SPECTROSCOPIC STUDIES OF SPECIFICALLY DEUTERIUM LABELLED MEMBRANE SYSTEMS

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I. INTRODUCTION

The deuterium nucleus has a spin I = 1 and thus possesses an electric quadrupole moment. The interaction of this moment with the electric field gradient at the deuteron nucleus causes a weak perturbation of the nuclear Zeeman levels so that separate transitions corresponding $+1 \leftrightarrow 0$ and $0 \leftrightarrow -1$ are observed. From the magnitude of this "quadrupole splitting" it is often possible to extract information about the rate and type of motion of the deuterium nuclei involved (1-3). Recently, it has been shown that deuterium magnetic resonance (DMR) spectroscopy is an extremely powerful technique with which to study the structural organization of both model (4-6) and natural cell membranes (7-8). In this article we review some of our recent progress in the use of deuterium NMR spectroscopy in the study of the dynamic structure of both model and biological membranes. In particular, we focus our attention on the effects of "impurities", such as cholesterol, in model systems. In addition, we present some preliminary data on deuterium NMR of mammalian cell membranes, together with neutron diffraction results on specifically deuterium labelled phospholipid-cholesterol mixtures.

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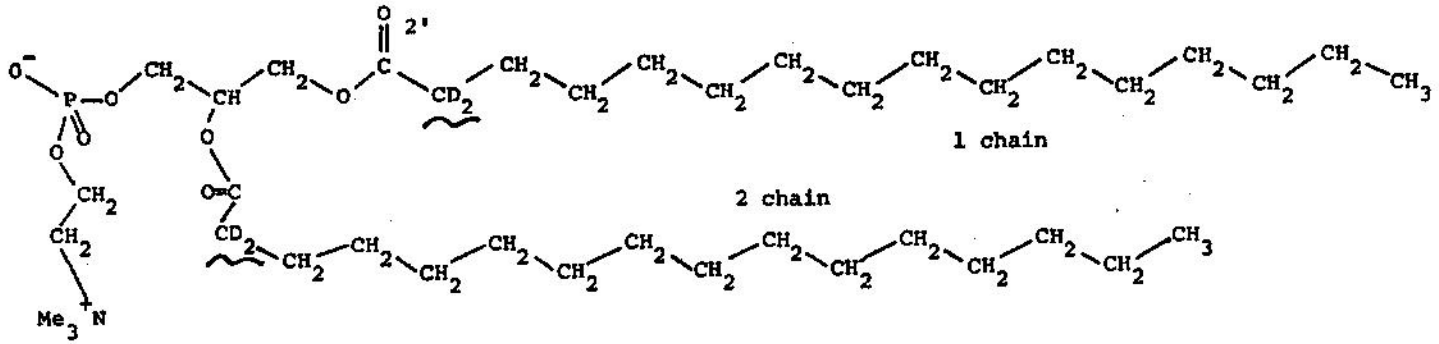

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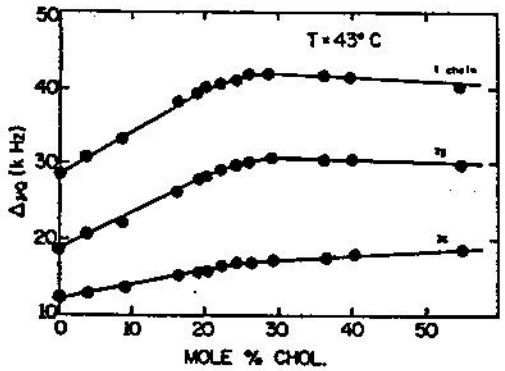

Fig. 1. Plot of deuterium quadrupole splitting $\Delta\nu_Q$ of 1,2[2',2'-d_2] dipalmitoylphosphatidycholine versus mole % cholesterol, for bilayer membranes in excess water at 43°C.

II. RESULTS AND DISCUSSION

Cholesterol. Recently Seelig and Seelig (9) have shown that the ^{2}H spectra of pure 1,2[2',2'-d_2]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. When cholesterol (CHOL) is added to I above T_c large changes in $\Delta\nu_Q$ are observed as is shown in Figure 1. At higher temperature the break in this curve occurs at higher cholesterol mole fraction (X_{CHOL}), and the temperature and X_{CHOL} at which it occurs corresponds approximately to the solid to fluid plus solid phase boundary proposed by Shimshick and McConnell (10). Below T_c the situation is quite different. In pure I below T_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. However, at X_{CHOL} >0.33 we recover the entire spectrum; a spectrum at X_{CHOL} = 0.40 at 25° C is shown in Figure 2a.

