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pH-dependent interaction of psychotropic drug with glycerophospholipid monolayers studied by the Langmuir technique

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ABSTRACT

We have earlier investigated the interaction of the antipsychotic drugs chlorpromazine(CPZ) and olanzapine (OLP) with glycerophospholipid monolayers. These experiments were carried out at high and low temperatures and showed that OLP had a more pronounced effect on the packing of the phospholipid (PL) monolayers than CPZ. At pH 7.36, where OLP consists of one positive and one neutral species. In the present work we have studied the interaction of the drugs with monolayers of PLs by the Langmuir technique at pH 6.00 and 10.00 at 37 °C. The PLs were palmitoylphosphatidyl-choline(DPPC), 1-stearoyl-2-arachinodonoylphoshatidylcholine(SAPC),dipalmitoylphosphatidyl-serine(DPPS) and 1-palmitoyl-2-oleoyl-phosphatidylserine(POPS). OLP has a pKa around 7.4, with one neutral and one positive species at pH 6.00 and pH 10.00, respectively. CPZ has pKa value around 9.4, and is positively charged at pH 6.00 and neutral at pH 10.00. Our studies revealed that the surface area of DPPC with CPZ in the subphase did not change at pH 6.00. In contrast, OLP increased the mean molecular area(MMA) of DPPC at pH 6.00, while CPZ caused distinct increase in MMA on the monolayer packing of all the other PLs, including monolayers of DPPC at pH 10.00. OLP, increased MMA of all PLs at both pHs. Further, OLP increased MMA of DPPC (pH 10.00), SAPC (pH 10.00), DPPS (pH 6.00) and POPS (pH 6.00) at 30 mN/m, the expected MMA of biological membranes. CPZ had the more pronounced effect at lift-off and gave an effect of the monolayers with negatively charged head groups in accordance our earlier experiments. However, CPZ affected the packing of the SAPC monolayer both at pH 6.00 and 10.00, and DPPC at pH 10.00. Both these PLs have neutral choline head group. Our results suggest that both drugs intercalate in the PL monolayers, and that the intercalation might involve electrostatic interaction with the head groups or hydrophobic interaction with the acyl chains of the PLs, or both. Probably the drugs intercalate to different extents depending on charge of both the drugs and the PL head groups. Our investigation may suggest that the interaction of CPZ and OLP with membrane PLs could be linked to both the psychotropic and the side effects.

1. Introduction

Chlorpromazine (CPZ, see Scheme 1), is an antipsychotic agent used for treatment of schizophrenia and bipolar disorders. The drug has severe side effects and has therefore been replaced by olanzapine (OLP, see Scheme 1). OLP has become one of the most commonly used atypical antipsychotics [1] that is believed to antagonize dopamine D_2, muscarinic M_{1-5}, α_1-adrenergic and histamine H_1 receptors [2].

OLP exhibits relatively high affinity for several neurotransmitter receptors, such as D_{1-5} dopaminergic, 5-HT_{2A-2C} and 5-$HT_{3,6,7}$ serotonergic, α_1-adrenoceptors and Histamine H_1 [1–7], as well as moderate affinity for the five muscarinic receptor subtypes, M_{1-5} [8]. The functional blockade of these receptors may contribute to its broad efficacy in the treatment of schizophrenia and related psychoses [9–12].

However, it has been shown that CPZ interferes with polyphosphoinositide metabolism in stimulated platelets [13–16]. These cells do not contain D_2 receptors [17], which are assumed to be the main target for the phenothiazines [18,19]. It has been found that micromolar concentrations of CPZ cause large increases in the mean molecular areas (MMAs), of acidic but not in neutral glycerophospholipids in monolayer [20–22]. These results have been confirmed by using magic angle spinning solid-state ^{13}CNMR on bilayer samples with CPZ of DPPC/DMPC and PBPS/DPPC [23–26], where CPZ affected the serine group by electrostatic attraction. Only minor effects were observed between CPZ and the choline head groups. These findings

Abbreviations: CPZ, chlorpromazine; OLP, olanzapine; DPPC, dipalmitoyl phosphatidylcholine; DPPS, dipalmitoyl phosphatidylserine; POPS, 1-palmitoyl-2-oleoyl phosphatidylserine; SAPC, 1-stearoyl-2-arachidonyl phosphatidylcholine; MMA, mean molecular area (in Å^2); PL, phospholipid.

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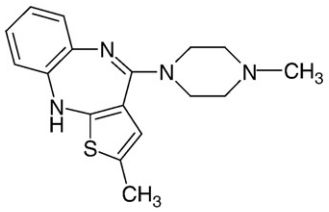
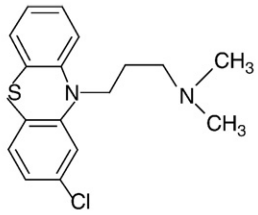


Olanzapine

{(2-Methyl-4-(4-methyl-1-piperazinyl)10H-thieno[2,3b][1,5]benzodiazepine}



Chlorpromazine

{2-chloro-N,N-diethyl-10H-phenothiazine-10-propanamine}

Scheme 1. Structural formulas of OLP and CPZ.

suggest that the psychotropic drugs may work through intercalation in membrane phospholipids (PL) in a receptor-unrelated fashion [27]. The intercalation of amphiphilic molecules like CPZ in mono-or bilayers of glycerophospholipid molecules leads to increase the intermolecular distances between the PL molecules and to alterations of the membrane structure. The serine head group is present only in the inner leaflet of biological membranes. However, all psychotropic drugs distribute between membranes and water with distribution coefficients in the range from 10,000 to 20,000 [28]. This suggests that the drugs will enter the membranes through the outer leaflet and diffuse through the acyl layer and thus be interacted with the serine in the inner leaflet. The structural changes caused by the amphiphilic drugs may thus affect the positioning of membrane-bound enzymes and receptors and thereby alter their functions. Thus in addition to act as antagonists for receptors, the drugs may also alter membrane protein activities. In a recent study, we investigated the interaction of CPZ and OLP with monolayers of DPPC, POPS and DPPS [29]. To our surprise, OLP increased the MMAs of both acidic and neutral LPs, indicating that OLP is better intercalated in neutral monolayers than CPZ. This work is confirmed by a ^{13}C and ^{31}P solid-state NMR study on bilayers (in liposomes) of DPPC/DPPS, and the results revealed that both the serine and the choline head groups are affected by OLP, and that the interaction appeared to be caused by electrostatic attraction to the serine head group carboxyl and repulsion of the choline head group positively charged nitrogen [30]. However, a molecular dynamic study of the interaction of CPZ in DPPC monolayers carried out at different surface densities, showed that CPZ is located in the acyl chain region, and the orientation of the ring structure varied with surface densities [31]. These studies indicate that the intercalation of an amphiphilic drug in biological membranes might be caused by electrostatic and hydrophobic forces or both.

