, also from Merck, was added to obtain 120 mM. Milli-Q water was used for the preparation of all the solutions.

2.2. Monolayer studies

Measurements were done with a KSV Langmuir instrument with a temperature-controlled Teflon trough ($75 \times 340 \times 5$ mm). A platinum Wilhelmy plate was used to measure the surface tension. The KSV software allows isobaric measurements by regulating the positions of the barriers that limit the film-covered area. Monolayers of the diacylphosphatidylserines were spread on the buffer solution at 37°C and set to the surface pressure of 20 mN/m. Chlorpromazine was then added to the subphase by injecting and mixing a concentrated CPZ solution at each sides of the trough. The induced area increase per lipid molecule was recorded against time.

2.3. Temperature studies

Lipid monolayers were spread on the buffer solution and surface pressure was plotted against the apparent molecular surface area of the lipids for different temperatures.

2.4. Liposome studies

Small unilamellar vesicles (SUV) were obtained by evaporation of the solvent under nitrogen and then under vacuum overnight. The buffer mentioned above was added to the lipids such that a final concentration of 0.1 mg lipids/ml was obtained. Vesicles were sonicated and various amounts of CPZ were added to the SUVs and incubated at 37°C. Zeta potentials were measured by microelectrophoresis in a Malvern Zetasizer 4 apparatus.

3. Results

3.1. Adsorption of CPZ on monolayers

CPZ was injected in the monolayer subphase such that the final concentration was under the critical micelle concentration of 7 mM [12]. The apparent molecular area of the lipid that was recorded at a surface pressure of 20 mN/m corresponded to the surface area of the monolayer divided by the number of lipid molecules that were initially spread at the air/buffer interface. The recorded values of the lipid area did not take in account the adsorption of CPZ in the monolayer, and therefore do not correspond to the lipid's own molecular surface area. This apparent molecular area rapidly increased with time after injection of CPZ and reached equilibrium after 10–15 min. The difference ΔA between the lipid apparent molecular area before and at equilibrium after CPZ injection was plotted against the initial drug bulk concentration (Figs. 1 and 2).

Two different models of adsorption were used, both take into account the electrostatic effects by means of the Gouy–Chapman theory.

In both cases, X_{CPZ} is the number of CPZ molecules that have penetrated the monolayer per lipid molecule ($X_{CPZ} = n_{CPZ}/n_L$ with n_{CPZ} being the number of CPZ and n_L being the number of lipid molecules in the monolayer) and the Langmuir adsorption isotherm can be expressed by [13]:

$$X_{CPZ}/C_M = K_p(1 - nX_{CPZ}) \quad (1)$$

with K_p the partition coefficient of the drug in the monolayer, n the number of lipid molecules bound per adsorbed CPZ and C_M the concentration of CPZ just beneath the phospholipid monolayer, expressed in the form:

$$C_M = C_{eq}\exp(-\Psi_0 z_{CPZ} F_0/RT) \quad (2)$$

where ψ_0 is the surface potential of the monolayer, z_{CPZ} the signed charge of CPZ, F_0 the Faraday constant, R the gas constant, T the temperature and C_{eq} the equilibrium concentration of the drug in the bulk. C_{eq} is approximated to the total drug concentration since only a very little fraction adsorbs the monolayer.

The surface potential ψ_0 is related to the surface charge density σ by the equation:

$$\sigma^2 = 2000\varepsilon_r\varepsilon_0 RT\Sigma c_{i,eq}[\exp(-\Psi_0 z_i F_0/RT) - 1] \quad (3)$$

where ε_r is the dielectric constant of water, ε_0 the vacuum permittivity, $c_{i,eq}$ the concentration of the ith electrolyte in the subphase and z_i its corresponding charge.

The surface charge density is calculated from the quotient between the total surface charge Q_T and the monolayer total surface area A_T.

