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SOME CHARACTERISTICS OF MONOLAYERS OF 1-PALMITOYL-2-OLEOYL-PHOSPHATIDYLGLYCEROL WITH AND WITHOUT DIPALMITOYLPHOSPHATIDYLCHOLINE DURING DYNAMIC COMPRESSION AND EXPANSION

BLAIR D. FLEMING, CHRISTINA M. RAYNOR and KEVIN M.W. KEOUGH *

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3X9 (Canada)

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Some properties of monolayers of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol (POPG) alone or of POPG in mixtures with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) have been measured near 35°C during dynamic compression and expansion at 3.6 $cm^2 \cdot s^{-1}$. (2) The mean values of minimum surface tension (corresponding to maximum surface pressure) which could be obtained with pure POPG monolayers at high compression ranged from 15 to 18 $mN \cdot m^{-1}$ in the presence of Na^+, Ca^{2+} or low pH (2.0) in the subphase. (3) The presence of Ca^{2+} or low pH in the subphase increased the collapse plateau ratios obtained on cyclic compression. This might represent enhanced respreading into the monolayer of pure POPG from a collapsed form during reexpansion of the surface. (4) Monolayers containing 10% or 30% POPG and 90% or 70% DPPC could be compressed to surface tensions approaching zero. (5) In such mixed monolayers, 10% or 30% POPG did not appear to enhance respreading, as measured by collapse plateau ratios, in the presence of Na^+ or Ca^{2+} in the subphase.

Phosphatidylglycerol (PG) is a component of pulmonary surfactant in mature mammalian lungs [1], but it is absent from surfactant in immature lungs [2]. PG has been used as an indicator of fetal lung maturity [3]. The isotherms obtained from monolayers of mature (with PG) and immature (without PG) forms of rabbit lung surfactant were found to be different, and thus PG may be a modulator of surface behaviour in surfactant [2]. PG may act to facilitate the spreading of lipids into the monolayer at the alveolar surface [4–7]. PG made from egg-yolk phosphatidylcholine has been found to be effective in promoting the spreading of a dry mixture of PG and dipalmitoylphosphatidylcholine (DPPC) into the air/water interface [4] and bacterial PG has been observed to have limited effectiveness in aiding respreading of mixtures of PG and DPPC after monolayers have been compressed beyond collapse [5]. Under varying conditions other investigators have also observed that the presence of PG can increase the effectiveness of tracheal instillates of lipid dispersions in restoring lung pressure-volume characteristics [6,7].

PG could promote spreading because it can act as a fluidizing agent for rigid DPPC [4,5], but an additional attribute of PG may also contribute to lipid insertion into the monolayer. It has been observed that dispersions of dilauroyl PG have a number of thermotropic forms, especially in the presence of Ca^{2+} [8]. Also a number of thermo-

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PG, phosphatidylglycerol; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol.

tropic forms have been observed for dispersions of the Ca^{2+}-salts of ditetradecyl PG [9], and of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol (POPG) [10]. At least one of these forms might be a hexagonal II phase [9,10]. Depending upon Ca^{2+} concentration and the history of the sample, ^{31}P-NMR spectra which have been associated with hexagonal phase by others, have been obtained for dispersions of Ca-POPG at 37°C (Fleming and Keough, unpublished observation). Hexagonal phases have been implicated in the formation of regions of unusual lipid packing or potential bilayer instability in processes such as membrane fusion [11]. Such regions of instability might aid in the promotion of lipid insertion from bilayers to monolayers.

Investigation of the fatty acid composition and molecular species of PG have indicated that varying amounts of saturated and unsaturated PG exist in different mammalian lungs [12–15]. Where identified, the predominant unsaturated PG class was monoenoic, and the monoene containing palmitate and oleate comprised the major portion of this class [15]. We have investigated the effect of the mixing of POPG with DPPC on the minimum surface tensions which can be achieved in interfaces containing monolayers of the lipid mixtures. The respreading of lipids into the interface after monolayer collapse has been measured using collapse plateau ratios as employed by Notter et al. [5] for mixtures with bacterial PG. The effects of lowering the pH and of different counterions in the subphase on these properties were also determined.