In the present work we have studied the effect of pH on drug intercalation in model membrane systems in order to differentiate between electrostatic and hydrophobic drug intercalation in the model membranes. We report monolayer behaviour of glycerophospholipids with CPZ or OLP in the subphase by the Langmuir technique at pH 6.00 and pH 10.00. At pH 6.00 both drugs are carrying a positive charge, whereas at pH 10.00 they are neutral. The PLs studied were dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphtidylserine (DPPS), 1-palmitoyl-2-oleoylphosphatidylserine (POPS) and 1-stearoyl-2-arachidonoylphosphatidylcholine (SAPC). The surfaces of the monolayer studied are systematically varied by the head groups and acyl chains of the PLs, and we have altered the surface by both addition of two different drugs and by changing the charge of the drugs.

2. Materials and methods

2.1. Lipids and chemicals

1,2-dipalmitoyl-sn-glysero-3-phosphocholine(DPPC), 1-stearoyl-2-arachidonoyl-sn-glysero-3-phosphocholine(SAPC), 1,2-dipalmitoyl-sn-glysero-3-phospho-L-serine (DPPS) and 1-palmitoyl-2-oleoyl-sn-glysero-3-phopho-L-serine(POPS) were all purchased from Avanti Polar Lipids Inc.(AL). The lipids were kept in the dark as powders or chloroform solutions at 20 °C. Chlorpromazine, (CPZ) was from Sigma Chemical Co. (St. Louis, MO), and Olanzapine, (OLP) was kindly provided by Eli Lilly Company (Indianapolis, IN). Stock solutions (10 mM) of CPZ and OLP were made in 0.9% NaCl and in ethanol, respectively. Working solutions were made by diluting the stock solutions with HEPES buffer in a ratio of 1:1000 (v/v). HEPES buffer (10 mM) was purchased from Sigma-Aldrich. Aliquots of NaOH or HCl of analytical grade was added to the HEPES buffer to obtain pH 10.00 or pH 6.00, respectively. MilliQ water with low ionic concentration (18.2 MΩ/cm) was obtained by an instrument (Academic) from Millipore.

2.2. Experimental

A KSV Minitrough (Helsinki, Finland) of dimensions 75 (w) × 364 (l) × 5 (h) mm was used in the study of the drugs on the monolayer at the air/water interface. The procedure and explanation of the compression phases are described elsewhere [22]. The trough was filled with HEPES buffer (10 mM, pH 6.00 or pH 10.00) with and without 10 μM drugs. Experiments were carried out using a thermostat bath at 37 °C. In each experiment 15 μl of PL dissolved in chloroform (1 mg/ml) was carefully spread on the aqueous surface with a Hamilton syringe, and the chloroform was allowed to evaporate before the compression started. During compression, the barrier speed was run at 5 mm/min. The surface pressure, Π, was determined using the Wilhelmy plate method. The lift-off areas were defined the MMA when the surface pressure has reached 1 mN/m. The experiments were usually performed with the amphiphilic drug dissolved in H_2O. OLP is not soluble in water, and was therefore dissolved in ethanol. Isotherms for all PLs were also obtained by adding 1 ml ethanol in 1 l Hepes buffer solution. No difference in the isotherms was found for any of the PLs with or without appropriate amounts of ethanol. The isotherms in the figures show the surface pressure as a function of the mean molecular area (MMA) during compression.

2.3. Statistics

All experiments were repeated three times, and SDs were calculated by the Excel or Sigma plot programs.

3. Results

Fig. 1 shows the isotherms of a) DPPC and b) SAPC with and without drugs at pH 6.00. At this pH, the PLs are neutral while both CPZ and OLP are positively charged. The figure shows that all monolayers of SAPC occupy larger MMA than DPPC, presumably due to the presence of four double bonds in the *sn*-2 acyl chain in SPAC. Furthermore, the collapse points of SAPC monolayers are at lower surface pressure than DPPC, indicating less stable packing of the SAPC lipids. In addition, the isotherms for DPPC with and without CPZ do

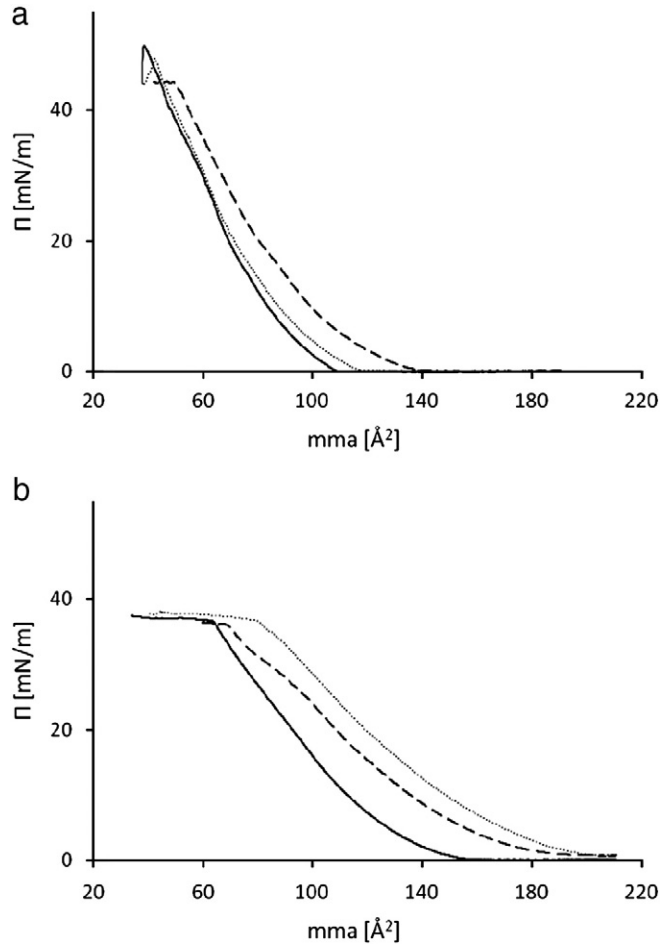


Fig. 1. The isotherms of a) DPPC and b) SAPC at pH 6.0. The isotherms of lipids without drug are shown in solid lines. The isotherms with CPZ and OLP are shown in dotted and hatched lines, respectively.

