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No Phospholipid Monolayer–Sugar Interactions†

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ABSTRACT: Studies by a number of workers using the Langmuir film balance have shown that when carbohydrates, such as sucrose or glycerol, are dissolved in a subphase on which a phospholipid is spread, film expansion occurs (Cadenhead & Demchak, 1969; Cadenhead & Bean, 1972; Maggio et al., 1976; Maggio & Lucy, 1978). Recently such effects have been observed again, particularly with the carbohydrates galactose and trehalose (Johnston et al., 1984). The origin of these film expansions was uncertain, and various suggestions have been made to explain them. One idea was that they might be due to interactions which these carbohydrates have with the water molecules close to the polar head groups of the lipids. Recent studies in our two laboratories, described here, show that the magnitude of the expansion effects is variable and that in general they arise from surfactant impurities in the sugars. These impurities are observed in carbohydrates which are reputedly of high grade; the amount of impurity present can vary from batch to batch, and sometimes they can be difficult to remove. Film balance techniques or subphase preparation can mask the detection of minor impurities. The presence of surfactant impurities in reputedly pure carbohydrates needs to be considered in other biochemical and biophysical studies of lipids and cell membranes.

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 biomembranes made up of the same type of lipid molecules (Phillips & Chapman, 1968). However, study of surface pressure–area isotherms can also throw light on the interaction between film molecules and other

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ions or molecules even if these are dissolved in the subphase. Information about recognition and adhesion phenomena can be obtained by studying the effect that solutes in the subphase have on glycolipid monolayers (Read et al., 1977). In principle stereoselective interactions between phospholipids and carbohydrates could also be studied in this way. Films of the pure enantiomers and racemic mixtures of chiral surfactants have been shown to have different properties (Arnett et al., 1978, 1982; Arnett & Thompson, 1981; Stewart & Arnett, 1982; Arnett & Gold, 1982).

Dissolving carbohydrates in a subphase on which monolayers of cell membrane lipids have been spread has provided an experimental arrangement that several groups have used for the study of both the protective and fusogenic effects of carbohydrates on membranes (Cadenhead & Demchak, 1969; Cadenhead & Bean, 1972; Maggio et al., 1976; Maggio & Lucy, 1978). Frequently film expansions are observed, and three explanations have been put forward to explain this: (a) by Cadenhead and Demchak (1972) that glycerol interacts with the hydrocarbon region of the monolayer; (b) by Maggio and Lucy (1978) and Cadenhead and Bean (1972) 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) by Crowe et al. (1984) and Rudolph et al. (1986) that carbohydrates coordinate to the polar head groups of the lipid monolayer and in so doing alter their mobility and/or packing density. It was suggested that coordination may take place directly to the ion or the aqueous solvation shell surrounding it. Studies by Johnston et al. (1984) also showed that carbohydrates such as trehalose and particularly galactose cause marked expansion of phospholipid monolayers. However, some of our recent studies show variable effects differing from earlier results. In this paper we reassess the various data on carbohydrate–phospholipid monolayer interactions.

Experimental Procedures

A number of film balances have been used. At London University film balance 1 is similar to the instrument described previously (Johnston et al., 1984); film balance 2 was arranged to provide a higher compression ratio. A higher compression ratio allows films to be compressed to a greater degree. At Duke two film balances were used: One, number 3, employs a highly sensitive torsion head with a floating barrier and Teflon ribbons to divide the trough, as described previously with the routine procedures for its use (Arnett et al., 1982), and is capable of detecting 0.005 dyn/cm differences in surface tension. Film balance number 4 employs a Wilhelmy plate suspended from a Cahn Model RTL microbalance and can detect differences of 0.1 dyn/cm in surface tension. Both film balances are enclosed in temperature-controlled cabinets and employ control systems that can be operated completely from outside.

At Duke University, interactions between monolayers of highly purified L and D phospholipids and two different carbohydrate-doped subphases (galactose and trehalose) were studied. Galactose (lot no. 44F-0093) and trehalose (lot no. 104F-3786) were obtained from Sigma Chemical Co. As supplied, the "purified grade" galactose showed evidence of surface-active impurities both through Du Nöuy tensiometry and the generation of surface pressure on both film balances 3 and 4. After seven recrystallizations from triple distilled water, a 30 g/L solution of galactose purified in this manner showed no surface activity. Thin-layer chromatography of the purified material showed only one component.

