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SELECTED LIPID MONOLAYERS ON AQUEOUS–GLYCEROL AND AQUEOUS–UREA SUBSTRATES

D. A CADENHEAD AND K E BEAN*

Department of Chemistry, State University of New York at Buffalo, Buffalo, N.Y 14214 (U.S.A.)

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SUMMARY

A comparison is made of the relative effects of glycerol and urea on a variety of selected lipid monolayers on aqueous substrates, through both surface pressure and surface potential changes. While both additives were found to relax or further expand most partially expanded films as well as to lower their surface potential, urea was found to produce the greatest expansion while glycerol produced the largest lowering in the surface potential.

The results are interpreted in terms of both hydrocarbon chain–substrate and dipole–dipole interactions and show that the latter alone cannot provide a satisfactory explanation. It is observed that the physical state of the film prior to addition of urea or glycerol is of prime importance and that films at, or close to, the liquid expanded–liquid condensed phase transition are particularly sensitive to change in substrate composition. The implications of these findings to other aqueous lipid aggregates and the gel–liquid crystalline phase transition, as well as to cell membrane structure, are pointed out.

INTRODUCTION

The physical state of a monomolecular film is determined, not only by film molecule interactions, but also by interactions between the film and its substrate. Our own investigations[1–3] have shown that on changing the nature of the substrate by adding glycerol[1,2] or ethanol[3], the physical state of a wide variety of films may undergo a transition to a more expanded state. We have typically observed that the addition of glycerol to a film substrate will lower the surface potential of all films be they condensed or expanded, and will further expand a partially expanded film. Condensed films, thus treated, show little or no expansion, and then only at low surface pressures.

We now propose to expand our studies to urea substrates for several reasons: (1) Sears[4] has observed effects with condensed films on urea substrates, similar to those observed by us with condensed films on glycerol substrates, and it would be interesting to see if this correspondence still exists with partially expanded films,

* Present address: Department of Chemistry, University of Northern Arizona, Flagstaff, Ariz., U.S.A

where the expansion of the film is much more significant. (2) Corner and Marquis[5] have shown that glycerol, alcohols or urea all exhibit a capability of relaxing bacterial protoplasts and thus increasing the degree of osmotic shock survival. Since both glycerol and alcohols[1–3] show expansion effects on monomolecular films, it would seem reasonable to expect that urea would behave in a similar fashion and that the two effects (monolayer expansion and bacterial protoplast relaxation) might have a common explanation. (3) The ability of glycerol, alcohols and urea to enhance low temperature cell survival[6], presumably arises, not only through their role as a cytoplasmic antifreeze, but also through their ability to fluidize a lipid bilayer structure (*i.e* to lower the lamellar liquid cyrstalline–gel transition temperature, T_c) Bearing in mind the excellent correspondence between physical states and phase transitions in monolayers at the air–water interface and those for lamellar lipid dispersions in excess water[7], it would again seem reasonable that monomolecular film studies should throw considerable light on the ability of such additives to increase cell survival.

EXPERIMENTAL

Materials

The monolayer substances myristic, pentadecanoic and palmitic acids, methyl pentadecanoate, and methyl palmitate, were obtained from Applied Science Laboratories, the stated purities of $> 99.5\%$ being verified by gas–liquid chromatographic analysis. These substances, rather than synthetic phospholipids, were chosen because of their ready availability, high purity and ease of handling, also because previous studies with glycerol had shown the effects under consideration to be determined by the physical state of the film (including phospholipids) and not by precise molecular structure. Research grade *n*-hexane (99.99 mole % purity) from the Philips Petroleum Company was used as the spreading solvent for the compounds as supplied after checking for the absence of surface active impurities on the film balance Research grade NaCl was roasted at 500 °C for at least 8 h before use to remove possible organic contaminants. The urea and glycerol used were both A C.S. certified grade compounds. Substrate solutions containing these compounds were passed through a column of granular activated carbon to remove surface active impurities. Reagent grade conc. HCl was redistilled before use. All water used was triply distilled, the second distillation being from alkaline potassium permanganate solution.

