Langmuir Film Balance Study of the Interactions between Carbohydrates and Phospholipid Monolayers†

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ABSTRACT: Using a Langmuir film balance, two types of experiments have been conducted to study the interaction between phospholipids and carbohydrates: carbohydrates were dissolved in the subphase and their influence on compression isotherms of the phospholipids was measured, and secondly, the compression isotherms of mixed films of simple glycolipids and charged amphiphiles were studied to see if they show a negative departure from ideal behavior. It was found that phospholipid monolayers were expanded by the presence of carbohydrate in the subphase; that is, they occupied a greater surface area at a given film pressure than a similar film spread on pure water. Although the magnitude of the effect depended on the nature of both the phospholipid and carbohydrate, the dependence on the carbohydrate was far greater, there being more than 2 orders of magnitude difference between the concentrations of glucose and galactose which cause similar film expansions. Consideration of the nature of this phenomenon suggests that carbohydrate is coordinated to the ionic head group of the lipid by hydrogen bonds and that the driving force for coordination is the rise in entropy resulting from the overall increase in translational freedom when several water molecules are replaced by one carbohydrate. Studies with mixed monolayers of simple glycolipids and charged amphiphiles revealed an interaction between ion and carbohydrate. However, the strength of the interaction was independent of the nature of the ion and the carbohydrate, suggesting that in this instance the forces involved are weaker. It is concluded that the interaction between a phospholipid head group and carbohydrate dissolved in the subphase is crucially dependent on the orientation of the carbohydrate hydroxyl groups to the ion. Comparison of the physical properties of monolayers on the subphase with and without dissolved carbohydrate showed that in the solid state films expanded by carbohydrate were considerably more compressible. It is suggested that there is an analogous difference between bilayers in the presence and absence of carbohydrate and that, even under anhydrous conditions, there may be a connection between the stability and compressibility of biomembranes. In fully hydrated systems at physiological temperatures, parallels may be drawn between the relative strengths of the interactions of the carbohydrates with charged monolayers and their properties in vivo. It was found that many of the carbohydrates involved in cell recognition and adhesion strongly expand lipid monolayers.

Carbohydrates are the most common group of chemical substances found in living organisms. The vast bulk present in the cell is in one of two forms, either as a structural material or as an energy store. However, other carbohydrates are associated with the cell surface, either covalently linked to membrane lipids and proteins where they serve as recognition and adhesion sites or adsorbed to the membrane, making it less susceptible to damage by freezing (Morris, 1981), desiccation (Crowe et al., 1984), or osmotic shock (Corner & Marquis, 1969). In both cases, the function of the carbohydrate requires that there be a specific interaction between it and the surface it contacts. Our interest is in the nature of this interaction and the molecular basis of its specificity.

Measurements of the relationship between the surface pressure and area of monomolecular films have long been used to obtain information about the forces operating within the film and, by extrapolation, to obtain information about biomembranes made up of the same type of lipid molecule (Phillips & Chapman, 1968). However, study of surface pressure–area isotherms can also throw light on the interaction between film molecules and other ions or molecules if these other ions or molecules are dissolved in the subphase. Thus, the Langmuir film balance can be used to investigate the interaction between carbohydrates and other cell constituents. Information about recognition and adhesion phenomena can be obtained by studying the effect solutes in the subphase have on glycolipid monolayers (Read et al., 1977), while dissolving carbohydrates in a subphase on which monolayers of cell membrane lipids have been spread might provide a suitable arrangement for a study of the protective effect of carbohydrates on membranes (Cadenhead & Demchak, 1969; Cadenhead & Bean, 1972; Maggio et al., 1976; Maggio & Lucy, 1978).

Crowe et al. (1984a) have used this second approach in an attempt to discover how the disaccharide trehalose is able to preserve the structure of membranes subjected to extreme desiccation. They found that monolayers of dimyristoylphosphatidylcholine were expanded when a carbohydrate was dissolved in the subphase; that is, the films occupied greater areas at a given surface pressure than they would have if spread on pure water. The magnitude of the monolayer expansion could be correlated with the protective effect of the carbohydrate. Thus, trehalose, which of the carbohydrates examined was most effective at preventing desiccation damage, was also found to cause the greatest expansion of the phosphatidylcholine monolayer. Hence, Crowe et al. (1984a) postulate that the unique protective effect of trehalose may derive from its ability to replace water molecules bound to the ionic head groups of membrane phospholipids.

The work reported in this paper is also concerned with the interaction between carboydrates and phospholipid monolayers, and it can be divided into three sections. First, we have remeasured the isotherms of dimyristoylphosphatidylcholine on trehalose-containing subphases. In addition, we have studied the influence of a wide range of carbohydrates on representatives from the other common phospholipid classes found in

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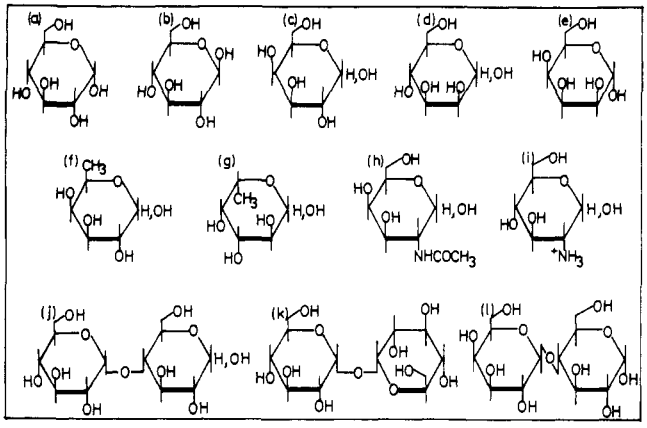

