Effects of albumin and erythrocyte membranes on spread monolayers of lung surfactant lipids

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Abstract

Dipalmitoyl phosphatidylcholine (DPPC), one of the main constituents of lung surfactant is mainly responsible for reduction of surface tension to near 0 mN/m during expiration, resisting alveolar collapse. Other unsaturated phospholipids like palmitoyloleoyl phosphatidylglycerol (PG), palmitoyloleoyl phosphatidylcholine (POPC) and neutral lipids help in adsorption of lung surfactant to the air–aqueous interface. Lung surfactant lipids may interact with plasma proteins and hematological agents flooding the alveoli in diseased states. In this study, we evaluated the effects of albumin and erythrocyte membranes on spread films of DPPC alone and mixtures of DPPC with each of PG, POPC, palmitoyloleoyl phosphatidylethanolamine (PE), cholesterol (CHOL) and palmitic acid (PA) in 9:1 molar ratios. Surface tension–area isotherms were recorded using a Langmuir–Blodgett (LB) trough at 37° C with 0.9% saline as the sub-phase. In the presence of erythrocyte membranes, DPPC and DPPC + PA monolayers reached minimum surface tensions of 7.3 ± 0.9 and 9.6 ± 1.4 mN/m, respectively. Other lipid combinations reached significantly higher minimum surface tensions >18 mN/m in presence of membranes (Newman Keul’s test, p < 0.05). The relative susceptibility to membrane inhibition was [(DPPC + PG, 7:3) = (DPPC + PG, 9:1) = (DPPC + POPC) = (DPPC + PE) = (DPPC + CHOL)] > [(DPPC + PA) = (DPPC)]. The differential response was more pronounced in case of albumin with DPPC and DPPC + PA monolayers reaching minimum surface tensions less than 2.4 mN/m in presence of albumin, whereas DPPC + PG and DPPC + POPC reached minimum surface tensions of around 20 mN/m in presence of albumin. Descending order of susceptibility of the spread monolayers of lipid mixtures to albumin destabilization was as follows: [(DPPC + PG, 7:3) = (DPPC + PG, 9:1) = (DPPC + POPC)] > [(DPPC + PE) = (DPPC + CHOL)] > [(DPPC + PA) = (DPPC)]. The increase in minimum surface tension in presence of albumin and erythrocyte membranes was accompanied by sudden increases in compressibility at surface tensions of 15–30 mN/m. This suggests a monolayer destabilization and could be indicative of phase transitions in the mixed lipid films due to the presence of the hydrophobic constituents of erythrocyte membranes.

Keywords: Phospholipid; Surface tension; Erythrocyte membranes

1. Introduction

Lung surfactant consists of 45–50% of dipalmitoyl phosphatidylcholine (DPPC), which is mainly responsible for its property of reducing surface tension inside the alveoli to near 0 mN/m during expiration. Other phospholipids and neutral lipids, which are present in relatively smaller amounts, are also necessary for the functioning of lung surfactant as they help DPPC to access the interface in repeated cycling during respiration. It is well known that below the transition temperature of 41°C, liposomes of DPPC are in the condensed gel state from which monolayers form very slowly hence, other fluidizing phospholipids are present in the lung surfactant along with DPPC to aid in adsorption [1]. The presence of phosphatidylcholines containing unsaturated fatty acids and other lipids promote the transfer of DPPC from a stable bilayer to a monolayer [2]. Presence of these phospholipids lowers the transition temperature of the mixture allowing a fluid state at 37°C and hence results in quicker adsorption.

Surfactant dysfunction due to hematological agents has been reported and can play a role in the pathogenesis of adult respiratory distress syndrome (ARDS). Our previous study had established a graded inhibitory potential of different hematological agents on spread DPPC monolayers with the maximum inhibition being due to erythrocyte membranes [3]. Though, albumin was non-inhibitory for spread DPPC monolayers [3],
it caused inhibition in studies of adsorbed surfactant mixtures [4,5]. Further, there is no gradation of the relative susceptibility of different phospholipid mixtures towards albumin and erythrocyte membrane inhibition. The present study was aimed at extending our previous work by comparing the effects of albumin and erythrocyte membranes on spread films of DPPC in the presence of other unsaturated phospholipids and neutral lipids and by grading the susceptibility of different lipid mixtures towards these inhibitors.

