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Modulation of the bilayer to hexagonal phase transition of phosphatidylethanolamines by acylglycerols

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The effect of mono-, di- and triacylglycerols on the bilayer to hexagonal phase (H_{II}) transition was studied by differential scanning calorimetry and ^{31}P-NMR spectroscopy. The acylglycerols were mixed with either dielaidoylphosphatidylethanoline or with 1-palmitoyl-2-oleoylphosphatidylethanolamine. Acylglycerols of lauric, oleic and stearic acids were utilized. All of the acylglycerols lowered the bilayer to H_{II} phase transition temperature. Diacylglycerols were much better H_{II} phase promoters than monoacylglycerols while triacylglycerols were the most potent bilayer phase destabilizers. Fatty acid composition generally had less of an effect except for the monoacylglycerols where bilayer destabilization increased from monolaurin to monostearin to monoolein. The most marked difference in behaviour resulting from changes in the fatty acid composition of the acylglycerol occurred with tristearin. This was the only acylglycerol which decreased the bilayer to H_{II} phase transition temperature only below a mol fraction of 0.005. Above this mol fraction, further addition of tristearin had no effect on the bilayer to H_{II} phase transition. These results suggest that the tristearin has limited solubility in phosphatidylethanolamine.

Introduction

Several of the properties of membranes are thought to be regulated by their propensity to convert from a stable bilayer arrangement of the phospholipids to non-bilayer structures [1]. The effect of additives on the rate of membrane fusion in model systems can often be correlated with the effect of these additives on the bilayer to hexagonal (H_{II}) phase transition temperature [2]. Additives which raise the bilayer to H_{II} phase transition temperature generally inhibit membrane fusion [3]. Several of these bilayer stabilizing compounds show antiviral activity [4–8]. Monolaurin has antiviral activity [9]. It is not known how monoacylglycerols affect the bilayer to H_{II} phase transition temperature. The related compounds, diacylglycerols, lower the bilayer to H_{II} phase transition temperature [10]. Does the extra hydroxyl group on monoacylglycerols make them better bilayer stabilizers than diacylglycerols or does the particularly short C_{12} chain of lauric acid make the molecule a poor H_{II} phase promoter?

Another correlation exists between H_{II} phase promotion and the activity of a membrane-bound enzyme. Uncharged and zwitterionic compounds which are bilayer stabilizers inhibit the activity of protein kinase C while H_{II} phase promoters are activators of this enzyme [11,12]. Diacylglycerols are potent hexagonal phase promoters and are activators of protein kinase C in agreement with

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this generalization. However, there are fairly specific structural requirements for diacylglycerol-related activators [13]. For example, triacylglycerols have little effect on the activity of this enzyme [14]. How does the effect of triacylglycerols on the bilayer to H_{II} phase transition compare with the effects of diacylglycerols?

To answer these and other questions, we have undertaken a more systematic study of the effects of several mono-, di- and triacylglycerols on the bilayer to H_{II} phase transition of dielaidoylphosphatidylethanolamine (DEPE) and 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE). We have used acylglycerols containing lauric (12:0), oleic (18:1_c) and stearic (18:0) acids.

Materials and Methods

Materials

DEPE and POPE were purchased from Avanti Polar Lipids, Birmingham, AL. All acylglycerols were purchased from Nu Chek Prep. Inc., Elysian, MN. Acyl groups are on position 1 of glycerol for the monoacylglycerols and on positions 1 and 2 for the diacylglycerols.

Sample preparation

The DEPE or POPE was dissolved together with an acylglycerol in a solution of chloroform and methanol (2:1, v/v). The solvent was evaporated with a stream of dry nitrogen so as to deposit the lipid as a film on the walls of a glass test tube. Last traces of solvent were removed into a liquid nitrogen trap by placing the samples in a vacuum oven at 40°C. The apparatus was maintained under high vacuum for at least 90 min. The lipid film was then suspended in a pH 7.40 buffer of 20 mM Pipes, 150 mM NaCl, and 0.02 mg/ml NaN_3. The tube was warmed to about 45°C and vortexed vigorously for about 30 s. The final concentration of DEPE or POPE was 5 mg/ml with varying amounts of acylglycerol admixed. The buffer and lipid suspensions were degassed under vacuum before loaded into the calorimeter.