FIGURE 1: Haworth structures for (a) α-glucose, (b) β-glucose, (c) α,β-galactose, (d) α,β-mannose, (e) α-talose, (f) D-fucose, (g) L-fucose, (h) *N*-acetylgalactosamine, (i) α,β-glucosamine hydrochloride, (j) α,β-maltose, (k) α,α-trehalose, and (l) α-lactose.

cells: dipalmitoylphosphatidic acid, dilauroyl- and dimyristoylphosphatidylethanolamine, and a mixture of phosphatidylserines extracted from bovine brain. The aim of this series of experiments has been to establish the relationship between monolayer expansion and the structures of phospholipid and carbohydrate. Second, we have studied the influence of selected carbohydrates on monolayers of behenylammonium, behenyltrimethylammonium, and stearyl carboxylate salts. Combined with the results from the previous section on phosphatidic acid, these data have allowed us to rank the different ions which comprise the phospholipid head groups in order of the strength of their interaction with carbohydrates. Finally, in an attempt to establish what role the orientation between carbohydrate and phospholipid plays in film expansion, we have synthesized simple glycolipids and measured pressure–area isotherms for films made from mixtures of these with the behenylammonium ions, the behenylammonium ions serving as models for the charged phospholipids. In these films, carbohydrate and ammonium ion are constrained to two dimensions. Plots of film area vs. film composition show if there is an interaction between carbohydrate and ammonium ion. Some deductions about the role of orientation in film expansion can be made by comparison of these results with isotherms obtained with carbohydrate free in the subphase.

Experimental Procedures

Materials. 1,2-Dimyristoyl- and 1,2-dipalmitoyl-L-phosphatidylcholines (DMPC and DPPC, respectively)[1] purchased from Fluka AG were puriss. grade ~99%). 1,2-Dilauroyl- and 1,2-dimyristoyl-L-phosphatidylethanolamines and 1,2-dipalmitoyl-L-phosphatidic acid (DLPE, DMPE, and DPPA, respectively) were purchased from Sigma Chemical Co. The phosphatidyethanolamines were stated to be 98% pure and the phosphatidic acid 99% pure. A phosphatidylserine (PS) sample was also purchased from Sigma (~98%). It contained several lipids differing from each other in their acyl chain types. The purity of the phospholipids was checked by thin-layer chromatography and, where it was accessible, by the sharpness of the main phase transition of their monolayers. Material which did not give a sharp single spot when the TLC plate was developed or have a compression isotherm with a sharp phase transition was discarded. The carbohydrates studied (see Figure 1 for structures) were α-glucose, β-glucose, α,β-galactose, α,β-mannose, α,β-glucosamine hydrochloride, L- and D-fucose, *N*-acetylgalactosamine, α-lactose, α,β-maltose, α,α-trehalose (all from Sigma), and α-talose, (from Koch Light). The manufacturer's premium grade material was purchased and used as supplied. We were unable to obtain a single anomer in each case, and so the galactose, glucosamine, fucoses, *N*-acetylgalactosamine, and maltose contained a mixture of anomers of unknown proportion. This must be borne in mind when interpreting the results. Arachidic alcohol and stearic and behenic acids (all 99% pure) were purchased from Sigma Chemical Co.

Behenylammonium and behenyltrimethylammonium trifluoroacetates were synthesized from the alcohol. Behenyl alcohol (Sigma) in dry dichloromethane was treated with a molar equivalent of trifluoroacetic anhydride. The acid formed was neutralized with sodium bicarbonate and solvent evaporated. The residue, behenyl trifluoroacetate, was dissolved in tetrahydrofuran and treated with excess lithium amide to form the alkyl ammonium salt and treated with excess trimethylamine to form the alkyl trimethylammonium salt. Both compounds were recrystallized from methanol and their identity and purity confirmed by thin-layer chromatography and infrared spectroscopy.

The method used to synthesize alkyl glycosides was essentially that described by Pascher (1974). The saccharide was protected and brominated by dissolving it (20% w/v) in hydrogen bromide saturated acetic anhydride. The resulting acetobromo sugar was freed from acetic anhydride and hydrogen bromide by rotary evaporation at 90 °C followed by pumping under oil pump vacuum for several hours. Two molar equivalents of the acetobromo sugar and mercury cyanide were added to stearyl alcohol dissolved in hot nitromethane (80 °C), 1 equiv at the start of the reaction and the rest in equal portions after 2 and 3 h. After 4 h, the product was isolated and dissolved in a dilute solution of potassium hydroxide (0.2 M) in 95% ethanol to remove the ester-protecting groups. The crude glycosides were purified by column chromatography on silica using a gradient of chloroform–methanol. Their identity and purity were checked by thin-layer chromatography, infrared spectroscopy, and mass spectrometry (stearyl glycosides had a molecular ion mass of 433).

Water for the Langmuir film balance was prepared by passing singly distilled tap water through a Milli Q filtration system (Millipore, London). Unless it is specifically stated otherwise, no chemicals, other than the carbohydrates, were added to this water. Its resistivity was always greater than 18 M Ω/cm, its pH was 5.5, and it was used immediately. *n*-Hexane (Merck, Uvasol) and ethanol (Merck, pro analysi) were used as spreading solvents. Lipids were spread, depending on solubility, in either 9:1 or 4:1 hexane:ethanol solutions. The solution concentrations were approximately 1 mg/mL.