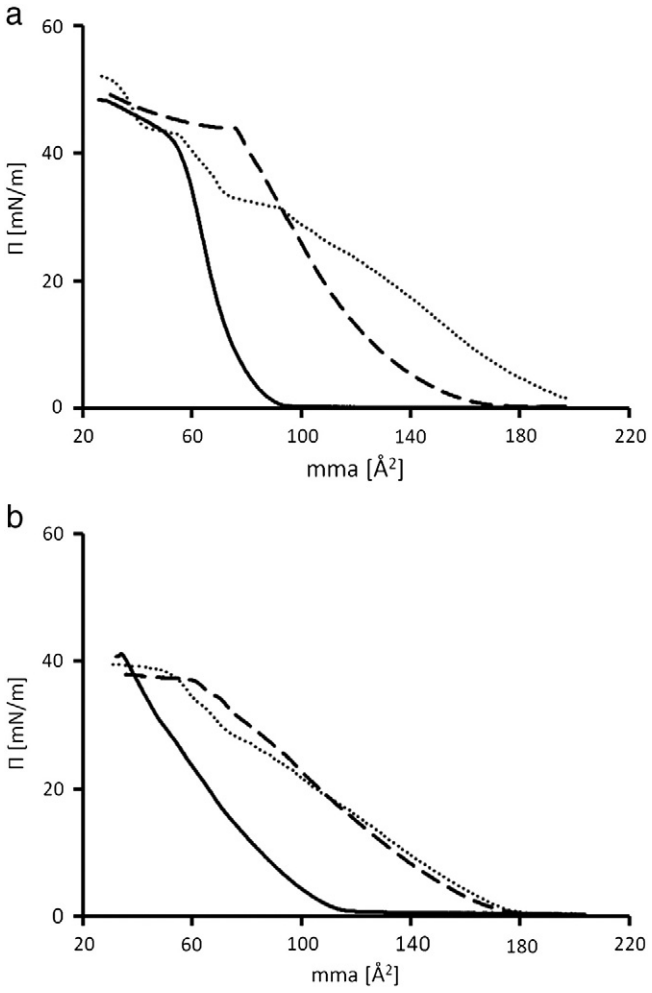


Fig. 2. The isotherms of a) DPPS and b) POPS at pH 6.00. The isotherms of lipids without drug are shown in solid lines. The isotherms with CPZ and OLP are shown in dotted and hatched lines, respectively. The isotherm of a) DPPS with CPZ (dotted line) shows the presence of two plateaus in the solid state, one at a surface pressure of 31.5 mN/m and the next at 42.4 mN/m. The isotherm of a) DPPS with OLP (hatched line) shows a plateau at a surface pressure of 44.1 mN/m. The isotherm of POPS shows that OLP and CPZ increase the MMAs.

not differ significantly. In contrast, the isotherms of DPPC with OLP on the subphase showed a distinct increase in the MMA. As shown in Table 1, OLP increased the MMA of DPPC with 24% at lift-off, 14% at surface pressure of 30 mN/m and reduces the collapse point by 24%. A reduction of the collapse point indicates that the monolayer is destabilized by OLP. The results indicate that CPZ did not change the monolayer packing of DPPC. OLP, on the other hand, may intercalate in the monolayer of DPPC and the intercalation induces destabilization of the DPPC monolayer. Both drugs increased the MMA of SAPC. Table 1 and Fig. 1 show that when CPZ was in the subphase; the MMA of SAPC increased by 31% at lift -off, 28% at surface pressure of 30 mN/m and reduce the collapse point by 17%. The changes in the SAPC monolayer with OLP in the subphase are 26%, 11% and 3%, respectively. These results indicate that CPZ has a more pronounced effect on SAPC monolayers than OLP. Fig. 2 shows the isotherms of a)

Table 1
Mean molecular area (MMA), in Å^2 of the glycerophospholipid monolayers at pH 6.00 with and without drugs at lift-off, at 30 mN/m and at the collapse point is shown. The SDs are included in the table.

Glycerophospholipid	Mean molecular area, Å^2			Mean molecular area change, %		
	Lift-off	Π = 30	Collapse	Lift-off	Π = 30	Collapse
DPPC	106.2 ± 2.0	59.1 ± 1.0	38.6 ± 0.1			
DPPC with CPZ	113.5 ± 1.0	61.4 ± 0.7	42.1 ± 2.1	7	4	9
DPPC with OLP	131.7 ± 1.7	67.2 ± 0.6	47.9 ± 0.9	24	14	24
SAPC	149.8 ± 3.2	74.8 ± 2.0	61.4 ± 0.5			
SAPC with CPZ	196.5 ± 5.2	95.8 ± 2.1	72.1 ± 0.7	31	28	17
SAPC with OLP	188.1 ± 8.0	83.0 ± 2.1	63.4 ± 0.7	26	11	3
DPPS	90.5 ± 2.5	61.9 ± 1,1	26.8 ± 0.5			
DPPS with CPZ	*	97.6 ± 1.7	27.7 ± 0.8		58	3
DPPS with OLP	162.9 ± 0.4	95.1 ± 0.7	26.8 ± 0.2	80	54	0
POPS	123.2 ± 15	49.6 ± 1.1	31.3 ± 2.1			
POPS with CPZ	173.0 ± 3.7	72.3 ± 2.9	50.1 ± 1.3	40	46	60
POPS with OLP	171.4 ± 1.6	80.3 ± 1.0	55.8 ± 2.9	39	62	78

The value noted with an asterisk is not defined.