The surface pressure and surface potential as a function of area per molecule were obtained simultaneously using an automated Langmuir–Wilhelmy trough which has been described elsewhere[8]. When either glycerol or urea was used in the substrate solution, the calomel electrode, used in conjunction with a radioactive electrode to measure surface potential, was replaced by a similar electrode in which both the inner and outer chambers were filled with a solution containing the appropriate substrate solution saturated with KCl

All isotherms were obtained at a compression rate of approximately 13 cm^2/min. Since varying amounts of monolayer forming compounds were used, the corresponding compression rate per molecule varies from 4 8–20 $Å^2$/molecule per min All isotherms described here were determined at 15 0 $\pm$ 0 2 °C

Areas were reproducible to $\pm$ 0 7 $Å^2$/molecule near the collapse points of

the monolayers and to $\pm$ 1.6 Å^2/molecule in the region of the transition point from the liquid expanded to liquid condensed state[8]. Surface pressures were reproducible to $\pm$ 0.5 dyne/cm and surface potentials were reproducible to $\pm$ 6 mV near collapse and at areas equal to or greater than that of the transition point. There were, however, greater fluctuations in the measured surface potentials in the intermediate region and reproducibility in this region was no better than $\pm$ 15 mV.

The stability of these monolayers at various surface pressures was tested by measuring the rate of change of surface pressure at a constant area/molecule. In cases where poor stability was found, the role of evaporation was tested by measuring the rate of change of surface pressure while nitrogen was blown gently over the film surface. Similarly, the extent of solubility of the monolayer forming compound in the substrate solution was tested by comparing both stability and isotherms for monolayers on substrates with and without 2 M NaCl added.

Surface tensions of substrate solutions were determined in the film balance with the temperature being held to $\pm$ 0.1 °C.

RESULTS

The isotherms obtained for monolayers of myristic, pentadecanoic and palmitic acids, also methyl pentadecanoate, and methyl palmitate on various substrate solutions are given in Figs 1, 2, 3, 4, and 5, respectively. In each figure, the surface pressure *versus* area plots (identified π–A) are in the lower portion and the corresponding surface potential *versus* area/molecule plots (identified ΔV–A) are in the upper portion of the figure. The pressure at which such monolayers became "unstable" was approximately 15–20 and 25–30 dynes/cm for esters and acids, respectively. The instability point being arbitrarily defined as the surface pressure at which, immediately after compression is stopped, the decrease in surface pressure at constant area/molecule became greater than 1 dyne/cm per min[8]. Substrate solutions for myristic acid, pentadecanoic acid and palmitic acid were adjusted to pH 2 with HCl to reduce the ionization of the long chain acids.

NaCl was added to a 2 M concentration to each substrate solution on observing that myristic acid and methyl pentadecanoate were slightly soluble in the mixed substrate solutions at higher surface pressures. The surface pressure curves were almost identical with or without the added NaCl, although there was a slightly greater expansion of the monolayers at low pressures when NaCl was present[9,10]. Surface potentials were from 55–75 mV higher at low areas/molecule in the presence of the NaCl; however, the relative surface potential results obtained for different substrate solutions were comparable.

As shown in Fig. 1, myristic acid forms a typical expanded monolayer on water at 15 °C in agreement with previously published values[11]. A sharp transition point from the liquid condensed to liquid expanded state occurs at 34.2 Å^2/molecule with a surface pressure of 9.2 dynes/cm and a surface potential of 312 mV The addition of 3 M glycerol to the substrate results in a further expansion, which is especially evident in the π-A curve at high areas. In addition, a decrease in surface potential is apparent at all areas/molecule. The addition of 3 M urea is seen to result in an even greater expansion with the transition point some 5.2 dynes/cm higher. However, there is a comparatively smaller decrease in the surface potential with urea in the substrate than with glycerol.