Methods. The film balance has been comprehensively described elsewhere (Albrecht & Sackman, 1980). It was fitted with a Wilhelmy pressure pickup system similar to that described by Fromherz (1975). The output of the pressure pickup was calibrated by recording the well-known isotherm of palmitic acid. This isotherm is characterized by a sharp phase transition at which the isotherm has a nearly horizontal slope. For pure water (pH 5.5) at 20 °C, this transition occurs at 22.4 dyn/cm (Albrecht & Sackmann, 1980). The Teflon trough was regularly cleaned with hot chromic acid. Films were spread on the water surface from a microsyringe, and

[1] Abbreviations: DMPC, 1,2-dimyristoyl-L-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-L-phosphatidylcholine; DLPE, 1,2-dilauroyl-L-phosphatidylethanolamine; DMPE, 1,2-dimyristoyl-L-phosphatidylethanolamine; DPPA, 1,2-dipalmitoyl-L-phosphatidic acid; PS, phosphatidylserine; TLC, thin-layer chromatography.

at least 15 min was allowed for evaporation of the solvent. Films were then compressed at a rate of 68.5 cm^2/min (in a typical experiment, this would correspond to 8.0 $Å^2$ $molecule^{-1}$ min^{-1}). Below this limit, a change in the compression rate did not alter the shape of the isotherm. Kinetic hysteresis effects can thus be excluded. All isotherms were run twice in the direction of increasing pressure. Each run was performed with a freshly prepared film. Under optimum conditions, two isotherms taken successively were completely superimposable. However, reproducibility values more typical under the conditions in which the bulk of the reported measurements were made would be ±0.25 dyn/cm, for surface pressure and ±0.2 $Å^2$/molecule for area. These values refer to measurements made on a single solution.

All the surface-active molecules used in this work form stable films. Stability was assessed by compressing monolayers to a pressure of 30 dyn/cm, stopping the barrier, and observing the pressure decay. Films were considered stable if the decay rate did not exceed 1 dyn/min. This criterion dictated the length of the alkyl chains of the fatty alcohol, glycolipids, and ammonium salts. Stearyl alcohol was found to be volatile, and at low temperatures, behenyl alcohol was subject to three-dimensional crystallization on the water surface. Hence, we have used arachidyl alcohol. Stearyl ammonium salts were found to be soluble in the subphase. Behenylammonium salts formed stable monolayers that, unlike behenyl alcohol, did not crystallize. Dodecyl monosaccharides and arachidyl disaccharides were also found to be soluble in the subphase. Stearyl and behenyl monosaccharides formed stable highly expanded monolayers. All measurements were made at a subphase temperature of 30 °C (±0.2 °C).

Since the Wilhelmy plate technique measures surface tension (which when subtracted from the surface tension of pure water gives the film pressure), the influence the various carbohydrates have on the surface tension of the subphase in which they are dissolved must be considered. Cadenhead & Bean (1972) have reported that a subphase containing 2 M sodium chloride had a surface tension of 77.8 dyn/cm and that the surface tension was decreased by only 0.5 dyn/cm on addition of 3 M glycerol. As we will show later, the carbohydrate which has the greatest effect on phospholipid monolayers is galactose. However, we were unable to detect any difference between the surface tensions of pure water and solutions containing 200 g/L galactose (1.1 M). Since the carbohydrate concentrations in our experiments rarely exceeded 0.5 M, we can be sure that differences between lipid isotherms measured on pure water and carbohydrate solutions are not the result of changes in the surface tension of the subphase.

Compression isotherms for mixed films of arachidyl alcohol, the stearyl glycosides, and the behenylammonium ions were obtained by spreading solutions containing amounts of each component calculated to give mole fractions of 0.25, 0.5, and 0.75. The average area per molecule (in angstroms squared) at a specific surface pressure was calculated by using the following relationship:

$$\frac{[\text{const } 1 - (\text{const } 2)x](n_a M_{r,a} + n_b M_{r,b})}{\text{vol} \times \text{concn}}$$

where x = the distance the pen traveled on the chart recorder (in centimeters), n_a and n_b = the mole fractions of components a and b, respectively ($n_a + n_b = 1.0$), $M_{r,a}$ and $M_{r,b}$ = the molecular weights of components a and b, respectively, concn = the concentration of lipids in spreading solvents (in milligrams per cubic centimeter), vol = the volume of the lipid solution spread (in microliters), and const 1 and const 2 are constants determined by the chart recorder and barrier speeds and the trough dimensions.

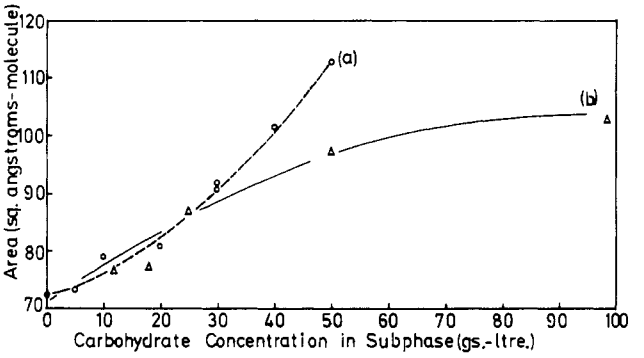

FIGURE 2: Plots of the area occupied by a film of dimyristoylphosphatidylcholine vs. the concentrations of (a) α,β-galactose and (b) α,α-trehalose in the subphase. All areas were measured at a film pressure of 10 dyn/cm.

Table I: Differences between Lipid Area per Molecule on Pure Water and Subphases Containing 30 g/L Carbohydrate[a]

lipid	carbohydrate			
	glucose	galactose	trehalose	glycerol
DMPE	3.5	44.9	16.0	1.4
DLPE	1.1	23.7		nil
DPPC	nil	14.6		
DMPC	0.6	19.6	14.5	0.1
DPPA	0.4	26.6		
PS (bovine brain)	2.3	20.2		

[a] Areas are in square angstroms per molecule and are measured at 10 dyn/cm surface pressure. Each value is the average of measurements from two experiments.

More general considerations underlying the calculations of the areas of mixed films have been described by Gaines (1966). The average areas per molecule so obtained were then plotted against component mole fractions. A negative deviation of this plot from ideality, i.e., the plotted points falling below a straight line joining the pure component areas, indicates a greater attractive force between carbohydrate and ion than between the molecules of the pure components.

