Spectroscopic Studies of Specifically Deuterium Labeled Membrane Systems. Nuclear Magnetic Resonance Investigation of Protein–Lipid Interactions in *Escherichia coli* Membranes†

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ABSTRACT: Deuterium nuclear magnetic resonance (NMR) spectra of membrane vesicles and lipid extracts from the fatty acid auxotroph *Escherichia coli* L48-2, grown on hexadecanoic acid specifically deuterated in the terminal methyl position, have been obtained at 34.1 MHz by using the quadrupole-echo technique. At temperatures of 0–30 °C, the spectra of both samples exhibit two components, the broader component with a quadrupole splitting ($\Delta\nu_Q$) of ~10 kHz from lipid molecules in a gellike phase and the narrower component ($\Delta\nu_Q$ of ~2–4 kHz) from a liquid-crystalline-like phase. The broad component is only ~20% of the signal at 30 °C, but by 0 °C it becomes 85% for the lipid extracts and 75% for the membrane vesicles. Its width is comparable for both samples and, within experimental error, is temperature independent from 0 to 30 °C. On the other hand, the narrow component is consistently broader and has a doublet structure for the lipid extracts ($\Delta\nu_Q$ ~ 3.4 kHz at 30 °C) but is a featureless line (of ~2.2-kHz width) for the membrane vesicles. Also, it is significantly broader at 0 °C than at 30 °C for both samples. These results indicate that in the region of the terminal methyl group, the protein in the membrane vesicles interacts preferentially with the lipids in the fluid state. The interaction produces a "dynamic disordering" of the lipid, in which the motions of the lipid hydrocarbon chains are more effective in averaging out the quadrupole splitting of the methyl group. Also, at 0 °C the interaction increases the fraction of lipid in the disordered phase. There is no evidence for formation of a rigid "boundary layer" of lipid around the membrane proteins.

Deuterium NMR spectroscopy of specifically labeled lipid molecules at high field provides a sensitive, nonperturbing probe of the structure of model membranes (Oldfield et al., 1978c, and references cited therein). Although the goal of most such investigations is to obtain information relevant to the structure and function of intact cell membranes or to develop methods which will facilitate their study, relatively few results have been reported for membranes per se (Oldfield et al., 1972, 1976, 1978b; Stockton et al., 1975, 1977). An early study (Oldfield et al., 1972) showed that perdeuterated fatty acids, such as lauric (*n*-dodecanoic-d_{23}) and palmitic (*n*-hexadecanoic-d_{31}), could be incorporated biosynthetically into the plasma membranes of the pleuropneumonia-like organism *Acholeplasma laidlawii* B (PG9). The resulting ^{2}H NMR spectra, obtained by the continuous wave method at 8 MHz, indicated that the hydrocarbon chains of membrane glycolipids and phospholipids are in a relatively rigid gellike state at their growth temperature. Although the ^{2}H line widths and line shape were not those expected of a rigid crystalline solid, where a quadrupole splitting of ~127 kHz would be observed, recent studies on perdeuterated lipids in the gel state using the more sensitive and reliable quadrupole-echo technique (Davis et al., 1976) have shown that these early spectra are quite characteristic of a "high-temperature" gel-phase lipid (J. H. Davis and M. Bloom, private communication; Oldfield et al., 1978a; Kang et al., 1979).

Deuterium labeling of biological membranes is, of course, not restricted to fatty acid labeling of *Acholeplasma*. For example, it has been possible to biosynthetically incorporate ^{2}H-labeled choline molecules into the membranes of mammalian cells, such as the LM line of mouse cells (Oldfield et al., 1976), and into the myelin of rat sciatic nerve (R. Skarjune and E. Oldfield, unpublished experiments) and to readily detect ^{2}H NMR signals from the intact membranes. A surprising result of the LM cell experiments was that the quadrupole splitting of a choline CD_3 residue in a lipid extract is *greater* than that in the intact cell membrane (Oldfield et al., 1976). Qualitatively, this suggests more ordering of the choline methyl region of lecithin (and sphingomyelin) in extracted lipids—in the absence of protein. When compared with similar data presented in the previous paper (Kang et al., 1979) and elsewhere (Oldfield et al., 1978a), this suggested to us that one general effect of protein in a *biological* membrane may be to cause disorder of the lipid molecules. We have therefore investigated the ^{2}H NMR quadrupole splittings of CD_3-labeled palmitic acid, which has been biosynthetically incorporated into the cell membranes of the bacterial fatty acid auxotroph *Escherichia coli* L48-2. The spectra of intact cell membranes are compared with those of the extracted lipids [mainly phosphatidylethanolamines; see, for example, Cronan (1978)].

