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MONOLAYER CHARACTERISTICS OF SOME 1,2-DIACYL, 1-ALKYL-2-ACYL AND 1,2-DIALKYL PHOSPHOLIPIDS AT THE AIR–WATER INTERFACE

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SUMMARY

Surface pressure and surface potential-molecular area data have been obtained for some 1,2-diacyl, 1-alkyl-2-acyl and 1,2-dialkyl phospholipids at the air–water interface. Replacement of the ester linkages in lecithins by ether links has no significant effect upon the molecular packing in fully expanded or condensed monolayers and only a small effect upon the phase transition from condensed to expanded monolayer. Analogous effects are predicted for lecithins dispersed in excess water. Ether phospholipids have surface potentials which are 30–200 mV lower than those of the corresponding ester compound. This occurs because the carbonyl dipoles play a large role in determining the surface potential of phospholipid monolayers. Estimates are derived for the orientation of the carbonyl groups within the film at various molecular areas. It is concluded that, during the crystallisation of phospholipids in the transitions from expanded to condensed monolayer and liquid crystal to gel phase, the carbonyl groups are forced further out of the plane of the monolayer or bilayer. The biological significance of these findings is discussed.

INTRODUCTION

1-*O*-Alkyl glycerol lipids are ubiquitous constituents of animal cells as well as of certain microorganisms (for a review, see ref. 1). Dialkyl glycerol phospholipids are the major phospholipids of a halophilic bacterium, *Halobacterium cutirubrum*[2] and dialkyl glycerol phospholipids occur also in human heart[3], rat brain and ox heart[4]. The biochemical reactions leading to the formation of alkyl glycerol lipids have been investigated only recently[5]. However, the biological significance of this class of lipids is still completely unknown.

With regard to the essential role of phospholipids in biological membranes it is of interest to compare the physical properties of alkyl phospholipids and acyl phospholipids. To our knowledge such investigations have not been performed previously. SHAH AND SCHULMAN[6] studied the surface properties of the structurally related 1-*O*-1'-alkenyl 2-acyl glycerophosphoryl choline (plasmalogen). However, the lipid employed was isolated from natural sources and was a mixture of several molecular species which contained hydrocarbon chains of different lengths and degree of unsaturation. For exact comparisons between ether and ester phospholipids

the use of well defined synthetic substrates is essential. In the present study, monolayer characteristics of synthetic alkyl-acyl and dialkyl phospholipids and of the corresponding diacyl phospholipids carrying the same hydrocarbon chains are reported.

EXPERIMENTAL

Apparatus and procedure

A surface balance similar to that described previously[7] by one of us was employed to obtain continuous simultaneous surface pressure (π) and surface potential (ΔV) measurements as a function of surface area/molecule (A). The trough (40 cm × 15 cm × 1.5 cm) and barriers were milled from Teflon and the surface area was altered by moving the barriers at a constant rate with a motor controlled worm-drive. Surface pressures were measured with a glass Wilhelmy plate suspended from the arm of a Cahn recording microbalance. The output from the balance was fed to one channel of a two-channel recorder. The second channel received the output from a Keithley 610B electrometer which measured the potential between an ionising ^{241}Am electrode suspended above the air–water interface and a reference (platinum) electrode in the aqueous substrate.

The phospholipids were spread from hexane–ethanol solutions[8] onto 0.1 M NaCl solutions (pH 5). The barriers took about 4 min to traverse the whole length of the trough, but the time required to obtain the isotherms shown in Figs. 1–3 ranged from 1–2 min depending upon their degree of expansion. The trough was placed in a thermostatted box so that the temperature was maintained at 22.5 ± 0.5°. The temperature control during a particular run was better than ± 0.2°. It was possible to detect changes in π of less than 0.1 dyne/cm, but the reproducibility of a given surface pressure on respreading a monolayer was only ± 1.5 dynes/cm. This rather large error occurs because sometimes the contact angle on the Wilhelmy plate increased slightly during spreading causing the measured π to be upto 3 % low. The areas could be reproduced to ± 1.5 Å^2 per molecule and the surface potentials to ± 10 mV.

Materials

The substrate materials and spreading solvents have all been described previously[8].