Differential scanning calorimetry (DSC)

Lipid suspension or buffer was loaded into the sample or reference cell, respectively, of an MC-2 high-sensitivity scanning calorimeter (Microcal Co., Amherst, MA). A scan rate of 39 K/h was generally employed. Second heating scans on the same sample were very similar to the first scan. Transition enthalpy values for phosphatidylethanolamine are inaccurate because of the difficulty in transferring these non-homogeneous suspensions to the calorimeter cell. Additions of acylglycerols did not alter the enthalpy values which remained within ±20% of the values previously reported [15].

^{31}P-NMR

NMR spectra were recorded on a Bruker WM-250 spectrometer operating at 101.26 MHz. The probe temperature was maintained to within ±0.8 K by a Bruker B-VT 1000 variable temperature unit. Temperatures were checked by thermocouple measurements. A 10 mm broad band probe was used. A vortex plug was inserted above the sample in the NMR tube. A spectral width of 29.4 kHz was employed with an acquisition time of 0.279 s (16 K data points). The 90° pulse width was 29.0 μs with composite pulse proton decoupling. FID's were processed using exponential multiplication (line broadening 50.0 Hz). Chemical shifts are expressed in ppm from an external reference of 85% phosphoric acid in ^2H_2O.

Results

For each acylglycerol tested, DSC heating curves were measured for a series of 6–8 samples with increasing mol fraction of additive. The bilayer to H_{II} phase transition was fitted to a single van't Hoff component for each scan. The transition temperature of this component was plotted against the mol fraction of acylglycerol in the lipid preparation. In general, these plots were linear with regression coefficients ≥ 0.99. Only in the case of the triacylglycerols (Figs. 1 and 2) and of diolein (Fig. 3) are the curves markedly non-linear at higher mol fraction of additive. For these cases, the slope was determined at lower mol fraction of additive where the relationship was linear. These slopes are somewhat arbitrary since they depend on the range of mol fractions included for the analysis. They are presented to allow comparison with other compounds tested. The values for the

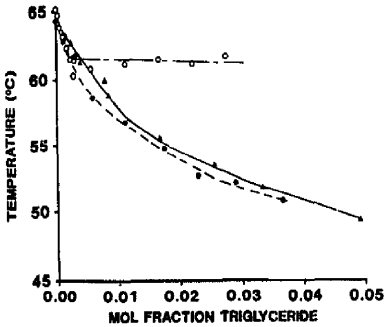

Fig. 1. Effect of triacylglycerols on the bilayer to H_{II} phase transition temperature of DEPE. ▲——▲, trilaurin; ●— — —●, triolein; ○— — —○, tristearin.

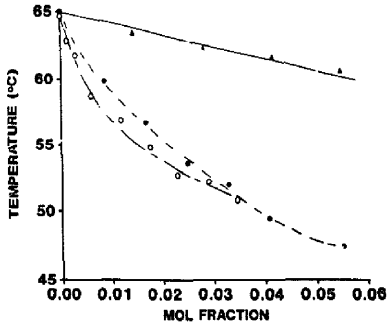

Fig. 3. Effect of monoolein (▲——▲), diolein (●— — —●) and triolein (○— — —○) on the bilayer to H_{II} phase transition temperature of DEPE.

slopes of these plots are given in Table I. The negative slopes indicate that the acylglycerols lower the bilayer to H_{II} phase transition temperature and are bilayer destabilizers.

The effect of additives on the bilayer to H_{II} phase transition temperature are generally at least an order of magnitude greater than their effects on the gel to liquid crystalline phase transition temperature. This is also the case for di- and triacylglycerols. Monoacylglycerols, however, which have relatively weak effects on the bilayer to H_{II} phase transition of DEPE have comparable effects on the gel to liquid crystalline transition. Monolaurin depresses the gel to liquid crystalline transition temperature 1.5-times as much as it lowers the bilayer to H_{II} phase transition temperature. The extent of the shift in transition temperature is dependent on the mol fraction of acylglycerol used. Monolein lowers the main transition only 0.2 as much as it lowers the H_{II} phase transition. In contrast, monostearin which also lowers the bilayer to H_{II} phase transition temperature, raises the gel to liquid crystalline transition temperature by 0.4-times as much. These results demonstrate that monolaurin can have substantial effects on the phase transition properties of DEPE and is therefore partitioning into the membrane, at least at lower temperatures. The behaviour of mono-