Materials and Methods

DPPC and POPC were purchased from Sigma Chemical Co., St. Louis, MO. The DPPC gave only one spot on thin-layer chromatography. POPC contained about 2–3% of the reverse *sn*-1,2 positional isomer [16].

POPG was prepared from POPC by transphosphatidylation with phospholipase D in the presence of 50% glycerol according to the method of Comfurius and Zwaal [17]. Pure POPG was obtained by column chromatography on carboxymethylcellulose CM 52 (Whatman Inc., Clifton, NJ) [17]. The column was eluted with 10 bed volumes of chloroform, 6 bed volumes of chloroform/methanol (95 : 5, v/v), 6 bed volumes of chloroform/methanol (87.5 : 12.5, v/v) and finally chloroform/methanol (80 : 20, v/v) until POPG eluted.

Conversion of POPG to the sodium form was achieved by dispersing the lipid in 1 M NaCl at room temperature. The Na^+ : PG ratio was 2.76 : 1 (mol/mol). The dispersion was partially dried by evacuation. The material that remained after evacuation was taken up in 10 ml of chloroform/methanol (2 : 1, v/v). The resulting solution was shaken with 2 ml of water and, after centrifugation at 1000 rpm for 2 min, the upper phase was removed. The lower phase was brought up to 10 ml again with chloroform/methanol (2 : 1, v/v) and was then shaken against 2 ml of theoretical upper phase [18]. The phases were separated as before and the extraction was repeated once more.

The POPG-Na salt thus obtained was repurified by thin-layer chromatography on Silica Gel H using chloroform/methanol/water (65 : 25 : 4, v/v) as solvent. The gel was extracted twice with chloroform/methanol (1 : 1, v/v) and once with methanol. The pooled extracts were evaporated and the lipid was dissolved in chloroform and then passed through a combination of a 0.45 μm plus a 0.22 μm filter (Millipore Type MF). Aliquots of the resulting solution of POPG-Na in chloroform were brought to dryness and taken up in hexane/methanol (98 : 2, v/v) for surface measurements. Lipid phosphorus was measured by small modifications of the methods of Fiske and SubbaRow [19] or Bartlett [20].

Surface balance experiments were carried out on a Kimray-Greenfield Surfactometer [21]. In some experiments, the platinum dipping plate was roughened with fine emory paper and heated to a dull red colour over a Bunsen burner prior to use. In other measurements, dipping plates which had been sandblasted (e.g. Ref. 5) and cleaned in chromic acid and water were employed. The surfactometer was enclosed in a small chamber and the air temperature was maintained at 37 ± 1.5°C. Subphase temperature was measured at the end of each experiment and varied between 33–35.5°C. Exact surface temperature was unknown, but could possibly be below 33–35°C due to some evaporation. The presence of the lipid

monolayer would be expected to reduce evaporation over that of a clean surface, however, and consequently minimize the difference between the bulk and surface temperatures. Subphase solutions were prepared from water that had been deionized, distilled and then redistilled over alkaline potassium permanganate. The balance was routinely checked by compressing 7–8 nmol of DPPC. Barriers were changed until such monolayers were compressible to 0–1 $mN \cdot m^{-1}$ and occupied an area at least 44 $Å^2$/molecule at a surface tension of 20 $mN \cdot m^{-1}$ [21]. Solvents and chemicals were reagent grade or better and were obtained from Fisher Scientific Co., Dartmouth, Nova Scotia. Before adding subphase to the trough, the recorder zero was set to a surface tension of zero. After subphase was added it was compressed and aspirated several times before addition of lipid to ensure the surface was clean. Lipids were applied to the surface in $5 \cdot 10^{-6}$ or $1 \cdot 10^{-5}$ l of 'Pesticide grade' hexane(Fisher)/methanol (98 : 2, v/v) or of hexane/ethanol (9 : 1, v/v) and the solvent was allowed to evaporate from the surface for 5 min before the monolayer was compressed. Each monolayer was compressed at least twice at a rate of 3.6 $cm^2 \cdot s^{-1}$. The total available surface area of the balance was 53.4 cm^2 and the minimum area was 8.0 cm^2.