Results

Isotherms of Phospholipid Films on Carbohydrate-Containing Subphases. Figure 2 contains plots of area per molecule vs. the concentration of carbohydrate in the subphase for DMPC films spread on subphases containing trehalose and galactose. The areas per molecule are reported at a film pressure of 10 dyn/cm. This pressure was selected since all the lipids are in an expanded state at this point and the rate of change of pressure with area is great enough to make measurement errors insignificant. Comparison of areas at virtually any other pressure would lead to similar conclusions. The curve obtained with trehalose is hyperbolic, showing a clear saturation of the expansion effect at high concentrations of the carbohydrate. The data obtained agree reasonably well with those reported earlier by Crowe et al. (1984a). However, we could detect no falling off in the increase in film area with increasing concentrations of galactose in the subphase. A saturation will occur at high enough galactose concentrations, but because of the geometry of our film balance, we were unable to make measurements at galactose concentrations greater than 50 g/L.

Table I lists the increase in area per molecule (at 10 dyn/cm surface pressure) of various phospholipid films spread on subphases containing fixed concentrations of glucose and galactose and in a few instances trehalose and glycerol. It can

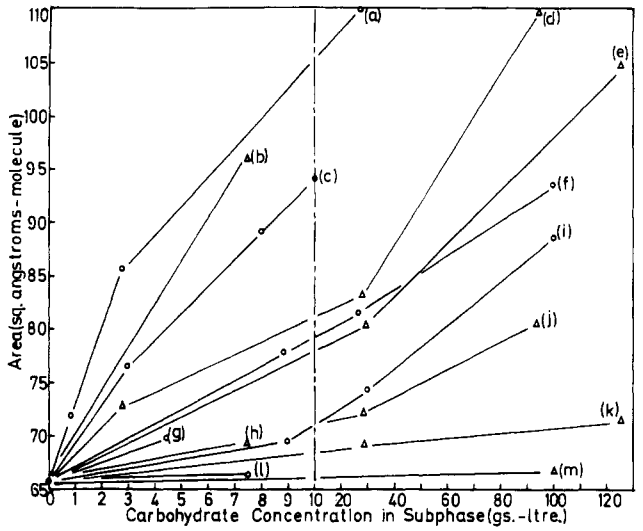

FIGURE 3: Plots of the area occupied by a film of dimyristoylphosphatidylethanolamine vs. the concentrations in the subphase of (a) α,β-galactose, (b) *N*-acetylgalactosamine, (c) α-lactose, (d) α,β-mannose, (e) α,β-glucosamine, (f) α,α-trehalose, (g) α-talose, (h) L-fucose, (i) β-glucose, (j) α,β-maltose, (k) α-glucose, (l) D-fucose, and (m) glycerol. All areas were measured at a film pressure of 10 dyn/cm.

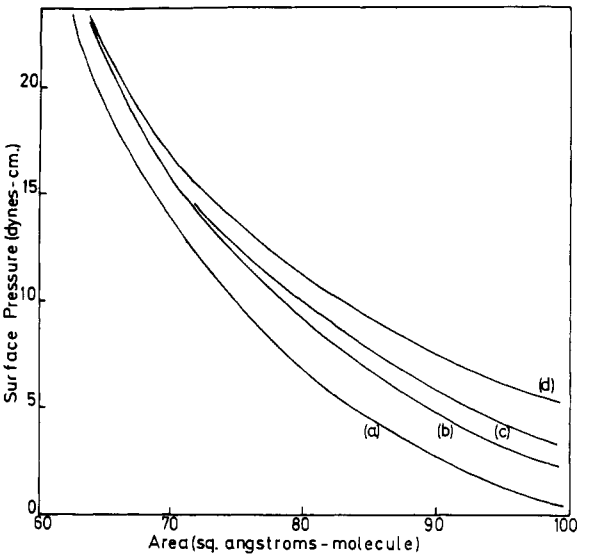

FIGURE 4: Isotherms of DMPC measured on subphases containing various concentrations of glycerol: (a) 0, (b) 150, (c) 300, and (d) 600 g/L.

be seen that the film expansion observed depends on the nature of both the carbohydrate and the phospholipid. All the phospholipids examined are much more expanded on galactose-containing subphases than glucose. The expansion on galactose-containing subphases is least for phosphatidylcholine, being about 50% greater than the area on pure water, and greatest for phosphatidylethanolamine, being almost 100% greater than the area on pure water.

An assessment of the effect the length of the phospholipid acyl chains has on its interaction with carbohydrates can be made by comparing DMPE, DPPC, DLPE, and DMPC isotherms. At 30 °C on pure water, the isotherms of DPPC and DMPE show breaks characteristic of fluid–solid phase transitions while DMPC and DLPE films are fully expanded at all surface pressures. Yet as the results in Table I show, the areas of DMPE films increase more than the areas of DLPE films when the subphase contains carbohydrate, while conversely for phosphatidylcholines it is the lipid with the shortest acyl chain, DMPC, which is most affected by the presence of carbohydrate. It would appear that the nature of the polar head group is more important in determining the interaction of phospholipids with carbohydrates than the length of the lipid acyl chain. The only difference we could detect between the isotherms of fully expanded phospholipids and those which undergo a phase transition was that in the second case the increase in film area produced by dissolving saccharides in the subphase fell markedly as the film pressure was increased. Isotherms of behenylammonium trifluoroacetate illustrate this behavior clearly (see Figure 6).

