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Phosphatidylcholine/7-ketocholesterol Interactions: II. Effect of Phospholipid Acyl Chain Length in Gel State Bilayers

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Oxygenated sterol compounds have recently been shown to interact in a manner different from that of cholesterol with phospholipid bilayers and red blood cell membranes. Part of the difference between mixtures of dipalmitoylphosphatidylcholine with cholesterol and with 7-ketocholesterol is in the number of gel state lamellar phases formed at low sterol concentrations [W. Tamura-Lis, L. J. Lis and J. M. Collins, *Mol. Cryst. Liq. Cryst.*, 132, 209–219 (1986)]. In this report, we examine mixtures of 7-ketocholesterol and distearoylphosphatidylcholine in water at 25°C using X-ray diffraction. Two gel state lamellar phases with different bilayer repeat spacings are observed at low sterol concentrations (1:19, 7-ketocholesterol/DSPC). These results indicate a difference in the miscibility of cholesterol and 7-ketocholesterol in gel state bilayers made from phosphatidylcholines of different acyl chain lengths. These results may have implications for the composition of domains in cell membranes.

INTRODUCTION

Cholesterol interactions with single and multi-component phospholipid systems have been well documented.[1–3] However, the effects of sterol derivatives such as oxygenated cholesterol on similar lipid bilayer systems have recently become of interest because of the implication that oxygenated cholesterols are associated with membrane function or morphology.[4] The presence of oxygenated cholesterols has been shown to synergistically interact with cholesterol to increase the sterol perturbation of lipid acyl chains and protein helical structure in red blood cell membranes.[5] Similar studies on model membranes

have shown that 7-ketocholesterol can synergistically interact with cholesterol to disrupt the acyl chain packing of dipalmitoylphosphatidylcholine (DPPC) bilayers.[6]

Lipid bilayers containing DPPC and 7-ketocholesterol in molar ratios (19/1, 9/1, 4/1, 7/3, 3/2 and 1/1) have been examined in water at 25°C using X-ray diffraction.[7] In contrast to DPPC/cholesterol gel state bilayers[8] at the same temperature, the presence of low contents of 7-ketocholesterol (i.e., 5 mole%) did not produce two DPPC gel state lamellar phases. The swelling of the DPPC/7-ketocholesterol bilayer in water was greater than that for either DPPC/cholesterol bilayer phase. However, the substitution of 19-hydroxycholesterol as the oxygenated sterol compound incorporated into the DPPC liquid crystal state bilayer at a mole ratio of 1:1 produced a more complicated phase relationship as evidenced by the appearance of two lamellar phases.[7] These results indicate that the position of the oxidized moiety is important in bilayer packing and morphology. In addition, it could be inferred that DPPC/7-ketocholesterol complexes have an increased miscibility in the DPPC gel state bilayer compared with DPPC/cholesterol complexes.

In this study, we report the effects of acyl chain species on the interaction of 7-ketocholesterol and gel state phosphatidylcholine bilayers in water at 25°C. It is more difficult to examine these interactions in liquid crystalline state bilayers because the high mobility of the molecules is less influenced by perturbations. Since we had not observed two gel state lamellar phases when low concentrations of 7-ketocholesterol were mixed with DPPC in water, we chose to examine the mixing of 7-ketocholesterol with a longer chain length phosphatidylcholine. It has been shown that cholesterol mixed with dimyristoylphosphatidylcholine (DMPC) produces one lamellar phase,[9] whereas cholesterol mixed with the longer acyl chain DPPC produces two lamellar phases.[8] It cannot be ruled out that this difference is influenced by the state of the lipid since DMPC is at or near the gel to liquid crystalline state transition at room temperature. We have chosen to examine the miscibility of 7-ketocholesterol in bilayers which are in the gel state at the same temperature but are not near a phase boundary. This selection minimizes the influence of thermal energy on the structures involved, but still allows us to directly compare our results with studies of DPPC-cholesterol mixtures. It was found that distearoyl phosphatidylcholine (DSPC)/7-ketocholesterol mixtures at low molar ratios (i.e., 19/1 and 9/1) do indeed produce two gel state lamellar phases similar to those produced by comparable amounts of cholesterol in DPPC. Thus 7-ketocholesterol is less miscible in gel state PC bilayers as the phospholipid chain length increases

but in a series different from that of cholesterol. These findings suggest that the substitution of cholesterol by oxidized cholesterols (i.e., 7-ketocholesterol) should influence the composition of membrane domains owing to their dissimilar miscibilities with lipids of different acyl chain lengths. Of course, these dissimilarities in miscibility may be reduced in the presence of a liquid crystalline state bilayer.