The total surface charge is:

$$Q_T = (z_L n_L + z_{CPZ} n_{CPZ})e \quad (4)$$

where e is the elementary charge. At pH 7.4, the protonated form of CPZ ($pK_a = 8.6$) [14] must overwhelm the unprotonated form and all phosphatidylserine molecules are expected to carry one negative charge, since their carboxyl group has a pK_a of 3.4 [15]. Therefore, the charge per

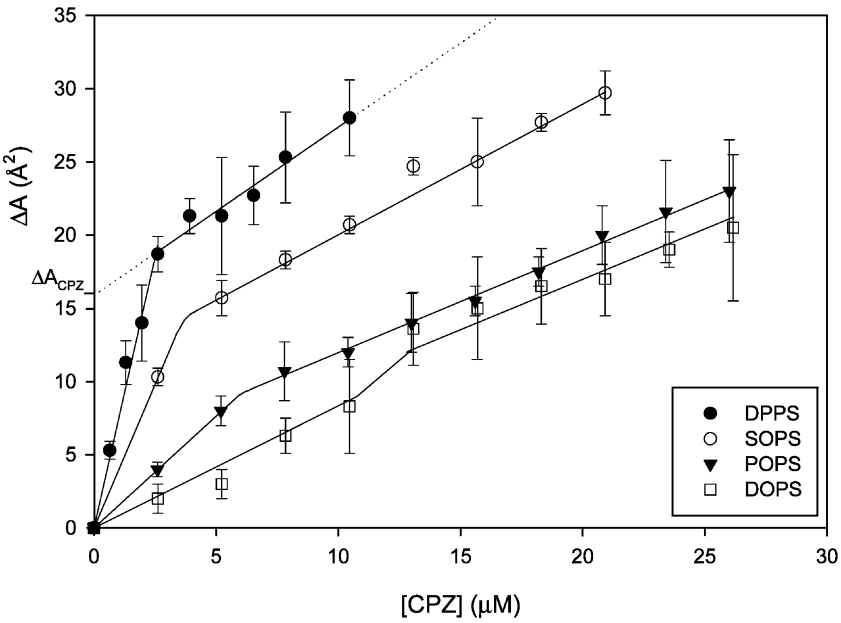

Fig. 1. Increase of the apparent molecular surface area ΔA (the difference between the surface area per lipid molecule when CPZ was present in the subphase and the area without CPZ) of DPPS, POPS, SOPS and DOPS, induced by different CPZ concentrations.

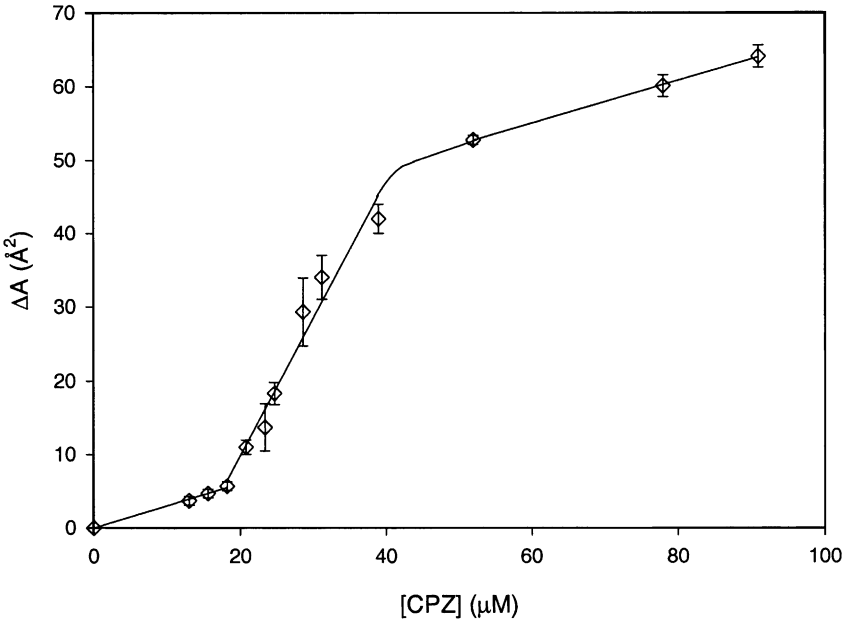

Fig. 2. Increase of the apparent molecular surface area ΔA of DSPS induced by different CPZ concentrations.