Since the greatest increases in area per molecule were obtained with DMPE, it was selected as the lipid with which to compare the capacity of various carbohydrates to expand monolayers. The results of these experiments are plotted in Figure 3. It can be seen that the capacity of carbohydrates to expand monolayers varies considerably and that this variation can be produced by relatively small changes in the structure of the carbohydrate. For instance, compared to pure water, there is a similar increase in the film area on subphases containing 0.75 g/L galactose and 125 g/L α-glucose. α-Galactose and α-glucose differ solely in the orientation of the hydroxyl group on carbon 4; it is equatorial on galactose and axial on glucose (see Figure 1 for sugar structures). The position of the disaccharide lactose on the graph is presumably a consequence of it possessing a galactose unit. A comparison of the results for glucose and glucosamine hydrochloride shows that the possession of a charge enhances the monolayer-expanding properties of the sugar. β-Glucose expanded DMPE monolayers more than α-glucose. With an equimolar mixture of anomers dissolved in the subphase, the increase in film area lay approximately halfway between the values measured for the pure anomers (data not shown).

Glycerol, unlike the sugars, expanded DMPE monolayers only very slightly. On this subject, our results differ from those of Crowe et al. (1984a), who found that the area occupied by a DMPC film at 30 °C was doubled when 30 g/L glycerol was added to the subphase. At this glycerol concentration, we were unable to measure any significant increase in film area (see Table I). It appears that the glycerol they used was contaminated. Two other groups have shown that glycerol has a minimal effect on the isotherms of phospholipids (Maggio et al., 1976; Cadenhead & Demchak, 1969). DMPC isotherms on subphases containing glycerol are shown in Figure 4.

Isotherms of Single Ion Amphiphile Films on Carbohydrate-Containing Subphases. In addition to measurements on glycerophospholipids, we have also studied simpler single ion amphiphiles in an attempt to establish the role individual ions play in the expansion of films. Measurements already reported on dipalmitoylphosphatidic acid indicate that the presence of a single phosphate ion is sufficient for film expansion to occur. In fact, the phosphatidic acid film was proportionately expanded more by the presence of galactose in the subphase than phosphatidylcholine or phosphatidylserine. Figure 5 shows that films of the behenylammonium ions and stearic acid increase in area as the concentration of galactose in the subphase rises. However, the increase in area per unit increase in carbohydrate concentration is twice as much for the behenylammonium salt than for either the behenyltrimethylammonium salt or the carboxylate salt. Like phospholipids, films of the ammonium and carboxylate salts are expanded far more by addition of galactose to the subphase than glucose. Isotherms for both behenylammonium salts on subphases containing various concentrations of galactose are traced in Figure 6. The trimethylammonium salt isotherms are atypical in that they exhibit what appear to be broad phase

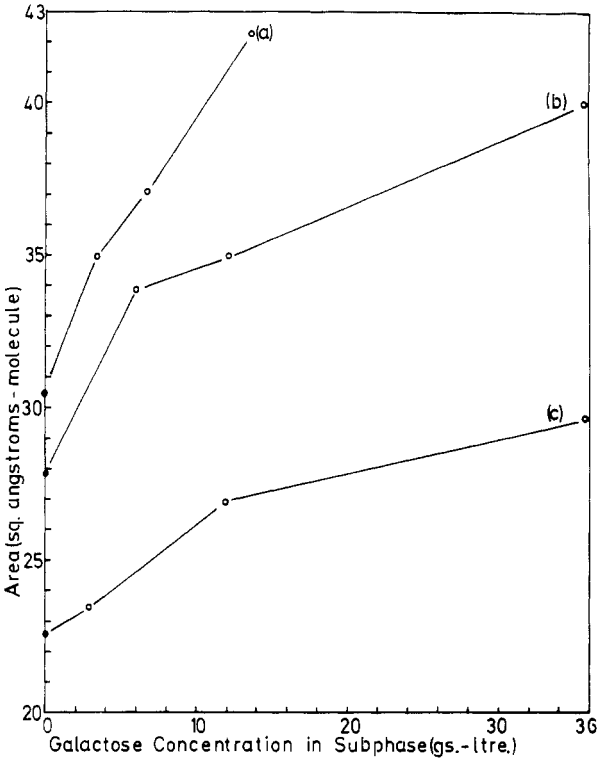

FIGURE 5: Plots of the area occupied by films of (a) behenylammonium trifluoroacetate, (b) behenyltrimethylammonium trifluoroacetate, (c) stearic acid vs. the concentration of α,β-galactose in the subphase. All areas were measured at a film pressure of 10 dyn/cm. In (a), the subphase contained sufficient HCl to reduce its pH to 4.0.

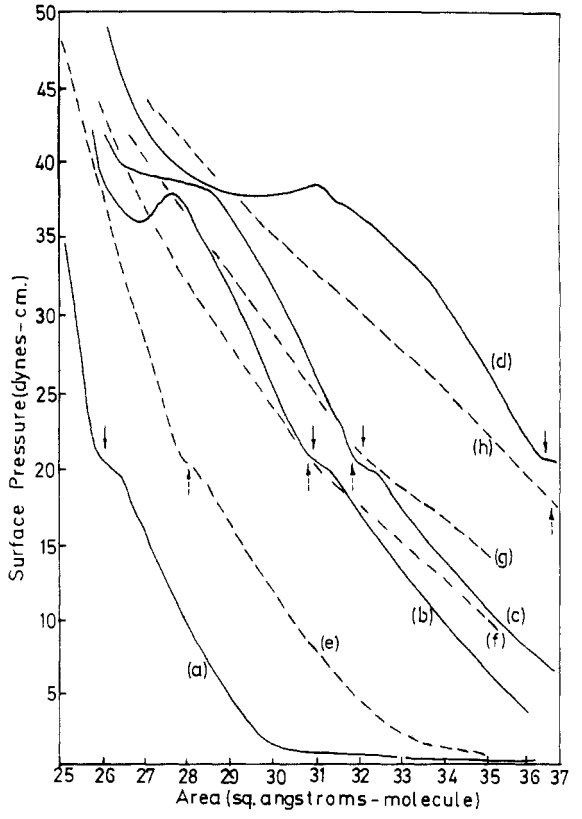

FIGURE 6: Isotherms of behenyltrimethylammonium trifluoroacetate (solid lines) and behenylammonium trifluoroacetate (dashed lines) on subphases containing various concentrations of α,β-galactose: (a and e) 0, (b) 6, (c) 12, (d) 36, (f) 3, (g) 5.5, and (h) 11 g/L. Arrows indicate the liquid condensed–solid phase change.