Experimental Section

Nuclear Magnetic Resonance Spectroscopy. Deuterium Fourier transform NMR spectra were obtained at 34.1 MHz by using the quadrupole-echo pulse technique (Davis et al., 1976). We used a "homebuilt" instrument for data acquisition (Oldfield et al., 1978c); it has single-phase detection combined with a spectrum reverse method (Nicolet Instrument Corp., Madison, WI; software package FT 74) which permitted the use of a 100-kHz effective spectral bandwidth. The 90° pulse width was ~6.5 μs, which corresponds to a radio frequency field strength (γH_1) of ~40 kHz; this is more than adequate to cover the relatively narrow gel-state line widths being studied. Sample volume was typically 200 μL.

Spectral simulations were carried out on a CDC Cyber-175 system by using the laboratory peripherals described previously (Kang et al., 1979).

Bacterial Strain and Growth. The fatty acid biosynthetic (*fab*) mutant L48-2, possessing *F*⁻, *fabD*, *fabE*, *gltA*, *ara*⁺,

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lac$^+$, and *gal* K-2 markers, was the generous gift of Dr. D. F. Silbert, Washington University. Cells were grown according to Harder's method (Harder et al., 1974) with minor modification, in minimal medium 63 (Pardee et al., 1959) supplemented with 1% glycerol, 0.2% casamino acid, 5 mM potassium glutamate, 1 μg/mL thiamine, and 1 μg/mL yeast extract. When indicated, 0.01% deuterium-labeled palmitate or 0.005% oleate in the presence of 0.1% Brij-58 was included in the medium.

Terminal methyl-labeled hexadecanoic acid was synthesized by Kolbe electrolysis as described previously (Kang et al., 1979), and a second sample was obtained from Serdary Research Laboratories (London, Ontario, Canada).

Inocula were prepared from L48-2 cultures grown overnight at 30 °C in liquid minimal medium supplemented with 0.005% oleic acid (*cis*-Δ^9-18:1) and 0.1% Brij-58. Cells were harvested and washed at room temperature, in medium 63 with no supplements, and were then inoculated to ~0.05 absorbance (at 600 nm) into minimal medium containing 0.01% deuterated palmitic acid and 0.1% Brij-58. Cultures were grown at 38 °C on a Gyrotory shaker, and absorbance at 600 nm was monitored with a Gilford spectrophotometer. The cells were harvested by centrifugation at an absorbance of 1.2 at 600 nm (~10^9 cells/mL) and were washed twice with 0.01 M Tris buffer, pH 8, at 4 °C.

Preparation of Membrane Vesicles. A slight modification of the procedure of Hertzberg & Hinkle (1974) was used. One gram (wet weight) of washed bacteria was suspended in 5 mL of a solution containing 10 mM potassium phosphate buffer (pH 7), 5 mM $MgCl_2$, 5% methanol, and 1 mM DL-dithiothreitol. The cells were broken by a single passage through an Aminco French Pressure Cell at 8000 psi, and the mixture was then centrifuged at 29000*g* for 10 min to remove unbroken cells. Vesicles were sedimented by centrifugation at 165000*g* for 2 h in a Beckman L5-50 ultracentrifuge using a 50-Ti rotor. The pellet was then washed twice in deuterium-depleted medium 63 at 4 °C by gentle homogenization and centrifugation prior to ^{2}H NMR spectroscopy.