With I at X_{CHOL} = 0.29 at 25°C we detect only the 1 chain

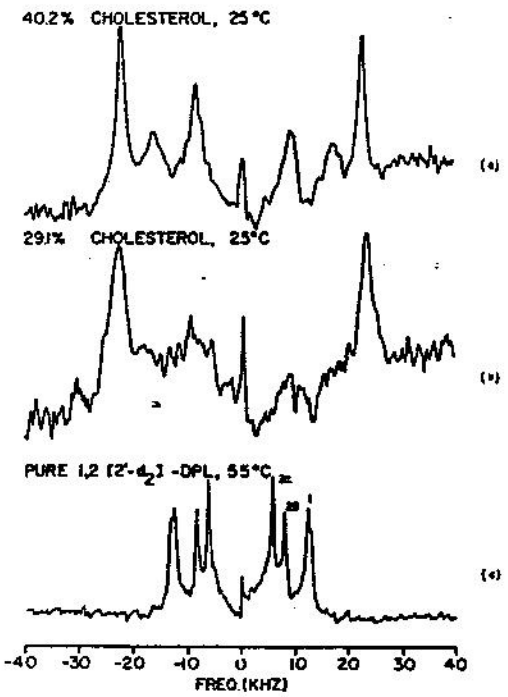

Fig. 2. Deuterium NMR spectra of 1,2-[2',2'-d_2] dipalmitoylphosphatidyl choline in excess water (a) at 25° with X_{CHOL} = 0.40 (b) at 25° with X_{CHOL} = 0.29 and (c) at 55° with X_{CHOL} = 0.

lines, as shown in Figure 2b. Thus, we conclude that the phase boundary observed in DSC (11-13) and X-ray diffraction experiments (14) at X_{CHOL} = 0.33 mainfests itself in our ^2H NMR experiments as a disappearance of the 2 chain lines in compound I. As we further decrease X_{CHOL} the second change occurs, and that is the disappearance of the 1 chain lines at X_{CHOL} = 0.20.

In an X-ray study of a single crystal of 1, 2-dilauroylphosphatidylethanolamine (15), 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 this plane. Such a conformation places the 2-2' position in a hindered configura-

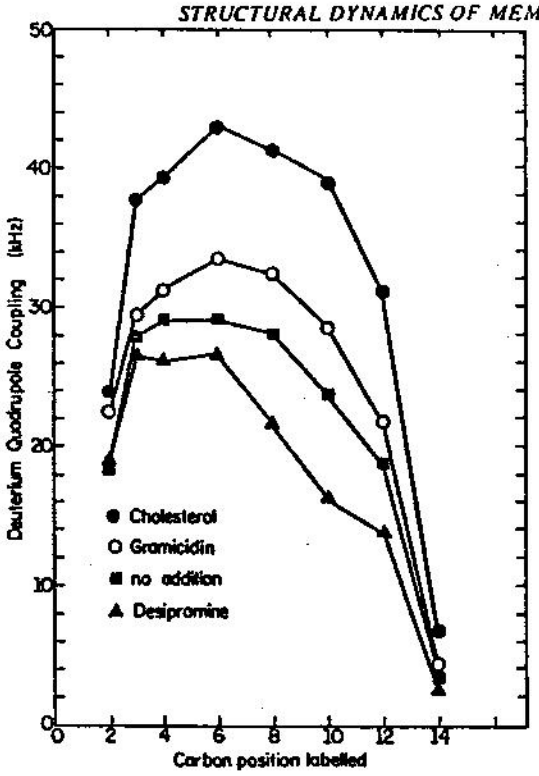

Fig. 3. Ordering profile for pure DMPC (at 35°C) and in the presence of cholesterol (20 mole %, 35°C), gramicidin A (5 mole %, 35°C) and desipramine (20 mole %, 31°C); all in excess water.

tion and thus two pairs of ^2H 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 could enjoy greater motional freedom since it is not sterically hindered and thus 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 $X_{CHOL} < 0.33$ one might expect the 2-2' lines to disappear before the 1-2' lines. The disappearance of the 1 chain line at $X_{CHOL} = 0.2$ would then reflect a retardation of internal molecular motion of the chains.

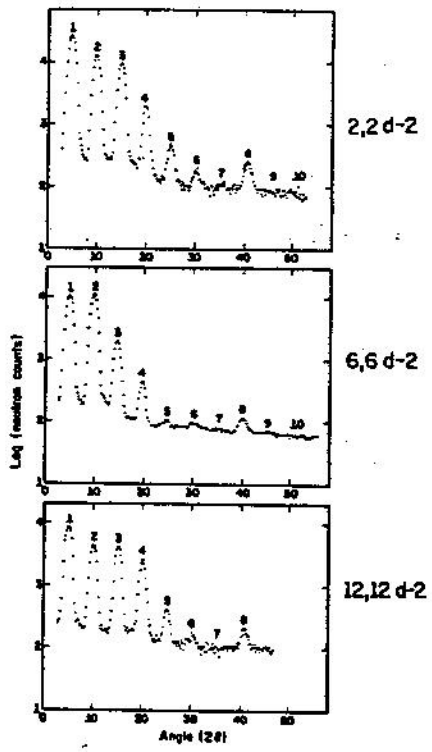

Fig. 4. Neutron diffraction data for oriented bilayers of specifically deuterated DMPCs in the presence of 30 mole % cholesterol, 23°.