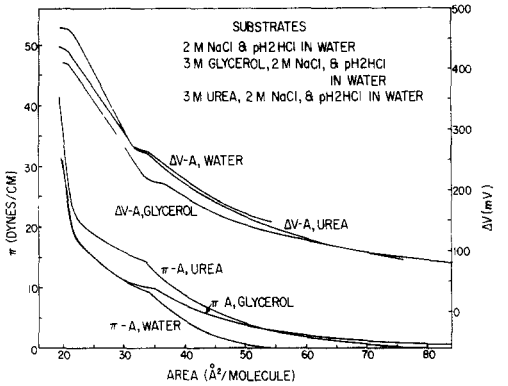

Fig 1 Surface pressure (π) and surface potential (ΔV) as a function of area/molecule(A) for monolayers of myristic acid on different substrates at 15 °C

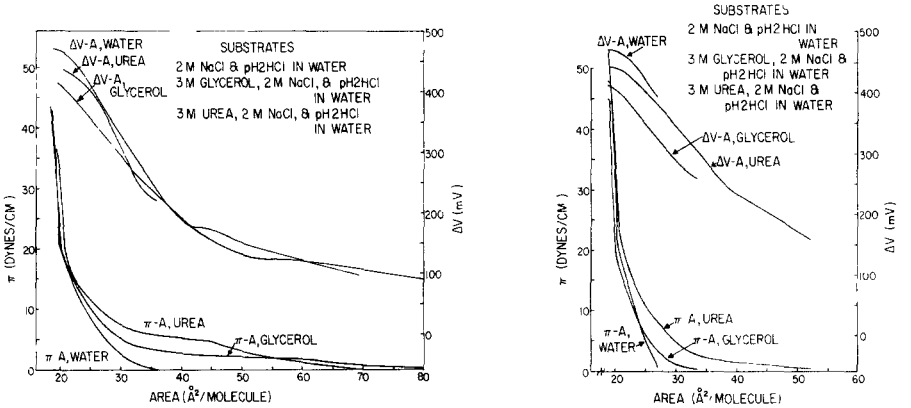

Fig. 2 Surface pressure (π) and surface potential (ΔV) as a function of area/molecule (A) for monolayers of pentadecanoic acid on different substrates at 15 °C.

Fig 3. Surface pressure (π) and surface potential (ΔV) as a function of area/molecule (A) for monolayers of palmitic acid on different substrates at 15 °C

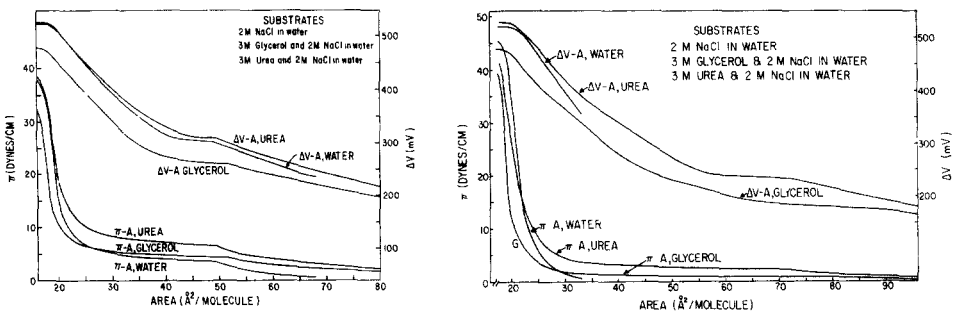

Fig. 4. Surface pressure (π) and surface potential (ΔV) as a function of area/molecule (A) for monolayers of methyl pentadecanoate on different substrates at 15 °C

Fig. 5. Surface pressure (π) and surface potential (ΔV) as a function of area/molecule (A) for monolayers of methyl palmitate on different substrates at 15 °C

The general features discussed above for myristic acid monolayers are also evident in the figures (Figs 2, 4, 5) for all but palmitic acid (Fig. 3). Neither pentadecanoic acid (Fig. 2) nor methyl palmitate (Fig. 5) form expanded monolayers on water at 15 °C, but a change in the physical state is evident in both the π–A and ΔV–A curves for these compounds on substrate solutions containing either glycerol or urea. For methyl palmitate (Fig. 5) the transition points to a liquid expanded state are at very high areas and low surface pressures (88 Å^2/molecule and 0.6 dyne/cm for 3 M glycerol solution and 72 Å^2/molecule and 2.3 dynes/cm for the 3 M urea solution).