Results

Effects of low pH and counterions on monolayers of POPG

All samples were examined at two surface loads (initial packing densities). Since it was anticipated that the nature of the subphase might have effects on the properties of monolayers of POPG, the surface load conditions were established using monolayers of pure DPPC. The loads chosen were 7.4 nmol and 14.8 nmol, which give packing densities of 120 $Å^2$ per molecule and 60 $Å^2$ per molecule, respectively. Monolayers containing POPG were more expanded than those of DPPC alone, and even the low loads resulted in an initial surface tension of less than 70 $mN \cdot m^{-1}$ (Figs. 1–3).

In all cases, the POPG was used in the lipid solutions in the Na^+-form. Typical isotherms obtained from monolayers of POPG alone spread from solution on 0.154 M NaCl (pH 6.2), on 0.154 M NaCl (pH 2.0), or on 0.154 M NaCl/0.005 M $CaCl_2$, (pH 6.0) are shown in Fig. 1. Under no conditions could monolayers be compressed to achieve surface pressures such that the surface tension was less than 9 $mN \cdot m^{-1}$ and mean values were between 15 and 18 $mN \cdot m^{-1}$. The minimum surface tensions (γ_{min}) reached during compression are listed in Table I. Within the limits of precision and accuracy of measurements on monolayers compressed on this balance, all values of γ_{min} were equivalent. Adding Ca^{2+} to the subphase or lowering the pH to 2 did not significantly change the area per molecule at collapse in comparison to the value obtained at pH 6.2 in 0.154 M NaCl. ($0.1 > P > 0.05$ for Ca^{2+}; $P > 0.5$ for pH 2; *t*-test).

As an index of respreading of POPG alone, for monolayers at low initial loads, it was possible to obtained ratios of the lengths of the collapse plateaux from the points of collapse to the minimum surface area for compression 1 and 2 [5]. In monolayers at high initial loads, a reliable estimate of the length of the plateau could not always be obtained on the first compression, so the percentage of pool area at collapse for the second compression was taken as an indication of respreading. There is general agreement between these two measures of respreading (Table I). At low surface loads it was observed that collapse plateau ratios were greater at low pH or in the presence of 5 mM Ca^{2+} in the subphase in comparison to the ratios found in the presence of Na^+ alone. With 14.8 nmol on the surface, the same tendency toward increased respreading in the presence of 5 mM Ca^{2+} or pH 2 could be seen in the values of the percent area at monolayer collapse in cycle 2 (Table I, last column). At high loads, the difference between Na^+ alone and pH 2 was just marginally not statistically significant ($0.05 > P > 0.025$; *t*-test) whereas the difference between Na^+ alone and Na^+ plus Ca^{2+} in the subphase was significant ($P < 0.001$; *t*-test).

In Fig. 1a we have shown a dashed and a solid isotherm for the first expansion cycle, which represent a range of returns seen over a number of samples. While these differences could have arisen from an inherent characteristic of the mechanism of the force transducer in the Kimray balance, it is more likely that they occurred either because the

TABLE I

PROPERTIES OF MONOLAYERS OF THE SODIUM SALT FORM OF POPG

Each figure represents the $\bar{x} \pm SD$ for 3–8 experiments. Each subphase contained 0.154 M NaCl. The pH was adjusted and $CaCl_2$ added as indicated. [A,B,a]Superscript letters indicate significantly different pairs (t-test): lower case, $P < 0.005$; upper case, $P < 0.001$.