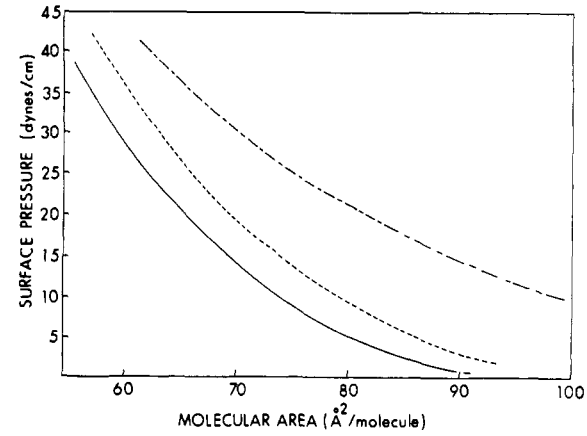

FIGURE 1: Film pressure/area isotherms (30 °C) for L-dimyristoylphosphatidylcholine monolayers spread on (—) pure water, (---) Sigma-grade galactose (batch 1), 60 g/L, and (-·-) Sigma-grade galactose (batch 2), 60 g/L.

Sigma trehalose used as supplied as a 30 g/L aqueous solution showed considerable contamination by Nöuy ring tensiometry, surface compression, and the presence of three components by thin-layer chromatography. No purification steps were taken with this sugar.

Water and spreading solvents were purified exhaustively as described elsewhere (Arnett et al., 1982).

L-Dipalmitoylphosphatidylcholine (no. P6267), D-dipalmitoylphosphatidylcholine (no. P1652), and L-dimyristoylphosphatidylcholine (no. P0888) were purchased from Sigma and purified by reverse-phase high-performance liquid chromatography (Altech, RSILC18-10u, 50 cm × 10 mm) eluted with 95% MeOH mobile phase. Just prior to use, the phospholipids were recrystallized twice from MeOH/Et_2O, recovered by centrifugation, and dried in vacuo over P_2O_5 at 100 °C for 12 h. Spreading solutions were prepared as described previously (Arnett et al., 1978).

At London University, four carbohydrates were added to the subphase: galactose, trehalose, lactose, and sucrose. Samples of galactose were purchased from Sigma Chemical Co. Ltd. (designated Sigma grade galactose) and Fluka AG (puriss grade). Samples of trehalose were purchased from Sigma, Aldrich Chemical Co. Ltd.; Koch Light Ltd., and Fluka AG. Lactose and sucrose were purchased from Aldrich and BDH Chemical Co., respectively. All the carbohydrates were the purest on offer from the supplier. Samples of lactose, trehalose, and Sigma galactose were recrystallized repeatedly from distilled water. Adding activated charcoal to the hot sugar solution before filtration was found to be an effective method of removing surfactant impurities. Sucrose subphases were allowed to stand over activated charcoal before use.

L-Dimyristoylphosphatidylcholine (puriss grade) was purchased from Fluka AG and used as supplied. All measurements were made at 30 °C.

Results and Discussion

Figure 1 contains isotherms of dimyristoylphosphatidylcholine (DMPC) measured on subphases of pure water and galactose solutions. The isotherm on the pure water subphase is in good agreement with that reported by Phillips and Chapman (1968). It can be seen that the phospholipid films spread on galactose solutions occupy larger areas at a given film pressure than the film on pure water. The larger increase in area is similar to that measured and reported earlier (Johnston et al., 1984). However, it is clear from the figure that the expansion observed is dependent on the batch of

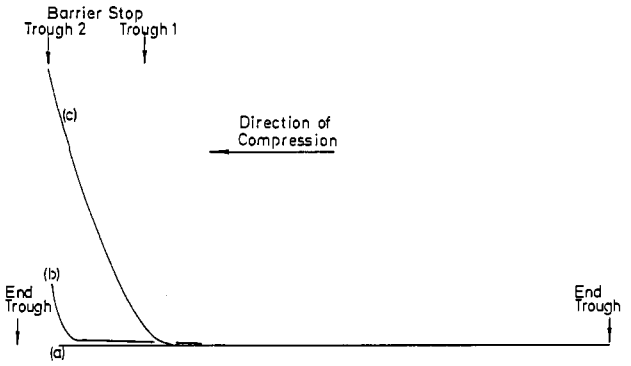

FIGURE 2: Surface pressure readings while barrier swept across the surface of (a) Sigma-grade galactose (batch 1), 30 g/L, (b) Sigma-grade galactose (batch 2), 30 g/L, and (c) as for (b), 90 g/L.

galactose used. It was also found that when the galactose was recrystallized 6 times from water before use, it no longer caused film expansion in either DMPC or DPPC.