DPPS and b) POPS with and without drugs at pH 6.00. At this pH both lipids are negatively charged while both drugs are positively charged. Both lipids without drugs had the lowest MMA, indicating that both drugs increased the MMA (i.e. the distance between neighboring lipid molecules) when present in the subphase. At lift-off CPZ increased the MMA of both lipids more than OLP, and more pronounced in the DPPS monolayers. The isotherms of DPPS with CPZ in the subphase increased the MMA area at 30mN/m with 58% and with 3% at the collapse point (Table 1). The isotherms of DPPS with CPZ in the subphase showed no defined lift-off. The changes in the MMAs for DPPS with OLP in the subphase were 80%, 54% and 0%, respectively. However, at surface pressure of 31.6 mN/m, the isotherms of DPPS with OLP in the subphase showed higher MMAs than the isotherms of DPPS with CPZ. Furthermore, the isotherms of DPPS with CPZ exhibited two plateaus, one at 31.6 mN/m and the second at 42.4 mN/m. Formation of plateaus indicate that lipids are expelled from the monolayer or are involved in coexisting phases [32].

The isotherms of DPPS indicated that both drugs did intercalate in the monolayer. However, the results might indicate that the mechanisms of intercalation may be different for the two drugs. Fig. 2 also shows the isotherms of POPS with and without drug at pH 6.00. The presence of drugs increased the MMA of POPS. At lift-off, CPZ increased the MMA of POPS more than OLP. However, at 16.6 mN/m, the isotherms of CPZ and OLP in the subphase have the same MMA of 115.5 Å^2. At 30 mN/m, OLP affected the monolayer of POPS more than CPZ. Table 2 also shows that change in MMA when CPZ is in the subphase is 40% at lift-off and 46% at 30 mN/m and 60% at the collapse point. When OLP is in the subphase, the changes in MMA are 39% at lift-off, 62% at 30 mN/m and 78% at the collapse point. The results indicate that both drugs were intercalated in the POPS monolayer. At pH 10.00 both drugs as well as DPPC and SAPC are all neutral, while DPPS and POPS are negatively charged. The isotherms of DPPC at pH 10.00 are shown in Fig. 3a, while the isotherms of SAPC are shown in Fig. 3b. The isotherms of pure DPPC and SAPC have the lowest MMA, and SAPC has the lowest collapse point of these two lipids. The isotherms for DPPC with both drugs increased the MMA of the monolayer, and no lift-off was observed in these isotherms under the experimental conditions used, suggesting that the monolayer packing arrangement do not include the gaseous phase when one of these drugs was present. The lipids in the monolayer are organized directly to the liquid phase when one of the drugs is present in the subphase. CPZ increased the MMA of the DPPC monolayer in the liquid phase more than OLP. Furthermore, at a surface pressure of 15.3 mN/m, both isotherms with drugs showed identical MMA of 131.7 Å^2. By further compression of the barriers to a surface pressure of 30 mN/m, the isotherm with OLP in the subphase show more increase of the MMA of DPPC monolayer than CPZ. Table 2 shows that the MMA increased

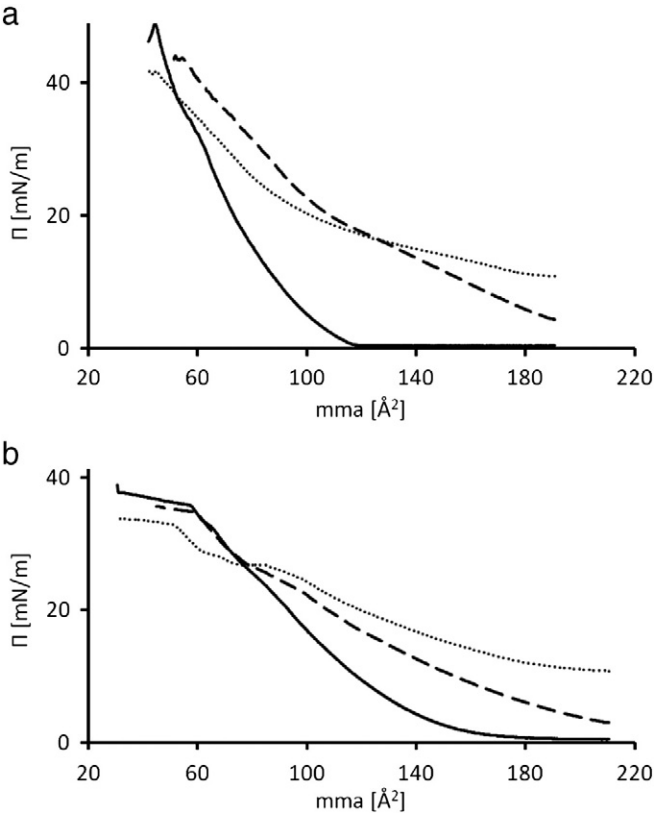


Fig. 3. The isotherms of a) DPPC and b) SAPC at pH 10.00. The isotherms of lipids without drug are shown in solid lines. The isotherms with CPZ and OLP are shown in dotted and hatched lines, respectively. The isotherm of b) SAPC with CPZ (dotted line) show the presence of a plateau at a surface pressure of 27.2 mN/m. The isotherms of DPPC with CPZ and OLP shows lower monolayer stability than the pure monolayer.

with CPZ by 12% at 30 mN/m and with 5% at the collapse point. The corresponding results with OLP in the subphase is 33% and 24%, respectively.

These results showed that CPZ is affecting the DPPC monolayer packing more than OLP in the liquid phase; however, the effects of OLP is more pronounced than CPZ at the surface pressure of 30 mN/m, which is the surface pressure of a biological cell membranes. Fig. 3b shows the isotherms of SAPC at pH 10.00. No lift-off was observed when one of the drugs were present in the subphase, and the increase in MMAs of SAPC monolayers was most pronounced with CPZ. However, upon compressing the barriers to a surface pressure of 26.1 mN/m, both drug-containing monolayers of SAPC were

Table 2
Mean molecular area (MMA), in Å^2 of the glycerophospholipid monolayers at pH 10.00 with and without drugs at lift-off, at 30 mN/m and at the collapse point is shown. The SDs are included in the table.

