molecule lies along the z axis and the second molecule lies along the intermolecular y axis. The most important contribution to the dispersion energy involves a pure $d_{x^2-y^2}$ virtual orbital of the first molecule. The d_{xy} orbital of the first molecule is also important. Other important virtual orbitals of the first molecule generally have a large coefficient corresponding to some d components. The d functions are of less interest when the molecule lies along the intermolecular y axis. For instance in the linear case, the largest contribution involves the first δ_u virtual orbital of each molecule. In this orbital the coefficient corresponding to the d_{yy} component is only 0.27. Other important virtual orbitals are of the type p_{xu} or p_{zu}, the coefficient corresponding to a d component (d_{xy} or d_{yz}, respectively) being not very large in such orbitals. Thus, though the importance of the d functions may not be negligible in the linear case, it is obviously of less importance than in the T-shaped configuration. This probably explains why the linear configuration is more stable than the "T" configuration in ref 3. Since the stability of the $(Cl_2)_2$ dimer is due mainly to the dispersion energy, a bad description of this dispersion energy may lead to misleading results.

Finally, we can see from the total energy E_{tot} (Table I) that the T-shaped configuration exhibits a much deeper van der Waals minimum (about -1.68 kcal/mol) than the linear configuration (about -0.511 kcal/mol). The corresponding intermolecular distance is about 3.44 Å. This is compatible with the observed polar character of this dimer.[1,3] Work on the L-shaped configuration is in progress. From our preliminary results, it seems that the L-shaped configuration is slightly more stable than the T-shaped one.

Acknowledgment. The calculations have been performed on the Univac 1110 of the Centre de Calcul de Strasbourg-Cronenbourg (Centre de Recherches Nucléaires du CNRS).

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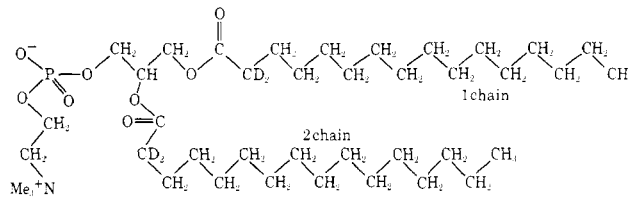
Deuterium Nuclear Magnetic Resonance Investigation of the Dipalmitoyl Lecithin–Cholesterol–Water System

Sir:

There has recently been considerable interest in using a variety of physical techniques to investigate the interactions between cholesterol and lipid membranes.[1] Early studies demonstrated that cholesterol could either increase or decrease the fluidity of lipid monolayers, depending on the initial degree of order of the hydrocarbon chains involved.[2,3] More recent investigations using differential scanning calorimetry,[4-6] x-ray diffraction,[7,8] spin-labeling,[9-11] freeze-fracture electron microscopy,[12] deuterium NMR,[13-15] carbon-13 NMR,[16-18] proton NMR,[19-20] and neutron diffraction[21,22] have been aimed at elucidating the precise nature of the phase separations induced by cholesterol in lipid bilayers, and of the effects of the bulky steroid nucleus on the segmental motion of the lipid hydrocarbon chains.

Below the chain melting temperature (T_c), spin label,[11] freeze fracture electron microscopy,[12] ^{13}C NMR,[18] and very recent calorimetric experiments[23] detect a phase boundary at a cholesterol (CHOL) mole fraction (χ_{CHOL}) of about 0.2. In contrast, earlier calorimetric data,[4-6] x-ray diffraction experiments,[8] and recent ^{13}C NMR experiments[24] detect changes at $\chi_{CHOL} \simeq 0.33$, but not at 0.2. In an attempt to understand these two groups of apparently conflicting experiments, we have undertaken a systematic study of the ^2H NMR spectra of the dipalmitoyl phosphatidylcholine (DPPC)–CHOL and dimyristoyl phosphatidylcholine (DMPC)–CHOL systems in excess water ($\geq$50 wt % H_2O), and we present a preliminary account of our results here. We detect significant changes in our NMR spectra at both $\chi_{CHOL} \simeq 0.2$ and 0.33, and offer possible explanations as to some of the molecular changes occurring at these compositions.

The parameters we study in our experiments are the residual quadrupole splittings, $\Delta\nu_Q$, of some specifically deuterated phosphatidylcholines. These splittings, which were monitored as a function of both χ_{CHOL} and temperature, reflect the motional state of the acyl chains. While one expects discontinuities in these parameters at phase boundaries, it does not necessarily follow that abrupt changes in motional parameters imply a phase boundary. This is so because thermodynamic phases are macroscopic and it is conceivable that one can have abrupt variations in structural/motional parameters, as the concentration or temperature is varied, at the microscopic level within the same macroscopic phase. This is a difficulty inherent in many of the approaches which have been used to map the phase diagram of the lecithin–cholesterol–water system, and it may account for some of the different conclusions which have been obtained using different methods. Thus, identification of the discontinuities and breaks in our data with phase boundaries is by inference only.