Chemical synthesis of phospholipids:

(i) Phosphatidylcholines: *rac*-1,2-dioctadecyl, 1,2-dihexadecyl and 1,2-dioctadecen-9′-yl glycerophosphorylcholine were prepared from the appropriate 1,2-dialkyl glycerols[9], following the procedure of HIRT AND BERCHTOLD[10] as modified by EIBL *et al.*[11]. *Rac*-1-octadecyl 2-oleoyl glycerophosphorylcholine was prepared by the same method. The intermediate 1-octadecyl 2-oleoyl glycerol was prepared by the action of pancreatic lipase[12] on 1-octadecyl 2,3-dioleoyl glycerol; the latter was synthesized from 1-octadecyl glycerol and oleoyl chloride by standard procedures[13]. The *rac*-1,2-diacyl glycerols utilized in the synthesis of diacyl glycerophosphoryl cholines were synthesized by acylation of 3-*O*-tetrahydropyranyl glycerol[14] with either two identical fatty acids (stearic, palmitic or oleic acid) or by stepwise acylation with stearic acid in position 1 and oleic acid in position 2, followed by acidic hydrolysis to remove the protecting group. (ii) Phosphatidylethanolamines: The *rac*-1,2-dialkyl

glycerophosphoryl ethanolamines were synthesized *via* the 1,2-dialkoxy-3-iodo propanes as described earlier[15]. *Rac*-1-octadecyl 2-oleoyl glycerophosphoryl ethanolamine was prepared in an analogous manner, the intermediate 1-octadecyl-oxy 2-oleoyloxy-3-iodopropane was synthesized according to BAYLIS *et al.*[16]. Diacyl glycerophosphoryl ethanolamines were prepared according to the procedure of DAEMEN *et al.*[17].

Intermediates and final products were purified by preparative thin-layer chromatography as described earlier[15]. To remove traces of silica gel the substances were passed through Sephadex G-25 columns[18]. The purity of phospholipids was checked by thin-layer chromatography, using 3 different solvent systems: chloroform–methanol–water (65:25:4, by vol.)[19], chloroform–acetone–methanol–water–acetic acid (50:20:10:5:10, by vol.)[20] and chloroform–methanol–7 M NH_3 (65:35:5, by vol.)[20]. In each case only one spot with an R_F identical to that of authentic standards was detectable after spraying with molybdic acid or charring after spraying the plate with 50 % H_2SO_4. In addition, the phosphate content of the phospholipids was determined and found to agree with the calculated values. With phospholipids containing ester bonds the correct ester: P ratio was found. Ester bonds were determined by the method of EGGSTEIN[21].

For the remainder of this paper the convention is adopted of referring to ester linked fatty acids as alkanoyl and ether linked fatty acids as alkanyl.

RESULTS

Figs. 1 and 2 depict the π–A isotherms at room temperature for the di-ester and di-ether lecithins containing palmitic, stearic and oleic acid chains. Force–area curves for the di-ester compounds have been published before from this laboratory[8,22], but the isotherms for the di-ether phospholipids are original. Because of alterations in experimental conditions, the present isotherms show minor differences from those

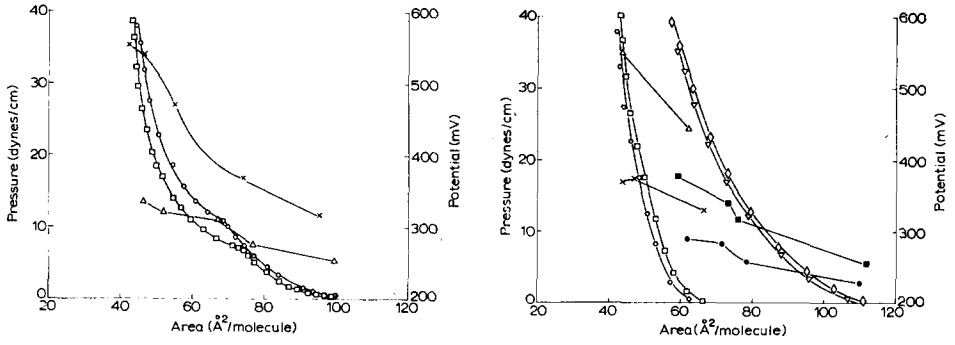


Fig. 1. Surface pressure (π) and surface potential (ΔV) – molecular area curves for dipalmitoyl (ester) and dipalmityl (ether) lecithins on 0.1 M NaCl at room temperature. □, π for dipalmitoyl lecithin; ○, π for dipalmityl lecithin; ×, ΔV for dipalmitoyl lecithin; △, ΔV for dipalmityl lecithin.