transitions at about 37 dyn/cm. Stearic and behenic acids behave similarly (data not shown). Such behavior was not

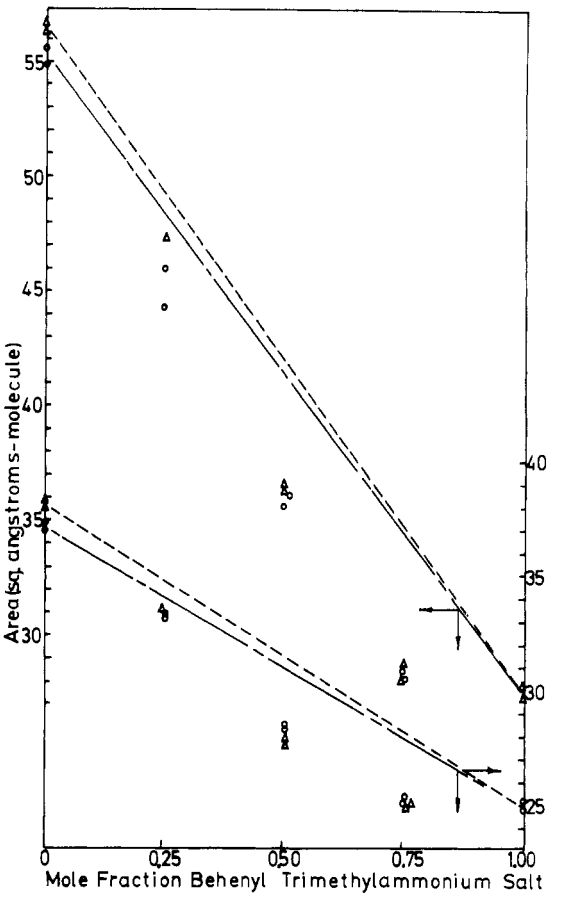

FIGURE 7: Plots of the areas of mixed films of behenyltrimethylammonium trifluoroacetate and the glycosides stearyl glucoside (triangles) and stearyl galactoside (circles) vs. the mole fraction of behenyltrimethylammonium trifluoroacetate. The upper sets of areas were measured at a film pressure of 5 dyn/cm and the lower at 25 dyn/cm.

observed with any of the glycerophospholipids. The isotherm on the trehalose-containing subphase also shows a discontinuity, in this instance beginning at about 29 dyn/cm. No discontinuity was observed on the glucose-containing subphase.

Isotherms of Mixed Films of Behenylammonium Salts and Stearyl Glycosides. Plots of area per molecule vs. film composition are graphed in Figure 7 for mixtures of stearyl glucoside and galactoside with the behenyltrimethylammonium salt. Plots with behenylammonium trifluoroacetate are virtually identical. The upper sets of results show film areas at a surface pressure of 5 dyn/cm and the lower at 25 dyn/cm. In all four sets of results, the areas of the mixtures are less than mean areas calculated for them by adding together the pure component areas multiplied by their mole fractions; i.e., in Figure 7, the mixture points fall below straight lines joining the pure component areas. The departure from straight-line, ideal, behavior is greatest at a film composition around 3 mol of behenylammonium salt to 1 mol of glycolipid. There do not appear to be any significant differences between the properties of the two sets of mixtures. By contrast, fatty alcohol–ammonium ion mixtures behaved ideally.

Discussion

Isotherms of Phospholipid Films on Carbohydrate-Containing Subphases. Three explanations for the capacity of carbohydrates to expand lipid monolayers have been put forward in the literature. (a) The first explanation by Cadenhead & Demchak (1972) is that glycerol interacts with the hydrocarbon region of the monolayer. (b) Maggio & Lucy

(1978) and Cadenhead & Bean (1972) proposed the second explanation that the presence of carbohydrates in the subphase water changes its long-range order. This affects the hydration, orientation, and interactions taking place in and between phospholipid polar head groups, resulting in an expansion of the film. (c) The third explanation by Crowe et al. (1984a) is that carbohydrates coordinate to the polar head groups of the lipid monolayer, and in so doing alter their mobility and/or packing density. Coordination may take place directly to the ion or to the aqueous solvation shell surrounding it.

Explanation a was put forward chiefly to account for the influence of glycerol on monolayers. It is doubtful that this explanation is applicable to saccharides. Saccharides are very polar molecules, only slightly soluble in alcohol, and it is difficult to envisage how they could partition into a hydrocarbon phase from water. Lipid bilayers have been shown to be permeable to glycerol but are not permeable to saccharides (Hargreaves & Deamer, 1978). In addition, such a postulate does not explain the large differences in expansions seen on subphases containing glucose and galactose. The structures of these two sugars are so similar that their affinities for a hydrocarbon phase could not differ by the margin necessary to explain the large difference between the properties of films spread on their aqueous solutions. Note also that the charged sugar glucosamine expands monolayers more than its uncharged homologue glucose.

For a similar reason, we also doubt explanations b. The differences between the bulk properties of dilute glucose and galactose solutions are not likely to be sufficient to account for the differences in the area of films spread on their surfaces. Also, if these sugars did perturb the bulk structure of water, differences between the physical properties of sugar solutions and pure water should be apparent. However, even at the highest concentrations of galactose, we could not distinguish between the surface tensions of solutions and pure water.