We evaluated the surface properties of spread monolayers of DPPC in combination with palmitoyloleoyl phosphatidylglycerol (PG), palmitoyloleyl phosphatidylethanolamine (PE), palmitoyloleyl phosphatidylycholine (POPC), palmitic acid (PA) and cholesterol (CHOL) in the presence of albumin and erythrocyte membranes. PG, POPC and PE were studied as examples of unsaturated phospholipids that are present in varying amounts in the lung surfactant. PA, though not a constituent of lung surfactant, was also evaluated as it is a constituent of a commonly used therapeutic surfactant and of model lung surfactants [6,7]. Hence its susceptibility to albumin and erythrocyte membranes are of interest. All the lipid mixtures were evaluated in a ratio of nine parts of DPPC to one part of non-DPPC (molar ratio). Though, lung surfactant consists of 45–50% of DPPC in bulk, the lung surfactant monolayer behaves like that of an enriched DPPC film [8]. Therefore, the effect of 10% non-DPPC allowing DPPC enrichment to 90% was evaluated in this study. Films containing 9:1 ratio of DPPC to non-DPPC are commonly used models of lung surfactants [9]. Further, the neutral lipids, predominantly cholesterol, in the lung surfactant constitute around 10 weight% of the total lipids [10]. Thus DPPC to cholesterol was evaluated in a 9:1 ratio. All other lipids were also evaluated in this same ratio for comparison. Apart from the 9:1 ratio films, DPPC:PG was also evaluated in an additional ratio of 7:3 as these lipids are present in this ratio in one of the protein-free therapeutic surfactants (Pumactant).

2. Materials and methods

2.1. Chemicals

2.1.1. Lipids and surfactants

l-a-Dipalmitoyl phosphatidylcholine (DPPC), palmitoyloleyl phosphatidylglycerol (PG), palmitoyloleyl phosphatidylethanolamine (PE), palmitoyloleyl phosphatidylcholine (POPC) and cholesterol (CHOL) all >99% pure were obtained from Sigma Chemicals Ltd. (St. Louis, USA). Palmitic acid (PA) (98% purity) was purchased from SISCO Research Laboratory (Mumbai, India). Bovine serum albumin (98% pure) was purchased from Sigma Chemicals Ltd. (St. Louis, USA). Palmitic acid (PA) and cholesterol (CHOL) all >99% pure were obtained from Sigma Chemicals Ltd. (St. Louis, USA). Palmitic acid (PA) (98% purity) was purchased from SISCO Research Laboratory (Mumbai, India). Bovine serum albumin (98% pure) was obtained from Sigma Chemicals Ltd. (St. Louis, USA). Palmitic acid (PA) and cholesterol (CHOL) all >99% pure were obtained from Sigma Chemicals Ltd. (St. Louis, USA). Palmitic acid (PA) and cholesterol (CHOL) all >99% pure were obtained from Sigma Chemicals Ltd. (St. Louis, USA).

2.1.2. Common chemicals

Sodium chloride (99.9% pure), and solvents used for cleaning of the Langmuir–Blodgett trough (acetone and methanol) were purchased from SISCO Research Laboratory (Mumbai, India). Ethylenediamine tetra-acetic acid (EDTA) was used as an anti-coagulant and was obtained from Loba Chemie Ltd. (Mumbai, India). Solvents (methanol and chloroform, 99.9% pure) used to spread phospholipid monolayers, were purchased from Spectrochem, Mumbai, India. The water used in all experiments was purified by means of a Milli-Q Plus water system (Millipore Corp., USA), having a surface tension of 70 mN/m at 37 °C and a resistivity of 18.2 MΩ cm.

2.1.3. Hematological inhibitors

Membranes obtained from whole blood, its various derivatives and bovine serum albumin were used as inhibitors to evaluate their effects on surfactant surface activity as described below.

2.1.3.1. Membranes (Mem). Blood was withdrawn from the ante-cubital vein of healthy human volunteers and was collected in plastic vials containing ethylenediamine tetra-acetic acid (1.5 mg/ml, final concentration in blood) as an anti-coagulant. Whole blood was then haemolysed at room temperature for 1 h in distilled water. This caused lysis of cells present in blood due to endosmosis. After centrifugation at room temperature, 500 g for 15 min, the cell membranes were separated. The pellet (membranes) obtained after centrifugation was suspended evenly in 0.9% saline before mixing into the sub-phase.

