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Structure and Morphology of Dipalmitoylphosphatidylcholine/7-keto Cholesterol Mixtures

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Lipid bilayers containing dipalmitoylphosphatidylcholine (DPPC) and 7-ketocholesterol or 19-hydroxycholesterol were examined in water using X-ray diffraction and scanning calorimetry. In contrast to DPPC/cholesterol bilayers, the presence of low contents of 7-ketocholesterol did not produce two DPPC lamellar phases. The swelling properties of the DPPC/7-ketocholesterol bilayers were also different from DPPC/cholesterol bilayers. However, the substitutions of 19-hydroxycholesterol as the oxygenated sterol compound incorporated into the DPPC bilayer produced a more complicated phase relationship. Thus the interaction of oxygenated sterol compounds with DPPC bilayers is compound specific.

Keywords: phospholipid-sterol mixtures, x-ray diffraction, phosphatidylcholine, 7-keto cholesterol, bilayer structure

INTRODUCTION

Cholesterol is known to be an important component in cell membranes and tissues. In order to fully determine how cholesterol interacts with a cell membrane, there have been many studies on the effect of cholesterol and cholesterol derivatives in model membrane systems. Oxygenated sterol compounds (OSC) have been associated with various biological processes including cholesterol synthesis.[1] In

addition, OSC have been inferred to be associated in some manner with cell membrane function and morphology.[2]

There have been a number of recent studies describing OSC interactions with phosphatidylcholine.[3-7] It has been established that 25α-hydroxycholesterol has a low solubility in DPPC bilayers.[4-7] In addition, sterols oxidized at the 7-position appear to have a greater influence on bilayer packing than sterols oxidized at the 20- and 25-positions.[7] None of the studies (including DSC[6]) has been able to confirm whether small concentrations of OSC in the DPPC bilayer lead to a multi-phase system as previously observed with cholesterol.[8-12]

In this study, we have probed the influence of 7-keto and 19-hydroxycholesterol on the number and dimensions of DPPC bilayer phases. We have previously reported that 10 mole% 7-ketocholesterol mixed with DPPC produced only one bilayer phase[7] while 10 mole% cholesterol mixed with DPPC produces two phases. The phase present in the 7-ketocholesterol/DPPC bilayer has a greater water layer thickness but the same lipid bilayer thickness as DPPC bilayers in water,[13] and one of the DPPC/cholesterol bilayers in water.[12] We report here our data for 19/1, 9/1, 4/1, 7/3, 3/2, and 1/1 DPPC/7-ketocholesterol mixtures and 1/1 DPPC/19α-hydroxycholesterol mixtures. The DPPC/7-ketocholesterol mixtures produce bilayer phases similar to those present in DPPC/cholesterol mixtures except for 19/1 which produces a single phase. However DPPC/19-hydroxycholesterol (1:1) mixtures produce two lamellar phases over a significant hydration range which are different from those observed in DPPC/cholesterol and DPPC/7-ketocholesterol mixtures. These results indicate that the position of the oxidized moiety is important in bilayer packing and morphology. In addition, we can infer that DPPC/7-ketocholesterol complexes have an increased miscibility in the DPPC bilayer compared with DPPC/cholesterol complexes.

MATERIALS AND METHODS

L-α dipalmitoylphosphatidylcholine was obtained from Avanti Polar Lipids (Birmingham, Alabama). 7-ketocholesterol and 19-hydroxycholesterol were obtained from Sigma Chemical Co. (St. Louis, Missouri). All lipids were used without further purification.

Lipid mixtures were obtained by dissolving DPPC and OSC 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 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 $K\alpha_1$ 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 our samples to provide an internal camera standard. The lattice repeat spacing, d, is directly calculated from our film readings. With less than full hydration, the d-spacing 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:

$$\phi = \left[1 + \frac{(1 - c)\, v_w\, (1 + K)}{c(K v_S + \bar{v}_L)}\right]^{-1}$$

where c is the weight fraction of lipid in the sample, v_w, $\bar{v}_L$ and v_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 (14).

Calorimetry measurements were made on a Perkin-Elmer DSC-2C with Data Station. Samples were run at 2.5°K per minute. Collection and analysis of the data were performed using software produced by Perkin-Elmer. Lipid samples were typically hydrated to at least 80% solvent before mounting in aluminum sample cans. Enthalpy and temperature were calibrated using data from indium as the standard.

