^{1}H NMR OBSERVATION OF SMALL MOLECULES IN PERDEUTERATED PHOSPHATIDYLCHOLINE VESICLES

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1. Introduction

Proteins and small molecules in phospholipid bilayer membranes can be studied by a variety of methods. Nuclear magnetic resonance (NMR) studies have, for the most part, been limited to observing changes in the NMR spectrum of phospholipids induced by such substances as valinomycin [1], alamethicin [2], cholesterol [3], various anesthetics [4], vitamin E [5] and other membrane-active substances [6]. Recently, cholesterol NMR signals have been studied, using ^{13}C- or ^{2}H-labeled cholesterol [7,8]. A contrasting strategy is to perdeuterate the phospholipid, thereby attenuating the background ^{1}H NMR signals, and then to observe the ^{1}H NMR spectrum of the molecule of interest. The latter method takes advantage of the sensitivity of the NMR experiment for ^{1}H, eliminates the need to synthesize a labeled version of each molecule of interest, and also provides signals from several parts of the molecule.

The use of phospholipids with perdeuterated acyl chains has permitted the observation of certain ^{1}H NMR signals from cholesterol [9] and from gramicidin A′ [10] in phosphatidylcholine bilayer vesicles. A further improvement in isolating the spectrum of the compound of interest can be expected by using phospholipids with both the head group and the acyl chains perdeuterated. We have recently prepared perdeuterated 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC-d_{72}) [11]. We report here that several small membrane-soluble compounds reveal well-resolved ^{1}H NMR signals when incorporated into DMPC-d_{72} vesicles.

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2. Experimental

The DMPC-d_{72} was dissolved in $CHCl_3$ either alone or together with 5 wt % of another compound. Solvent was removed under vacuum and 0.5 ml D_2O was added. Small unilamellar vesicles were prepared by sonication in a bath-type sonicator for 10 min at 40°C. ^{1}H NMR spectra were obtained on a Bruker WH-360 NMR spectrometer operating at 360 MHz. Sample temperatures were 24°C for samples in $CDCl_3$ and 50°C for aqueous vesicle preparations. Chemical shifts are reported relative to an internal tetramethylsilane (TMS) standard in $CDCl_3$ and an internal sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP) standard in D_2O. Samples in $CDCl_3$ had 10 mg/ml of the compound of interest.

3. Results and discussion

The 360 MHz ^{1}H NMR spectrum of a deuterochloroform solution of DMPC-d_{72} is shown in fig.1a. The peaks in the solvent spectrum are readily assigned [12], facilitating the assignment of resonances from DMPC-d_{72} aqueous bilayer vesicles (fig.1B). With the exception of the acyl αCHD, all positions on the phospholipid have <0 7% residual protons. The αCHD has ~7% residual protons.

Figure 2 shows the ^{1}H NMR spectra of cholesterol in $CDCl_3$ and in vesicles of DMPC-d_{72}. The peaks in

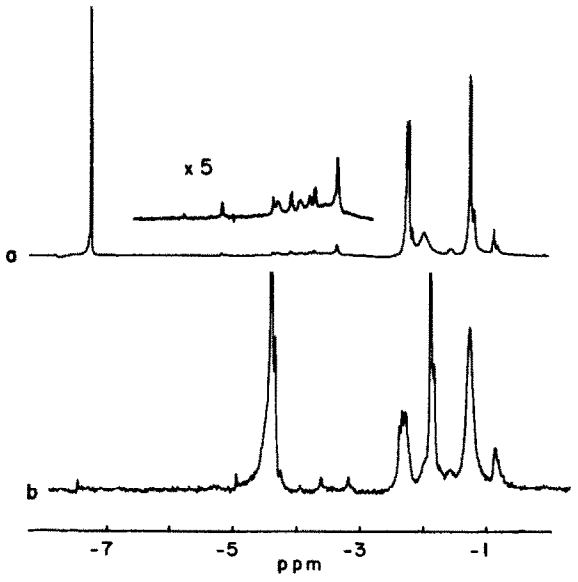

Fig.1. (a) DMPC-d_{72} in dry $CDCl_3$, 1000 transients with an acquisition time of 2 s and a sensitivity enhancement giving 0.5 Hz line broadening. Assignment of peaks [12]: −0 90 ppm, acyl CH_3, −1.25 ppm, acyl CH_2, −1.60 ppm, βCH_2, −2 00 ppm, residual acetate; −2.24 ppm, αCH_2, −3.35 ppm, choline $N(CH_3)_3$, −3.72 ppm, residual ethanol CH_2; −3 81 ppm, choline CH_2N, −3.96 ppm, glycerol CH_2OP; −4.10 and −4.38 ppm, glycerol CH_2OCO; −4 31 ppm, choline $POCH_2$, −5.19 ppm, glycerol HCOCO, −7 24 ppm, $CHCl_3$. (b) DMPC-d_{72} vesicles, 400 transients with an acquisition time of 1 s and a sensitivity enhancement giving 2 Hz line broadening. The large peak at −1.89 ppm in this and all other vesicle spectra is from a trace of residual acetate.