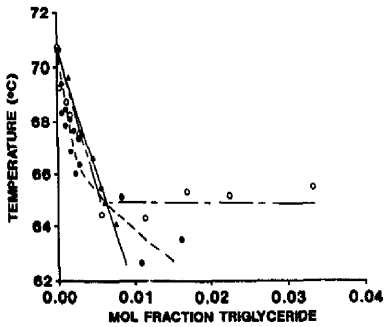

Fig. 2. Effect of triacylglycerols on the bilayer to H_{II} phase transition temperature of POPE. ▲——▲, trilaurin; ●— — —●, triolein; ○— — —○, tristearin.

TABLE I

EFFECT OF ACYLGLYCEROLS ON THE BILAYER TO H_{II} PHASE TRANSITION TEMPERATURE OF PHOSPHATIDYLETHANOLAMINE

Additive	Slope [a], DEPE
Monolaurin	−13 ± 1
Dilaurin	−234 ± 13
Trilaurin	−889 ± 56 [b]
Monoolein	−79 ± 5
Diolein	−450 ± 39 [b]
Triolein	−925 ± 56 [b]
Monostearin	−39 ± 2
Distearin	−270 ± 10
Tristearin	−968 ± 32 [b]
	Slope [a], POPE
Trilaurin	−740 ± 47
Triolein	−680 ± 41 [b]
Tristearin	−1000 ± 200 [b]

[a] Units are degrees/mol fraction additives (see text).
[b] Marked deviation from linearity at higher mol fractions.

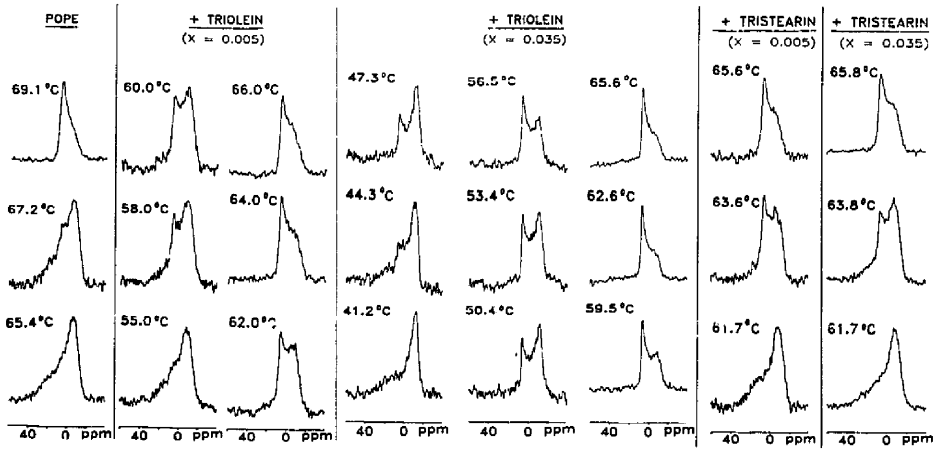

Fig. 4. Effect of triacylglycerols on the ^{31}P-NMR spectra of POPE as a function of temperature and mol fraction of additive. One thousand scans were accumulated for each spectrum. Each sample contained 100 mg POPE in 0.7 ml Pipes buffer.

stearin is unusual as most hydrophobic or amphiphilic compounds lower the gel to liquid crystalline phase transition temperature of DEPE.

The effects of the acylglycerols on DEPE and on POPE are similar. For a given fatty acid, triacylglycerols have the greatest negative slope, followed by diacylglycerols and then Monoacylglycerols (Fig. 3 and Table I). There is less of an effect of different fatty acids, particularly for triacylglycerols. However, there is a tendency for acylglycerols of oleic acid to have the largest negative slope, followed by those of stearic acid and then the acylglycerols of lauric acid. The behaviour of tristearin is unusual in that although it is a potent H_{II} phase promoter, its effect is manifested only below mol fractions of 0.005. Above this low mol fraction, tristearin has no further effect on either DEPE (Fig. 1) or POPE (Fig. 2). This is also observed with ^{31}P-NMR spectroscopy (Fig. 4). The NMR results show that triolein but not tristearin markedly lowers the bilayer to H_{II} phase transition temperature of POPE between a mol fraction of 0.005 and 0.035. The POPE transitions at higher mol fractions of triolein could not be observed by DSC because of the broadness of the transition.