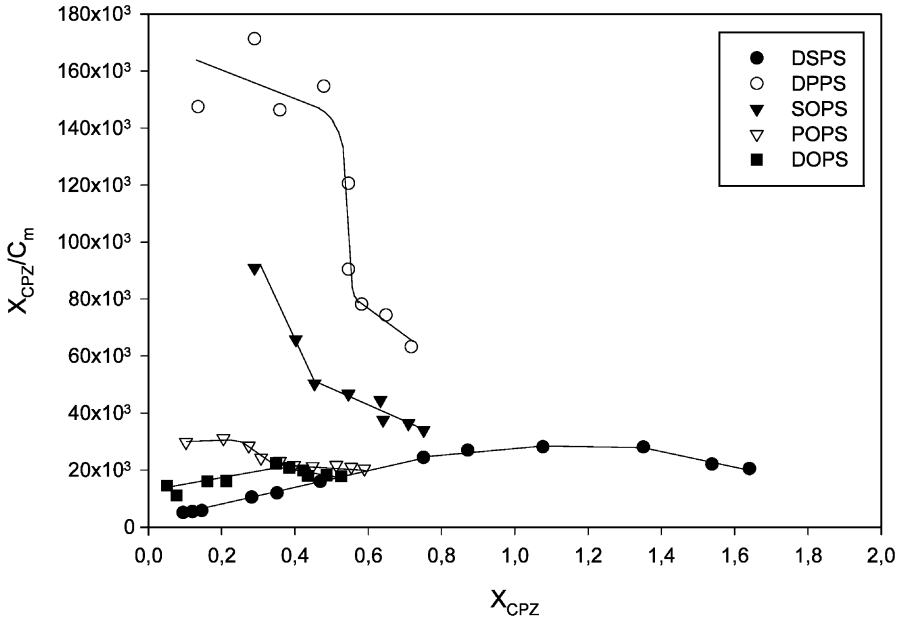

Fig. 3. Scatchard plots for adsorption of CPZ on different phosphatidylserine monolayers when ΔA is supposed to be directly proportional with the amount of adsorbed drug.

lipid molecule can be taken as $z_L = -1$ and CPZ's charge as $z_{CPZ} = +1$.

The measured total surface area is:

$$A_T = n_L(A_0 + \Delta A) \quad (5)$$

with A_0 the molecular interfacial area of the lipid molecule spread on CPZ free buffer at 20 mN/m.

The surface charge density is then:

$$\sigma = (z_L + X_{CPZ} z_{CPZ})e/(A_0 + \Delta A) \quad (6)$$

The surface charge density σ can be calculated from the adsorption data, the surface potential ψ_0 is then estimated from Eq. (3), and the value of C_M is calculated.

Values of X_{CPZ}/C_M are then plotted as a function of X_{CPZ} in Scatchard plots, and the partition coefficient K_p is determined.

For the first model, the induced increase in area ΔA is assimilated with the area of inserted chlorpromazine molecules per lipid:

$$\Delta A = X_{CPZ} A_{CPZ} \quad (7)$$

The Gibbs absorption isotherm of CPZ at the surface of the buffer solution at 37°C gave a value of 39 Å^2 for A_{CPZ}, the area of CPZ molecule, close to the value of 42 Å^2 measured by Frentzel [16].