Samples of membrane vesicles were assayed for triglyceride content prior to ^{2}H NMR spectroscopy by thin-layer chromatography (TLC). The membrane preparation was freeze-dried, and the lipid was extracted several times with $CHCl_3$–MeOH (2:1, v/v) followed by thin-layer chromatography on Merck silica gel 60 F-254 plates (EM Laboratories, Elmsford, NY), eluting with petroleum ether (60–80 °C)–$CHCl_3$ (10:1, v/v) and visualizing with I_2, rhodamine 6G, or molybdenum phosphate reagent (Dittmer & Lester, 1964). Samples were also analyzed by TLC after ^{2}H NMR spectroscopy to determine if any detectable lipid breakdown had occurred. In this case $CHCl_3$–MeOH–7 M NH_4OH (230:90:15, v/v) was used to develop the chromatograms.

Lipid phosphorus was determined by the method of Ames & Dubin (1960), and protein concentration was determined by using the method of Lowry et al. (1951).

Lipids were extracted by using the method of Bligh & Dyer (1959) as modified by Ames (1968). Lipids were freeze-dried and then resuspended in deuterium-depleted water for ^{2}H NMR spectroscopy.

Cells for autoradiography were grown and harvested essentially as described above except that 50 μCi of [U-^{14}C]-palmitate (New England Nuclear, Boston, MA) was added together with nonlabeled palmitate. Two-dimensional thin-layer chromatography and autoradiography were carried out according to Ames (1968).

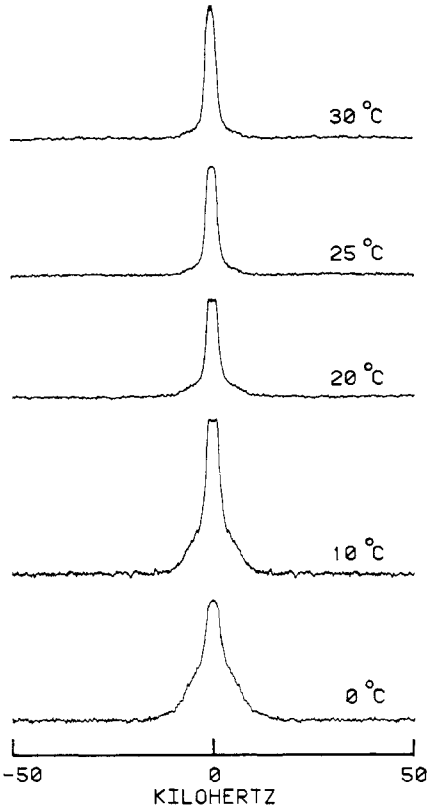

FIGURE 1: Deuterium NMR spectra of membrane vesicles from *E. coli* L48-2 grown in the presence of hexadecanoic-*16,16,16-d*$_3$ acid. Spectra were recorded at 34.1 MHz at the temperatures indicated by using a 100-kHz spectral width, a recycle time in the 0.054–1.1-s range, 2K data points, $t_1 = t_2 = 40$ μs, 6-μs 90° pulse widths, 5000–25 000 scans, and 150-Hz line broadening. Sample volume was 200 μL.

Results and Discussion

The theoretical aspects of the ^{2}H NMR spectra are discussed in detail elsewhere (Oldfield et al., 1978c, and references cited therein; Seelig, 1977, and references cited therein; Kang et al., 1979). For the ^{2}H nucleus, with a spin $I = 1$, the allowed transitions correspond to $+1 \leftrightarrow 0$ and $0 \leftrightarrow -1$ and give rise to a quadrupole splitting of the NMR absorption line, given by

$$\Delta\nu_Q = \frac{3}{2}\frac{e^2qQ}{h}\langle P_2(\cos\theta)\rangle$$

where e^2qQ/h is the deuterium quadrupole coupling constant, which has been found to be ~170 kHz for ^{2}H nuclei in aliphatic C–D bonds (Derbyshire et al., 1969; Burnett & Muller, 1971), θ is the angle between the applied dc magnetic field H_0 and the principal component of the electric field gradient tensor at the deuterium nucleus, and $\langle P_2(\cos\theta)\rangle$ is the value of $^1/_2(3\cos^2\theta - 1)$ averaged in time over any motions that are rapid compared with 170 kHz. The line shape is governed also by the distribution in $\langle\theta\rangle$, usually that of a "crystal powder".