the solvent spectrum were assigned by comparison with published spectra [9,13] and by comparison with model compounds. The vesicle spectrum reveals remarkable mobility for protons of the steroid nucleus. The H3 and H6 resonances have linewidths of 80 Hz

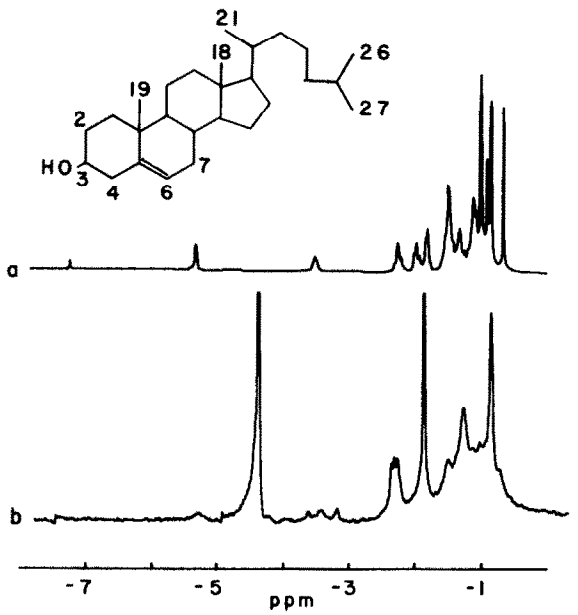

Fig.2. (a) Cholesterol in $CDCl_3$, 24 transients, other spectral parameters as in fig.1a. Assignment of peaks from [9,13] −0.69 ppm, H18; −0.88, H26 and H27, −0.93, H21, −1.02 ppm, H19; −1.05 to −1.7 ppm, not yet assigned; −1.84 ppm, H2 plus one other proton, −2.00 ppm, H7; −2.28 ppm, H4, −3.52 ppm, H3, −5.34 ppm, H6, −7 24 ppm, $CHCl_3$. (b) Cholesterol (10 mol %) in DMPC-d_{72} vesicles, spectral parameters as in fig.1b.

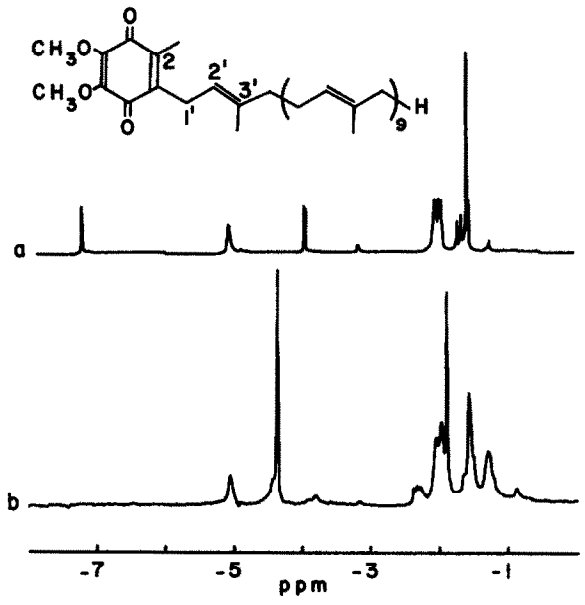

Fig.3. (a) UQ_{50} in $CDCl_3$, spectral parameters as in fig.2a Assignment of peaks from [14]: −1.28 ppm, impurity; −1 61 ppm, isoprenoid methyls, −1.69 ppm, terminal methyl, −1.75 ppm, methyl on C3', −1.97 to −2.11 ppm, isoprenoid methylenes, and methyl on C2; −3.19 ppm, H1', −3 99 ppm, OCH_3, −5.11 ppm, isoprenoid vinyl protons, −7.24 ppm, $CHCl_3$. (b) UQ_{50} (5 mol %) in DMPC-d_{72} vesicles, data acquisition parameters as in fig.1b with a sensitivity enhancement giving 0 5 Hz line broadening

and 60 Hz, respectively. These resonances are still observable down to 30°, with linewidths > 100 Hz. The H18, H19, H21 and H26,27 methyl resonances are also resolved down to 30°, with linewidths of 20–30 Hz. The observation of resolved and assigned cholesterol ^{1}H NMR signals from bilayer vesicles can provide a basis for studying the interaction of cholesterol with other molecules, e.g., phospholipids and polyene antibiotics, in membranes.

Figure 3 shows the spectra of ubiquinone-50 (UQ_{50}) in $CDCl_3$ and in vesicles of DMPC-d_{72}. The linewidths of the resonances in the vesicle spectrum range from 20–30 Hz. Studies of the mobility of UQ_{50} in phospholipid bilayer membrane systems might be useful in elucidating the role of UQ_{50} in oxidative phosphorylation.