At a surface pressure of 20 mN/m, adsorption of CPZ on monolayers of five different molecular species of phosphatidylserine give rise to non-linear Scatchard plots (Fig. 3). Values of K_p were estimated by regression to zero X_{CPZ} and are presented in Table 1. According to this model, K_p appears to be poorly coherent with the unsaturation state of the lipids forming the monolayer. CPZ seems to adsorb to a larger extent on the saturated species DPPS, while its partition coefficient decreases in the order of the respective monolayer composition: SOPS > POPS > DOPS > DSPS. Though the effective charge of CPZ can have a smaller absolute value, it might influence the shape of the Scatchard plots, the order in which K_p decreases in amplitude with the different PS monolayers would still be the same.

In the second model, the change of the lipid's own area upon adsorption of CPZ was proposed.

Table 1
Partition coefficients and works of insertion of CPZ in phosphatidylserine monolayers at 20 mN/m[a]

	K_p (M^{-1})		ΔW ($\times 10^{-20}$ J)
	A	*B*	
DOPS	$(1.5 \pm 0.5) \times 10^4$	$(1.7 \pm 0.3) \times 10^4$	0
POPS	$(3 \pm 0.5) \times 10^4$	$(1.5 \pm 0.2) \times 10^4$	4.3
SOPS	$(1.5 \pm 0.2) \times 10^5$	$(1.9 \pm 0.2) \times 10^4$	9.1
DPPS	$(1.7 \pm 0.3) \times 10^5$	$(2.2 \pm 0.3) \times 10^4$	19.4
DSPS	$(5 \pm 1) \times 10^3$	$(6.5 \pm 0.8) \times 10^3$	24.1

[a] Partition coefficients, K_p (M^{-1}) in monolayers at 20 mN/m for the five phosphatidylserine molecular species studied, (a) with the assumption that ΔA is directly proportional to the amount of adsorbed CPZ; and (b) when the CPZ-induced increase of the lipid's own area is taken into account. In this last case, the extrapolated work of insertion ΔW of one molecule CPZ done on the area of one lipid molecule is determined.

Except for DOPS which showed a relatively slow and constant increase in ΔA with increasing CPZ concentration (Fig. 1), the other molecular species appeared to have a biphasic or even a triphasic adsorption. The degree of change of ΔA with CPZ concentration for DPPS, POPS and SOPS became smaller at a critical CPZ concentration of 2.5, 6 and 5 μM, respectively (Fig. 1). For DSPS, the rate of change of ΔA with the CPZ concentration was triphasic and largest for concentrations between 19 and 37 μM (Fig. 2).

Following the idea of Beurer and Galla [17], who suggested that monolayers spread on a subphase containing CPZ were comparable to monolayers at a higher temperature without CPZ, we supposed that the two different apparent adsorption phases seen with DPPS, POPS and SOPS could be consistent with a single partition coefficient of the drug in the monolayer: a large ΔA increase at low drug concentration could be due primarily to the expansion of the lipid's own surface area induced by the presence of CPZ. At a critical CPZ concentration, this mechanism would be saturated, and for larger drug concentrations, the increase in ΔA would be due only to the area of the CPZ molecules that penetrate the monolayer. Isothermal compressions of the same molecular species (DPPS), on the same buffer subphase, free of CPZ, at different temperatures are presented in Fig. 4. At a surface pressure of 20 mN/m, the molecular area increased with increasing temperature until saturation (inset Fig. 4). The increase in the molecular areas of all lipids were measured as the temperature was raised from 37°C. The maximum increase in area, denoted ΔA_T, for DPPS, SOPS, and POPS, were 14, 11.5, 5.5 Å^2, respectively, all within ± 1 Å^2. The area difference could not be measured for DSPS because the temperature had to be increased above the maximum temperature allowed by the temperature regulation device in order to reach the saturation area at 20 mN/m (Fig. 5). ΔA_T was then compared with the expansion of the lipid's own surface area induced by CPZ, denoted ΔA_{CPZ}. This value was determined for each monolayer composition by extrapolating the second phase of adsorption, i.e. after the initial break in the slope, to zero CPZ concentration as shown in Fig. 1. DSPS, DPPS, SOPS and POPS were estimated to have a ΔA_{CPZ} value of 31, 16, 11.5, and 5 Å^2, respectively. Thus, we find that the maximum expansion of the lipid's own surface area due to temperature, ΔA_T, is close to the expansion of the lipid's surface area due to the drug, ΔA_{CPZ}. For DSPS, ΔA_{CPZ} was obtained by subtracting ΔA at the first break point in the slope (at 19 μM CPZ) from the extrapolated ΔA of the third phase of adsorption to zero CPZ concentration. The value of ΔA_{CPZ} was estimated to be 31 Å^2 for the DSPS monolayer.