We show in Figure 1 deuterium NMR spectra of *E. coli* L48-2 membrane vesicles containing biosynthetically incorporated terminal methyl ^{2}H-labeled palmitic acid (16,-16,16-trideuteriohexadecan-1-oic acid). At temperatures just below that of growth, the ^{2}H NMR spectrum consists predominantly of a single narrow resonance with a poorly resolved quadrupole splitting, $\Delta\nu_Q$, of ~2.2 kHz and a full line width at half-height of ~3 kHz. There is also a much broader component which increases in relative intensity as the sample temperature is lowered. Spectral simulations indicate that it

Table I: Quadrupole Splitting and Line Width Parameters Used in Computer Simulations of Experimental Data

temp[a] (°C)	membranes[b] fluid $\Delta\nu_Q^d$ (Hz)	membranes[b] fluid W^e (Hz)	membranes[b] solid $\Delta\nu_Q^d$ (Hz)	membranes[b] solid W^e (Hz)	membranes[b] % fluid[f]	extracted lipid[c] fluid $\Delta\nu_Q^d$ (Hz)	extracted lipid[c] fluid W^e (Hz)	extracted lipid[c] solid $\Delta\nu_Q^d$ (Hz)	extracted lipid[c] solid W^e (Hz)	extracted lipid[c] % fluid[f]
30	2200	300	9 500	1800	75	3400	140	11 000	1100	80
25	2500	300	9 500	1800	70	3500	150	11 000	1100	72
20	2600	350	10 000	1800	60	3550	150	11 000	1100	68
10	2800	350	10 000	1800	45	3800	120	10 500	1300	50
0	2800	350	10 000	1850	25	3900	140	10 000	1500	15

[a] Accuracy is ±2°. [b] Membrane vesicles containing biosynthetically incorporated palmitate-*16,16,16-d*$_3$; see Experimental Section for details. [c] Bligh–Dyer extract (Bligh & Dyer, 1959) as modified by Ames (1968); hand dispersed in excess deuterium-depleted water. [d] Quadrupole splitting; the splitting given has an uncertainty of about ±10%. [e] Lorentzian line width convoluted with theoretical powder pattern [see Kang et al. (1979) for details]; the width given has an uncertainty of about ±10%. [f] 100 × (area fluid component signal/total signal intensity).

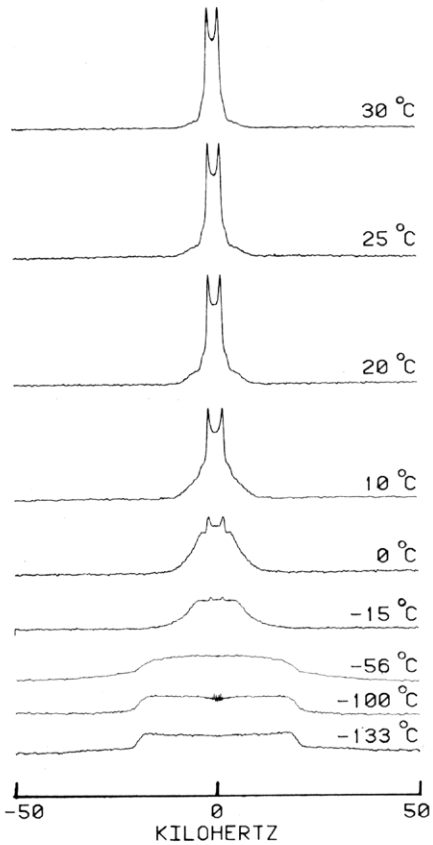

FIGURE 2: Deuterium NMR spectra of a lipid extract from the batch of membrane vesicles used for Figure 1. Lipids were dispersed in deuterium-depleted water; other conditions were as given in Figure 1.

has a quadrupole splitting of ~10 kHz (Table I). By analogy to our results for model systems (Oldfield et al., 1978a; Kang et al., 1979), the broad component is assigned to hydrocarbon chains in a relatively rigid, crystalline, gellike state while the narrow signal which predominates at higher temperatures is attributed to hydrocarbon chains in a more disordered, liquid-crystal-like environment with greater motional averaging, i.e., a smaller value for the time-averaged $\langle P_2(\cos\theta)\rangle$.