It is noteworthy that the further expansion of monolayers in the presence of glycerol or urea is approximately the same for the methyl esters as for the acids, although the methyl ester of a given chain length is already more expanded on water than its parent acid. In each case the urea causes a greater increase in the surface pressure, but the glycerol causes a greater decrease in the surface potential; indeed, the decrease in the surface potential on urea substrates for myristic acid and for pentadecanoic acid is evident only at low areas/molecule.

Monolayers of palmitic acid were also studied on these substrate solutions. Glycerol and urea as substrate additives each cause a small expansion at low surface pressures, that for urea again being greater, however, no change in physical state occurs at 15 °C, which is well below the temperature at which palmitic acid becomes expanded on a water substrate[11].

For films already in a liquid expanded state glycerol and urea each tend to change the physical state of the monolayers at high areas from liquid expanded to vapor expanded. This can be deduced from the more asymptotic approach of the π–A curves to the abscissa at low surface pressures and from the absence of rapid fluctuations in surface potential at high areas[8].

Surface tension measurements of the substrates at 15 °C were primarily dictated by the 2 M NaCl. Thus, either with or without addition of HCl to attain pH 2, a 2 M NaCl aqueous substrate had a surface tension of 77.8 dynes/cm (pure water 74.2 dynes/cm at 15.0 °C). The further addition of 3 M urea raised the surface tension of the aqueous salt substrate by 0.6 dynes/cm while the alternate further addition of 3 M glycerol showed a decrease of 0.5 dynes/cm.

DISCUSSION

Before comparing the effects of glycerol and urea on the various lipids, it is important to first establish any relative shift in the substrate surface tension[3]. The reason for this is that the surface pressure:

$$\pi = \gamma_0 - \gamma$$

where γ_0 = the surface tension of the pure substrate and γ = the surface tension of the film covered substrate, is a measure of changes in the film only when γ_0 remains constant. In the studies reported here while this is not quite true, the difference is small (approx. 1.0 dynes/cm), and film effects are usually significantly greater than this.

A decrease in surface potential values occurs on adding either glycerol or urea to the substrate with the maximum effect being observed either when the film is

in a close packed state or undergoing a phase transition, the shift decreasing with increasing area/molecule. In every case, the effect of glycerol on the film surface potential is greater than that of an equivalent amount of urea. Since, as an approximation, one may write:

$$\Delta V = 12\pi n\mu$$

where ΔV is the volta surface potential in mV, n the number of dipoles/cm^2 and μ the vertical component of the surface dipole in mD. μ may be interpreted as being made up of three components[13,14]: the hydrocarbon chain end group, the polar-head group and the oriented substrate. Of these the latter two components clearly predominate. The decrease observed in the surface potential values thus presumably arises through a changed polar group–substrate interaction. Regrettably it is not possible to completely separate out the changes in the two χ potentials of the polar group and substrate in evaluating the altered volta potential, but it is clear that equivalent amounts of glycerol have a greater effect on the polar group environment than urea.

In contrast, when films are already partially expanded, it is the urea that produces the greater effect on the physical state of the film even after allowing for changes in γ_0. Expansion effects occur with all films previously in a liquid expanded state (myristic acid, methyl pentadecanoate and methyl palmitate) also for one film in a liquid condensed state (pentadecanoic acid) but not for the other (palmitic acid) We have previously explained such expansion effects in terms of a hydrocarbon chain–substrate enhanced interaction based on the observations that:

(1) Liquid condensed films show little or no expansion effect on addition of either urea or glycerol and with a condensed film chain–substrate interactions are minimized In pentadecanoic acid we have an exception to this rule and this particular case requires further discussion

(2) The expansion effect shows independence of the precise nature of the polar group, a wide variety of lipids including phosphatidylethenolamine, choline and cholesterol, having been studied[2]. Even here the change in polar group from acid to ester is not a determining factor, instead it is the initial physical state of the lipid film which establishes the likelihood of further expansion.