Subphase	Initial concentration (Å²/molecule)	γ_{min} (mN·m⁻¹)	Area/molecule at monolayer collapse on cycle 1 (Å²/molecule)	Cycle 2/cycle 1 collapse plateau	Percent of pool area at monolayer collapse on cycle 2
pH 6.2	120	18±2	57±12	0.52±0.11 [A,a]	
pH 6.2	60	16±1			42±4 [B]
pH 2.0	120	18±1	59±6	0.74±0.04 [a]	
pH 2.0	60	15±3			65±15
5 mM $CaCl_2$, pH 6.0	120	16±1	70±4	0.78±0.02 [A]	
5 mM $CaCl_2$, pH 6.0	60	16±2			71±7 [B]

rate of respreading of the collapsed monolayer was sufficient to produce a significant surface pressure, or because there was a small change in contact angle. The inclusion of 1 mM EDTA in the subphase did not abolish this effect. Sometimes expansion isotherms showed small abrupt changes in surface pressure, as can be seen in the different returns shown in Fig. 2d. These dislocations were not correlated with other parameters such as γ_{min}, γ_{max} (minimum, maximum surface tension) or the collapse plateau ratio. In some recent experiments we have attempted to observe the meniscus at the dipping plate during cycling of monolayers on subphases containing Na^+ alone, Na^+ plus Ca^{2+}, or NaCl/1 mM EDTA. Very slight movements of the meniscus could be seen when the surface tension was near collapse values for monolayers of POPG on all subphases. No differences could be seen before and after compression in the positions of the meniscus. The quality of the optical system was such that we could not assure ourselves that we could really measure the true contact angle. No gross changes such as tilting of the plate occurred. During compressions of mixtures of DPPC/POPG, 9:1, on the various subphases, a large drop in the meniscus on the dipping plate could be observed at high surface pressures (over 50 mN·m⁻¹), but the meniscus returned to its original position on re-expansion of the films. No correlation of this effect with any specific subphase was seen.

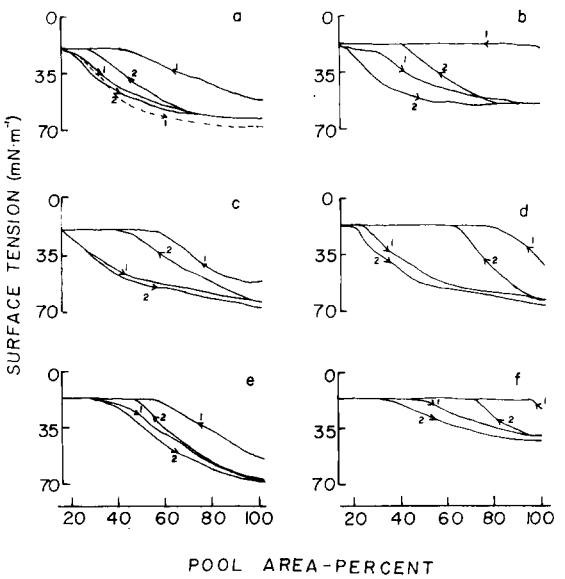

Fig. 1. Surface tension-area isotherms for monolayers of POPG-Na on various subphases. Cycles 1 and 2 are shown. (a) 7.4 nmol on 0.154 M NaCl (pH 6.2); (b) 14.8 nmol on 0.154 M NaCl (pH 6.2); (c) 7.4 nmol on 0.154 M NaCl (pH 2.0); (d) 14.8 nmol on 0.154 M NaCl (pH 2.0); (e) 7.4 nmol on 0.154 M NaCl/5 mM $CaCl_2$ (pH 6.0); (f) 14.8 nmol on 0.154 M NaCl/5 mM $CaCl_2$ (pH 6.0). These lipid loads correspond to initial concentrations of 120 Å²/molecule and 60 Å²/molecule. The solid and dashed lines shown on the first expansion represent the range of expansion isotherms observed over all samples.

