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Protein-Lipid Interactions in Biological and Model Membrane Systems

DEUTERIUM NMR OF *ACHOLEPLASMA LAIDLAWII* B, *ESCHERICHIA COLI*, AND CYTOCHROME OXIDASE SYSTEMS CONTAINING SPECIFICALLY DEUTERATED LIPIDS*

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Deuterium nuclear magnetic resonance spectra of *Acholeplasma laidlawii* B (PG9) membranes and lipid extracts enriched biosynthetically in the presence of avidin, with either [14-$^2H_3$]tetradecan-1-oic acid, [16-$^2H_3$]hexadecan-1-oic acid, [4-$^2H_2$]-, [6-$^2H_2$]-, or [8-$^2H_2$]-tetradecan-1-oic acids, have been recorded at a variety of temperatures. The results indicate that at their growth temperature (37°C) the *A. laidlawii* membrane lipids are ~90% in a rigid gel-like state. Plasma membranes which had been lyophilized, then rehydrated, behaved in the ^2H-NMR experiment as did fresh plasma membranes. The ^2H-NMR quadrupole splittings ($\Delta\nu_Q$) were very similar for all of the fluid phase spectra recorded. These results indicate that protein has little effect on lipid order in the *A. laidlawii* B membrane system. The ^2H-quadrupole splittings observed for the 4, 6, 8, and 14-labeled tetradecanoic acid-enriched membranes were within experimental error the same as those observed previously for bilayers of pure 1,2-myristoyl-*sn*-glycero-3-phosphocholine (DMPC) (Oldfield, E., Meadows, M., Rice, D., and Jacobs, R. (1978) *Biochemistry* 17, 2727–2740) when examined immediately above the end of the solid-to-fluid phase transition temperature range. Relatively small decreases in order in the DMPC molecule were seen using cytochrome oxidase as a model membrane protein at high protein to lipid ratio, the effects being largest near the chain terminus (C12–C14).

By contrast, ^2H-NMR spectra of the [6-$^2H_2$]- or [10-$^2H_2$]-hexadecan-1-oic acid-enriched *Escherichia coli* L48-2 cell membranes showed extreme line broadening compared to spectra of their lipid extracts, and $\Delta\nu_Q$ values were slightly decreased. Results with intact *E. coli* cell membranes show essentially the same NMR line shapes as those seen previously with the DMPC-gramicidin A′ system (Rice, D., and Oldfield, E. (1979) *Biochemistry* 18, 3272–3279) including collapsed terminal methyl group quadrupole splittings and large (4 to 6 kHz) line widths of methylene segment chain resonances.

The plasma membrane of *Acholeplasma laidlawii* and the cell membranes of various *Escherichia coli* strains are some of the natural biological membranes studied most frequently using physical techniques. The cell wall less *Acholeplasma* is particularly attractive since the plasma membranes can be readily isolated as a pure preparation (1) while *E. coli* is attractive because of its ease of culture, its well understood genetics, and the ready availability of a variety of mutants.

Some of the earliest physical studies of these systems involved the use of differential scanning calorimetric methods (2–4) and for *A. laidlawii* B it was concluded (5) that 90 ± 10% of the lipids were in an extended bilayer configuration organized in a Danielli-Davson sandwich structure (6). The assumptions used in arriving at this conclusion were later questioned (7). The early differential scanning calorimetric studies were followed shortly by x-ray diffraction investigations (8, 9) which attempted to delineate the gel to liquid crystal phase transition undergone by these systems, although it proved to be difficult to monitor the low temperature end of the transition, which corresponds to a loss of a broad 4.6 $Å^{-1}$ reflection.

At about the same time, the first ^2H- and ^{13}C-NMR studies of membrane structure, using ^2H- or ^{13}C-labeled species, were reported (10, 11). However, these early NMR studies, together with essentially all of those reported to date, were limited, allowing only incomplete comparisons of the intact biological membrane (with protein) and its lipid extract. For example, Metcalfe *et al.* (10) compared spectra of intact ^{13}CO-palmitate-labeled *A. laidlawii* B (PG9) with *sonicated* lipid extracts, which are known to have narrower line widths than unsonicated dispersions, while Oldfield *et al.* (11) did not make any comparisons with *Acholeplasma* lipid extracts. Smith *et al.* (12) later also observed the ^2H-NMR spectra of ^2H-labeled *A. laidlawii*; however, only freeze-dried membranes were studied, and spectra were poorly resolved. Subsequently, with new instrumentation, Stockton *et al.* (13) reported improved ^2H-spectra of lyophilized membranes 5°C above their growth temperature, together with a profile of chain ordering and a spectrum showing the effect of cholesterol incorporation on lipid ordering, although again a comparison with the membrane's lipid extract was not made. Most recently these authors have repeated their earlier work at additional temperatures (14) and by analogy with the work of others concluded that phospholipid, and presumably glycolipid, molecules exhange rapidly between sites in the membrane and that the average perturbation of the local orientational order of the acyl chain of phospholipid (and glycolipid) molecules by proteins must be small at 45°C. However, no measurements on lipid extracts or purified lipid fractions were reported so although these observations were consistent with more detailed results in model systems (15–17) no direct independent