Fig. 2. Surface pressure (π) and surface potential (ΔV) – molecular area curves for distearoyl, distearyl, dioleoyl and dioleyl lecithins on 0.1 M NaCl at room temperature. ○, π for distearoyl lecithin; □, π for distearyl lecithin; ◇, π for dioleoyl lecithin; ▽, π for dioleyl lecithin; △, ΔV for distearoyl lecithin; ×, ΔV for distearyl lecithin; ■, ΔV for dioleoyl lecithin; ●, ΔV for dioleyl lecithin.

published previously. However, the essential features are unchanged and these correlate well with the observations of other workers. Thus both the dipalmitoyl and dipalmityl lecithins undergo a two-dimensional condensation[8,23]. The stearic acid containing compounds give fully condensed isotherms[8,24,25] whilst the introduction of double bonds as in the oleic acid containing compounds gives rise to fully expanded isotherms[22,25]. The data of Fig. 3 for some mixed chain phospholipids show that introduction of a single double bond as in 1-stearoyl-2-oleoyl lecithin is sufficient to give a fully expanded isotherm (*cf.* DEMEL *et al.*[25]). The 1-stearyl-2-oleoyl phosphatidylethanolamine which contains one ether and one ester linkage undergoes a two-dimensional phase transition as does the equivalent di-ester compound[26].

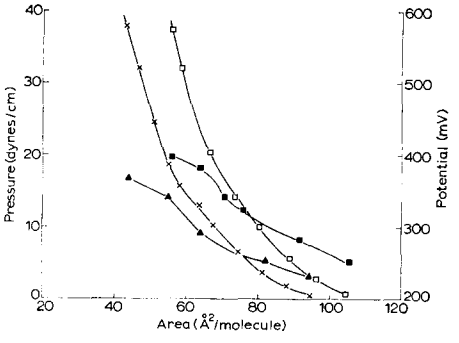


Fig. 3. Surface pressure (π) and surface potential (ΔV) – molecular area curves for 1-stearoyl-2-oleoyl lecithin and 1-stearyl-2-oleoyl phosphatidylethanolamine on 0.1 M NaCl at room temperature. □, π for 1-stearoyl-2-oleoyl lecithin; ×, π for 1-stearyl-2-oleoyl phosphatidylethanolamine; ■, ΔV for 1-stearoyl-2-oleoyl lecithin; ▲, ΔV for 1-stearyl-2-oleoyl phosphatidylethanolamine.

The ΔV–A curves for the above phospholipids are also presented in Figs. 1–3. The original data were obtained as continuous recorder tracings and the symbols in Figs. 1–3 are simply used as labels and to mark inflections. It is clear that the slopes of these curves for the dipalmitoyl and dipalmityl lecithins change at about 70 Å²/molecule in the region of the two-dimensional condensation. At lower areas/molecule where the monolayers are condensed the ΔV–A curves diverge, with that of the di-ester compound reaching much higher values of ΔV. The ΔV–A variation for dipalmitoyl lecithin is similar in form and magnitude to that on 0.15 M NaCl obtained by VILALLONGA *et al.*[27]. The ΔV–A curves (Fig. 2) for the distearoyl and distearyl lecithins have been smoothed to give the straight lines shown. In fact the recorder tracings showed small deviations from linearity during compression but these small fluctuations were not reproducible. The data for distearoyl lecithin are consistent with those reported for condensed lecithin films[8,28]. ΔV–A curves for dioleoyl and dioleyl lecithins have not been published previously. The values of ΔV for the dioleoyl lecithin are similar to those observed with other fully expanded lecithin monolayers[7,8,23]. ΔV increases more or less linearly with decreasing molecular area except for the region between 70–80 Å²/molecule where the curves show an inflection. The reason for this effect is not known, but it may be associated with the presence of double bonds, since it does not seem to occur with saturated lecithins. The data of Fig. 3 do not exhibit this phenomenon, but otherwise they are similar. It is apparent that the replacement of one ester link by an ether link can decrease ΔV and that

decreases are observed with phosphatidylethanolamines as well as with lecithins. With all the ΔV–A curves presented, it was found that at areas greater than "lift-off" ΔV showed random variations of the order of 100 mV which indicates that the films were liquid-expanded and not vapour-expanded.