MATERIALS AND METHODS

L-α-dipalmitoylphosphatidylcholine and L-α-distearoylphosphatidylcholine were obtained from Avanti Polar Lipids (Birmingham, Alabama). The 7-ketocholesterol was obtained from Sigma Chemical Co. (St. Louis, Missouri). All lipids were used without further purification.

Lipid mixtures were obtained by dissolving the PC and 7-ketocholesterol 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. Diffraction patterns were obtained at room temperature (25°C) and atmospheric pressure. Under these conditions, both DPPC and DSPC are in the gel or non-fluid state. The Cu $K\alpha_1$ line ($\lambda = 1.540$ Å) from a Dunlee X-ray tube connected to a Picker Instruments 6238 diffraction generator was isolated using nickel foils. A Phillips X-ray film reader was used to measure the diameters of the circular diffraction patterns. Powder teflon was mixed in the samples to provide an internal camera standard. The lattice repeat spacing, d, is directly calculated from our film readings. Diffraction patterns from non-equilibrated samples which produced diffuse or slanted diffraction lines were not used in these determinations. 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:

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

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 these gel state phospholipids was taken as 0.95.[11]

RESULTS AND DISCUSSION

Mixtures of DSPC and 7-ketocholesterol were examined as a function of water content at 25°C using X-ray diffraction (Figures 1–3). Two gel state lamellar phases were observed over an extensive range of water contents, including excess water, for DSPC/7-ketocholesterol mixtures with mole ratios of 19/1 and 9/1. This is in contrast to the previous observation of only one gel state lamellar phase in similar mixtures of DPPC/7-ketocholesterol at 25°C.[7] There is obviously a difference in miscibility of 7-ketocholesterol in these two gel state lipid bilayers; 7-ketocholesterol is more miscible in DPPC than in DSPC bilayers. A phase complex between DSPC and 7-ketocholesterol cannot be ruled out as the cause of the relative immiscibility of 7-ketocholesterol in DSPC bilayers.

The significance of these findings becomes apparent when they are compared with previously obtained results on the number of phases found in PC/cholesterol mixtures. Cholesterol has been found to mix differently with dimyristoylphosphatidylcholine (DMPC) than with DPPC at 25°C. It has been observed that at low sterol contents, DMPC/cholesterol mixtures form one phase in water,[9] while DPPC/cholesterol mixtures form two lamellar phases.[8] Cholesterol clearly is more miscible in the myristoyl derivative of phosphatidylcholine although the phase state of the lipid may have an influence on this result. By the same token, it appears likely that cholesterol and 7-ketocholesterol each mix differently in PC bilayers but that this property is dependent in some manner on the acyl chain length of the lipid.

The increase in 7-ketocholesterol content from 5 to 10 mole% in the DSPC bilayer results in increases in the bilayer repeat spacings