The narrow component in the spectra of the membrane vesicles (Figure 1) does not possess the well-resolved, somewhat larger quadrupole splitting characteristic of model (lecithin) liquid-crystalline phases (Seelig & Seelig, 1974; Oldfield et al., 1978a,c). Instead, it resembles very closely the spectra of protein–lipid complexes (Oldfield et al., 1978a,b; Kang et al., 1979). If these differences are caused by protein–lipid interactions, removal of the proteins should lead to spectra characteristic of the normal liquid-crystalline bilayer phase.

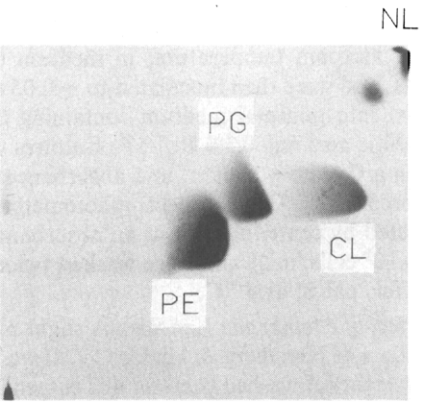

FIGURE 3: Two-dimensional autoradiogram of lipid extract of [U-^{14}C]palmitate-labeled cells (Ames, 1968) showing ~3–4% neutral lipids (NL). The main constituents are phosphatidylethanolamine (PE, 58%), phosphatidylglycerol (PG, 13%), and cardiolipin (CL, 18%), indicating that little if any ^{2}H NMR signal arises from the neutral lipid fraction.

In Figure 2 we present ^{2}H NMR spectra as a function of temperature for such a lipid extract from the sample of membrane vesicles used for Figure 1. The extract is diglyceride- and triglyceride-free and consists almost exclusively of phosphatidylethanolamine, cardiolipin, and phosphatidylglycerol as judged by thin-layer chromatography. In addition, we have obtained autoradiograms of two-dimensional thin-layer chromatograms of [^{14}C]palmitate-labeled lipid extracts (see Experimental Section) as shown in Figure 3, which indicate the very low levels (~3–4%) of neutral lipids present.

In the spectra of the lipid extract the quadrupole splitting of the narrow doublet increases from ~3.4 ± 0.2 kHz at 30 °C to ~3.9 ± 0.2 kHz at 0 °C. Also, a broader component develops. At very low temperatures (−133 °C in Figure 2), as expected, a quadrupole splitting of ~36 kHz is obtained for the ^{2}H in the lipid extract. This splitting is characteristic of a solid with "rigid" hydrocarbon chains, in which only reorientations of the CD_3 group are fast enough to average out part of the quadrupole coupling. Similar results have been obtained on pure bilayers (Oldfield et al., 1978a) and ethane (Burnett & Muller, 1971).

The experimental spectra given in Figures 1 and 2 for the two samples between 0 and 30 °C are accurately simulated by two overlapping ^{2}H powder patterns. The parameters obtained are given in Table I. The results indicate that the gel-to-liquid crystal phase transition of the isolated lipids occurs over a temperature range of ~30–40 °C and over a somewhat larger range for the membranes (Figure 2 and Table I).

The functional membrane vesicles used to obtain Figure 1 consist of about 60 wt % protein and only 40 wt % lipid.

Although their spectra are similar in several ways to those for the lipid extract in Figure 2, there are significant differences in the 0–30 °C temperature range. Both samples have two components in their spectra, the broader component from lipid molecules in the gel-state phase and the narrower component from a liquid-crystalline phase. The splitting of the broader component is, within experimental error (1 kHz), the same for both samples in the 0–30 °C temperature range (Table I) and changes little if any with temperature. In contrast, the narrower component is about half again as broad in the lipid extracts as in the membrane vesicles, and $\Delta\nu_Q$ for both samples is appreciably greater at 0 °C than at higher temperatures. Also, the narrower component is a smaller fraction of the total signal for the lipid extracts at low temperature; for example, at 0 °C it comprises 15% for the lipid extracts but nearly double that (~25%) for the membrane vesicles.