Summarising the data of Figs. 1–3 it is apparent that replacement of the two ester linkages in lecithins by ether links has no significant effect upon the π–A curves of fully expanded or condensed monolayers and only a small effect upon the phase transition from condensed to expanded monolayer. In contrast, there is a dramatic effect upon the magnitude of ΔV in all cases, and the ether compounds have surface potentials which are 30–200 mV lower than those of the corresponding di-ester phospholipid.

DISCUSSION

Comparison of π–A curves

The physical states of phospholipid monolayers at the air–water interface and their correlation with the structures occurring in the lyotropic mesomorphism of lecithins have been described by one of us[8]. Bearing this correlation in mind, the π–A curves of Figs. 1–3 indicate that the packing of di-ester and di-ether lecithins in the gel and liquid–crystalline phases in excess water are essentially the same. This would suggest that the heat of chain-melting would be the same for both the di-ester and di-ether compounds. The only significant difference in the force–area curves of the two types of compounds is shown in Fig. 1. It seems that the dipalmityl lecithin has to be compressed to a lower area/molecule and higher pressure before it crystallises. The initiation of a phase transition within a monolayer is extremely sensitive to variations in experimental conditions such as rate of compression and temperature. Nonetheless the data in Fig. 1 coupled with knowledge of the temperature coefficient of pressure at which two-dimensional condensation occurs[8], suggest that the liquid–crystalline transition temperature for the di-ether compound is about 3° lower than that of the equivalent di-ester compound. Differential scanning calorimetry confirmed this expectation because 50 % (w/w) dispersions of these two lecithins gave gel to liquid–crystal transition temperatures of 41 and 38° for dipalmitoyl and dipalmityl lecithins, respectively. ABRAMSON[29] has recently compared the liquid–crystalline transition temperatures of dipalmitoyl and dipalmityl lecithins in excess water and found that the temperatures differ by 3°, but that the di-ether compound melts at a higher temperature. The reason for this discrepancy is perhaps due to differences in sample purity.

We can conclude that the interaction energies between phospholipid molecules in monolayers and bilayers are hardly affected by replacement of ester links by ether links and that the molecular packing is essentially the same for both types of compounds.

Comparison of ΔV–A curves

It has become conventional[30] to express ΔV for neutral monolayers in terms of a surface dipole moment (μ) where

$$\Delta V = 4\pi n\mu \qquad (1)$$

This overall perpendicular moment μ is comprised of three components μ_1, μ_2, μ_3 (ref. 31). μ_1 is the change in moment which arises from reorientation of water dipoles around the film-forming molecules. μ_1 cannot be measured and is usually incorporated into μ_2, the vertical component of the permanent dipoles of the insoluble molecules. μ_3 arises from the bond at the upper limit of the monolayer and will have a value[32] of (—)0.3 D (Debye) for the paraffinic chains discussed in this paper. It is possible for μ_3 to assume much larger values than this and for ω-monohalogenated compounds μ_3 can be as great as (—) 1 D. Such substitutions in close-packed hexadecanoic acid monolayers decrease ΔV by 1100 to 1300 mV (ref. 32). Apart from this large change and that caused by the introduction of charge (the double-layer potential is taken as constant throughout this discussion), ΔV for neutral *n*-alkane compounds is relatively insensitive to minor changes in structure (*e.g.* chain length) and particularly to variations in the headgroup[33] (*e.g.*–OH for –COOH) and therefore μ_2.