Discussion

A theory to quantitatively predict the relative stability of the bilayer and H_{II} phases is currently being developed. Among the factors which affect the position of equilibrium between these phases are the intrinsic radius of curvature of a monolayer, hydration of the head group and hydrocarbon packing constraints [16]. Monoacylglycerols with two hydroxyl groups would have a larger polar region than diacylglycerols and be more hydrated. They would therefore tend to favour the bilayer phase compared with di- or triacylglycerols. This predicted relative order is found with triacylglycerols being the best H_{II} phase promoters and monoacylglycerols the worst. There may also be a contribution resulting from the different molecular weights of the additives. Thus, if monoacylglycerols and triacylglycerols were present at equal mol fractions then the triacylglycerols would be present at a greater weight fraction and therefore be able to expand the hydrocarbon phase of the membrane to a greater extent. This factor, however, cannot explain the larger differences in bilayer destabilization that we observed among the acylglycerols (Table I).

Oleic acid should have much more tendency toward hexagonal phase formation than the saturated acids since the cis double bond introduces a kink in the acyl chain leading to expansion of the center of the bilayer. The diacylglycerol, 1-oleoyl-2-arachidonyl glycerol has been found to be particularly potent in promoting H_{II} phase formation [17]. However, this factor

does not result in great differences among di or triacylglycerols in their effect on the bilayer to H_{II} phase transition temperature. Another factor influencing the effect of these compounds is their partitioning into the phosphatidyethanolamine-rich phase of the membrane. Monolaurin being the least hydrophobic and most polar of the compounds studied may have little effect on the phase transition temperature because it does not mix well with DEPE. However, monolaurin is capable of lowering the gel to liquid crystalline phase transition temperature and therefore must partition, at least to some extent, into the membrane phase.

Monolaurin has been shown to have antiviral activity [9]. This activity is not a result of a potent bilayer stabilizing ability of this compound. Neither is monolaurin a H_{II} phase promoter; its lack of substantial effect on lipid polymorphism in this model system would not rule out a bilayer stabilizing effect in the more complex environment of a biological membrane.

Based on their promotion of H_{II} phase formation, triacylglycerols would be expected to be activators of protein kinase C [11,12]. These substances have no effect on the activity of protein kinase C [14] compared to their H_{II} phase-promoting activity. Triacylglycerols are not exceptions to the rule which states that uncharged substances which affect the activity of protein kinase C will be activators if they are H_{II} phase promoters. There is no quantitative relationship between the slope of a mole fraction vs. transition temperature plot and effects on protein kinase C activity [11,12].

Perhaps the most interesting and unexpected result of the present study is the marked difference between the effects of triolein and tristearin on the bilayer to H_{II} phase transition. At very low mol fractions below 0.005, both triacylglycerols affect the phase transitions of DEPE and POPE in a similar fashion. As the concentration of triacylglycerol in the membrane increases above 0.005, triolein continues to lower the phase transition temperature while tristearin has no further effect. It is possible that only a small amount of tristearin is soluble in the phosphatidylethanolamine membrane and that further addition of tristearin results in the formation of tristearin-rich domains. This is consistent with the lack of effect of tristearin on the cooperativity of the bilayer to H_{II} phase transition between a mol fraction of 0.005 and 0.05. It has recently been shown that plasma membranes of some cell lines contain substantial amounts of triacylglycerol [18]. This triacylglycerol is contained in non-bilayer domains and may increase membrane permeability [18]. Little is known about the fatty acid composition of this triacylglycerol. It is also interesting that consumption of saturated fats is more correlated with cardiovascular disease than is the consumption of unsaturated fats. If saturated fats are more prone to form separated domains in phosphatidylethanolamine-rich areas of the membrane, they may cause more profound changes in membrane properties because of the existence of phase boundaries between phospholipid-rich and triacylglycerol-rich domains. The saturated triacylglycerols would therefore be more damaging to the cell and would be more prone to form triacylglycerol-enriched plaques.

Acknowledgements

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