We propose to separate the Langmuir adsorption data in Fig. 1 into two parts: for CPZ concentrations below and above the critical CPZ concentration for each PS molecular species.

For CPZ concentrations larger than the critical concentration which correspond to the break in

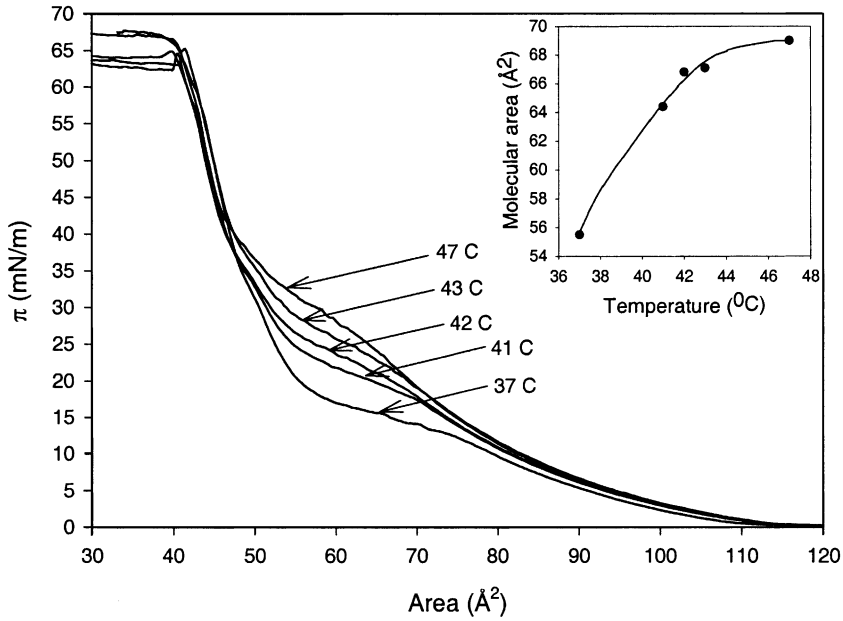

Fig. 4. Isotherms of DPPS monolayer at various temperatures, with the molecular areas measured at the surface pressure of 20 mN/m plotted against temperature in the insert.