Recently Seelig and Seelig[25] have shown that the ^2H spectra of pure 1,2-[2′,2′-D]DPPC (I) exhibit three major quadrupole splittings, and by selectively deuterating either the 1 or 2 chains they assigned the largest splitting to the deuterons on the 1 chain and the two smaller splittings to the deuterons on the 2 chain. We have confirmed this result. Figure 1c illustrates a typical spectrum obtained at 55 °C of pure I in excess water. In addition to the major splittings between the 1 and 2 chains mentioned above, we also observe a small splitting of the 1 chain lines not reported by Seelig and Seelig: this splitting is observed either with or without high power proton irradiation. The splittings observed in the ^2H spectra could be due to magnetic inequivalence of the two deuterons on each chain, or to two different conformations of the lipid molecules, which are interconverting at a rate slow compared to the splittings. In temperature-dependent studies on DPPC and DMPC in the absence of cholesterol, the 2-chain lines remain of approximately equal intensity over about a 40 °C range above T_c. In addition, we have observed split equal intensity lines from 2-

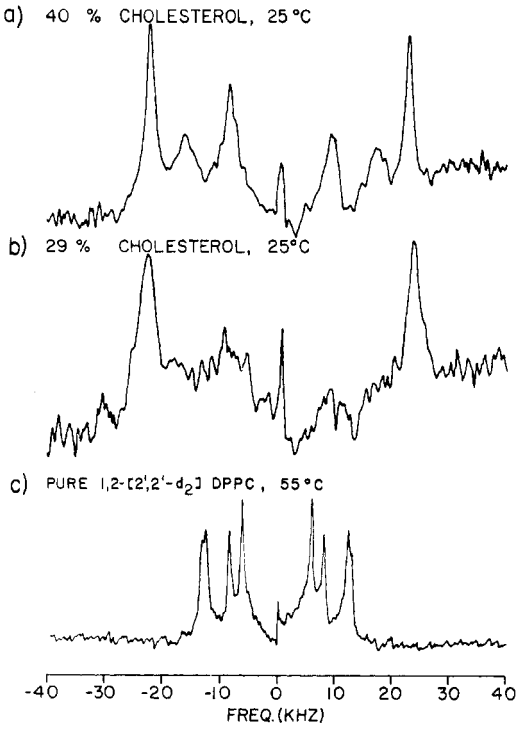


Figure 1. Deuterium NMR spectra of 1,2-[2′,2′-D]dipalmitoylphosphatidylcholine in excess water (a) at 25 °C with $\chi_{CHOL} = 0.40$, (b) at 25 °C with $\chi_{CHOL} = 0.29$, and (c) at 55 °C with $\chi_{CHOL} = 0$.

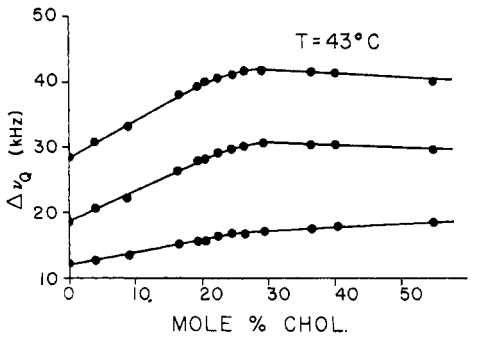


Figure 2. Plot of deuterium quadrupole coupling $\Delta\nu_Q$ of 1,2-[2′,2′-D]-dipalmitoylphosphatidylcholine vs. mol % cholesterol, for bilayer membranes in excess water at 43 °C.

[2′,2′-D]dipalmitoylphosphatidic acid above T_c (pH 8.5, 58 °C),[26] results which may favor the hypothesis that each deuteron gives rise to a separate pair of lines. In any case, it is not essential to resolve the ambiguity over the origin of these resonances for the purpose of interpreting the results described below.