Glycerophospholipid	Mean molecular area, Å^2			Mean molecular area change, %		
	Lift-off	Π = 30	Collapse	Lift-off	Π = 30	Collapse
DPPC	114.2 ± 0.5	62.1 ± 0.6	45.5 ± 0.9			
DPPC with CPZ	*	69.6 ± 1.0	49.7 ± 0.7		12	5
DPPC with OLP	*	82.7 ± 1.0	56.6 ± 1.3		33	24
SAPC	174.6 ± 6.6	68.7 ± 1.0	54.7 ± 0.2			
SAPC with CPZ	*	60.1 ± 1.6	48.9 ± 0.6		−13	−11
SAPC with OLP	*	69.0 ± 1.1	50.2 ± 0.6		0	−8
DPPS	188.0 ± 3.9	69.1 ± 1,4	49.1 ± 1.4			
DPPS with CPZ	*	61.3 ± 1.9	37.0 ± 0.9		−11	−25
DPPS with OLP	*	55.3 ± 1.9	30.8 ± 1.0		−20	−37
POPS	139.0 ± 2.0	62.3 ± 1.0	49.1 ± 2.3			
POPS with CPZ	*	105.9 ± 2.3	40.6 ± 2.6		70	−17
POPS with OLP	191.9 ± 1.0	64.1 ± 0.5	35.4 ± 0.6	38	3	−5

Values noted with an asterisk are not defined.

concurrent at a MMA of 82.5 Å^2. Furthermore, at this surface pressure (26.1 mN/m), the CPZ isotherms showed only a small plateau. At 30 mN/m, OLP had a more pronounced effect on the SAPC monolayers. The CPZ isotherms had the lowest collapse point of the two isotherms, indicating that the SAPC monolayers with CPZ were less stable than the SAPC monolayers with OLP. These results suggested that both drugs became intercalated in the SAPC monolayers. Fig. 4a shows the isotherms of DPPS with and without drugs at pH 10.00. The isotherms with only pure DPPS showed a remarkable high collapse point as compared to the DPPS isotherms with drugs and the pure POPS isotherm (Fig. 4b). The results also indicated that the difference in stability of the two serine PL isotherms could be due to effects of different acyl chains. The results indicated that an extremely dense and stable lipid packing can occur in pure DPPS monolayers, and may be due to stabilization of the head group charge by Na^+ ions in the HEPES buffer and effective packing of the saturated acyl chains in DPPS. The results also indicated that both drugs increased the MMA of DPPS, and most with CPZ. The isotherms with CPZ present, did not show any lift-off. In contrast, the isotherm for DPPS with OLP in the subphase, a gaseous phase was observed. In the liquid and solid phases the isotherms with CPZ or OLP in the subphase, have the same course, and the CPZ isotherm increased the MMA of DPPS slightly more than OLP. In Fig. 4, the most striking results, were that the collapse points with DPPS with both drugs were dramatically reduced. Furthermore, the collapse point was reduced by 25% and 37% for the CPZ and OLP isotherms, respectively (Table 2). These results indicated that both drugs might be intercalated in the DPPS monolayers. The isotherms of POPS at pH 10.00 are shown in Fig. 4b. The isotherms of POPS with CPZ in the subphase, had no lift-off, and the effect of CPZ on the POPS monolayer showed a remarkable high increase in MMA. Consequently, CPZ had a more pronounced effect on the monolayer packing than OLP. When OLP was present in the subphase, a gaseous phase was observed in the isotherm. Furthermore, a plateau was observed in the isotherms with CPZ at a surface pressure of 32.4 mN/m. The difference between these isotherms can also be seen in Table 2, where at a surface pressure of 30mN/m, the MMA change was 70% and 3% for CPZ and OLP, respectively. The results indicated that both drugs intercalates in the monolayer of POPS. As shown in Fig. 5a, at pH 6.00, with a surface pressure of 30 mN/m, CPZ and OLP increased the MMA of the LPs. However, the effects of the drugs on the LP monolayers with serine head groups were most pronounced. At pH 6.00, OLP affected the POPS monolayers more than CPZ, and DPPS was more affected by addition of CPZ in the subphase. The effect of the drugs on monolayers with choline head groups, showed that CPZ increased the MMA of SAPC more than OLP, and that OLP in the subphase had a more pronounced effect on the DPPC monolayer than CPZ. At a surface pressure of 30 mN/m at pH 10.00 both drugs affected the monolayer packing of the PLs (Fig. 5b). CPZ caused the most pronounced increase in the MMA of POPS, but not in the SAPC monolayer. The former also showed an increase in the MMA, while the latter shows a decreasing MMA. OLP, on the other hand, produced a small increase in the MMA of POPS. However, OLP increased the MMA of DPPC more than CPZ. In Fig. 5b, two of the investigated PLs, DPPS and SAPC, showed decreasing MMA upon addition of CPZ to the subphase, most pronounced effect on SAPC monolayers. However, with OLP in the subphase, the MMA of the DPPS monolayer did also decrease. No change in the MMA was observed at 30 mN/m in the monolayers of SAPC upon addition of OLP to the subphase.

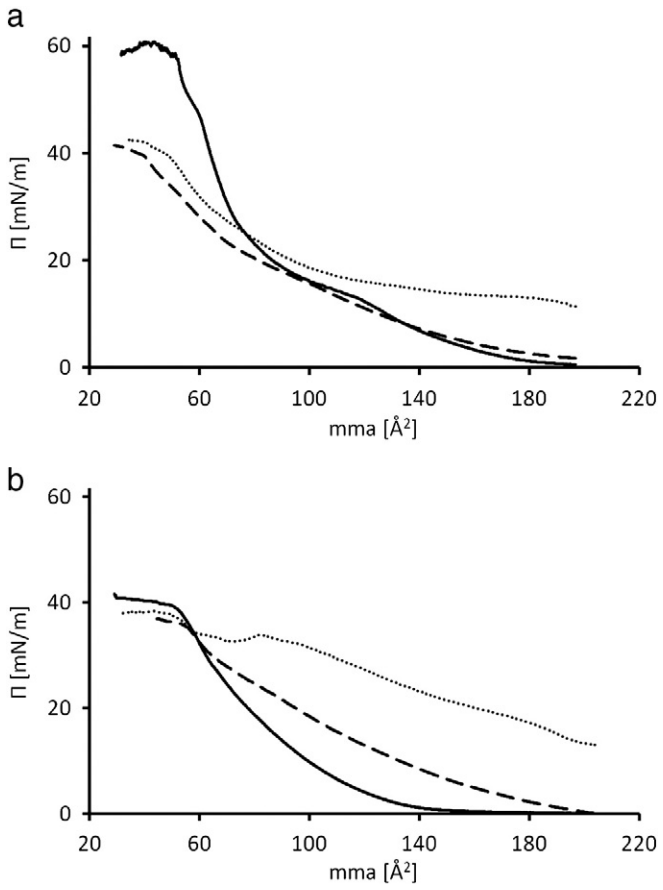


Fig. 4. The isotherms of a) DPPS and b) POPS at pH 10.00. The isotherms of lipids without drug are shown in solid lines. The isotherms with CPZ and OLP are shown in dotted and hatched lines, respectively. The isotherm of b) POPS with CPZ (dotted line) show the presence of a plateau at a surface pressure of 32.4 mN/m. The isotherms of DPPS with CPZ and OLP shows increased MMA.