In the isotherms of the expansion of POPG on the various subphases (Fig. 1), the surface tension remained near the collapse value for a significant expansion of the area. This effect was more pro-

nounced in the presence of Ca^{2+}, or in the presence of pH 2. This property may have reflected a change in contact angle. We were unable to observe any differences in the meniscus during cycles when this effect occurred or when it did not. In some recent experiments, the effect was also observed in some monolayers on NaCl plus 1 mM EDTA. We have employed two types of platinum plate, one type roughened by scratching and the other by sandblasting, and have found that there was no consistent or systematic correlation between the plates and any of the properties of these monolayers.

Monolayers of mixtures of POPG and DPPC

Isotherms typical of those obtained from monolayers of mixtures of POPG and DPPC spread from solution on either 0.154 M NaCl or on 0.154 M NaCl plus 0.005M $CaCl_2$ are shown in Figs. 2 and 3 together with isotherms for monolayers of DPPC alone. It can be seen in these figures and in the summary of data in Table II, that at this cycling speed, monolayers of DPPC alone or mixed with 10 or 30% POPG all reached very low surface tensions during compression. The minimum surface tension reached was independent of whether or not 5 mM $CaCl_2$ was present in the subphase. The areas per molecule at $\gamma = 20$ $mN \cdot m^{-1}$ for the four classes of monolayers of DPPC (Table II) were found not to be different from one another by analysis of variance. Similarly, the area per molecule at $\gamma = 20$ $mN \cdot m^{-1}$ for eight classes of monolayers containing DPPC plus POPG were found to be not significantly different from one another. The average of the areas per molecule at $\gamma = 20$ $mN \cdot m^{-1}$ of the total sample of monolayers of DPPC alone (48 ± 6 $Å^2$/molecule, $\bar{x} \pm$ SD, $n = 18$) was found by a t-test to be different from the average of all monolayers containing DPPC plus POPG (54 ± 6 $Å^2$/molecule, $\bar{x} \pm$ S.D., $n = 31$, $P < 0.005$).

Obtaining reliable collapse plateau ratios for the isotherms shown in Fig. 2 and 3 was more difficult than for those in Fig. 1. In the case of POPG alone, the monolayers collapsed sharply and remained flat (parallel to the abscissa) at surface tensions of 16–20 $mN \cdot m^{-1}$. For monolayers of lipid mixtures which reached very low surface tensions there were usually changes of

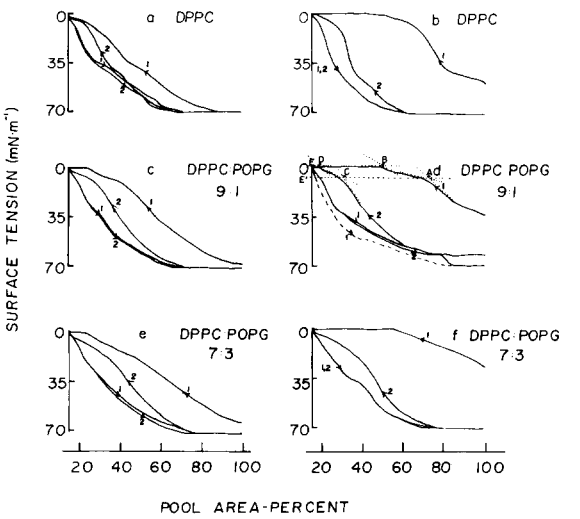

Fig. 2. Surface tension-area isotherms for monolayers of DPPC and DPPC:POPG-Na at molar ratios of 9:1 and 7:3 on a subphase of 0.154 M NaCl (pH 6.2). Cycles 1 and 2 are shown. In (a,c,e) the initial load of lipid was 7.4 nmol; in (b,d,f) the initial load was 14.8 nmol. These loads correspond to initial concentrations of 120 $Å^2$/molecule and 60 $Å^2$/molecule. The dashed and solid lines for the first expansion isotherm shown in (d) represent a range of isotherms seen over all samples. The two estimates of collapse plateau ratios given in Table II are obtained as shown in (d) where:

$$\frac{\text{length DE}}{\text{length BE}} = \text{estimate 1} \qquad \frac{\text{length CE}'}{\text{length AE}'} = \text{estimate 2}$$