In Figure 1 we present the deuterium NMR spectra of DPPC specifically labeled with deuterium in both acyl chains, above T_c, and below T_c but in the presence of cholesterol. When CHOL is added to I, large changes in $\Delta\nu_Q$ are observed. At constant $T > T_c$ the splittings increase indicating that the chains are more highly ordered, and the increase is temperature and composition dependent. For example, at 43 °C we observe an increase in $\Delta\nu_Q$ for the 1 chain from 28 to 41 kHz on going from $\chi_{CHOL} = 0$ to $\simeq 0.25$, and a small decrease in $\Delta\nu_Q$ above this χ_{CHOL}, as is shown in Figure 2. At higher temperature the break in this curve occurs at higher χ_{CHOL}, and the temperature and χ_{CHOL} at which it occurs correspond approximately to the solid to fluid-plus-solid phase boundary proposed by Shimshick and McConnell.[11] We also note that Figure 2 indicates that the chain inequivalence in DPPC is not removed by addition of CHOL.

Below T_c the situation is quite different. In pure I below T_c

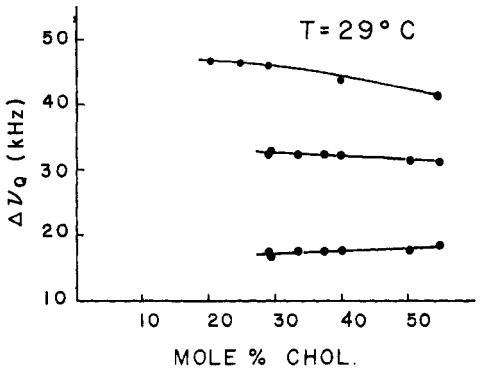


Figure 3. Plot of deuterium quadrupole coupling $\Delta\nu_Q$ of 1,2-[2′,2′-D]-dipalmitoylphosphatidylcholine vs. mol % cholesterol, for bilayer membranes in excess water at 29 °C.

we have not observed spectra in our 80-kHz spectral windows, which is consistent with the DPPC molecules being in a highly ordered state.[27] However, at $\chi_{CHOL} > 0.33$ we recover the entire spectrum; a spectrum at $\chi_{CHOL} = 0.40$ at 25 °C is shown in Figure 1a. We also note that at 25 °C $\Delta\nu_Q = 45$ kHz for the 1 chain lines (compared with 41 kHz at 43 °C) and that the spectra of both the 1 and 2 chain lines are broadened. As we decrease χ_{CHOL} to $\simeq 0.33$, we still observe resonances from both the inner (α) and the outer (β) components of the 2 chain resonance. However, the β component is significantly broader than the α, and in temperature runs it is very clear that the β lines "disappear" 2–3° above the α. Identical behavior has been observed with 1,2-[2′,2′-D]dimyristolyphosphatidylcholine a few degrees below the lipid T_c, at $\chi_{CHOL} = 0.30$.[28]

With I at $\chi_{CHOL} = 0.29$ we detect only the 1 chain lines, as shown in Figure 1b. It could be, of course, that at $\chi_{CHOL} = 0.29$ the 1 and 2 chain splittings are identical, giving rise to a single doublet; however, since we have examined a $\chi_{CHOL} = 0.29$ sample of 2-[2′,2′-D]DPPC and observed no lines, we may exclude this possibility. Thus, we conclude that the "phase boundary" observed in DSC[4-6] and x-ray diffraction experiments[8] at $\chi_{CHOL} \simeq 0.33$ manifests itself in our ^{2}H NMR experiments as a disappearance of the 2 chain lines in compound I. As we further decrease χ_{CHOL} the second change occurs, and that is the disappearance of the 1 chain lines at $\chi_{CHOL} \simeq 0.20$. Thus, the "phase boundary" that is observed in spin label,[11] electron microscope[12] and ^{13}C NMR experiments,[18] appears to be associated with the phenomenon which "freezes out" the 1 chain ^{2}H lines. We should also mention that the disappearance of the 2 chain signals is temperature dependent; at 29 °C we observe the 2 chain signals at $\chi_{CHOL} = 0.29$, at 35 °C we observe both the 1 and 2 chain signals at $\chi_{CHOL} = 0.20$, and below $\chi_{CHOL} = 0.20$ at 35 °C, they both disappear. A typical plot of $\Delta\nu_Q$ vs. χ_{CHOL} at $T = 29$ °C is shown in Figure 3.