Figure 4 shows the spectra of linoleic acid in $CDCl_3$

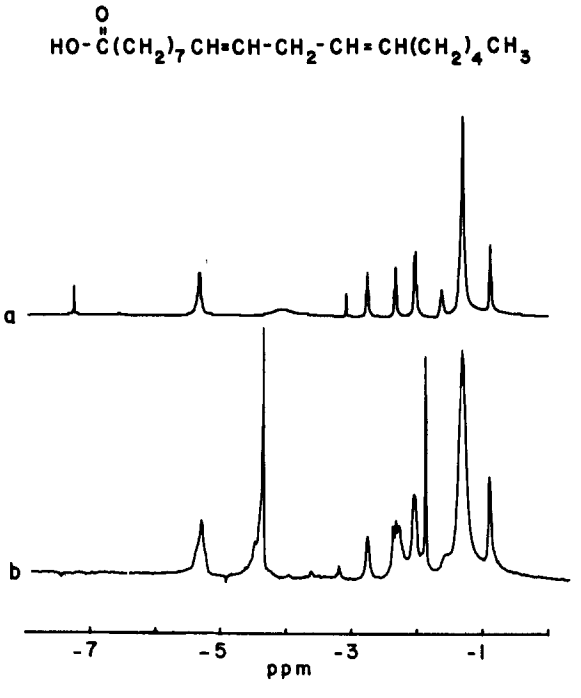

Fig.4. (a) Linoleic acid in $CDCl_3$, 12 transients, other spectral parameters as in fig.1a. Assignment of peaks: –0.90 ppm, terminal CH_3, –1.32 ppm, CH_2, –1.64 ppm, βCH_2; –2.05, $\underline{CH}_2CH=$, –2.37 ppm, αCH_2; –2.77 ppm, $=CH-\underline{CH}_2-CH=$; –3.09 ppm, impurity, –4.09 ppm, H_2O, –5 34 ppm, CH=C, –7.24 ppm, $CHCl_3$ (b) Linoleic acid (14 mol %) in DMPC-d_{72} vesicles, data acquisition parameters as in fig 1b with a sensitivity enhancement giving 1 Hz line broadening.

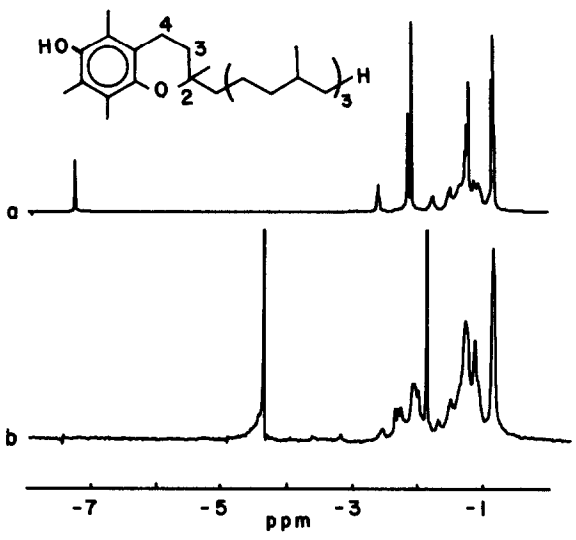

Fig.5 (a) Vitamin E (D-α-tocopherol) in $CDCl_3$, spectral parameters as in fig.2a. Assignment of peaks from [15,16]: –0.86 ppm, isoprenoid methyls, –1 00 to –1 7 ppm, isoprenoid methylenes and methines, and methyl on C2, –1.79 ppm, H3, –2.12 and –2.16 ppm, aryl methyls, –2.60 ppm, H4, –7.24 ppm, $CHCl_3$. (b) Vitamin E (9 mol %) in DMPC-d_{72} vesicles, spectral parameters as in fig.4.

and in vesicles of DMPC-d_{72}. In vesicles the linewidths of the $=CH-\underline{CH}_2-CH=$, $-CH_2CH=$ and $\underline{CH}_3$ resonances are all ~20 Hz. The CH=C are ~35 Hz in width.

Figure 5 shows the spectra of D-α-tocopherol (vitamin E) in $CDCl_3$ and in vesicles of DMPC-d_{72}. Linewidths of the pyranyl CH_2 protons and isoprenoid CH_3 protons are 20–30 Hz in the vesicle spectrum. The resonances in the 1.0–1.7 ppm region are not resolved at 360 MHz and have not yet been completely assigned. Since both linoleic acid and vitamin E show several well-resolved resonances in their ^{1}H NMR spectra in vesicles, this system provides a good model for studying the interaction of polyunsaturated fatty acids and vitamin E. It has been suggested that an interaction between tocopherol and lipids with polyunsaturated fatty acid residues may be important in biological membranes [17], but so far studies of this interaction have generally been limited to phospholipid monolayers [18,19] or high concentrations of α-tocopherol [5,20].

One of the interesting features of these spectra is that cholesterol, UQ_{50}, linoleic acid and vitamin E all

yield well-resolved ^{1}H NMR signals. Because resonances are observed from all parts of these molecules, they must be undergoing relatively rapid and relatively unrestricted molecular motion A detailed study of molecular motion will involve measuring the temperature and frequency dependence of the relaxation times The observations reported here illustrate the potential usefulness of perdeuterated phospholipds for ^{1}H NMR studies of model membranes.

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