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The Effect of Pregnanedione on the Structure and Packing of Dipalmitoylphosphatidylcholine Bilayers

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X-ray diffraction analyses of a mixture of dipalmitoylphosphatidylcholine (DPPC) and 5α-pregnanedione (9:1 molar ratio) in water indicated the presence of two lamellar phases. High resolution calorimetry of dilute solutions of the same mixture in greater than 99.5 wt% water indicate only a slight change in the thermogram when compared to pure DPPC bilayers. Increasing the amount of sterol in the mixture did not alter the thermogram significantly. These results indicate that 5α-pregnanedione acts in a similar manner to cholesterol when present in low molar percent mixtures with DPPC and in less than 70 wt% water. As the water content is significantly increased, the sterol is no longer associated with the bilayer in contrast to similar studies done with cholesterol in DPPC bilayers.

Keywords: phase transitions, mesophase structures, pregnanedione, lipid bilayers, x-ray diffraction, calorimetry

INTRODUCTION

Knowledge of the interactions with and assimilation of sterols into biological membranes is important in understanding the basic structure and function of the membranes. In recent years, a general trend has been observed for the effect of sterols (i.e., cholesterol and ox-

idized sterol compounds) on the DSC thermodynamic properties of phosphatidylcholine (PC) model membrane (bilayer) systems [1-3]. Specifically, the thermograms representing the pre- and main phase transition for PC bilayers in the presence of sterols are broadened and shifted to lower temperatures. The degree of change is dependent on the sterol concentration in the bilayer. Less well defined is the effect of cholesterol on the PC mesophase structure and packing. X-ray[4-7] diffraction studies have shown that in the presence of low concentrations of sterols (<10 mole%), bilayers made from saturated PC's contain two lamellar phases. The appearance of two phases is chain length dependent with longer chained PC molecules generally producing two bilayers when mixed with sterols. For example, only one lamellar phase[6] is formed when dipalmitoylphosphatidylcholine (16 unit acyl chain) is mixed with low concentrations of 7-ketocholesterol, whereas two lamellar phases[7] are observed when the sterol is mixed with distearoylphosphatidylcholine (18 unit acyl chain). In both cases, the fully hydrated bilayers are in the gel (L_β) thermodynamic state.

A recent calorimetric and Raman spectroscopic study[8] has indicated that a variety of anesthetic and non-anesthetic sterols also cause the dipalmitoylphosphatidylcholine (DPPC) thermograms to broaden and shift to lower temperatures. In the interest of expanding our examination of the effect of sterols on the PC bilayer structure and packing, we have chosen to study mixtures of DPPC and 5α-pregnanedione in water using high resolution calorimetry and x-ray diffraction. Since 5α-pregnanedione is one of the few anesthetic sterols which is non-toxic, we were not required to upgrade our sample handling facilities. It was determined that the partition of 5α-pregnanedione into DPPC bilayers (and therefore its influence on the lipid bilayer) is dependent on the water content of the mixture.

MATERIALS AND METHODS

L-α-dipalmitoylphosphatidylcholine was obtained from Avanti Polar lipids (Birmingham, Alabama). The 5α-pregnane-3,20-dione (allo) was obtained from Sigma Chemical Co. (St. Louis, Missouri). All lipids were used without further purification.

Lipid mixtures were obtained by dissolving the DPPC and 5α-pregnanedione in chloroform at room temperature. The chloroform was removed by placing the mixture in a rotovaporator, with final drying done under a dry vacuum to remove all traces of chloroform.

X-ray samples were prepared by mixing known amounts of the lipid mixtures in distilled water and allowing equilibration to occur at room temperature over 48 hours. The lipid-water samples were then transferred to x-ray sample holders and placed in Guinier-type cameras to obtain the x-ray powder pattern. The Cu $K\alpha_1$ ($\lambda = 1.540$ Å) from a Dunlee x-ray tube connected to a Picker Instruments 6238 diffraction generator was isolated using nickle foils. A Phillips x-ray film reader was used to measure the diameters of the circular diffraction patterns. Powdered teflon was mixed in the samples to provide an internal camera standard. The lattice repeat spacing, d, is calculated directly from our film readings. With less than full hydration, the d-spacing from a single bilayer phase can be converted into the bilayer thickness, d_L, and water layer thickness, d_w, from the volume fraction of the lipid in the sample (ϕ) where: $d_L = \phi d$ and $d_w = d - d_L$. The volume fraction of the lipid is determined by the expression:

$$\phi = \left[1 + \frac{(1-c)\,\nu_w\,(1+K)}{c(K\nu_S + \nu_L)}\right]^{-1}$$

where c is the weight fraction of lipid in the sample, ν_w, ν_L and ν_S are the partial specific volumes of water, phospholipid and sterol, respectively, and

$$K = \frac{MW_S}{MW_L} f$$

where f is the mole ratio of sterol to phospholipid, and MW_L and MW_S are the molecular weights of the phospholipid and sterol, respectively. The average specific volume of the phospholipid was taken as 0.95.[11]

Calorimetry samples were prepared by dissolving known weights of ratios of DPPC and 5α-pregnanedione in chloroform in a test tube. The chloroform was evaporated off under a stream of nitrogen gas, simultaneously rotating the test tube by hand creating a thin coat of lipid mixture on the bottom third of the tube. The lipid mixture was then dried under vacuum for one hour to completely remove the chloroform. An appropriate amount of water was added to the lipid mixture to make a 5 mg per ml dispersion. The dispersion was formed by sonicating the lipid mixture-water samples in a bath sonicator (Bransonic 220) for 30 second bursts. Some samples were heated in

a 60°C hot water bath for 10 minutes before re-sonication to completely disperse the lipid mixture.

The sample and reference (water) solutions were delivered into their respective calorimetric cells in the MicroCal MC-2. The calorimeter cells were tightly sealed and then equilibrated for a minimum of thirty minutes before measurements were taken. High sensitivity differential scanning calorimetry was performed on the MicroCal MC-2. All calorimetric scans were performed at a scan rate of 12°C/hr and started at least 20°C below Tm. All samples were run under a nitrogen gas pressure of 15 psi to minimize bubble formation in the sample and reference.

Following the completion of the scan, the lipid dispersion was extracted from the calorimeter cells using methanol and dried to an ash in a warm oven (approximately 54°C). The lipid concentration was determined from the dry weight of the ash.

Thermograms obtained with the MicroCal MC-2 were analyzed with the MicroCal DA2 software package using an IBM PC interfaced to the calorimeter. Enthalpy was calibrated using an internal electronic calibration. The onset temperature of the thermogram was used as the transition temperature.

RESULTS AND DISCUSSION

The bilayer structural parameters for a mixture of dipalmitoylphosphatidylcholine and 5α-pregnanedione (9:1 mole ratio) were determined as a function of water content using x-ray diffraction. As shown in Figure 1, two lamellar repeat spacings were observed for water contents of 15 to 70 wt%. The limiting values for these structures were approximately 61 and 67 Å. At water contents less than 15 wt%, only one lamellar repeat spacing was observed. It cannot be determined if this result is due to a single bilayer, or to two bilayers with repeat spacing outside the limit of our resolution.

A previous calorimetric and Raman spectroscopic report[8] of the interaction between DPPC and anesthetic steroids indicated that a 9:1 molar mixture of dipalmitoylphosphatidylcholine and 5α-pregnanedione had a broader thermogram than that produced by DPPC in water. This result is consistent with the general influence of sterols, i.e., cholesterol,[1] or oxidized sterol compounds,[3] on DPPC bilayers at these hydration levels. The appearance of two lamellar phases is also a general phenomenon describing the influence of sterols on PC bilayers (e.g., cholesterol on DPPC bilayers[4] and 7-ketocholesterol