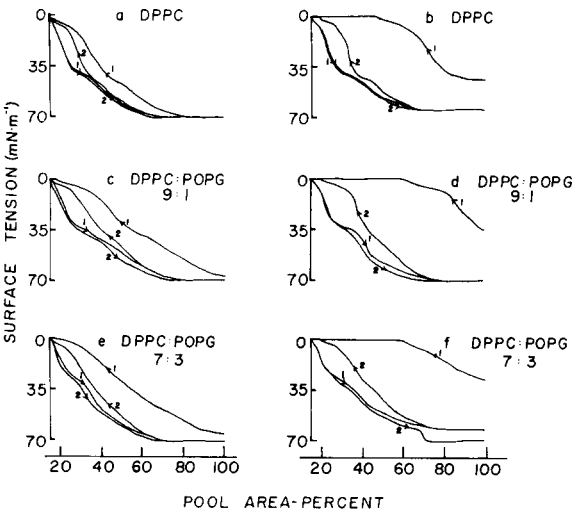

Fig. 3. Surface tension-area isotherms for monolayers of DPPC and DPPC/POPG-Na at molar ratios of 9:1 and 7:3 on a subphase of 0.154 M NaCl/5 mM $CaCl_2$ (pH 6.0). Cycles 1 and 2 are shown. In (a,c,e) the initial load of lipid was 7.4 nmol; in (b,d,f) the initial load was 14.8 nmol. These loads correspond to initial concentrations of 120 $Å^2$/molecule and 60 $Å^2$/molecule.

TABLE II

PROPERTIES OF MONOLAYERS OF MIXTURES OF DPPC AND POPG-Na ON VARIOUS SUBPHASES

Each figure represents the $\bar{x} \pm$ SD for 3–6 experiments. Monolayer compositions given as molar ratios. Each subphase contained 0.154 M NaCl. The pH was adjusted and $CaCl_2$ added as indicated. n.p. indicates that no collapse plateau was observed on cycle 2.

Monolayer composition	Subphase	Initial concentration (Å^2/molecule)	γ_{min} (mN·m^{-1})	Area/molecule at γ = 20 mN·m^{-1} (Å^2/molecule)	Cycle 2/cycle 1 collapse plateau length ratio	
					Estimate 1	Estimate 2
DPPC (100:0)	pH 6.2	120	1±1	50± 6	0.02±0.04	0.54±0.10
DPPC (100:0)	pH 6.2	60	0±0	48± 5	0.12±0.17	0.30±0.10
DPPC (100:0)	5 mM $CaCl_2$, pH 6.0	120	1±1	48± 9	0.14±0.28	0.63±0.09
DPPC (100:0)	5 mM $CaCl_2$, pH 6.0	60	0±0	48± 5	0.17±0.07	0.31±0.04
DPPC/POPG-Na (9:1)	pH 6.2	120	2±2	51± 5	n.p.	0.41±0.24
DPPC/POPG-Na (9:1)	pH 6.2	60	1±1	50± 2	0.13±0.12	0.33±0.06
DPPC/POPG-Na (9:1)	5 mM $CaCl_2$, pH 6.0	120	1±1	55± 3	n.p.	0.50±0.03
DPPC/POPG-Na (9:1)	5 mM $CaCl_2$, pH 6.0	60	0±0	52± 0	0.14±0.02	0.34±0.06
DPPC/POPG-Na (7:3)	pH 6.2	120	3±2	63±12	n.p.	0.56±0.16
DPPC/POPG-Na (7:3)	pH 6.2	60	0±0	55± 1	0.03±0.06	0.35±0.09
DPPC/POPG-Na (7:3)	5 mM $CaCl_2$, pH 6.0	120	1±1	53± 6	n.p.	0.55±0.11
DPPC/POPG-Na (7:3)	5 mM $CaCl_2$, pH 6.0	60	0±0	53± 2	0.13±0.05	0.28±0.05