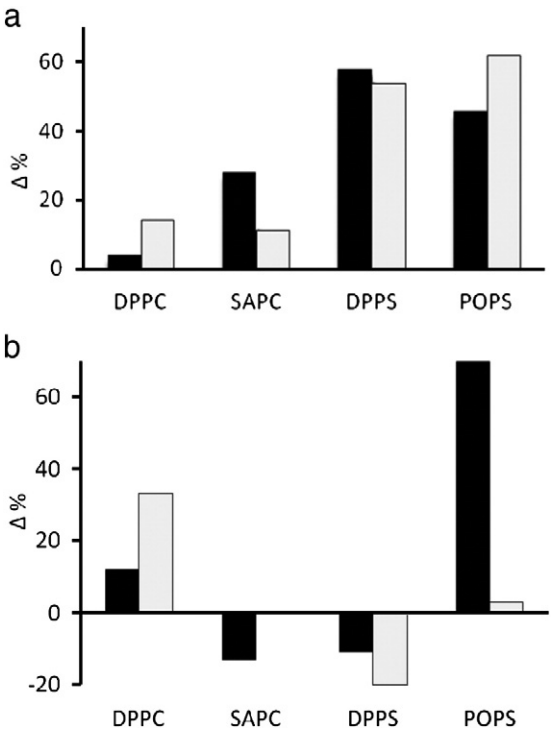


Fig. 5. a. At pH 6.00, CPZ (black columns) and OLP (grey columns) the MMAs of the LPs increases. The effects of CPZ and OLP on PLs with serine head group are most pronounced. OLP is affecting the POPS monolayer to a larger degree than CPZ, in contrast DPPS which is more affected by CPZ. b. At pH 10.00, both drugs influence the LP monolayer packing. CPZ (black columns) causes the most pronounced increase in MMA of POPS. OLP (grey columns) causes largest increase in MMA of DPPC. The drugs cause a decrease in MMA when added to DPPS and SAPC monolayer.

4. Discussion

Both CPZ and OLP are positively charged at pH 6.00 while at pH 10.00 OLP is neutral. CPZ has a pKa of ~9.2; however, the pKa value may change depending on the chemical environment. At pH 10.00, might consist of a mixture of neutral and positively charged species. The lipids with choline head groups are neutral at both pH values, and the lipids with serine head group are negatively charged. The isotherms of the monolayers of lipids with choline head groups (DPPC and SAPC) without drugs in the subphase at pH 6.00 and pH 10.00 do not differ significantly. This indicates that packing of these lipids is not pH-dependent. At both pH values, the choline head group is neutral, and changing the pH of the subphase does not affect the lipid organization in the monolayer of these lipids. However, the isotherms of SAPC monolayers show increased MMA compared to the DPPC monolayer due to the four double bounds in one of the SAPC acyl chains. Furthermore, the lipids with choline head groups both showed higher MMA than the lipids with serine head groups. This is presumably either caused by the size of the choline head group, which is larger than the serine head group, or higher mobility in the choline head group than the serine head group, or both. The isotherms of the PLs with serine head groups, POPS without drug, show slightly MMA at pH 6.00, while DPPS, on the other hand, showed pH-dependent monolayer packing, due to the observation of larger MMA at pH 10.00 than at pH 6.00. Further, the collapse point of the DPPS monolayer is significant higher at pH 10.00 than at pH 6.00, indicating that the DPPS monolayer packing is more stable at pH 10.00. The serine head groups are negatively charged at both pH values, and the subphase changes charge from positive to negative at pH 6.00 and pH 10.00, respectively. Findings in an earlier NMR study of bilayers of phosphatidylserine [33], are that the ^{31}P and ^{2}H relaxation times of phosphorus and deuterium in the phosphatidylserine head group are dramatically shorter than in the phosphatidylcholine head group. Shorter relaxation times are due to lower mobility in a rigid head group structure of the serine head group. The rigidity of the serine head group is explained by electrostatic interaction or hydrogen bonding between or within the phosphatidylserine head groups. At pH 6.00, the negatively charged serine head group might be stabilized by hydrogen bonding to excess H^+ ions in the subphase thus making a stable monolayer of DPPS lipids. At pH 10.00, the observed mean molecular area is enlarged, which means that the serine lipids are further apart in this monolayer. This might be explained by Na^+ ions present in the subphase, that interfere with the serine head group, presumably at the phosphate oxygen, to neutralize the repellent forces from the negatively charged OH^- ions in the subphase, making looser packed DPPS monolayers in all phases. The collapse point of these monolayers are remarkable high, and we suggest that this monolayer collapse when Wan der Waals's distance between Na^+ ions and the oxygen is reached. That the monolayer packing of DPPS is more stable than POPS is not concurrent whit the earlier NMR study [33], which showed that POPS is more rigid. In Langmuir terms this means that POPS monolayer should have higher collapse point. Our results showed the opposite. The explanation might be that the NMR studies are based on bilayers, while the Langmuir method is based on monolayer, where the acyl chains are solved in air. In NMR, the acyl chains in one leaflet are solved in the acyl chains in the other leaflet. The different results might be explained by the different acyl chain environment in the two methods. In a monolayer, the saturated acyl chains can pack closer than the unsaturated, which can explain the higher collapse point of DPPS.