(3) Recent calculations of dipole–dipole interactions[13] suggest that, at least in the close packed state, such interactions are not a significant factor in determining film packing. This does not rule out the possibility that, at or near a transition point where chain–chain interactions approach a point of balance with thermal energy, dipole interactions would determine the precise transition point from one physical state to another. There is little doubt, however, that chain interaction is the primary factor in determining when a condensed film will expand and when, either water will penetrate between the hydrocarbon chains or the chains will be drawn into the interface. Comparison of monolayers and lamellar liquid crystalline aqueous dispersions and differential scanning calorimetric measurement on the latter suggest that liquid condensed–liquid expanded (gel–liquid crystalline) transitions will be determined primarily by chain interactions, while liquid expanded–gas (liquid crystalline–liquid–gas) transitions wil depend on both chain and dipole interactions.

The new information in this report is that the substrate additive which

produces the greater decrease in surface potential has a smaller effect on the physical state of the film (at least this is the case from about 5–20 dynes/cm). Since the shift in the surface potential arises primarily through polar group substrate interactions, it is clear that film expansion must involve some factor other than dipole interactions, and the only likely candidate is a chain–substrate interaction. At low pressures the situation is less clear and it may be that, as the film is expanded, chain–substrate interactions decay faster than dipole–dipole interactions, however, small changes in γ_0 and increasing lack of precision at low pressures and large areas/molecule make such interpretations hazardous.

A precise molecular picture of the changes taking place in these systems is clearly not possible, however, certain generalizations may be made. Thus glycerol[16], urea[17] and alcohols[18] are all known to change water structure in the bulk phase. For glycerol and urea some correspondence between the bulk and surface may be expected but for aqueous-alcohol systems drastic changes in surface tension are indicative of a considerable difference between bulk and surface structure. Glycerol in aqueous solution would appear to be partially capable of replacing water and, although some long range order is lost, the transition from pure glycerol to pure water would appear to be continuous as indicated by changes in both the dielectric constant and the viscosity. Urea on the other hand has only very short-lived hydrogen bonded interactions with water and since urea self-association does not appear to occur, a considerable reduction in long-range order is brought about.

The only interaction possible between saturated hydrocarbon chains and an aqueous substrate is one involving dispersive forces. If only enthalpy changes were involved it would then be logical to expect that equivalent amounts of different substrate additives would produce similar effects However, it is clear that significant entropy changes must also contribute to the overall free energy change shown by the expanded isotherm shift and urea would seem to be a more likely structure breaker than glycerol, an observation in line with a greater preservation of an expanded state for films on aqueous-urea substrates. Needless to say, the 2 M salt and the presence of the lipid film may modify conclusions based on substrate structure, certainly they prevent any quantitative evaluation.

The only exception to the above would appear to be the case of pentadecanoic acid. Comparison with palmitic acid reveals that whereas the C_{16} acid shows almost no expansion the C_{15} acid undergoes a significant change. It would, therefore, appear that we should revise our original correlation of physical state and substrate expansion effect thus: The physical state of monomolecular films close to their transition temperature will show a sensitivity to a wide variety of substrate additives. Films well below, or well above a phase transition will show little or no effect. This observation is in excellent agreement with observations of sensitivity to substrate salt additives[9] or the ability of cholesterol to condense expanded films[19].

The correspondence between monolayer liquid condensed–liquid expanded transitions and bilayer gel–liquid crystalline transitions[7], also the recent emphasis on the bimolecular lipid layer as a general membrane component[20,21], indicates that the molecular interpretations presented here are of relevance in understanding cell membrane survival under osmotic and low temperature shock conditions as well as lipid–protein interactions. One final point should be made clear, however, and that is that while our studies show that chain–substrate interactions play a

role in establishing film physical states, they in no way prove whether the substrate "penetrates" the lipid or the lipid hydrocarbon chain is drawn into the film–substrate interface. This in turn means that membrane models indicating protein or glycoprotein penetration[22] based on film expansion or surface pressure increases for fixed area films require further evidence before acceptance.

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