2.1.3.2. Separation of hydrophobic and hydrophilic components of membranes. Separation of hydrophobic and hydrophilic components of blood cell membranes was done using Bligh and Dyer’s method of lipid extraction, by extracting membranes with chloroform and methanol [11].

2.1.3.3. Albumin (Alb). Bovine serum albumin (98% pure) was purchased from SISCO Research Laboratory (Mumbai, India). Albumin was used corresponding to its concentration in whole blood when used in a ratio of 10 parts of surfactant per million parts of whole blood.

2.2. Protocols and parameters

All the surface activity studies were conducted in a Langmuir–Blodgett trough (KSV Ltd., Finland). The trough is equipped with two Delrin barriers that can be moved inward and outward at the same speed during compression and expansion cycles. The temperature of the subphase in the Langmuir–Blodgett trough was maintained at 37 ± 1 °C by an external circulating water bath. Wilhelmy plate made of platinum was used to sense the change in surface pressure during the experiment. It was roughened each time before the start of an experiment to ensure complete wetting of the plate. The trough was cleaned with deionized water, methanol, acetone and deionized water (again), in sequence several times. The surface of the sub-phase was cleaned with the help of an aspirator. Cleanliness was confirmed by a zero reading of surface pressure. Physiological saline (0.9% sodium chloride, pH 7.0) was used as the sub-phase for all surface activity experiments.

To study the effect of the inhibitors, the inhibitors were evenly mixed into the sub-phase by repeated, gentle pipetting and stirring of the sub-phase. Before spreading the surfactant
monolayer, 15 min were allowed for inhibitor adsorption. Surfactant solution was spread as tiny drops on the surface of the sub-phase to a concentration corresponding to 110 Å²/molecule. In those cases where organic phase (OP) obtained from membranes was used with surfactant, the required amount of OP was mixed with surfactant solution and spread on the surface with the help of a micro syringe. Surfactant solutions for film formation were prepared in a mixture of chloroform and methanol (2:1, v/v). After allowing 0.5 h for solvent evaporation, the monolayers were compressed and expanded at the same rate of 0.002 m/s without any wait time between compression and expansion. The number of cycles of compression and expansion completed per second with this speed is lower than the adult rate of respiration. However, this is the maximum speed with which, the instrument can be used without causing any mechanical disturbance to the monolayer. The monolayer was compressed up to 85% of the initial available surface area (maximum compression) to record the minimum surface tension.

The isotherms were recorded by compressing the monolayer at a constant rate of 0.002 m/s while continuously monitoring the surface pressure. After recording surface pressure–area isotherms, various other parameters were calculated from these isotherms to analyze the effect of inhibitors on the surface activity of surfactants.

All the parameters were calculated from the second compression cycle of the isotherms. The parameters studied were minimum surface tension, surface tension at 50% compression, percentage area change and compressibility.

2.3. Parameters studied

2.3.1. Minimum surface tension

The minimum surface tension attained at maximum compression was calculated by subtracting the value of maximum surface pressure from the initial surface tension of the sub-phase.

2.3.2. Surface tension (ST) at 50% compression

During a normal respiratory cycle, a total of 54% area reduction takes place. Simulating natural respiratory cycle in in vitro conditions, surface tension at 50% compression was recorded. For the calculation of percentage area change and compressibility, areas were normalized relative to that at 30 mN/m.

2.3.3. Percentage area change

Percentage compression required for the monolayer to reach a surface tension of 10 mN/m from that of 30 mN/m was calculated. The equilibrium surface tension of lung surfactant is around 27–30 mN/m [12] and a surface tension of <10 mN/m is essential for a functional surfactant [13]. This parameter is important to analyze the effect of inhibitors on lung compliance during respiration.

2.3.4. Compressibility

Compressibility was evaluated throughout the physiologically relevant range of surface tension 30 to the minimum surface tension reached. Trends in the changes in compressibility during compression were evaluated as a measure of monolayer stability. The absolute value of compressibility at a surface tension of 15 mN/m was also compared to study the efficiency of lung surfactant in reducing surface tension with minimal area change. An ideal lung surfactant should have low compressibility. A low compressibility ensures that a large surface area is available in the lungs for gas exchange.

\[ \text{Compressibility} = \left( \frac{1}{A} \right) \times \left( \frac{1}{m} \right) \]

Where as, \( A \), relative area; \( m \), slope of the surface tension–area curve. Inhibitory effects of various blood components on this parameter were studied.