A convenient method for testing for surfactant impurities is to sweep the barrier of the balance across subphases containing various concentrations of carbohydrate. This was done earlier with galactose in the subphase using film balance 1 (Johnston et al., 1984). No increase in surface pressure or reduction in surface tension was observed. However, when the barrier of film balance 2 was swept across the surface of the galactose solution on which the large film expansion shown in Figure 1 was recorded, a rise in surface pressure was detected (see Figure 2). Film balance 2 has a higher compression ratio than balance 1. Clearly this sugar contains surface-active impurities. Thin-layer chromatography also showed that this sugar sample was contaminated. No increase in surface pressure was detected when the galactose solution that caused the smaller film expansion was swept. Impurities could not be detected in previously impure galactose samples after six recrystallizations.

Other carbohydrates have been reported to cause lipid film expansion when dissolved in the subphase. These include glucose, glycerol, and trehalose (Crowe et al., 1984), sucrose and glycerol (Maggio & Lucy, 1978), and lactose (Johnston et al., 1984). However, Johnston et al. (1984) did not detect film expansion with subphases containing glucose and glycerol which were free of contamination. We have now measured the isotherms of dimyristoylphosphatidylcholine films spread on solutions of the other three carbohydrates, and the Duke group has studied purified dipalmitoylphosphatidylcholine with purified galactose. Isotherms run on solutions of Fluka (puriss grade) trehalose and pure water are traced in Figure 3. While some batches of trehalose are capable of causing a monolayer expansion, the figure shows that with others there is no difference between the isotherms obtained on pure water and the sugar solution. Furthermore, trehalose carefully recrystallized in the presence of charcoal does not cause film expansion. Pure lactose (e.g., Aldrich) is capable of causing an expansion of lipid monolayers. However, it was also found that after this sugar had been carefully recrystallized from water to which charcoal had been added, the isotherms of DMPC run on its solutions and pure water were identical. Previous measurements of the effects of sucrose on phospholipid monolayers (Maggio & Lucy, 1978; Crowe et al., 1984) were made at solution concentrations between 0.1 and 3 M. These workers showed that sucrose was capable of causing marked expansion of monolayers. However, after the sugar was boiled with charcoal we were unable to measure any significant monolayer expansion.

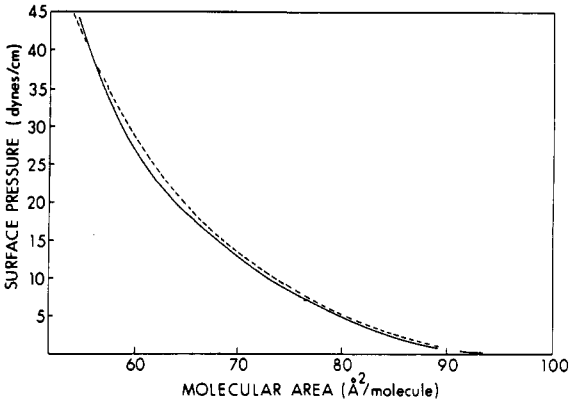

FIGURE 3: Film pressure/area isotherms (30 °C) for L-dimyristoylphosphatidylcholine monolayers spread on (—) pure water and (- - -) Fluka (puriss grade) trehalose, 60 g/L.

CONCLUSIONS

Film balance expansion effects observed previously by a number of workers when monolayers are compressed on subphases containing carbohydrates are probably due to the presence of surfactant impurities in the carbohydrate, despite the fact that reputedly pure carbohydrates were used. This indicates the extreme care that is necessary when dealing with compounds which are dissolved in high concentrations in subphases for monolayer studies. It also points to the necessity to consider the effects that variable amounts of these surfactant impurities in reputedly pure carbohydrates, including sucrose, may have in other studies of biological and model membrane systems. This is especially important where high concentrations of the carbohydrate are required in the experimental procedure. Finally, it raises the interesting question as to the nature of the impurities that have such a striking effect on phospholipid films although present at such relatively low concentrations.

The significance of measurable interactions between phospholipid monolayers and subphase carbohydrate solutions has been described in previous articles on this topic. The present report does not eliminate the possibility of demonstrating such effects but indicates the extreme difficulty of differentiating them from the effects of impurities.