RESULTS AND DISCUSSION

The bilayer structural parameters d, d_L and d_w are plotted as a function of lipid content in Figure 1 for a variety of DPPC/7-ketocholesterol mixtures. Even at the lowest concentration of 7-ketocholesterol in the DPPC bilayer studied (19/1, DPPC/7-ketocholesterol), only one lamellar phase was observed at full hydration with a repeat spac-

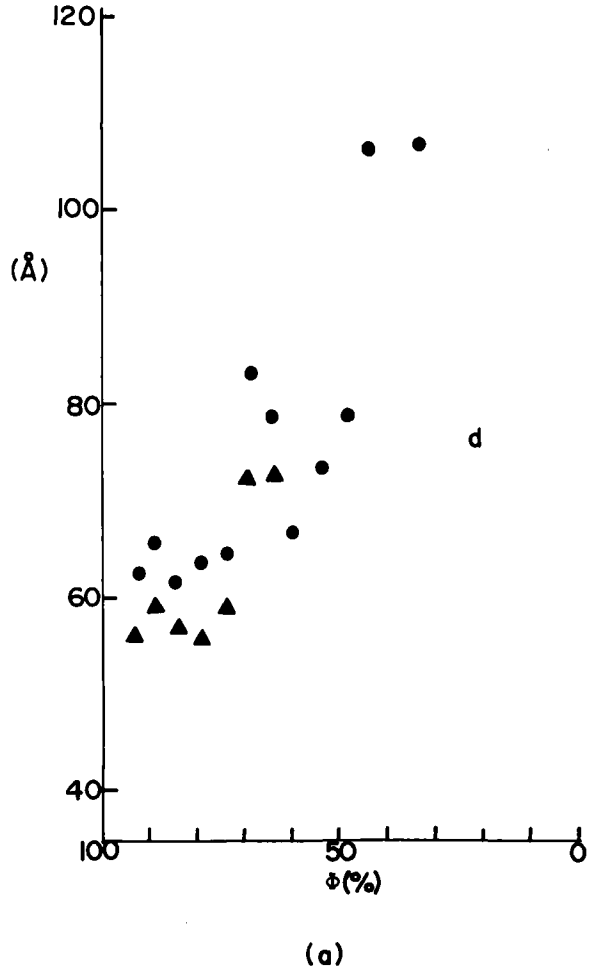

FIGURE 1 Bilayer structural parameter d (▲ and ●) and d_L (o when one phase is observed) as a function of lipid content for DPPC/7-ketocholesterol mixtures [a) 19:1, b) 9:1, c) 4:1, d) 7:3, e) 3:2, and f) 1:1 moles DPPC:moles 7-ketocholesterol].

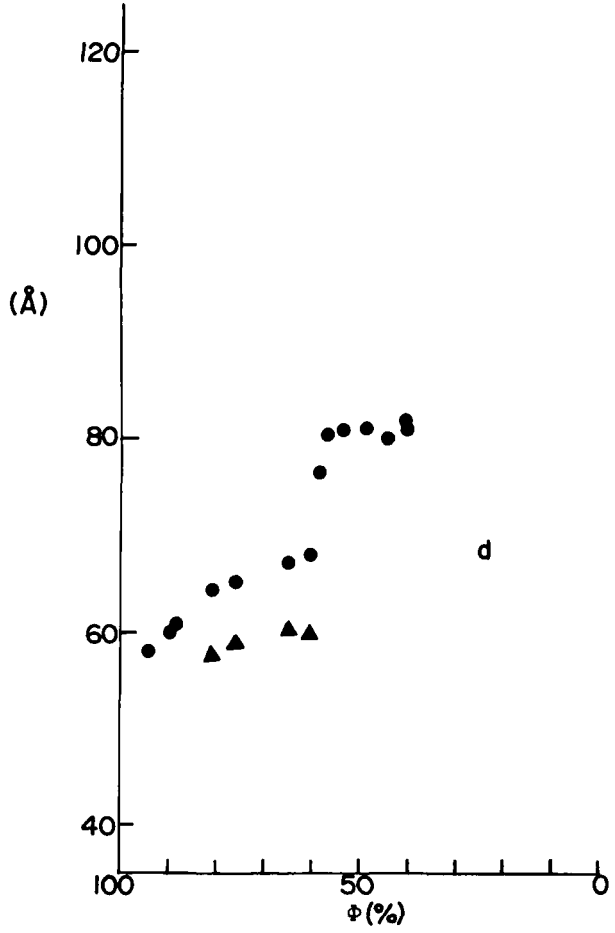

FIGURE 1(b)

ing of approximately 106 Å. This is in contrast to previous observations of the formation of two lamellar phases in DPPC/cholesterol mixtures at low cholesterol content. When the mole ratio of DPPC/7-ketocholesterol is 19/1 or 9/1, two lamellar phases are observed at less than full hydration, and only one phase at full hydration. The other DPPC/7-ketocholesterol mixtures studied produced single lamellar phases at all water contents.