2.4. Statistical analysis

Results obtained for minimum surface tension were analyzed for statistical significance using one-way analysis of variance (\( \alpha = 0.05 \)) and Newman’s Keul’s test (\( p < 0.05 \) as the cut off) for statistical significance.

3. Results

3.1. Susceptibility of lipid mixtures for membrane inhibition

Fig. 1A and B are the control isotherms for DPPC and lipid mixtures and Fig. 2A and B represent the surface tension–area isotherms in presence of membranes. DPPC + PE control was more surface active than pure DPPC and it reached a MST of 0.0 mN/m with a lower percentage area change that that of pure DPPC. However, this mixture was more susceptible for membrane inhibition than DPPC alone.

In the presence of the membrane inhibitors all the lipid mixtures studied were significantly inhibited. DPPC + PG (7,3), DPPC + POPC, DPPC + PA, DPPC + PA were more susceptible to inhibition by membranes in comparison to DPPC and DPPC + PA. DPPC + PA mixture was as susceptible as pure DPPC to membrane inhibitors. The results were analyzed using one way analysis of variance and were significant with \( F = 38.7, F_{\text{crit}} = 18, p < 0.05 \). Further Newman-Keul’s test confirmed that each of DPPC + PG (7,3), DPPC + POPC, DPPC + PA, DPPC + PA mixture was equally susceptible and significantly inhibited by membranes as compared to DPPC and DPPC + PA (\( p < 0.05 \)).

The susceptibilities of these phospholipid combinations was also checked with isolated organic phase of membranes (OP) in two different concentrations (ratio of phospholipids: OP = 1:1 and 1:100, w/w) as depicted in Fig. 3A and B. DPPC in presence of organic phase (1:1, w/w) achieved a MST of 13.0 ± 0.7 mN/m, DPPC + PG (9:1) was significantly more susceptible for OP inhibition (MST = 23.4 ± 0.7) in comparison to all combinations and pure DPPC. After DPPC + PG (9:1), other susceptible surfactants were DPPC + PG (7,3), DPPC + CHOL, DPPC + POPC and DPPC + PE, which were also significantly inhibited by OP (MST more than 18 mN/m) in comparison to pure DPPC and DPPC + PA (MST = 13.0 ± 0.7 and
15.4 ± 0.9 mN/m, respectively. The aqueous phase of membranes was not inhibitory (data not shown).

It is evident from Fig. 4 that all combinations of phospholipids were inhibited to the maximum extent by 1:100 ratio of PL to OP and the minimum surface tension achieved at the end of full compression was in the range of 20–23 mN/m. These values were achieved on 50% compression and further compression did not cause any reduction in these values.

3.2. Susceptibility of lipid mixtures for albumin inhibition

Albumin was not significantly inhibitory for DPPC surface activity but it inhibited the surface activity of all the lipid mixtures significantly except DPPC + PA (MST = 2.4 ± 0.7 mN/m) (Fig. 5A and B). Descending order of susceptibility of the lipid mixtures to albumin inhibition is as follows: [(DPPC + PG, 7:3) > (DPPC + PG, 9:1) > (DPPC + POPC)] > [(DPPC + PE) = (DPPC + CHOL)] > [(DPPC + PA) = (DPPC)]. DPPC + CHOL was more significantly inhibited by albumin than DPPC and DPPC + PA but it is interesting to note that in absence of albumin CHOL was more inhibitory to DPPC surface activity than in presence of albumin. (Summary of ANOVA: $F = 23.5, F_{\text{crit}} = 2.9, p < 0.001$.)

Inhibitory effects of albumin on compressibility at 15 mN/m were also observed. The values of compressibility
in decreasing order for different combinations were DPPC + PE > DPPC + CHOL > DPPC + PA > DPPC + PE. It was not possible to calculate this parameter in case of DPPC + PG (both ratios) and DPPC + POPC in the presence of albumin as they did not achieve a value of 15 mN/m. It is interesting to note that it was not possible to calculate compressibility at 15 mN/m for DPPC + CHOL in absence of albumin as it reached a ST of >15 mN/m but in presence of albumin, a MST of 10.6 ± 1.7 mN/m was reached and the compressibility at 15 mN/m was 0.0231 ± 0.003 m/mN.