Thus, the protein in the *E. coli* membrane vesicles reduces $\Delta\nu_Q$ of lipids in the liquid-crystal phase, indicating a "dynamic disordering" of the lipid hydrocarbon chains, at least in the region of the terminal methyl groups. This is a rather surprising result when viewed in the context of recent theories of "boundary lipid" in model systems (Jost et al., 1973). However, as discussed in the previous article (Kang et al., 1979) and elsewhere (Oldfield et al., 1978a), slow but large amplitude motions of the lipid hydrocarbon chain in the presence of protein may be invoked to reconcile ^{2}H NMR and ESR spin-label pictures of protein–lipid interaction, both in model systems and in intact biological membranes.

In addition, our results indicate that the protein in *E. coli* membranes interacts preferentially with the lipids which are in the fluid liquid-crystalline state. This is shown not only by the much greater effect of the protein upon the $\Delta\nu_Q$ and the line shape of the narrow component in the spectra but also by the fact that it increases the fraction of lipid with a smaller splitting ($\Delta\nu_Q$), as has been found previously for protein–lipid interactions in model systems (Oldfield et al., 1978a; Kang et al., 1979). Similar behavior has been observed previously by using freeze-fracture electron microscopy with several proteins including (Mg^{2+} + Ca^{2+})-ATPase (ATP phosphohydrolase, EC 3.6.1.3) from sarcoplasmic reticulum (Kleeman & McConnell, 1976) and with the erythrocyte membrane sialoglycoprotein glycophorin (Grant & McConnell, 1974), when incorporated into heterogeneous solid plus fluid binary lipid mixtures.

Implications for Models of Protein–Lipid Interactions. In the past 5 years a number of theories have been proposed for protein–lipid interactions (Marčelja, 1976; Schröder, 1977; Owicki et al., 1978). A general feature of the models is that lipids are "ordered" within an annulus around a protein molecule, extending in some cases to as much as three layers, and that the lipids may thereby mediate protein–protein interaction. We, however, find no evidence in our ^{2}H NMR spectra (at 0–30 °C) for an ordering effect of protein on the lipid hydrocarbon chain in the *E. coli* cell membrane, nor have we found any such evidence for a wide variety of proteins and polypeptides added to dimyristoylphosphatidylcholine (DMPC) labeled with deuterium in the terminal methyl group of the 2-chain or for cytochrome oxidase interacting with an unsaturated lipid (Oldfield et al., 1978a; Kang et al., 1979; Rice & Oldfield, 1979).

Each of the systems we have studied exhibits either a decrease in $\Delta\nu_Q$ of the labeled lipid on addition of protein or polypeptide (at ~1:1 lipid–protein or lipid–polypeptide weight ratios) or essentially no change in $\Delta\nu_Q$. In some instances, however, there are line broadening effects which may indicate either a distribution of order parameters or an effective relaxation process. In none of these systems have we yet obtained ^{2}H NMR evidence for formation of a boundary lipid class of lipid molecules characterized by a larger quadrupole splitting $\Delta\nu_Q$. On the other hand, when cholesterol is added to the lipid, a large increase is seen in $\Delta\nu_Q$ of the lipid above T_c, the gel-to-liquid crystal phase transition temperature, indicating ordering of the hydrocarbon chain in this instance (Oldfield et al., 1971, 1978c). Thus, as viewed by ^{2}H NMR spectroscopy, the effects of proteins and cholesterol on hydrocarbon chain organization in model and biological membranes (Stockton et al., 1977) are completely different. New theories of protein–lipid interaction in membranes should take these observations into account.