compressibility $[(1/A) \cdot (dA/d\gamma)]$ at various surface tensions below 20 mN·m^{-1}. For most low initial loads, the isotherm did not achieve a flat portion on the second cycle. We have made two estimates of the collapse plateau ratios for each monolayer. The exact manner in which the estimates were obtained is given in Fig. 2d. The first, which was based upon only the portions of the isotherms which were flat, are shown in column 6 of Table II. A second estimate was made by taking the point of collapse as the intersection of the tangents to the isotherms in the regions of low and high compressibility in the range of 20 mN·m^{-1}. For the high initial loads it was not always easy to determine these points on the first compression. The collapse points taken are likely to give fairly conservative estimates of the cycle 2/cycle 1 lengths. These are shown in column 7 of Table II, and, like the values in column 6, show no significant differences (t-test) between DPPC alone and DPPC in the presence of POPG and/or Ca^{2+}.

Discussion

For pure POPG monolayers, the mean minimum surface tension achievable during compression was not lower than 15–18 mN·m^{-1}, no matter what subphase composition or initial lipid loads were employed. This observation is consistent with the presence of fluid fatty acid chains in the lipid in the monolayers in either the Na^+, Ca^{2+} or protonated forms. These monolayers were like monolayers at 23°C of bacterial PG which contained substantial amounts of unsaturated and cyclopropane fatty acids [5], and like monolayers of PG from rabbit lung which also had a fairly large amount of unsaturated lipid [12]. Differential scanning calorimetric studies of dispersions of Na-POPG indicate that it has a gel to liquid-crystalline phase transition near −3°C and thus it would have fluid acyl chains at 35°C [10]. The thermotropic properties of the protonated form of POPG have not been studied. For saturated PG the protonation of the headgroup raises the transition temperature by about 20°C above that of the Na form [23]. By analogy, the protonated form of POPG would still have fluid chains at 35°C. Calorimetric [10] and ^{31}P-NMR (Fleming and Keough, unpublished observations) studies of dispersions of Ca-POPG indicate that it has a complex, history-dependent thermotropic mesomorphism, and under some circumstances hexagonal or liquid crystalline phases may be present in the range of temperatures employed here. Thus fluid chains may also be present in the monolayers of POPG spread over the subphase containing 0.005

M $CaCl_2$. However, monolayer collapse at surface tensions in the range of 15–20 $mN \cdot m^{-1}$ can occur in the presence of rigid acyl chains also. Monolayers of dipalmitoylphosphatidylethanolamine, which has a transition temperature of 65°C [24], collapse at about 20 $mN \cdot m^{-1}$ [25,26]. Monolayers of dipalmitoylphosphatidylethanolamine will sustain high surface pressures equivalent to surface tensions near zero when the subphase pH is high (Ref. 26 and Hawco, M.W. and Keough, K.M.W., unpublished observations). Thus surface properties are affected by both chain fluidity and headgroup type and organization. All these molecular properties may influence the characteristics of the pure POPG films on different subphases.

Although the monolayers were spread using POPG in the Na form, the differences observed on the three different subphases (see Fig. 1 and Table I) would indicate that the bulk properties of the subphase caused a change in the counterions associated with the headgroup. We note that it has been found that, because of the preferential binding of Ca^{2+} over Na^+, an Na^+/Ca^{2+} ratio of 100 : 1 was necessary to abolish all the effects of Ca^{2+} using monolayers of dilauroyl PG [27]. It would seem reasonable to assume that most, if not all, of the POPG was converted to the respective protonated or Ca^{2+} form on the corresponding subphases.