We favor explanation c. The replacement of water molecules by bulky sugar molecules will increase the effective area of the lipids and cause an increase in film pressure. Such an idea would account for the marked difference in the capabilities of structurally similar sugars to expand films. The coordination of sugars to the cation and anion of lipids which will proceed primarily by hydrogen bonding through several hydroxyl groups would be expected to be strongly influenced by the arrangement of hydroxyl groups around the tetrahydropyran ring. However, if we are to accept this postulate, we must explain in thermodynamic terms how carbohydrates can effectively compete with water for lipid head groups when the water is present in molar concentrations 3–4 orders of magnitude higher. The enthalpy contribution to the free-energy change on solvation of an ion by water or carbohydrate will be similar since in both cases the bond formed is a hydrogen bond. Therefore, it would seem that solvation of the polar head group of a lipid with a sugar takes place with less of a fall in entropy than with water. Consideration of the nature of the ion–solvent "complex" suggests why this might be so. The hexose monosaccharides have five hydroxyl groups potentially available for hydrogen bonding and an ether oxygen atom. If these hydroxyl groups were favorably oriented, one sugar molecule could provide a degree of solvation equivalent to five water molecules. Considerably less translational freedom would be lost since one rather than five molecules would be bound to the monolayer. There is no clear relationship between the structures of the hexose sugars and their capacity to expand monolayers. Galactose and mannose are clearly superior to talose and glucoses. The only structural feature unique to these two sugars is the presence of two equatorial hydroxyl groups lying on adjacent carbons (3,4 and 2,3, respectively) on the side of the tetrahydropyran ring opposite the ether oxygen.

If coordination of sugar molecules to lipid head groups is the reason for the expansion of films on carbohydrate-containing subphases, then the magnitude of the expansion measured will be a product of the size of the lipid–sugar complex and the number of lipid molecules in the film to which the sugar is bound. This last factor will depend on the free-energy change associated with the replacement of water by sugar. Thus, while we could not measure any appreciable expansion of phospholipid films on glycerol-containing subphases, we cannot conclude that glycerol does not coordinate to phospholipid head groups. It may well do so but since it is smaller than saccharides, the glycerol–phospholipid "complex" may not be appreciably larger in area than phospholipid solvated solely by water. Conversely, there may be little difference between the amounts of glucose and galactose bound to films. Films on galactose-containing subphases may be more expanded simply because the orientation of its hydroxyl groups compels it to penetrate films more to achieve the same degree of solvation as glucose.

Isotherms of Single Ion Amphiphile Films on Carbohydrate-Containing Subphases. While unequivocal conclusions about the strength of binding between lipid and sugar cannot be drawn from monolayer expansion, some indication of the relative affinity of sugars for ions may be deduced from isotherms like that of the behenyltrimethylammonium ion shown in Figure 6. On carbohydrate-containing subphases, this lipid (and carboxylic acids, data not shown) exhibits what appears to be a phase change at a film pressure which depends on the nature of the carbohydrate in the subphases but not the length of the lipid alkyl chain. However, this cannot be a true phase change as the lipid has already passed through its liquid condensed–solid transition at 20 dyn/cm and the change in area is too large for a simple solid–solid transition. The area per molecule change during this transition can also be seen to depend on the carbohydrate concentration. It does not signify film collapse as continued compression of the film causes its pressure to rise steeply again. We believe that this behavior marks the exclusion of sugar from the film. The film pressure at which this occurs may be a more reliable parameter with which to judge the strength of the interaction between lipid and sugar. Trehalose begins to be excluded from the film at 29 dyn/cm and galactose at 37 dyn/cm, suggesting that galactose binds more tightly to the trimethylammonium ion than trehalose. It may well bind to other ions more effectively than trehalose, and this may explain why galactose invariably expands monolayers more than trehalose. Unlike behenyltrimethylammonium trifluoroacetate, galactose is not excluded from monolayers of behenylammonium trifluoroacetate at accessible film pressures. This implies a stronger interaction between galactose and ammonium ion than between galactose and trimethylammonium or carboxylate ion. This is plausible since of the three ions it is the ammonium ion which has the highest charge density. Stronger coordination to the ammonium ion would account for the following two observations: (1) the more rapid rise seen in plots of film area vs. carbohydrate concentration for the behenylammonium salt compared to the behenyltrimethylammonium salt and stearic acid; (2) the markedly greater expansion of phosphatidylethanolamine monolayers compared to phosphatidylcholine (see Table I). Isotherms of all the glycerophospholipids are strongly expanded on galactose-containing subphases in both the fluid and condensed regions. However, no loss of carbohydrate from these

films could be detected at any surface pressure. This suggests either that the carbohydrate–phosphate ion interaction is a strong one, as the measurements made on DPPA do indeed suggest, or that there is more room for carbohydrates around the ionic head group of glycerophospholipids. The fact that films are strongly expanded in the solid state demonstrates that the carbohydrate is strongly bound and it may be that even in expanded states there is no rapid exchange between bound carbohydrate and carbohydrate in the bulk phase.

While carbohydrates are capable of increasing the surface pressure in a film at a given area, they do not significantly change the surface pressure at which phase changes occur (see Figure 6). Thus, lipids enter the solid phase at much greater molecular areas. Since the degree of expansion falls as film pressure rises, the solid phase of lipids spread on subphases containing carbohdyrates which expand monolayers is considerably more compressible than the solid phase of films spread on pure water. For example, at 30 dyn/cm, to change the area of a DMPE film by 1 Å^2 per molecule the film pressure must be increased by 9.8 dyn/cm. If under comparable conditions the film is spread on a subphase containing 30 g/L galactose, a similar change in area occurs when the film pressure is increased by only 1.06 dyn/cm.