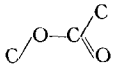
Since the change from ester to ether linkages in phospholipids leads to a decrease in ΔV, it follows that the carbonyl dipoles give a large positive contribution to $(\mu_1 + \mu_2)$. The dipole is orientated such that, in the vertical plane, the negative pole is further away from the radioactive electrode. The contribution of the two carbonyl dipoles at 60 Å^2/molecule causes the diester lecithins to have ΔV's 85–110 mV higher than their diether analogues. SHAH AND SCHULMAN[6] have shown that at 60 Å^2/molecule, ΔV for a natural plasmalogen sample was 140 mV lower than that of dipalmitoyl lecithin. However, in this work it was assumed that the carbonyl dipoles have a negligible vertical component and the difference in ΔV was attributed to the presence of an additional induced dipole in the double bond of the vinyl ether linkage of the plasmalogen. The role of the induced dipole was inferred by analogy with its effect in a 2-octadecenoic acid monolayer which has a μ equal to twice that of oleic acid[34]. The ΔV data for the di-ether phospholipids now indicate that the removal of a carbonyl group in the plasmalogen molecule would itself lead to a decrease in ΔV and the whole effect cannot be attributed to the presence of a double bond vicinal to the ether bond oxygen atom.

It is interesting to note that in monolayers of single chain paraffinic compounds (*e.g.* fatty acids) the situation is different and carbonyl groups do not make a big contribution to μ. Thus removal of the carbonyl group on changing from stearic acid to octadecanol or octadecyl methyl ether[35] hardly changes ΔV. Similarly in close-packed films of both ethyl palmitate[36] and cetyl ethyl ether[37] μ is about 0.2 D.

There are difficulties associated with the concept of a surface dipole moment and these have been discussed by GAINES[30]. Generally μ is one-third to one-tenth of the magnitude of the intrinsic molecular dipole moment of the film-forming molecules. Thus the magnitude of ΔV cannot be quantitatively accounted for. From a consideration of bond moments and orientations STANDISH AND PETHICA[38] concluded that, in a condensed phosphatidylethanolamine monolayer, the total normal dipole moment of 0.67 D can be principally attributed to the two fatty acid ester linkages alone. This conclusion is supported by the observation[7] that μ for a condensed diglyceride film is 0.61 D. The present observations on the effects of the removal of carbonyl groups confirm that the C = O dipole plays a large role in determining ΔV for phospholipids. Comparison of ΔV for the di-ester and di-ether lecithins gives a quantitative estimate of the contribution of the C = O dipoles to $(\mu_1 + \mu_2)$. The fact that the molecular packing of monolayers formed from these two types of

lecithins is essentially the same suggests that, on removal of the carbonyl groups, no gross structural rearrangements occur within the film. It may therefore be permissible to assume that $\mu_1 \ll \mu_2$ and attribute changes in ΔV caused by removal of C = O groups solely to changes in μ_2. If this is done it is then possible to estimate the orientation of the carbonyl groups within the film at various molecular areas.

Conversion of the ΔV data of Fig. 2 to μ–A plots *via* Eqn. 1 indicates that for distearoyl lecithin μ decreases from 0.72 D at 60 Å²/molecule to 0.64 D at 44 Å²/molecule. For distearyl lecithin the equivalent figures are 0.54 and 0.43 D, respectively. Therefore, if it is assumed that the two carbonyl groups are equivalent, at 44 Å²/molecule each C = O dipole contributes about 0.1 D to μ. If this 0.1 D is taken as a true dipole change within the phospholipid molecule, then with a knowledge of its bond moment, the orientation of the C = O dipole can be calculated. For these calculations, we shall employ the standard[39] bond moment for the C = O bond of 2.4 D and an "interfacial bond moment" of 0.3 D which has been used for similar treatments in the past[36,37]. If θ is the angle of the dipole to the vertical, then substitution of these bond moments gives Cos θ = 0.1/2.4 and Cos θ = 0.1/0.3, respectively. The C=O dipoles are therefore orientated at about 2 or 20° to the plane of the interface, depending upon which bond moment is employed. In the liquid-expanded state the difference in μ for the di-ester and di-ether lecithins is about 0.13 D. Repeating the above calculations with this value indicates that the C = O dipoles are either about 1 or 12° to the interface. It therefore seems likely that the C = O bonds are always orientated within 20° of the interface. A contribution to this restriction could arise because all five atoms of an ester group



are coplanar because of the partial double bond character of the O–C bond[40]. The configurational freedom will be greater in the equivalent region of an ether phospholipid.