Any explanation of our results must incorporate a mechanism whereby the 2′ positions of the 1 and 2 chains can appear magnetically inequivalent and can "freeze" independently. In an x-ray study of a single crystal of 1,2-dilauroylphosphatidylethanolamine,[29] it was found that the 2 chain is initially extended parallel to the bilayer plane, but after the 2′ position it is perpendicular to this plane, while the 1 chain is at all positions extended perpendicular to the plane. This conformation has been successfully employed in fitting the low-angle x-ray diffraction data for dimyristoylphosphatidylethanolamine bilayers.[30] Such a conformation places the 2–2′ position in a hindered configuration and thus two pairs of ^{2}H satellites could be observed from this position for DMPC, and DPPC. In addition, as a consequence of this hindered configuration, the residual quadrupole splitting exhibited by these deuterons would be primarily a reflection of the molecular motion present at the glycerol backbone, e.g., overall molecular rotation. In contrast the 1–2′ position would enjoy greater motional free-

dom since it is not sterically hindered, and internal as well as overall molecular motion would determine $\Delta\nu_Q$ of the 1-2′ lines. Assuming this to be the case, then if overall molecular rotation slowed at $\chi_{CHOL} = 0.33$ one might expect the 2-2′ lines to disappear before the 1-2′ lines. The disappearance of the 1 chain lines at $\chi_{CHOL} \simeq 0.2$ would then reflect a retardation of internal molecular motion of the chains.

Other experimental data can be interpreted to support this hypothesis. In the x-ray experiment of Engelman and Rothman it was observed that the in plane 4.15 Å spacing was replaced by a 4.7 Å spacing at $\chi_{CHOL} \simeq 0.33$ at 20 °C. Such an increase would facilitate molecular rotation about the bilayer perpendicular. Note, in addition, that our data predict this break point will be rather temperature dependent.

The spin-label data of Shimshick and McConnell[11] were obtained with a label at the 8 position of the 2 acyl chain. Thus, our hypothesis concerning the chain motion freezing at $\chi_{CHOL} \simeq 0.2$ is consistent with the spin-label results.

Finally, we should mention that we have studied the concentration and temperature dependence of the ^2H spectra of [3-α-D]CHOL in the range $\chi_{CHOL} = 0.1$–0.5 and $T = 20$–60 °C. In the case of DMPC we observe an essentially constant $\Delta\nu_Q \approx 50$ kHz at $T \sim 23$ °C over the entire concentration range, while at $T = 60$ °C $\Delta\nu_Q$ increases from 38 to 47 kHz in going from $\chi_{CHOL} = 0.1$ to 0.5. To date we have not observed two components in these spectra, as has been reported in ^{13}C spectra of [4-^{13}C]CHOL,[18] but this could be due to one species simply having a very broad spectrum. We will report more complete results in a future publication.

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Radical Ions in Photochemistry. 4. The 1,1-Diphenylethylene Anion Radical by Photosensitization (Electron Transfer)[1]

Sir:

A few years ago we described the formation of the 1,1-diphenylethylene (I) cation radical by photosensitization (electron transfer) using electron-accepting sensitizers (e.g., 1-cyanonaphthalene, II).[2a] Subsequent studies have shown that this procedure has synthetic utility; products formally derived from anti-Markownikoff addition of alcohols, carboxylic acids, water, and hydrogen cyanide to several aryl olefins are readily prepared by this reaction.[2] We now report that the corresponding anion radical can also be prepared by the photosensitized (electron transfer) technique through the use of electron-donating sensitizers (e.g., 1-methoxynaphthalene (III), 1,4-dimethoxynaphthalene (IV), and 1-methylnaphthalene (V)). The resulting products are those expected from Markownikoff addition to the olefin under *mild, nonacidic conditions*. Reactions 1 and 2 are illustrative.

$$\underset{\text{I}}{(C_6H_5)_2C{=}CH_2} + ROH \xrightarrow[CH_3CN]{h\nu\,(\text{III, IV, or V})} (C_6H_5)_2CCH_3 \quad (1)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad OR$$

VI, R = CH_3
VII, R = H

$$\underset{\text{I}}{(C_6H_5)_2C{=}CH_2} + KCN + CF_3CH_2OH \xrightarrow[CH_3CN]{h\nu\,(\text{III or IV})} (C_6H_5)_2CCH_3 \quad (2)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CN$$

VIII

Irradiation[3] of I (0.5 mmol) in acetonitrile–methanol (2 and 1.6 mL, respectively) with III, IV, or V (0.22 mmol) present as photosensitizer (electron donor) resulted in formation of 1,1-diphenylethyl methyl ether[4] (VI, 50–80%). When the irradiation was carried out using methanol-*O-d*, after ~50% conversion, analysis of the nuclear magnetic resonance and mass spectrum of the starting material indicates incorporation of deuterium (29% d_1, 1% d_2) in the vinyl position. Deuterium (81% d_1, 13% d_2, 0.1% d_3) was also incorporated in the methyl position of product VI. The photosensitizer (III, IV, or V) was largely recovered (50–60%). Scheme I accounts for these observations.