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Deuterium Nuclear Magnetic Resonance Studies of the Interaction between Dimyristoylphosphatidylcholine and Gramicidin A'†

David Rice‡ and Eric Oldfield*

ABSTRACT: Deuterium nuclear magnetic resonance spectra of dimyristoylphosphatidylcholines (DMPCs) specifically labeled with deuterium at one of positions 2', 3', 4', 6', 8', 10', 12', or 14' of the 2-chain have been recorded at 34.1 MHz in the presence of varying concentrations of the linear pentadecapeptide antibiotic gramicidin A'. Deuterium quadrupole splittings ($\Delta\nu_Q$) have been used to partially characterize the motion and hydrocarbon chain order of phospholipid in contact with the polypeptide surface. At lipid concentrations below 4 lipids/gramicidin molecule the quadrupole splitting of a terminal methyl-labeled DMPC collapses to a single line. The quadrupole splittings of the methylene labels are decreased, and the line shapes are dominated by large intrinsic line widths. The time constants characterizing the decays of the echo intensity (T_{2e}) are correspondingly reduced in the lipid–polypeptide complexes. At lipid–polypeptide molar ratios of greater than 15:1, the quadrupole splittings of the labels increase linearly with gramicidin concentration to a value ~30% greater than that of pure lipid. Between ratios of 15:1 and 4:1, the splittings decrease slowly. At a ratio of about 4:1 a rather abrupt transition occurs to the high gramicidin phase, and on the time scale of the deuterium NMR experiment (~5.0 μs) lipid adjacent to polypeptide appears disordered (smaller $\Delta\nu_Q$) compared to pure lipid. At all lipid–protein ratios investigated above the gel-to-liquid crystalline phase transition temperature of the pure lipid (T_c), the experimental spectrum is shown to be accurately simulated by using one quadrupole splitting together with a Lorentzian line broadening corresponding to the quadrupolar echo decay rate $(\pi T_{2e})^{-1}$. In some gramicidin–lecithin complexes, T_{2e} values as short as 49 μs are observed. This implies in general for studies of protein–lipid organization in both model and biological membranes that T_{2e} values should be determined routinely in order to eliminate spectral distortions due to relaxation.

In recent years there has been much interest in developing and applying physical methods to study the structure of model and intact biological membranes. Information on the static structures of both types of membrane has been obtained by using X-ray diffraction (Engelman, 1971; Tardieu et al., 1973) and neutron diffraction techniques (Worcester & Franks, 1976; Büldt et al., 1978; D. L. Worcester, M. Meadows, D. Rice, and E. Oldfield, unpublished experiments), while information on the dynamic structures of these systems has been gained predominantly from a variety of magnetic resonance techniques [for example, Chapman & Salsbury (1966), Oldfield et al. (1971, 1972, 1976, 1978a,b) Gaffney & McConnell (1974), Davis et al. (1976), Lawaczeck et al. (1976), Gent et al. (1978), Mantsch et al. (1977), Seelig (1977), Feigenson et al. (1977), Marsh et al. (1978), and Griffin et al. (1978)].

Although proteins normally constitute about one-half of a membrane's dry weight, there have been remarkably few studies of protein–lipid interaction using physical techniques. Electron spin resonance (ESR)[1] studies using nitroxide free-radical spin-labels have suggested that, at least on the time scale of $\sim 10^{-8}$ s, there is a reduction in the rate of motion of a fraction of the hydrocarbon chains in a protein-containing lipid bilayer, the so-called "boundary lipid" or "annulus" (Jost et al., 1973; Hesketh et al., 1976).[2] Calorimetric studies indicate a decrease in the enthalpy of the gel-to-liquid crystalline phase transition of zwitterionic phospholipids with intrinsic protein- or polypeptide-containing bilayers (Chapman et al., 1974; Papahadjopoulos et al., 1975; Curatolo et al., 1977), and Raman spectroscopy has shown a reduction of the number of gauche isomers at low concentrations of the polypeptide gramicidin A' (Chapman et al., 1977), which is in agreement with spin-label results which indicate an increase in chain segmental order parameters (Chapman et al., 1977; Cornell et al., 1978).

More recently, deuterium quadrupole-echo Fourier transform nuclear magnetic resonance (NMR)[1] studies of protein–lipid and polypeptide–lipid interactions in model and intact biological membranes have been reported (Oldfield et

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[1] Abbreviations used: DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; PPPC, 1-palmitoyl-2-palmitoleyl-3-*sn*-phosphatidylcholine; NMR, nuclear magnetic resonance.

[2] More recent studies by Jost & Griffith (1978) have interpreted the ESR results as indicating that "boundary lipid" is far more ordered than free bilayer lipid.

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