We have determined the quadrupole splitting (or order parameter) versus position of deuterium label in a series of synthetic high purity dimyristoyl phosphatidyl cholines, in the presence of 30 mole % cholesterol, as shown in Figure 3. Our results complement those obtained previously using perdeuterated phospholipid (4), but indicate a maximum ordering several carbons along the chain, rather than immediately adjacent the polar region. The simple-minded interpretation, that this profile arises from a "condensing" effect caused by the rigid steroid nucleus (with little contribution from the C_8 side chain), is to some extent shaken by our observation (see below) that a very

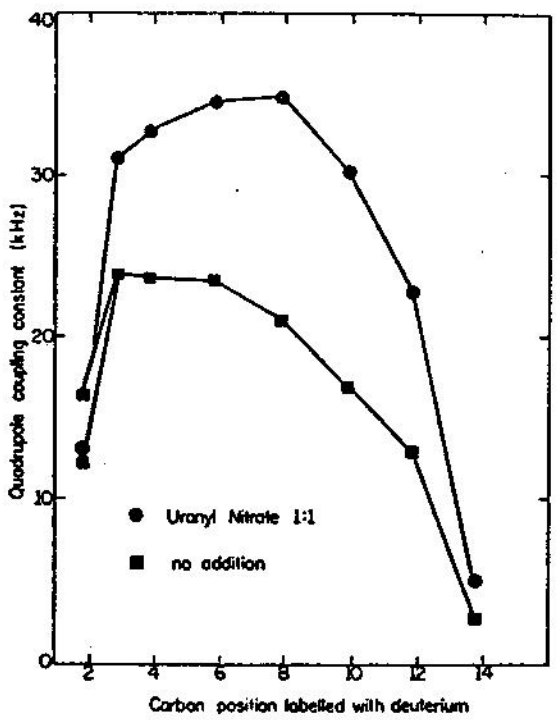

Fig. 5. Ordering profile for DMPC in the presence and absence of $UO_2(NO_3)_2$, in excess water at 58°.

similar profile may be generated by heavy metal (UO_2^{++}, as the nitrate) binding to the polar headgroup. In both cases the number of gauche rotamers per chain decreases, but the causes are clearly different—and could not be immediately derived from inspection of the order parameter profile.

An alternative approach to elucidating the organization of lecithin bilayers containing, for example, cholesterol, involves the use of neutron diffraction. Utilization of the large differences between the coherent scattering amplitudes of hydrogen and deuterium has already been used to determine the position of cholesterol in an egg lecithin bilayer (16). We have now obtained results on DMPC-cholesterol bilayers containing specifically deuterium-labelled lecithins, as shown in Figure 4,

which will now permit very accurate measurement of the positions of individual methylene groups in the bilayer.

Heavy Metal and Drug Interactions. The tricyclic antidepressant desipramine, and a variety of metal ions, are thought to bind primarilly to the polar region of phospholipid bilayer membranes (17,18). Using ^{2}H-NMR, we have found that desipramine causes a 12° depression in gel to liquid crystal phase transition temperature of DMPC when incorporated into the bilayer at the 10 wt % level. This effect is reflected in the bilayer ordering profile above T_c, Figure 3. Uranyl nitrate however, causes a dramatic increase in T_c and in bilayer order, as shown in Figure 5. The UO_2^{++}-DMPC profile is very similar to the cholesterol (or gramicidin)-DMPC profile, even though there is no similarity in the structure or location of these species. These results indicate to us the need for caution in interpreting ordering profile data in terms of specific molecular interactions. At present it appears that NMR, neutron diffraction, calorimetric, and statistical mechanical approaches will have to be brought together in order to give a complete description of model membrane organization.

Biological Membranes. We have recently incorporated choline completely deuterated in the methyl groups into two mammalian systems (19). The first was the chemically transformed mouse fibroblast LM cell line, grown in suspension culture. The second system consisted of mitochondria from Sprague-Dawley rats which were fed upon a choline-deficient diet enriched with deuterated choline chloride. Two classes of deuterium nuclear magnetic resonance signal were obtained from each system. The first class of signal exhibited no residual quadrupole coupling constant and was assigned to the natural abundance of ^{2}H in water, together with free labeled choline. The second class of signal had a residual quadrupole coupling of about 1 KHz and was assigned to ^{2}H-labelled choline headgroups of phospholipids. These results are in conflict with a previous study of deuterated

rat liver mitochondrial membranes (20). In addition, we have noted slight but reproducible differences in the magnitude of the quadrupole coupling of the deuteriomethyl groups in intact cell membranes and in isolated lipids. It appears that isolated lipids are consistently more ordered than the intact membrane lipids. Further studies are underway in order to clarify this point.

In temperature runs on both whole cells, mitochondrial membranes, and microsomal membranes, we have not yet seen evidence of thermal phase transitions (lateral phase separations) above 0°C. Below 0°C, we note that the ^{2}H-NMR signals rapidly broaden between about -10° and -20° C: this may correspond to an ordering of the lipids into the gel phase at this temperature.

ACKNOWLEDGEMENT

This work was supported in part by grants from the National Science Foundation (PCM 76-01491) and the National Institutes of Health (HL-19481, RR-07030, GM-23289, GM-21953, and GM-00193).

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