Deuterium Nuclear Magnetic Resonance Spectroscopic Study of the Fluorescent Probe Diphenylhexatriene in Model Membrane Systems†

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Received April 4, 1986

ABSTRACT: We have investigated the deuterium (^{2}H) nuclear magnetic resonance (NMR) spectra of two ^{2}H-labeled fluorescence probes (*trans,trans,trans*-1,6-diphenylhexa-1,3,5-trienes, DPHs) incorporated into model lipid bilayer membrane systems at various temperatures. The membranes consisted of multilamellar bilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) containing varying concentrations of cholesterol. The conventional one-order parameter approach often used in the analysis of the NMR data of lipid membranes does not explain the observed temperature variations of the spectral features. Consistent with the molecular symmetry, the results have thus been analyzed in terms of an ordering matrix with more than one independent element. The molecular order parameter (S_{NMR}), the order along the long molecular axis, in the pure lipid system varies from 0.49 to 0.26 as the temperature is increased from 25 to 57 °C. These values are somewhat larger than the order parameters obtained from fluorescence depolarization (S_{FLU}) on sonicated DMPC vesicles. Such discrepancies probably arise from the looser packing of the sonicated vesicles. Addition of cholesterol to the model membranes causes the order parameter of the probe molecules to increase. At 35 °C, S_{NMR} increases from 0.38 (with no cholesterol) to 0.92 (in the presence of 50 mol % cholesterol). These values are about 10% larger than those obtained from fluorescence depolarization studies on sonicated vesicles. The S_{NMR} for DPH are somewhat larger than those obtained in earlier NMR studies of ^{2}H-labeled cholesterol. However, they compare well with those obtained for ^{2}H-labeled DMPC. These results suggest that ^{2}H-labeled DPH, and by analogy DPH itself, can be a good probe for following the molecular ordering of lipids and for studying lipid–sterol interactions. We believe these studies should lay the groundwork for future studies of DPH/lipid chain organization in systems containing polypeptides and proteins.

The role of spectroscopic probe molecules in studying the nature of lipid–lipid, lipid–sterol, and lipid–protein interactions in model and biological membranes has been the subject of intense study in recent years. The spectroscopic techniques most often used for these investigations are electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), and fluorescence spectroscopies. The findings from these techniques on occasion have led to conflicting interpretations. For example, they do not all agree as to whether proteins order or disorder the hydrocarbon chains of lipid bilayers (Smith & Oldfield, 1984).

We thus decided to carry out a quantitative analysis of a simple lipid system, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)–cholesterol (CHOL), in excess water, using ^{2}H NMR of two ^{2}H-labeled fluorescence probes, [1,6-$^2H_2$]-diphenylhexa-1,3,5-triene ([1,6-$^2H_2$]DPH) and [*phenyl*-$^2H_{10}$]diphenylhexa-1,3,5-triene ([*phenyl*-$^2H_{10}$]DPH), to compare these results on the NMR time scale with those obtained from fluorescence studies on the same, or very similar, (sonicated) systems, and to compare both sets of results with those obtained by means of ^{2}H NMR spectroscopy of specifically ^{2}H-labeled DMPCs and CHOL. The DMPC–CHOL system was chosen because it is generally agreed that CHOL causes a condensing or ordering of the DMPC acyl chains, and we thus felt a quantitative analysis of the order parameters obtained from NMR and fluorescence on this simple system could provide a basis for future studies of more complex systems, containing polypeptides such as gramicidin A, or proteins, such as cytochrome oxidase. Clearly, if agreement on the DMPC–CHOL system cannot be obtained, future studies on proteins would be premature. Our results indicate, in general, good quantitative agreement between the NMR (S_{NMR}) and fluorescence (S_{FLU}) derived order parameters; e.g., for pure lipid systems, S_{NMR} changes from 0.49 to 0.26 as the temperature is raised from 25 to 57 °C, while S_{FLU} decreases from 0.54 at 25 °C to 0.21 at 48 °C. Similarly, reasonable agreement is obtained in DMPC–CHOL–DPH systems. For example, at 35 °C, S_{NMR} changes from 0.38 to 0.92 when the cholesterol concentration increases from 0 to 50 mol %, while S_{FLU} increases from 0.33 to 0.85 as the cholesterol concentration increases from 0 to 40 mol %. Small differences between the two data sets are ascribed to slight differences in packing between the multibilayer and unilamellar (sonicated) vesicles.

Materials and Methods

[^{2}H]DPH Syntheses. The [*phenyl*-$^2H_{10}$]DPH and [1,6-$^2H_2$]DPH were synthesized by coupling of the appropriately labeled benzaldehyde to a bifunctional Wittig reagent, using the procedure of Heitman et al. (1963). The bifunctional Wittig reagent was synthesized as follows: 13.1 g of triphenylphosphine (Aldrich Chemical Co., Milwaukee, WI) was dissolved in 150 mL of dimethylformamide in a 500-mL round-bottom flask. The flask was flushed with N_2, and an inert atmosphere was maintained throughout the reaction. A solution of 5.4 g of 1,4-dibromobutene (Aldrich) in 50 mL of dimethylformamide was added dropwise, with stirring, over

† This work was supported in part by the National Science Foundation Biophysics Program (Grant DMB 84-16771).
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0006-2960/86/0425-6517$01.50/0