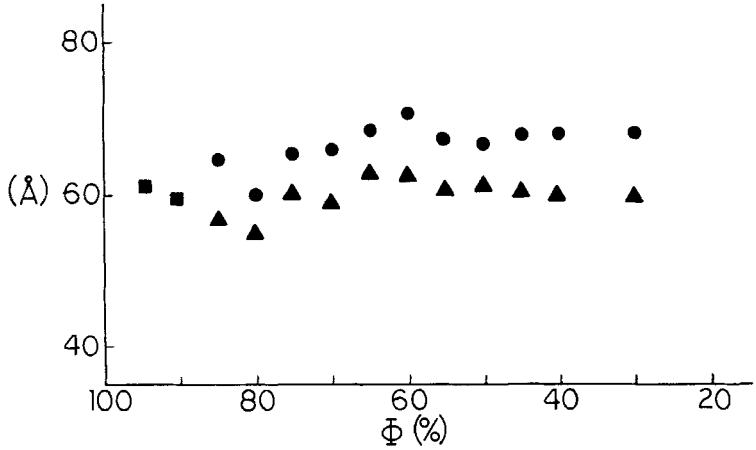

FIGURE 1 Bilayer repeat spacings (d) for 9/1 molar ratio of dipalmitoylphosphatidylcholine/5α-pregnane-3,20-dione (allo) as a function of water content. (● larger d of the two observed phases; ▲ smaller d of the two observed phases; ■ single d of only one observed phase).

on distearoylphosphatidylcholine bilayers[7]), and occurs simultaneously with the observation of a broadened calorimetry thermogram.

We have also examined the influence of higher water contents on various molar ratios of DPPC and 5α-pregnanedione using high resolution scanning calorimetry. It had been suggested by O'Leary and co-workers,[8] that there was a possibility that anesthetic sterols would partition to the aqueous phase from the bilayer as the water content is increased. In order to determine the extent of partitioning of 5α-pregnanedione to the aqueous phase in a biological system, we examined mixtures with less than 0.5 wt% lipid in water. High sensitivity calorimetric scans were compared for pure DPPC, and for DPPC-pregnanedione at various molar ratios between 9:1 to 1:1 (PC:sterol). All thermograms for mixtures of ratios between 9:1 and 1:1 DPPC:pregnanedione were essentially identical to that for DPPC in relation to the gel to liquid crystalline phase transition temperature (41°C), enthalpy (7.07 Kcal/mole) and peak width at one half the peak height (~0.25°C). The observation of no change in DPPC thermogram in the presence of 5α-pregnanedione in a large excess of water is contrary to the previous report for low water contents.[8] The results suggest that at high water contents (approx. 0.5% lipid mixture (W/V) in water) and perhaps using sonication, the sterol partitions from the lipid bilayer to the aqueous phase. This is supported by the additional observation that following sonication, some sediment was

observed for all mixtures of DPPC-pregnanedione studied. Neither heating in a water bath for 10 minutes nor heating in an oven for an hour (both at 60°C) followed by sonication had any effect on preventing the formation of the sediment. We can also infer that the 5α-pregnanedione is not associated with the DPPC bilayer at these high water contents since the presence of 25 mole% or less cholesterol causes the DPPC thermogram to be broadened.[1,2] A similar water content dependence for the partition of cholesterol[1,2] or oxidized sterol compounds[3,6] into the DPPC bilayer has not been observed.

CONCLUSIONS

A previous study[8] utilizing high resolution differential scanning calorimetry and Raman spectroscopy indicated that anesthetic sterols affect DPPC bilayer structure and thermodynamic properties in a manner similar to cholesterol when low water contents were used. We examined a 9:1 ratio of DPPC and 5α-pregnanedione in order to compare our results directly with those of O'Leary and co-workers.[8] We observed two lamellar repeat spacings for the water contents used (including excess) for hydrating this lipid mixture, which is consistent with our previous study of DPPC/cholesterol mixtures.[6] However, our calorimetric examination of mixtures of DPPC and 5α-pregnanedione with increasing sterol molar content from 9:1 to 1:1 (DPPC/5α-pregnanedione) suggests that there is little influence of the sterol on the bilayer phase transition properties. These results indicate that the partition of 5α-pregnanedione into the DPPC bilayer is dependent on the water content. The amount of 5α-pregnanedione in the bilayer decreases drastically as the water content is raised. These observations are consistent with the view that anesthetics do not interact either specifically or non-specifically with lipids, but probably do interact with specific proteins.[9,10]

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