Isotherms of Mixed Films of Behenylammonium Salts and Stearyl Glycosides. The shape of plots of area per molecule vs. the mole ratio of mixed monolayers of behenylammonium salts and stearyl glycolipids (Figure 7) indicates that there is a positive interaction between sugar and ion. The net interaction in the film is greatest at a ratio of behenylammonium ion to glycolipid of at least 3 to 1. This suggests that the saccharide hydrogen bonds to several ions at once and the contraction in the area occupied by the film is caused by the resultant limitation of movement of the behenylammonium ions with respect to one another. No contraction could be detected in mixed films of the behenylammonium ions and arachidic alcohol. Thus, while we are able to demonstrate interactions between ionic lipids and sugars in both experimental arrangements (the sugar free in solution or as the polar head of an amphiphile), the nature of the interaction is clearly different in each case. In mixed films, the interaction is independent of the nature of both the ion and the sugar, suggesting that the forces involved are weaker and operate over greater distances. Clearly, for there to be a strong interaction between carbohydrate and ion, the carbohydrate must be free to adopt a preferred orientation to the ion. This orientation will be the one in which hydrogen bonding and the consequent displacement of water molecules from the monolayer are at a maximum.

Conclusions

We have shown that irrespective of their physical states monolayers are expanded by saccharides dissolved in the subphase. Consideration of the nature of this phenomenon suggests that the expansion is caused by the comparatively bulky sugar replacing water molecules in the solvation shell of the polar head group of the lipid molecules which make up the film. The driving force for displacement of water by sugar against a concentration gradient is the translational freedom gained when one sugar replaces several waters. The volume of the solvation shell is thereby increased, and hence the pressure the film exerts at a given area. The film expansion observed depends on both the nature of the saccharide and the lipid ionic head group, the dependence on the saccharide being the most marked. Galactose-type sugars caused the greatest increase in film area. Film expansion is the product of the increase in area of the lipid–saccharide "complex" and the proportion of the film solvated by carbohydrate. It is not possible to separate the contributions each of these factors makes to the increase in film area. However, the behenyltrimethylammonium salt isotherms suggest that the strength of the interaction between the film and the carbohydrate certainly plays a role. The orientation of the carbohydrate's hydroxyl groups will influence both factors: the area, by determining the orientation the carbohydrate must take to the lipid ions to achieve effective solvation, and the interaction strength, by its influence on the number of waters displaced from the film per carbohydrate bound.

On the basis of the monolayer measurements, some speculations about the role that carbohydrates play in both membrane preservation and cell recognition/adhesion can be made. In a membrane undergoing dehydration or freezing, attractive forces between the lipid hydrocarbon chains are increased. This usually leads to the separation of lipid and protein molecules. The lipid will crystallize, and the bilayer structure may be disturbed. Such processes are sometimes irreversible, and membrane integrity is lost (Crowe & Crowe, 1984). However, if the membrane lipids have been expanded by coordination of a suitable carbohydrate to the ionic head groups, the forces between the hydrocarbon chains will be decreased. This may mean that upon dehydration the presence of carbohydrate prevents the lipid from entering a solid crystalline phase. Indeed, recent experiments (Crowe et al., 1984b) show that the main endothermic phase transition temperature of DPPC is decreased by mixing the lipid with trehalose, i.e., replacing water by carbohydrate. We have also demonstrated that the solid phase of phospholipid monolayers is significantly more compressible when the water of solvation is replaced by carbohydrate. We might expect a similar change in the compressibility of phospholipid bilayers solvated by carbohydrate. In the case of cells subject to osmotic shock, clearly the more compressible and the more elastic the membrane the more likely it will be to survive undamaged an abruptly applied shearing force. Crowe et al. (1984) did not include galactose among the carbohydrates they tested for membrane preservation. We will have to wait until experiments with galactose-type sugars are carried out before reaching a definite conclusion on how the monolayer expansions caused by carbohydrates relate to their capacities to protect membranes from dehydration damage.

An aspect of cell adhesion which has been widely studied is the agglutination of cells by protein molecules known as lectins. Lectins have binding sites for sugar residues, are polyfunctional, and may be very specific in the type of sugar with which they interact. One commercial supplier (Sigma Chemical Co.) lists 21 sugar-specific lectins in its catalog. Of these, 11 bind *N*-acetylgalactosamine, 7 galactose, 2 L-fucose, and 1 glucose–mannose. Of the sugars we have examined, *N*-acetylgalactosamine and galactose are the two which expand monolayers most strongly and therefore are probably the sugars which bind most strongly to the ionic groups of the monolayer lipids. Perhaps in these two cases the binding sites on lectins may, at least in part, consist of the ionic groups of peptides. These sites may be arranged spatially on the surface of the protein to match the distribution of galactose-type sugars in oligosaccharides attached to membrane proteins or lipids. Similar comments can be made about the carbohydrates which are thought to be responsible for cell recognition. The oligosaccharide chains attached to membrane glycoproteins are made up of some or all of a group of just six monosaccharides, *N*-acetylgalactosamine, D-galactose, D-mannose, L-fucose, *N*-acetylglucosamine, and sialic acid (Harrison & Lunt, 1980).

We have shown that the first three of these sugars strongly expand lipid monolayers.

It has been demonstrated that the area occupied by molecules in a DMPE film may be doubled by the presence of sufficiently high concentrations of galactose. The increase in spacing of the lipid acyl chains that would result from such a film expansion should make the film, and by analogy a phospholipid bilayer, far more fluid and permeable to polar solutes. Localized areas of the plasma membrane may undergo a similar expansion near glycoproteins or glycolipids which have a sufficiently high density of suitable sugars. Such localized areas of increased membrane fluidity and permebility may play a role in the function of membrane proteins or entry of polar molecules into the cell.

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