3.3. Comparison of susceptibility of lipid mixtures for albumin and membrane inhibition

The minimum surface tensions achieved by the lipid monolayers in the absence and presence of albumin and erythrocytic membranes have been compared in Fig. 6. It was found that in the presence of erythrocytic membranes the minimum surface tension was significantly higher than that in the presence of albumin for DPPC, DPPC + PA, DPPC + CHOL, and DPPC + PE. In the cases of DPPC + PG and DPPC + POPC there was no significant difference in the minimum surface tensions reached in the presence of albumin and erythrocytic membranes. Thus, overall, erythrocytic membranes were inhibitory to all the lipid mixtures regardless of their albumin susceptibility. Membranes were more inhibitory than albumin in the case of DPPC, DPPC + PA, DPPC + CHOL and DPPC + PE and equally inhibitory in the case of DPPC + PG and DPPC + POPC. A similar trend was also observed for surface tension at 50% compression and percentage area change.

3.4. Changes in compressibility during compression

Monitoring changes in compressibility through film compression can be a very sensitive parameter, which can be suggestive of phase transitions during film compression [14]. This parameter was calculated at different area points after achieving a surface tension of 30 mN/m during compression. Effects of the organic phase of erythrocyte membranes and albumin, on all phospholipid combinations were analyzed by evaluating compressibility changes during compression. The compressibility of the mixed phospholipids monolayers showed sudden changes from near 0 to 5 mN/m in the presence of OP which led to monolayer collapse at a surface tension ≥19 mN/m.
Appearance of peaks in the compressibility versus surface tension curves due to sudden increases in compressibility depict monolayer destabilization and are suggestive of possible phase transitions. In case of effects of albumin, the change in compressibility was similar to the destabilization caused by OP for susceptible monolayers like DPPC:POPC which collapsed between 17 and 21 mN/m during compression. In contrast, DPPC and DPPC + PA monolayers, which were less susceptible to albumin inhibition, did not show any sudden changes in compressibility at high surface tensions indicating the presence of more stable monolayers.

4. Discussion
4.1. Relative susceptibility to erythrocyte membrane inhibition

DPPC in the presence of erythrocyte membranes achieved a minimum surface tension of 7.3 ± 0.9 mN/m whereas mixed films of DPPC:PG in the presence of erythrocyte membranes achieved a minimum surface tension greater than 20 mN/m (Fig. 2A). There was no difference between the susceptibility of DPPC:PG in 7:3 and 9:1 ratios in this regard. Similarly, the minimum surface tensions achieved by DPPC:POPC, DPPC:CHOL, and DPPC:PE in the presence of membranes were 20.7 ± 0.2, 15.1 ± 1 and 18.8 ± 0.4 mN/m, respectively (Fig. 2B). In the presence of unsaturated phospholipids and cholesterol, DPPC monolayers become more susceptible for inhibition caused by membranes. This may be due to the fluidizing effect of these lipids or of the membrane lipids. Whole cell membranes also contain fluidizing lipids, which may further reduce the stability of the monolayers causing collapse at a lower surface pressure.

On comparing the relative surface activity of PG, PE and POPC added DPPC monolayers (Fig. 1A and B) it can be inferred that the headgroup plays an important role in determining the surface activity of the monolayers. As seen in our study, the glycerol headgroup formed more fluid monolayers than that due to unsaturated forms of choline and ethanolamine lipids in the absence of membranes. This can be explained by the relative size and charge of the head groups, the preferred molecular shapes and orientations which in turn affect the packing of the monolayer.

However, regardless of the surface activity, all the unsaturated phospholipids were equally susceptible to membrane inhibition.
indicating a role of the degree of unsaturation in determining membrane–surfactant interaction and fluidization. The susceptibility to membrane inhibition was not determined solely by the fluidizing effects of the additive unsaturated phospholipids and membranes individually but by their interactions. As seen in Figs. 1 and 2, DPPC:POPC and DPPC:PE in the absence of membranes were in fact able to form rigid monolayers which achieved near zero values of minimum surface tension on film compression but were susceptible to fluidization in the presence of membranes.