4.1. DPPC

As seen from Fig. 1a, CPZ does not affect the monolayer packing of DPPC at pH 6.00. The drug is positively charged, and the lipid is neutral. The choline head group is mobile, and CPZ, due to the structure, is mobile, and is presumably accommodated within the DPPC unit cell close to the head group and subphase interfaces. We assume that the positive part of CPZ is close to the choline head group, and the hydrophobic part is intercalated into the DPPC acyl chain region. This is consistent with a solid-state ^{31}P-NMR that the positive part of CPZ interact with the negatively charge on the phosphate group of PLs [23]. This interaction will reduce intercalation of the drug into the acyl chain region. At pH 10.00, where both drug and lipids are neutral, CPZ affects the DPPC monolayer packing by increasing the MMA of the monolayers. When CPZ is present in the subphase, no gaseous phase is observed, it causes phase transition of the lipids in the monolayer directly in the liquid phase. Furthermore, since no lift-off is observed, CPZ is instantly affecting the DPPC monolayer. These results strongly suggest that CPZ is intercalated among the acyl chains and is not interfering with the choline head group. It may be reasonable to assume that CPZ is more deeply intercalated in the acyl chain of the DPPC monolayer at pH 10.00 than at pH 6.00, and this could be caused by hydrophobic forces between the uncharged CPZ phenothizine moiety and the acyl chains of DPPC. OLP, on the other hand, changes the monolayer packing of DPPC at both pH values, and more pronounced at pH 10.00. The intercalation is less effective at pH 6.00 and might be due to interaction with the charge of the phosphate group of DPPC, and is therefore less intercalated among the acyl chains than at pH 10.00. Like CPZ, OLP does not show the presence of a gaseous phase at pH 10. However, OLP is a less effective intercalating agent than CPZ until a surface pressure of 15.3 mN/m. At higher pressures, OLP is better intercalated than CPZ in DPPC monolayer. In the DPPC monolayer, the intercalation of drugs by hydrophobic forces is more effective. The explanation for the different behavior of CPZ and OLP in monolayer, might be that the structure of CPZ is more flexible than OLP, and will more easily intercalate at lower surface pressures. The CPZ molecule is folded about the N–S axis and the angel of the C–S–C bonds are 97.3° [34]. The contraction of the C–S–C bonds, the angle of the C–S–C bonds and the folding of the molecule are characteristic for related compounds [34]. On the other hand, a thienobenzodiazepine, like OLP, show polymorphism and can form aggregates [35–37]. This might explain why OLP intercalate better in DPPC monolayer.

4.2. SAPC

Our results show that both drugs affect the monolayer packing of SAPC at pH 6.00. The effect of the monolayer is most pronounced when CPZ is present in the subphase. In addition, the collapse point for the isotherm with CPZ is slightly higher than for the isotherm of SAPC without drug. This indicates that CPZ is stabilizing the monolayer packing of SAPC. The intercalation of both drugs is less effective at pH 6.00 than at pH 10.00. At the latter pH, both drugs intercalate more deeply into the hydrophobic acyl chains of the SAPC lipid monolayer, due to hydrophobic forces between the drug and the acyl chains. Both drugs are intercalated instantly in the monolayer causing phase transition of the lipids from gaseous to the liquid phase. The intercalation of CPZ is better than OLP at this pressure, due to a more flexible structure of CPZ. At a surface pressure of 26.1 mN/m, this isotherm is concurrent with the isotherms of SAPC without drug and the isotherm with OLP in the subphase. Furthermore, a small plateau in the CPZ isotherm is observed. At this pressure (solid state), both drugs are intercalated in the acyl chain region without disturbing the pure SAPC matrix. The appearance of a plateau in the CPZ isotherm might be due to monolayer domain formation or lipids being expelled from the monolayer into the subphase [22]. For the former to occur, both positively and neutral species of CPZ have to be present in the subphase. However, in other CPZ isotherms at pH 6.00, plateaus appears. It is therefore likely that there is only neutral CPZ species present in the subphase at pH 10.00, and that the appearance of the plateau is due to keep the monolayer stability upon increased

pressure; both lipid and drug might be expelled from the monolayer into the subphase, resulting in reduced MMA [22]. As shown in Fig. 5b at a surface pressure of 30 mN/m, CPZ shows decreased MMA of the SAPC monolayer, which might be caused of reduced lipid molecular motion which causes denser packing of the lipids and a decrease in the membrane fluidity. At 30 mN/m, the OLP isotherm, on the other hand, can be superimposed on the pure SAPC isotherm. Thus, OLP does not change the fluidity. Both drugs appear to affect the choline-containing lipids more at pH 10.00, and drug intercalation in the SAPC monolayer is better at pH 10.00, probably due to the hydrophobic forces between the drugs and the lipid acyl chains.

4.3. DPPS

Both drugs change the monolayer packing of DPPS at pH 6.00, and most pronounced with CPZ. It is reasonable to suggest that the positively charged drug is electrostatic attracted to the negative charge on the phosphate oxygen in the lipid head group [25], and is thereby inhibited to further intercalation within the acyl chains in the gaseous and liquid phases. Intercalation of the drug close to the head group will force neighboring lipids further apart in the monolayer, which increases the mean molecular area when CPZ or OLP is present in the subphase. However, in the isotherm with CZP, two plateaus appear, one in the liquid-to-solid phase transition, and the other in the solid state. The explanation might be that CPZ, which is a more flexible molecule than OLP, is penetrating deeper into the acyl chain region of the monolayer, making the head groups to pack closer in the liquid–solid phase transition. Further, in the solid state plateau, CPZ might be further intercalated into the acyl chain region of the lipid, before the monolayer collapses. Both drugs increase the MMA of DPPS at pH 6.00 more than at pH 10.00. At the latter pH the CPZ isotherm do not show a lift-off, and the intercalation of CPZ force the monolayer of DPPS into the liquid phase. Furthermore, at pH 10.00 the collapse point of the monolayer of pure DPPS indicates presence of a very stable monolayer, most likely due to stabilizing Na^+ ions close to the negatively charged serine head groups. The lipid packing in this monolayer is thus more effective than in the pure POPS monolayer. The effectiveness of the packing is due to both charge stabilization of the head group with the subphase interface and the effect of the saturated acyl chains in the DPPS monolayer. Saturated acyl chains occupy lower MMA than unsaturated acyl chains. Both drugs reduce the stability of the DPPS monolayer, with OLP having a stronger effect than CPZ. The results indicate that when one of the drugs intercalates into the monolayer, it disturbs the stabilization effect of Na^+ ions. At a surface pressure of 30mN/m, both drugs increase the MMA of the DPPS monolayer at pH 6.00, CPZ more pronounced. At pH 10.00, on the other hand, both drugs decrease the MMA of the DPPS monolayer, with OLP being more pronounced. At this pressure, the intercalation of these drugs causing a denser packed monolayer.