The area per molecule occupied by Na-POPG was a little less than that found for bacterial PG [5] but was similar to that of unsaturated lecithins [21,22]. Compression rates and small leaks could have an effect on these area estimates. Changing the pH of the subphase did not change the area per molecule of POPG at collapse. The addition of Ca^{2+} tended to produce a larger area but the difference was not significant. It has been observed for monolayers of dilauroyl PG that, at lower surface pressures (under 40 $mN \cdot m^{-1}$), the presence of Ca^{2+} or low pH resulted in smaller areas per molecule than for the Na salt [27]. At a surface tension of 40 $mN \cdot m^{-1}$ (surface pressure = 30 $mN \cdot m^{-1}$) the isotherms of pure POPG indicated that there was no significant difference in area per molecule between the Na form (92.0 ± 8.9 $Å^2$, $\bar{x} \pm$ S.D.) and protonated form (85.3 ± 7.5 $Å^2$). There was an increase in the area per molecule of POPG to 109.3 ± 12.4 $Å^2$ per molecule in the presence of 5 mM $CaCl_2$ ($P = 0.025$). These differences may be due to kinetic effects associated with the compression rates, but they may also be a reflection of the differences in the complex mesomorphism of the lipids [8,10].

For the monolayers of pure POPG, the collapse plateau ratios for cycle 2/cycle 1 suggest that respreading is faster for the Ca^{2+} and the protonated forms than for the Na^+ form. Different excluded structures may arise depending upon the salt form of the POPG and respreading from each may be different. As noted above, there may be some possibility of hexagonal II and lamellar phase being formed by Ca-POPG at 35°C.

During the expansion phase of monolayers of POPG, especially those in the presence of Ca^{2+} or low pH, the surface tension remained at or near the collapse value until relatively high surface areas had been reached. It could be that the contact angle of the dipping plate was changed. Within our experimental limitations this possibility cannot yet be excluded. On the other hand, the phenomenon may reflect a special property of these monolayers such as an ability to respread very quickly and maintain dense packing for a longer time on expansion. There was no correlation ($r^2 = .126$) between the value of the collapse plateau ratio and the extent of the 'lag' in the surface tension expressed as a proportion of the first plateau. The monolayers of mixtures of POPG and DPPC did not show this unusual property.

When highly compressed, the monolayers of mixtures of 10% or 30% POPG with DPPC were all found to be capable of reaching very low surface tensions. This is consistent with the behaviour exhibited by monolayers of mixtures of DPPC with fluid phosphatidylcholines or with PG which have been studied previously [4,5,21,22,28, 29]. The values of γ_{min} were not affected by counterions, these being low under all conditions. When all monolayers containing POPG plus DPPC were compared with all those containing only DPPC, the mixed ones had slightly higher areas per molecule at $\gamma = 20$ $mN \cdot m^{-1}$. The values were not as high as those found for pure fluid phosphatidylcholines with this type of balance [21] and are consistent with the monolayers being composed of mixtures of fluid and rigid lipid at least up to a state of compression where $\gamma = 20$ $mN \cdot m^{-1}$.

The collapse plateau ratios as measures of respreading indicate that in the mixed monolayers, the presence of POPG was, at best, marginally effective in promoting this effect. Notter et al. [5] found that respreading of mixed films of DPPC and bacterial PG at 23°C was not large, and their preliminary absorption measurement at 37°C [5] also indicated the lack of a dramatic effect of PG in promoting insertion of lipid into the monolayer. The possibility of increased leak occurring at 37°C as opposed to 23°C could account for the small differences between our results and those obtained for the bacterial PG [5]. At 37°C there may also be more exclusion to nonmonolayer structures than at 23°C. Notter et al. [22] have observed with low loads of DPPC that films were more compressible at 37°C than at 23°C. Also, low cycle 2/cycle 1 ratios comparable to ours were obtained for DPPC alone at 37°C [23]. Increased compressibility at 37°C due to increased exclusion and/or leak could account for the small differences between the DPPC/PG mixtures studied here and those studied by Notter et al. [5]. Our cycling time (30 s/cycle) was substantially less than the one used by Notter et al. [5], and in our system there would have been less time during the expansion phase for respreading to occur. Although further studies will be required for a definite conclusion, the results suggest that if respreading of lipid from collapsed states is an important route for the replenishment of lipid at the air/water interface in lungs it would appear that unsaturated PG may not be the effective agent. The results would not preclude the possibility that PG may promote spreading of the surfactant into air/water interface during the initial insertion process.

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