DPPC:PA, on the other hand, formed rigid monolayers both in the absence and presence of membranes resisting collapse and inhibition (Figs. 1A and 2A) Our results indicate that though DPPC + PA combination was significantly inhibited by membranes with a minimum surface tension of 9.6 ± 1.4 mN/m, it was less susceptible for inhibition in comparison to other lipid combinations studied. A similar effect of reduced susceptibility to inhibition was also found by Cockshutt and Possmayer who have shown that addition of 10% PA to Survanta and lung surfactant extract (LES) counteracts the inhibition caused by lyso-PC [15].

In our study, in the presence of erythrocyte membranes which contain fluid lipids, lyso-lipids and/or fluid lipids, addition of PA to DPPC was helpful in preventing monolayer collapse during compression in comparison to other phospholipid combinations. This may be due to strong interactions between DPPC and PA and close packing of DPPC:PA monolayers which resist collapse. Palmitic acid is mostly protonated at physiological pH (pKa = 8.5) and such protonated molecules will have a cylindrical shape suitable for tight packing with DPPC. During expansion, the fatty acid will tend to fill the gaps and bare areas at the interface, while upon compression it will pack tightly with DPPC to form the gel phase. Similar interactions between palmitic acid and other lipids have been documented by Schullery et al. [16] and Ortiz and Gómez-Fernández [17] using differential scanning calorimetry which may explain the decreased susceptibility of DPPC:PA to membrane inhibition.

Overall susceptibility to membrane inhibition was as follows: [(DPPC + POPC) + POPC] > [(DPPC + CHOL) + CHOL] > [(DPPC + PA) + PA] > (DPPC). A similar trend was observed for all the parameters minimum surface tension, surface tension at 50% compression and percentage area change where the DPPC and DPPC + PA showed lower values as compared to the other lipid mixtures in the presence of erythrocyte membranes.

4.2. Effects of hydrophobic components of erythrocyte membranes

The addition of the organic phase of membranes led to an increase in the minimum surface tension achieved by all the phospholipids monolayers. Isolated hydrophobic components of membranes were more inhibitory than their hydrophilic components and were responsible for the inhibitory effects of whole membranes. As seen in Fig. 3A and B, when used in a 1:1 ratio with the surfactant lipids, the organic phase of membranes led to destabilisation of the monolayers with collapse plateaus at surface tensions of 15–25 mN/m. This suggests that the hydrophobic interactions between lung surfactant lipids and hydrophobic components of membranes, hinder the re-organization and packing of the surfactant lipids. Also the presence of phospholipases in the organic phase, might be responsible for the degradation of lipids. Degraded products of lipids/phospholipids are reported to be inhibitory for lung surfactant surface activity by several groups [18,19] and may be responsible for the inhibition observed in our study.

The presence of unsaturated phospholipids and cholesterol led to increased fluidization of the monolayers in the presence of organic phase of the membranes as compared to that of DPPC alone (Fig. 3A and B). The collapse plateau occurred by just 20% film compression for the monolayers DPPC:POPC, DPPC:PE and DPPC:PG 9:1 with the most part of the isotherms being horizontal. This could be due to flooding of the interface by the fluidising lipids of the membrane in these cases with desorption of the surfactant lipids.

The destabilization and fluidization due to flooding of membrane lipids at the interface was further confirmed by increasing the amount of membrane lipids used. As seen in Fig. 4, the monolayers of all the phospholipid mixtures showed collapse plateaus at the beginning of compression with collapse occurring within 25% of film compression. The only exception was that of DPPC with organic phase, which showed a collapse plateau at around 45% film compression. However, even in case of DPPC the shape of the DPPC monolayer changed from that in the presence of 1 part of OP being more similar to the DPPC membrane isotherm to that in the presence of 100 parts of OP which was similar to the isotherm of OP alone. The flooding of the interface with the organic phase of membranes led to a saturated response of fluid collapsed monolayers having surface activity similar to that of the membrane lipids.