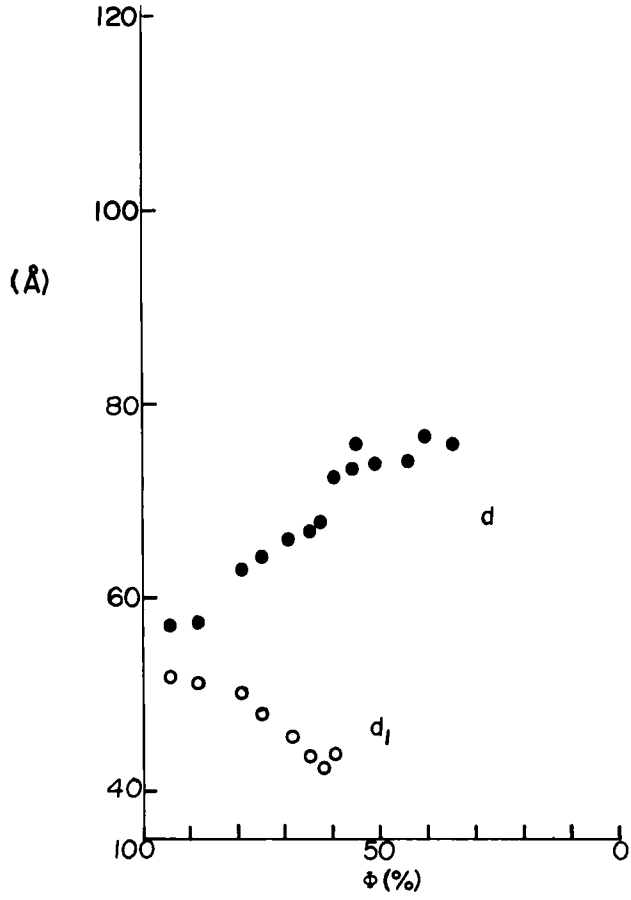

FIGURE 1(c)

The dramatic increase in d-spacing in mixtures with low sterol content is almost entirely made up of an increase in water separation between bilayers (see Table I). At full hydration, the limiting d_L is approximately 47 Å for 19/1 DPPC/7-ketocholesterol while d_w is 59 Å. This represents a 3 Å change in d_L when compared to pure DPPC bilayers in water. However, this is not a significant change because of the breadth of the X-ray lines in this system.

Increasing the 7-ketocholesterol content from 20 to 50 mole% pro-

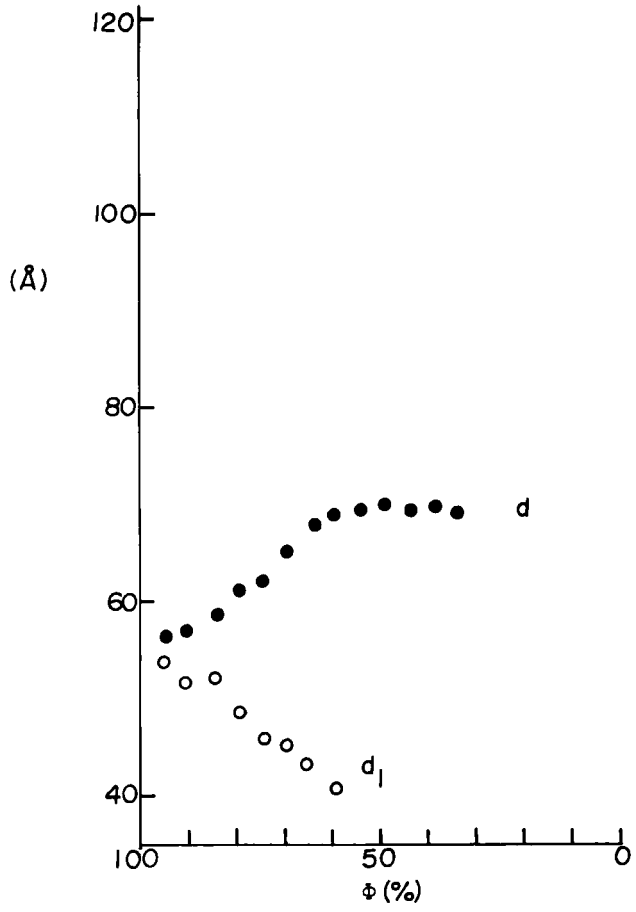

FIGURE 1(d)