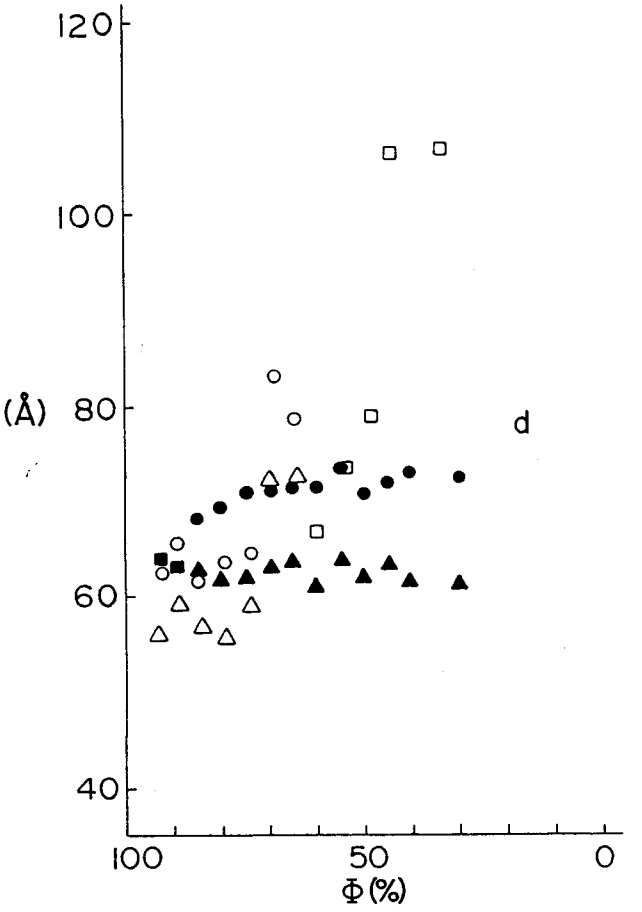

FIGURE 1 Bilayer repeat spacings (d) for 19/1 molar ratio of dipalmitoyl- (○, △, □) or distearoyl- (●, ▲, ■) phosphatidylcholine/7-ketocholesterol as a function of water content at 25°C. DPPC/7-ketocholesterol data from reference 7. (○, ●: larger d of the two observed phases; △, ▲: smaller d of the two observed phases; □, ■: single d of only one observed phase).

for the two observed phases from 61 and 72 Å to 67 and 83 Å (Figures 1 and 2). This increase is not consistent with previous observations of a slight decrease in the higher repeat spacing while the lower remains the same for gel state DPPC bilayers with increasing cholesterol contents.[8] The single DPPC/7-ketocholesterol gel state bilayer phase observed at low sterol contents[7] also slightly decreases in repeat spacing as the sterol content is increased in this range. It

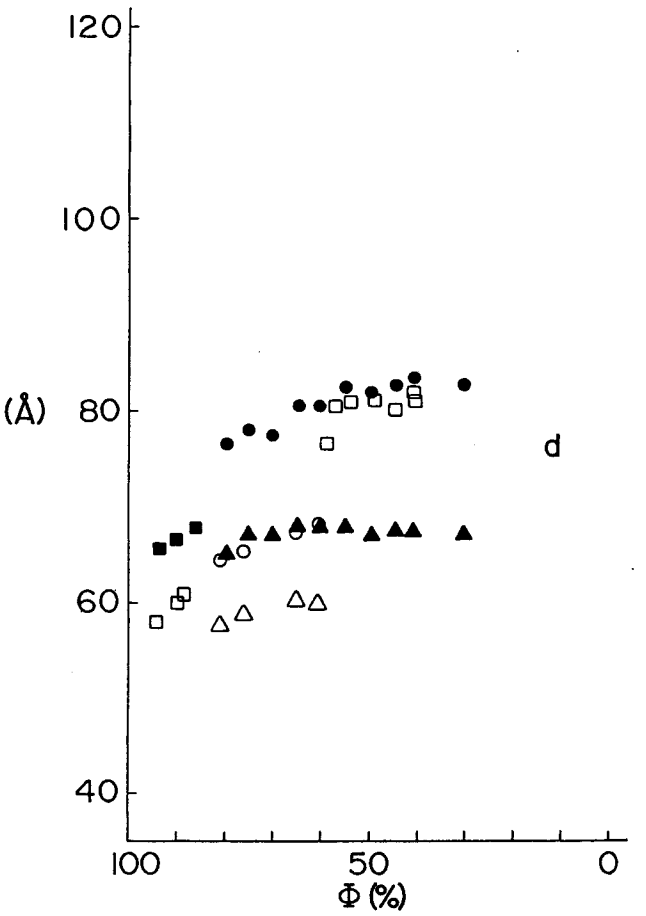

FIGURE 2 Bilayer repeat spacings (d) for 9/1 molar ratio of dipalmitoyl- (○, △, □) and distearoyl- (●, ▲, ■) phosphatidylcholine/7-ketocholesterol as a function of water content at 25°C. DPPC/7-ketocholesterol data from reference 7. (○, ●: larger d of the two observed phases △, ▲ smaller d of the two observed phases; □, ■ only single d of one observed phase).

appears that the presence of 7-ketocholesterol also influences the extent of the sterol induced modification of the gel state bilayer separation at low sterol contents which subsequently decreases dramatically with increasing sterol content. We do not have sufficient information to warrant an hypothesis that this modification is indeed continuous.