4.3. Relative susceptibility to albumin inhibition

In our study, albumin was not inhibitory to spread DPPC monolayers but in the presence of other phospholipids like POPC, PE and PG, the spread monolayers become susceptible to albumin inhibition (Fig. 5A and B). DPPC films containing PE, PG, POPC and cholesterol reached minimum surface tensions above 10 mN/m, in the presence of albumin. DPPC:PA and DPPC alone are able to achieve low minimum surface tensions in the presence of albumin in spread films indicating that they are able to desorb the albumin molecules from the interface and maintain a rigid closely packed monolayer. This implies that the DPPC monolayer becomes more susceptible to destabilization by albumin in the presence of other phospholipids, which are unable to squeeze out albumin from the interface.

Addition of cholesterol was inhibitory for the surface activity of DPPC but in the presence of albumin, the MST obtained was lower than that of the control (Figs. 1B, 5A and 6). The minimum surface tension reached by the DPPC:CHOL monolayer was 13.9 ± 1.3 mN/m whereas in the presence of albumin, the minimum surface tension reached was 10.6 ± 1.7 mN/m. Cholesterol inhibits the extent to which surface tension can be reduced by lung surfactant upon compression presumably
due to the increase in the fluidity of the mixture and the ability of cholesterol to resist squeeze out upon film compression. Our results of the fluidizing effects of cholesterol in surfactant monolayers are in accordance with previous studies [20–22].

The reason for the decrease in the MST of the DPPC:CHOL monolayer from 13.9 to 10.6 mN/m in the presence of albumin is perhaps due to cholesterol molecules binding with albumin and making them less available for inhibitory effects. This is in accordance with the reported literature regarding an affinity between cholesterol and albumin [23,24]. Serum albumin plays a major role in cholesterol transport in circulation and ~24% of the non-esterified cholesterol is bound to serum albumin. Ha et al. [25] speculated that human serum albumin might harbor at least two binding sites for cholesterol. Similar interactions between albumin and cholesterol in our monolayers could be responsible for the effects on the minimum surface tension observed.

The relative susceptibility of the spread lipid monolayers to albumin inhibition was [(DPPC + PG, 7:3) = (DPPC + PG, 9:1) = (DPPC + POPOP)] > [(DPPC + PE) = (DPPC + CHOL)]. Though, the minimum surface tensions achieved by PG and POPC added films were similar and higher than that of all other combinations in the presence of albumin, Fig. 5A and B indicate that DPPC:POPC monolayer is relatively more susceptible than the PG added monolayer as it collapses by just 30% film compression.

4.4. Comparison of susceptibility of lipid mixtures for albumin and membrane inhibition

On comparing the relative susceptibilities of the different lipid monolayers to albumin and membrane inhibition, it was found that the monolayers showed a differential susceptibility to each of these inhibitors (Fig. 6). The minimum surface tension achieved in the presence of membranes was higher than that in the presence of albumin for DPPC, DPPC:PA, DPPC:CHOL and DPPC:PE. The minimum surface tension achieved by DPPC:PG showed similar responses of least susceptibility to albumin and membrane inhibition. On comparing the relative susceptibilities of the different lipid monolayers to albumin and membrane inhibition, it was found that the monolayers showed a differential susceptibility to each of these inhibitors (Fig. 6). The minimum surface tension achieved in the presence of membranes was higher than that in the presence of albumin for DPPC, DPPC:PA, DPPC:CHOL and DPPC:PE. Though, the minimum surface tension achieved by DPPC:PA was similar and higher than that of all other combinations in the presence of albumin, Fig. 5A and B indicate that DPPC:POPC monolayer is relatively more susceptible than the PG added monolayer as it collapses by just 30% film compression.

5. Conclusions

We conclude that spread monolayers of DPPC in the presence of unsaturated lipids are more susceptible for membrane inhibition than DPPC and DPPC:PA. The organic phase of erythrocyte membranes can destabilize mixed lipid monolayers leading to their collapse at higher surface tensions in comparison to that of DPPC in the presence of membranes. Membranes are inhibitory to all the mixed lipid monolayers studied regardless of their albumin susceptibility. A graded response to albumin inhibition exists namely [(DPPC + PG, 7:3) = (DPPC + PG, 9:1) = (DPPC + POPOP)] > [(DPPC + PE) = (DPPC + CHOL)]. Such graded susceptibilities may influence the effect of hematological agents on different lung surfactant monolayers.

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