duces bilayers with decreasing lipid bilayer thicknesses and approximately the same water layer thicknesses. This increase in sterol content thus leads to a disordering of the bilayer with a concomitant decrease in bilayer thickness. When the bilayer contains equal proportions of 7-ketocholesterol and DPPC, it has undergone a phase change to the liquid crystal state due to this ordering.

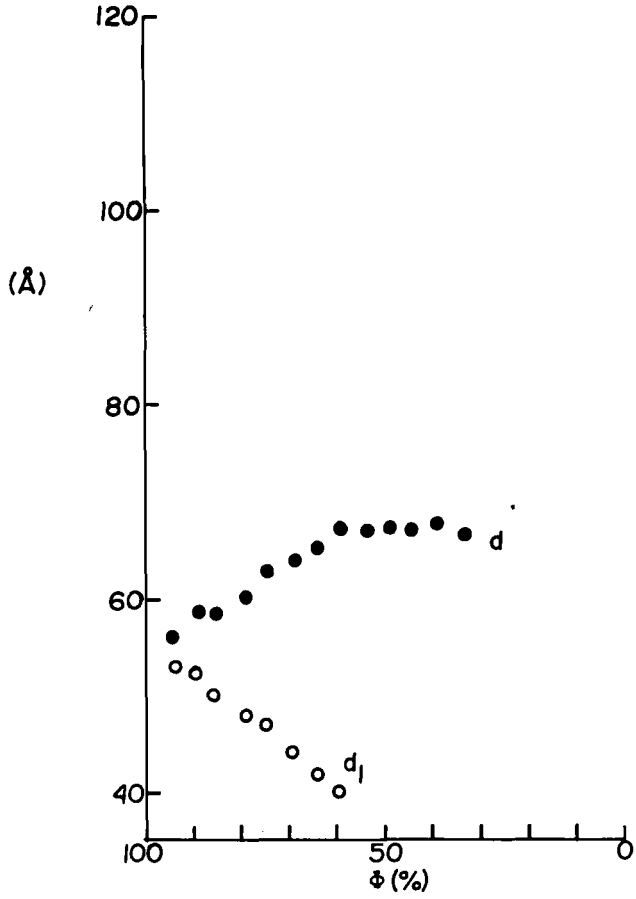

FIGURE 1(e)

The above results are not necessarily characteristic of all DPPC bilayers containing oxidized cholesterol. When DPPC is mixed with an equimolar content of 19-hydroxycholesterol, two lamellar phases are produced below full hydration. The interactions between oxidized sterols and DPPC bilayers are thus compound specific. The disappearance of two lamellar repeat spacings at low sterol content cannot be taken to indicate that lateral separation of DPPC and DPPC/7-ketocholesterol phases does not occur within a bilayer.

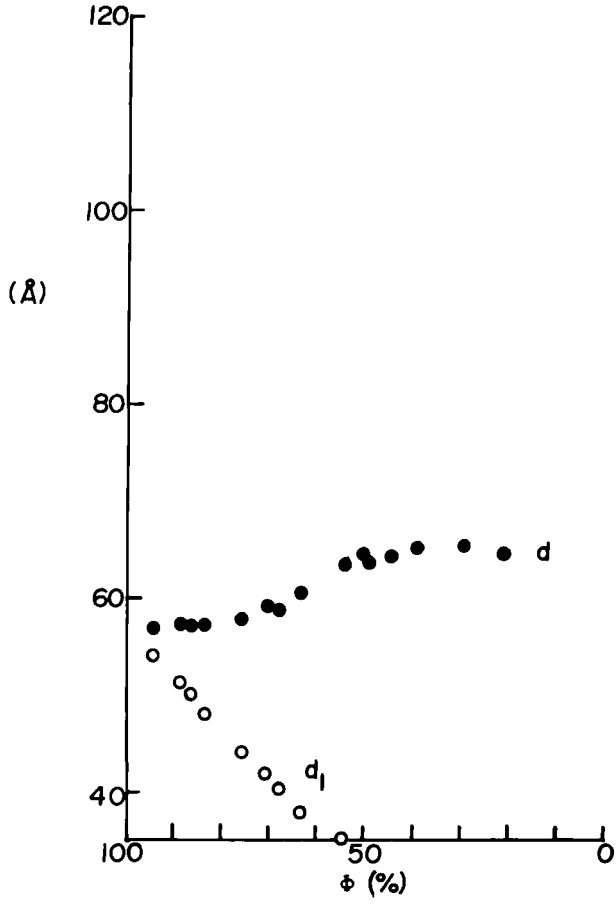

FIGURE 1(f)