Generally any detailed interpretation of the variation of μ with molecular area is difficult[30]. However, where there are no large changes in μ on compression of the monolayer, it is likely that no gross reorientation of the headgroup dipoles occurs. This condition appears to hold for liquid-expanded monolayers of fatty acids and alcohols[41,42], but in the condensed region μ usually decreases on compression. The lattter effect is probably due both to enhanced mutual polarisation of the dipoles as their packing density is increased and some structural rearrangement within the headgroup region. Where μ for liquid-expanded monolayers undergoes large changes on compression (*e.g.* fatty esters[36]) reorientation within the headgroup region of the film occurs.

Conversion of the ΔV–A data of Figs. 1–3 into μ–A curves indicates that while the monolayers are liquid-expanded μ decreases on compression. This suggests that some reorientation within the film is occurring. When the films become condensed, this effect is enhanced such that μ decreases even more sharply. However it is striking that in condensed films μ for the di-ester lecithins decreases more slowly than that of the di-ether lecithins. It is this effect that gives rise to the divergence in the ΔV–A curves of Fig. 1 at low areas/molecule. We may conclude that, during the condensation of

di-ester lecithin monolayers, the C = O dipoles become tilted more steeply to the interface and make a greater contribution to μ. The difference in μ for the di-ester and di-ether lecithins increases from 0.13 to 0.21 D on going from expanded to condensed films. The difference of 0.08 or 0.04 D per C = O dipole can be attributed to the change in angle of tilt of this bond. Making calculations similar to those described above, the swings away from the horizontal are about 1° if the bond moment is taken as 2.4 D and about 8° if the bond moment is taken as 0.3 D. In the light of the correlation[8] between monolayers and the smectic mesophase in water of lecithins, similar reorientations of the carbonyl groups might be expected during the liquid-crystal to gel transition.

Biological significance

Replacement of a 1-acyl group by an alkyl group affects the chemical behaviour of the whole phospholipid molecule. For example, the ester bond in position 2 is more resistant to the action of phospholipase A_2 in alkyl-acyl than in diacyl phospholipids (F. PALTAUF, unpublished result). Similar observations were reported with 1-*O*-1'-alkenyl 2-acyl glycerophospholipids (plasmalogens)[43]. The different behaviour in enzymic hydrolysis of alkyl-acyl and diacyl phospholipids might be explained by different molecular packing of the two types of lipids which could result in steric hindrance in the case of the alkyl-acyl derivative. However, the similarity in the force-area curves of alkyl and acyl phospholipids suggests that the molecular arrangement of phosphatidylcholines and phosphatidylethanolamines does not depend upon whether the molecule carries alkyl or acyl substituents.

As shown above, the absence of an ester C = O dipole leads to a decrease of the surface potential of alkyl phospholipids. This change in surface potential is not likely to have much effect on the total interaction energy of a phospholipase molecule with an ordered array of phospholipid molecules such as a monolayer or bimolecular lamella. Thus the C = O group plays some specific role in the enzymic hydrolysis and its removal leads to inactivation. Besides providing a particular electronic environment it is possible that preservation of the planar configuration of the five atoms of the ester group[40] by the carbonyl group is also essential for hydrolysis. The recent finding that 1-*O*-alkyl glycerols are acylated with fatty acids by microsomal enzymes of the rat small intestinal mucosa in a strictly stereospecific manner, in that only the *sn*-1 isomer acts as a substrate, while the acylation of 1-acyl glycerols to di- and triglycerides is not stereospecific[44,45] again suggests that C = O groups can also exert a neighbour group effect. Thus the ester bond in position 2 is protected by an alkoxy group in position 1 during both enzymic attack by phospholipase A_2 and non-enzymic alkaline hydrolysis[46,47].

The fact that ether phospholipids are stable over a wide pH range against hydrolysis could be a reason for the presence of high levels of these molecules in halophilic bacteria. The cell envelope of *Halobacterium cutirubrum* which is only viable in more than 3 M NaCl contains 13 % (w/w) of ether phospholipid[48]. The above monolayer data indicate that their packing is likely to be similar to that of the equivalent ester phospholipid. As a result it is chemical differences between the molecules that will be important.

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