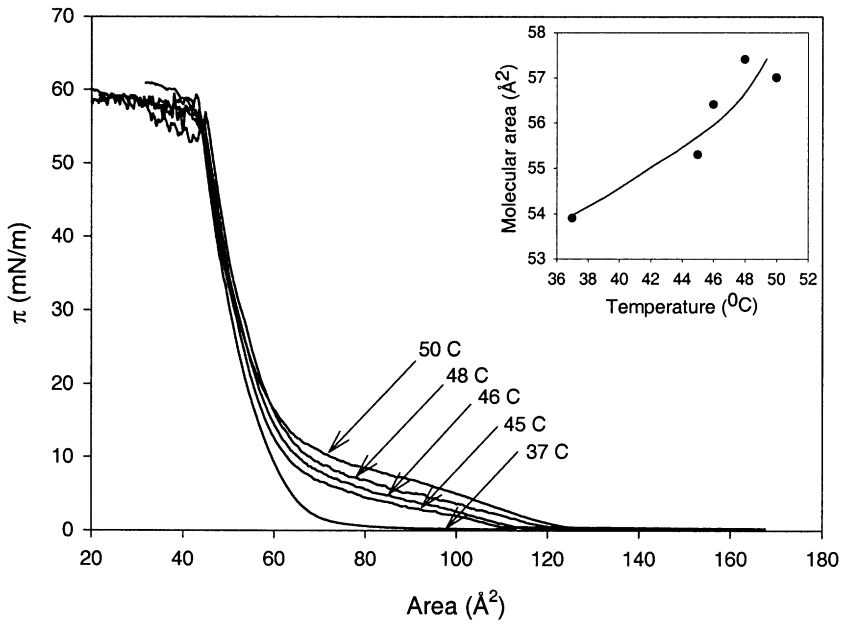

Fig. 5. Isotherms of DSPS monolayer at various temperatures, with the molecular areas measured at the surface pressure of 20 mN/m plotted against temperature in the insert.

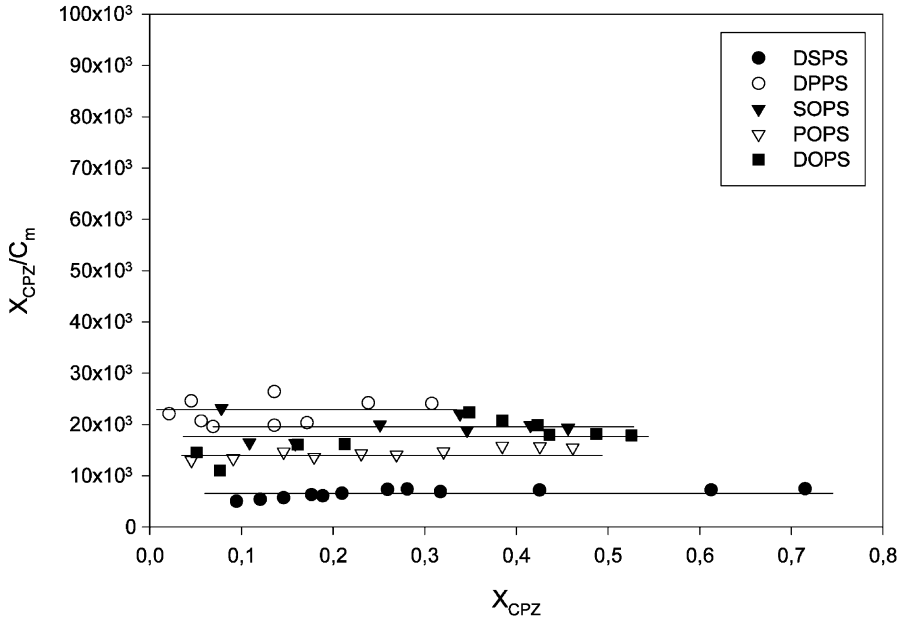

Fig. 6. Scatchard plots for adsorption of CPZ on different phosphatidylserine monolayers when the CPZ-induced increase of the lipid's own area is taken into account.

the adsorption curves and with the assumption that the molecules in the monolayer behave as hard cores, the drug-induced increase of the apparent surface area ΔA per lipid molecule can be expressed by:

$$\Delta A = \Delta A_{CPZ} + X_{CPZ} A_{CPZ} \tag{8}$$

At the critical CPZ concentration, the critical CPZ partition X_{CPZc} is expressed by:

$$X_{CPZc} = (\Delta A - \Delta A_{CPZ})/A_{CPZ} \tag{9}$$

For CPZ concentrations smaller than the critical concentration, the drug-induced increase of ΔA can be written:

$$\Delta A = \frac{\Delta A_{CPZ} X_{CPZ}}{X_{CPZc}} + X_{CPZ} A_{CPZ} \tag{10}$$

From Eqs. (8)–(10), the partition of CPZ in the monolayer at 20 mN/m can be expressed by:

$$X_{CPZ} = \frac{\Delta A}{((\Delta A_{CPZ}/X_{CPZc}) + A_{CPZ})} \quad (\text{for } X_{CPZ} < X_{CPZc}) \tag{11}$$

$$X_{CPZ} = (\Delta A - \Delta A_{CPZ})/A_{CPZ} \quad (\text{for } X_{CPZ} > X_{CPZc}) \tag{12}$$