Low resolution calorimetry was performed on DPPC bilayers with and without 7-ketocholesterol at a mole ratio of 9:1 in water. DPPC bilayers produced a pre-transition with an onset temperature of 304°K with an enthalpy of transition of 0.95 Kcal per mole of DPPC, and a main transition with an onset temperature of 310°K with an enthalpy of transition of 7.40 Kcal/mole. DPPC/7-ketocholesterol (9/1) mixtures in water produced no pre-transition but did produce a main transition with an onset temperature of 312°K and a transition en-

TABLE 1

Limiting values of structural parameters for fully hydrated DPPC/7-ketocholesterol bilayers in water

Mole Ratio DPPC/7-ketocholesterol	d	d_L	d_w
100/0	62.0	43.6	18.4
19/1	106.6	47.3	59.3
9/1	(81.1)	(43.2)	(37.9)
4/1	72.4	43.7	28.8
7/3	69.1	40.7	28.4
3/2	67.3	39.9	27.4
1/1	63.5	35.1	28.4

( ) This value is taken from the first single phase in this mixture after the two-phase region is observed.

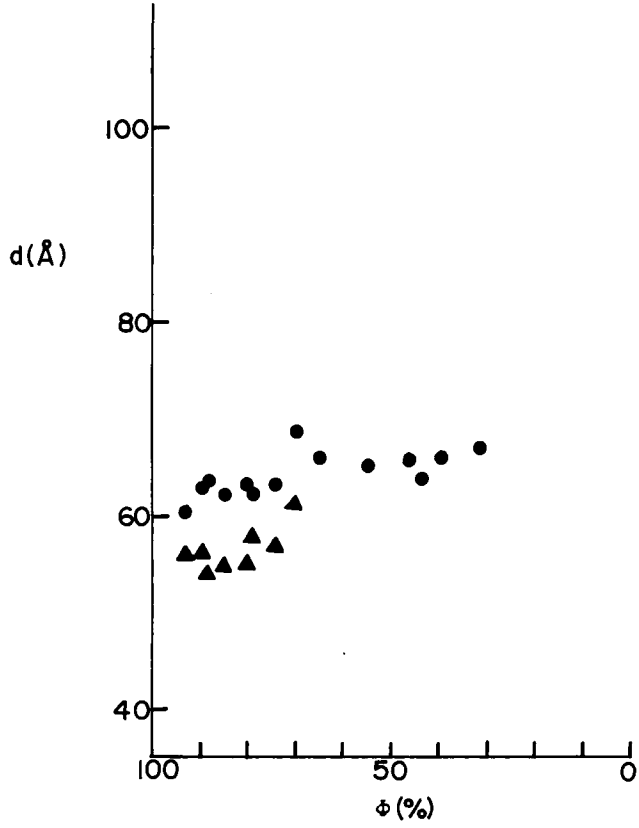

FIGURE 2 Bilayer repeat spacings d (●, ▲) as a function of lipid content for a (1:1) mixture of DPPC/19-hydroxycholesterol in water.

thalpy of 3.62 Kcal/mole of DPPC. The enthalpy results are lower than those previously reported by Engli et al.[6] owing to the fact that our scan rate was four times slower. However, the same general data trend was observed.

CONCLUSIONS

The oxygenated sterol 7-ketocholesterol is readily incorporated into DPPC bilayers resulting in a disorder of the phospholipid acyl chains. Additionally, the insertion of 7-ketocholesterol modifies the swelling properties (and thus the inter-bilayer interactions) of DPPC bilayers when compared to DPPC with and without cholesterol. Specifically, low concentrations of 7-ketocholesterol do not cause two lamellar phases to be produced in contrast to low concentrations of cholesterol in DPPC bilayers. The water uptake at full hydration is more for DPPC bilayers containing 5 mole% 7-ketocholesterol and less for the other mixtures studied than for bilayers containing cholesterol. However, these results are specific for the oxidized sterol compound used as DPPC bilayers containing 19-hydroxycholesterol have different phase relationships.

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