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evidence was presented to support these conclusions, and no results were reported on the effects of lyophilization on membrane structure. Other studies were made by Wieslander *et al.* (18) on ^{2}H-labeled *A. laidlawii* A lipid extracts, but they did not examine the intact membranes.

There have been fewer NMR investigations of *E. coli* cell membranes. Birdsall *et al.* (19) incorporated [1-^{13}C]acetate and [2-^{13}C]acetate into the membrane phosphatidylethanolamines, but no detailed interpretations of the results for intact membranes were possible since the spectra consisted of overlapping resonances from all of the membrane components. Experiments by Urbina and Waugh (20, 21) used selective enrichment of a fatty acid auxotroph (K-12 30E) with 9,10-[^{13}C]methylenehexadecanoic acid and a cross-polarization NMR method to investigate the solid-fluid membrane phase transition in this system, but again no membrane-lipid extract comparisons were made to assess the nature of protein-lipid interaction.

More recently, Davis *et al.* (22) have reported the results of incorporating perdeuterated palmitic acid into *E. coli* L51. They found that most of the phospholipid molecules participated in the phase transition and that the ^{2}H-NMR spectra of intact membranes were similar to those of their total lipid extracts, although profiles of molecular ordering were not obtained. In another study using *E. coli* Kang *et al.* (23) observed the ^{2}H-NMR spectra of biosynthetically incorporated 16-d_3-labeled palmitic acid and found for it that protein had the effect of disordering the hydrocarbon chain organization, although the ^{2}H-NMR spectra of other labeled positions were not investigated.

In this publication we report results of a detailed comparison between the ^{2}H-NMR spectra of intact *A. laidlawii* B (PG9) plasma membranes and their lipid extracts and of *E. coli* L-48 cell membranes and their lipid extracts, into which we have biosynthetically incorporated specifically chain-deuterated fatty acids. In this way we investigate the nature of protein-lipid interaction in these systems. We also investigate the effects of lyophilization on *A. laidlawii* membrane structure and assess the necessity of having fluid liquid-crystalline regions present in the *A. laidlawii* membrane in order to achieve good cell growth. Our results are compared with others recently obtained in these laboratories (15, 16), and models of protein-lipid interaction are proposed that involve either a small disordering or no ordering of membrane lipid by protein.

EXPERIMENTAL PROCEDURES

Nuclear Magnetic Resonance Spectroscopy

Materials and Methods—Deuterium NMR spectra were obtained at 34.1 and 55.3 MHz (corresponding to magnetic field strengths of 5.2 and 8.5 Tesla) using the quadrupole-echo Fourier transform technique (24). Spectra were proton coupled. The low-field spectra were obtained as outlined in the accompanying publication (25). The high-field spectra were obtained on another "home built" spectrometer, which consisted of an 8.45 Tesla 3½-inch bore Oxford Instrument Co. high resolution superconducting solenoid (Oxford Instrument Co., Osney Mead, Oxford, U.K.), together with assorted digital and radiofrequency components.[1] We used a Nicolet NIC-808 data system (Nicolet Instrument Corporation, Madison, WI) to acquire and process most ^{2}H-spectra, using a 100 kHz effective spectral width (25). For some spectra we used a home built 400 kHz data system, consisting of an LSI-11 microcomputer and dual floppy discs, to achieve increased spectral widths. The 90° pulse at 34.1 MHz was 6 to 7 μs and at 55.3 MHz ~7 μs.