The surface charge density, the surface potential and the concentration of CPZ close to the monolayer were then determined. Adsorption of CPZ according to this model is presented in the form of Scatchard plots in Fig. 6. For all PS monolayers, the Scatchard plots are linear within experimental uncertainty. The new values of K_p (Table 1) are decreased by approximately 10-fold for DPPS and SOPS, and are relatively identical for DSPS. The partition coefficients of CPZ in DPPS, SOPS, POPS and DOPS monolayers at 20 mN/m are all close to $1.9 \pm 0.4 \times 10^4$ while the partition coefficient is $6.5 \pm 0.8 \times 10^3$ with DSPS. These results show the importance of the shift of

the lipid's own area induced by the adsorption of CPZ.

To further study the importance of the CPZ-induced increase in the lipid's own area in a monolayer kept at 20 mN/m, we calculated the extrapolated work of insertion ΔW done by one molecule of inserted CPZ on the own area of one lipid molecule at the surface tension γ.

$$\Delta W = -\gamma \Delta A_{CPZ}/X_{CPZc} \quad (13)$$

The extrapolated work of insertion of a CPZ molecule in monolayers (Table 1) decreases as the chain length of the lipid decreases and it decreases even more when a double bond is present in one acyl chain. For the lipid containing two unsaturated acyl chains, DOPS, a value of null work of insertion within experimental uncertainty was measured. The presence of one double bond on one acyl chain decreases the measured ΔW by 15×10^{-20} J compared with the corresponding saturated species. Furthermore, the shortening of the saturated acyl chain by two carbons corresponds to a decrease of ΔW by 5×10^{-20} J. The work of insertion of CPZ decreases therefore dramatically by the introduction of a double bond in the lipid's acyl chains, and much more than by a reduction of the chain length.

3.2. Adsorption of CPZ on liposomes

The electric potential ξ at the shear plane of the liposomes was related to the electrophoretic mobility through the Helmholtz–Smoluchowski equation [18] and plotted against CPZ concentration in Fig. 7. For DOPS, POPS and SOPS, the magnitude of the negative zeta potentials ξ decreased by approximately 50% with 500 μM CPZ, while this value decreased only by approximately 15% for DPPS and by 5% for DSPS with the same drug concentration. The partition coefficient of CPZ is probably different than in monolayers at 20 mN/m since the lateral pressure in liposomes is approximately 30 mN/m. Adsorption of CPZ on liposomes of different molecular species of PS appears, however, to be related to the previously calculated extrapolated