Registry No. Galactose, 50855-33-9; trehalose, 99-20-7; L-dimyristoylphosphatidylcholine, 18194-24-6.

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Studies on the Substrate Specificity of Human and Pig Lecithin:Cholesterol Acyltransferase: Role of Low-Density Lipoproteins†

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ABSTRACT: The substrate properties of low-density lipoprotein (LDL) fractions from human and pig plasma and of lipoprotein *a* [Lp(a)] upon incubation with either pig or human lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) were investigated and compared with those of pig high-density lipoproteins (HDL) or human HDL-3. The cholesterol esterification using purified native pig LDL-1, human LDL, or Lp(a) as a substrate was approximately 36–42% that of pig HDL or human HDL-3, while cholesteryl ester formation with pig LDL-2 was 41–47%. No significant difference was found in the substrate activity between pig HDL and human HDL-3, and between human LDL and Lp(a), respectively. After depletion of pig LDL-1, pig LDL-2, and human LDL from apolipoprotein A-I (apoA-I), cholesteryl ester formation decreased to about 22–28% of the value found with pig HDL. Depletion of human LDL from apolipoprotein E (apoE) did not result in significantly different esterification rates in comparison to native LDL. Total removal of non-apoB proteins from human LDL resulted in esterification rates of approximately 10–15% that of HDL. Readdition of apoA-I to all these LDL fractions produced solely in apoA-I-depleted LDL fractions an increase of cholesteryl ester formation, whereas in those LDL fractions that were additionally depleted from apoE and/or from apoC polypeptides, a further decrease in the esterification rate occurred. When the phosphatidylcholine (PC)/free cholesterol (FC) ratio in LDL was raised by incubation with liposomes (PC/FC molar ratio 3.5:1 to 4.5:1) in the presence of human lipid transfer protein, the substrate properties of phosphatidylcholine-enriched LDL were nearly equivalent to those of HDL. From our results we conclude (1) that native LDL or Lp(a) also in vivo may serve as substrate for LCAT and (2) that enrichment of LDL with phosphatidylcholine leads to a reorientation of surface lipids in LDL, thus allowing activator proteins and/or LCAT to interact with this substrate in a similar manner as with HDL.

The cholesteryl esters found in plasma are mainly produced by the action of lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43), an enzyme synthesized by the liver (Glomset, 1973, 1979). Initially, these cholesteryl esters are incorporated into high-density lipoproteins (HDL)[1] (Akanuma & Glomset, 1968) and transferred to or exchanged between low-density lipoproteins (LDL) and very low density lipoproteins (VLDL) (Nichols & Smith, 1965; Fielding & Fielding, 1980; Barter et al., 1982a,b). Together with triglyceride-rich particles and cholesteryl ester transfer protein (CETP) LCAT plays a major role in the interconversion of HDL-3 to HDL-2 (Dieplinger et al., 1985; Knipping et al., 1985). However, it was shown that LCAT interacts also directly with apoB-containing lipoproteins (Barter, 1983; Barter et al., 1984).

HDL are believed to serve as the main substrate for LCAT because of the content of apoA-I, a necessary cofactor for the LCAT reaction (Fielding et al., 1972). There exist, however, other cofactors, e.g., apoC-I (Soutar et al., 1975) or apoA-IV (Steinmetz & Utermann, 1984).

In previous work on the HDL-3/HDL-2 interconversion using pig plasma (Knipping et al., 1985) we observed a significant increase of cholesteryl esters in LDL during the LCAT reaction. This increase could not be explained by cholesteryl ester transfer from HDL to LDL, since pig plasma does not contain CETP (Barter et al., 1981).

In contrast to human LDL, pig LDL comprises two subclasses designated LDL-1 and LDL-2 (Janado & Martin,

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[1] Abbreviations: HDL, high-density lipoproteins; LDL, low-density lipoproteins; pig LDL-1, buoyant density fraction 1.020–1.063 g/mL; pig LDL-2, buoyant density fraction 1.063–1.090 g/mL; apoA-I, apolipoprotein A-I; apoC, apolipoprotein C; apoE, apolipoprotein E; apoB, apolipoprotein B; PC, phosphatidylcholine; FC, free cholesterol; CETP, cholesteryl ester transfer/exchange protein; Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; SD, standard deviation.

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