When 20 mole% 7-ketocholesterol is added to the DPPC bilayers,

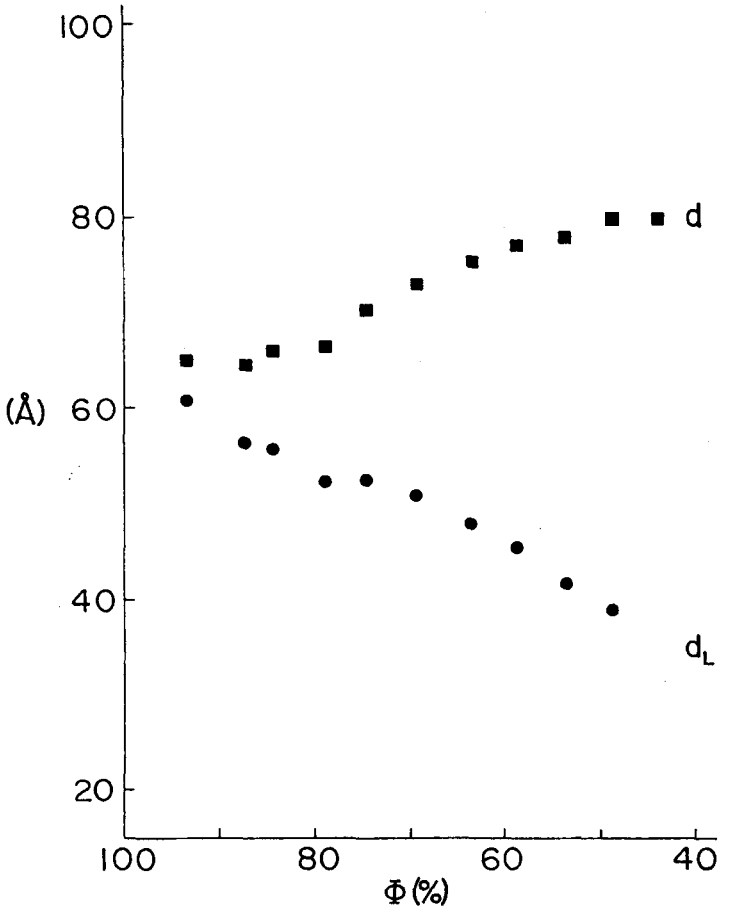

FIGURE 3 Bilayer structural parameters (d = ■; d_L = ●) for 8/2 molar ratio of distearoylphosphatidylcholine/7-ketocholesterol as a function of water content at 25°C.

only one gel state lamellar phase is observed at 25°C. The limiting bilayer repeat spacing is approximately 80 Å with a bilayer thickness of approximately 40 Å. This value for d_L is slightly lower than that for DSPC bilayers in the gel state (ca. 46 Å) and larger than that for PC bilayers in the liquid crystalline state (ca. 34–35 Å).[10] We would expect that the bilayer thickness might be more representative of a totally disordered bilayer since high sensitivity DSC measurements indicate that between 10 and 20 mole%, 7-ketocholesterol abolishes the DSPC phase transition (W. Tamura-Lis and L. J. Lis, unpublished observation). These observations are further evidence for a direct

complexation between 7-ketocholesterol and DSPC resulting in disordered DSPC molecules motionally constrained in a bilayer by the presence of the sterol.

In summary, we find that 7-ketocholesterol interacts with gel state DSPC bilayers in a manner different from its interaction with gel state DPPC bilayers. We can infer that the miscibility of 7-ketocholesterol in gel state PC bilayers is different from that of cholesterol and is a function of acyl chain type; i.e., 7-ketocholesterol disorders longer chained lipids at a lower sterol concentration. We can then hypothesize that the substitution of cholesterol by 7-ketocholesterol will result in different compositions of membrane domains based on total sterol content. The influence of 7-ketocholesterol on the bilayer surface as determined from the bilayer repeat spacings suggests that with a mole ratio of greater than 5/1 of DSPC/7-ketocholesterol, the sterol may form an array with the DSPC molecules.

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