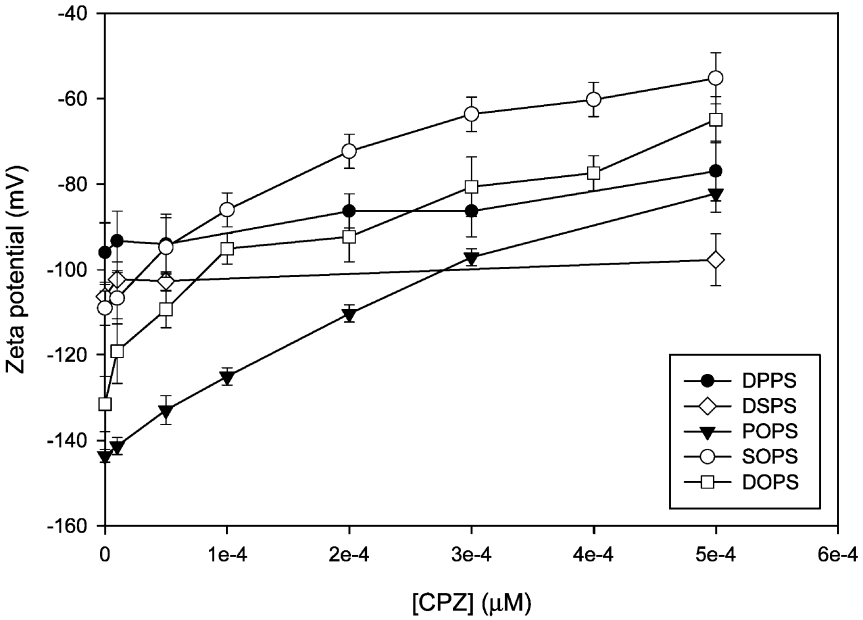

Fig. 7. Zeta potentials of phosphatidylserine liposomes in function of CPZ concentration for each phosphatidylserine molecular species.

work of insertion of CPZ in a monolayer. CPZ has the largest extrapolated work of insertion with the saturated species DSPS and it also adsorbs least on DSPS liposomes, as shown by the smallest CPZ-induced change in zeta potential. With the shorter saturated acyl chains specie DPPS, the work of insertion was the next largest among the five PS species studied, in agreement with the CPZ-induced decrease in zeta potential of DPPS liposomes. The small work of insertion of CPZ in the three unsaturated SOPS, POPS and DOPS monolayers could be also correlated to the large CPZ adsorption on the corresponding liposomes.

4. Discussion

For monolayers, the large increase of the lipid's own molecular area ΔA_{CPZ} induced by CPZ concentrations beyond a critical concentration was compared to an increase of the molecular area due to a temperature increase in the monolayer free of drug. For bilayers, no evidence of a similar effect could be found. In addition, the partition of CPZ according to lipid molecular species appeared to vary differently for monolayers and bilayers. But, the extrapolated work of insertion of CPZ determined by the monolayer study is correlated to the partition of CPZ in the corresponding liposomes. In bilayers and at neutral pH, DOPS, POPS, SOPS, DPPS and DSPS have been shown to have a gel/liquid-crystalline phase transition temperature T_m, respectively at -11°C [19], 14°C [20], 17°C [21], 53°C [19] and 62°C [22]. Though we cannot directly compare the states of bilayers and monolayers at a same temperature because of probable differences in lipid interfacial density, the values of ΔA_{CPZ} appeared to increase in the same order as the values for transition temperature (DOPS < POPS < SOPS < DPPS < DSPS). For monolayers in liquid-crystalline phase, ΔA_{CPZ} would be minimal, as in the case of DOPS monolayers. At the opposite, ΔA_{CPZ} would be maximal for a monolayer in gel phase such as DSPS. Furthermore, in our monolayer studies with DSPS, the adsorption phase where the lipid's own area increased, started at a drug partition corresponding to one molecule CPZ for 6,8 molecule lipids. At this ratio, the screening of interactions between adjacent DSPS molecules would appear, revealing a high cohesion between these long chain saturated phosphatidylserines. Monolayers of DPPS, SOPS and POPS might be in a state between the gel phase (as for DSPS) and the liquid-crystalline phase (as for DOPS) that could be a non-homogeneous phase, such as a gel containing liquid-crystalline domains. A two-dimensional cooperative transition could be induced by intercalation of CPZ between lipid molecules, thus shielding the cooperative interactions between the molecules, in agreement with the proposed mechanism of action of CPZ via disruption of hydrogen-bonds between adjacent headgroups [23]. In monolayers CPZ may cause a gel to liquid phase transition through a mechanism involving a disruption of cooperative intermolecular interactions and resulting in an increase of the lipid interfacial area.

The cooperative cohesion forces between lipid molecules can be related to the extrapolated work done by one molecule of inserted CPZ on the own area of one lipid molecule, calculated from the monolayer studies. Though it seems that the large disorder in the tail environment of DOPS makes the insertion of any hydrophobic or amphiphilic molecule easy, it can not be ruled out that the drug's structure might be specific for its adsorption on different phosphatidylserine molecular species.

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