4.4. POPS

Both drugs increase the MMA of POPS monolayers at pH 6.00, whereas CPZ giving a slightly more pronounced increase in the gaseous and liquid phases. However, in the solid phase, the isotherm with CPZ shows a possible plateau, probably caused by intercalation of CPZ deeper into the acyl chain region of the monolayer. At a surface

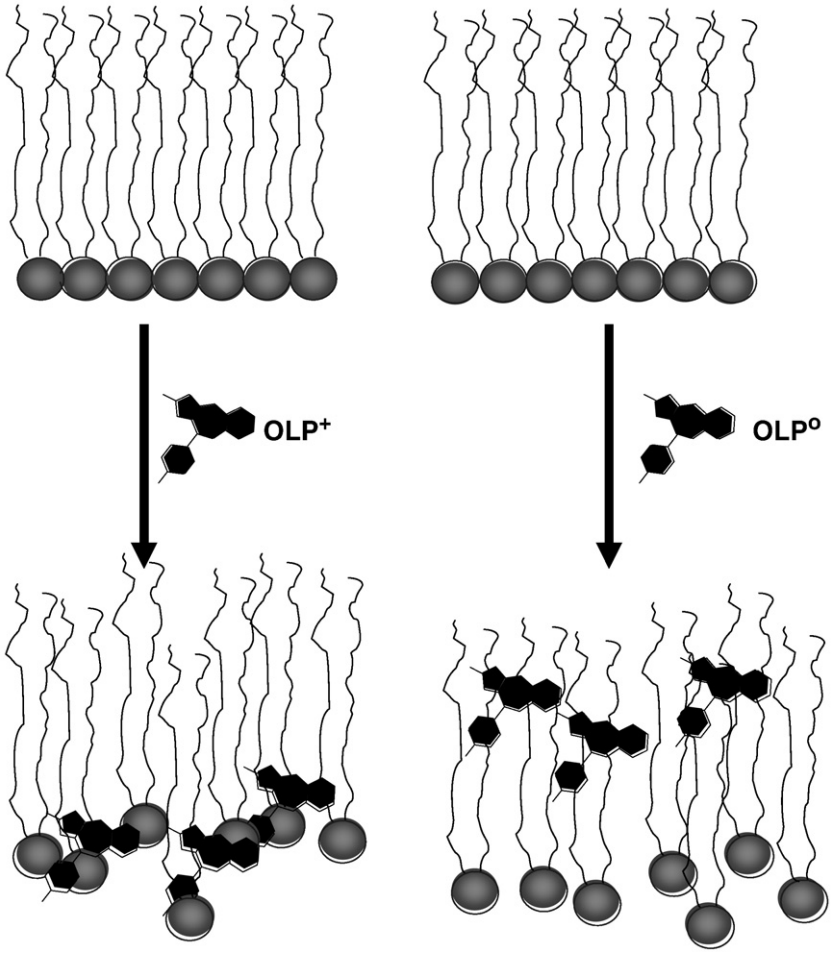


Fig. 6. The illustration shows how OLP may affect the PL monolayer by intercalating between the acyl chains of the PL. Left: OLP interacts with negative charged head groups, and the hydrophobic part of OLP is positioned intercalated between the acyl chains. Right: OLP has no net charge, and intercalates better in the acyl chain region of the PL monolayer.

pressure of 30 mN/m, OLP is affecting the monolayer packing of POPS more than CPZ. The POPS monolayer with OLP at this pressure induces a more loosely packed monolayer than the POPS monolayer with CPZ. At pH 10.00, the intercalation of CPZ brings the POPS monolayer into the liquid phase, in that no lift-off is observed. Further, in the CPZ isotherm, a plateau is appearing in the solid phase. It is reasonable to suggest that the flexible neutral CPZ molecule (34) is packed deeper into the acyl chains, and due to one unsaturated acyl chain, it is possible to compress the monolayer more tightly without increasing the surface pressure. Furthermore, at a surface pressure of 30 mN/m, OLP only gives a small increase in MMA of POPS, while CPZ show significant increase in MMA of the POPS monolayer.

In conclusion, the Langmuir technique used here shows that the monolayer packing of pure lipids with choline head groups did not show pH-dependent monolayer packing; however, in contrast, the lipids with serine head groups, demonstrate pH-dependent monolayer packing. DPPS at pH 10.00 is the most stable monolayer in the study, and both drugs have a destabilizing effect on the monolayer upon intercalation. At pH 6.00, where lipids with the serine head groups are negatively charged and the drugs are positively charged, the electrostatic attraction between the drugs and the serine head groups is the major contributor for the observed increase in the MMA. The drugs are located towards the head groups, and the uncharged part of the drug is located into the acyl chain region of the monolayer as illustrated in Fig. 6a. The monolayer packing of the lipids with choline head groups are at pH 6.00, show less effect on the monolayer packing upon drug intercalation. At pH 10.00, hydrophobic forces between the DPPC and the neutral drugs are the major contributor to increase in the MMA of DPPC caused by the intercalation of the drug more deeply into the acyl chains as shown in Fig. 6b. The hydrophobic interaction between SAPC monolayer and CPZ demonstrates that CPZ is deeply into the acyl chains of the SAPC monolayer, causing a denser packed SAPC monolayer upon intercalation. Drug intercalation in the DPPS acyl chain region induces denser packing of the monolayer. In POPS monolayer, the intercalation of drug induce looser monolayer packing. The appearance of plateaus upon CPZ in the subphase, is not due to the existence of two different charged CPZ species. Plateaus are observed in isotherms at both pH-values. Different structural features of the drugs might affect the intercalation. We suggest that CPZ might intercalate more easily due to a more flexible and smaller molecular structure than OLP. The results clearly show that the intercalation of CPZ and OLP into glycerophospholipid monolayer are pH-dependent.

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