Spectral Simulations—Deuterium spectral simulations were carried out on the University of Illinois Digital Computer Laboratory's Control Data Corporation Cyber-175 computer as described (25). Single-component spectra were fitted to a theoretical lineshape function $g(\omega,\Delta\nu_Q)$ where $g(\omega,\Delta\nu_Q) = \int_0^{\pi/2}(d\theta\sin\theta)(\delta/\pi)/(\delta^2 + (\omega \pm (\Delta\nu_Q/2) \times (3\cos^2\theta - 1))^2)$. δ is the half-width at half-height (HWHH) of the Lorentzian broadening function, and $\Delta\nu_Q$ is the quadrupole splitting. Two component spectra were fitted using linear combinations of such theoretical powder patterns.

Production of ^{2}H-Labeled Membranes—*A. laidlawii* B (PG9) were obtained from the National Institute of Allergy and Infectious Diseases Catalog of Research Reagents. *E. coli* L-48 was the kind gift of Professor David F. Silbert, Washington University, St. Louis, MO. The *A. laidlawii* were grown basically as described previously (11) except that avidin (grade II, Sigma Chemical Company, St. Louis, MO) was incorporated into the growth medium at a level of 25 units liter^{-1} (26, 27). Specifically deuterated fatty acids from the batches whose syntheses have been described previously (15, 28) were added at a level of 50 μg ml^{-1}. *A. laidlawii* plasma membranes were isolated using a hypotonic lysis method (11, 29). *E. coli* were grown and membranes isolated as described previously (23). Lipids were extracted from both *A. laidlawii* and *E. coli* membranes using a chloroform-methanol procedure (29). For ^{2}H-NMR spectroscopy, intact membranes were exchanged with a 50 mM pH 7.4 phosphate buffer made using ^{2}H-depleted H_2O (Aldrich Chemical Company, Milwaukee, WI) to reduce the intensity of the natural abundance HO^2H signal. The dried chloroform-methanol lipid extracts were dispersed at ~40°C in the same buffer on a Vortex mixer.

Protein Isolation and Reconstitution Method—The cytochrome oxidase samples were prepared using methods described previously (15, 16). We used a cholate dilution method with lipid-depleted cytochrome oxidase, removing excess cholate by extensive dialysis (30) over Amberlite XAD-2 resin (British Drug Houses, Poole, Dorset, U.K.). Enzyme activities were typically 15 to 20 μmol of cytochrome *c* oxidized min^{-1}·mg^{-1}.

RESULTS AND DISCUSSION

The Acholeplasma Phase Transition—We show in Fig. 1 the results of a series of ^{2}H Fourier transform NMR experiments at 34.1 MHz on 14-d_3 myristate-enriched *A. laidlawii* B (PG9) membranes (Fig. 1*A*), lyophilized membranes which have been resuspended in 50 mM pH 7.4 phosphate buffer (Fig. 1*B*), and chloroform-methanol (2:1, v/v) extracted lipids hand dispersed in excess water (Fig. 1*C*), as a function of temperature.

It is a straightforward matter to analyze quantitatively the results of Fig. 1 if we assume that the broad (~10 kHz) components of the spectra are characteristic of gel state lipid while the narrow (~4 kHz) quadrupole-split doublet is characteristic of lipids in a disordered, liquid-crystalline state, as we have done previously for *E. coli* labeled with [16-$^2H_3$]-palmitic acid (23). We then obtain the results shown in Table I which give the percentage of fluid phase lipids in freshly

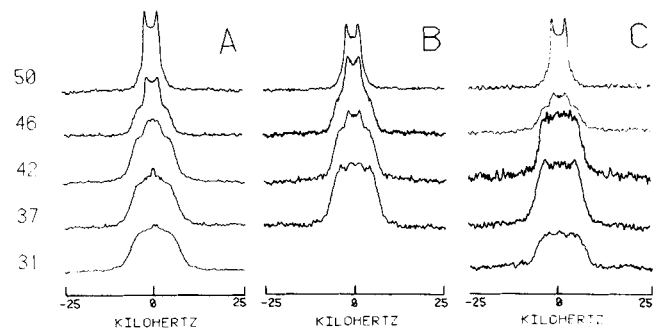

FIG. 1. **Deuterium NMR spectra of plasma membranes, lyophilized plasma membranes, and a lipid extract of the plasma membranes of *Acholeplasma laidlawii* B (PG9) grown in the presence of [14-$^2H_3$]tetradecanoic acid and avidin.** Spectra were recorded at 34.1 MHz at the temperatures indicated, by using typically a recycle time in the 0.054 to 1.1 s range, 2,048 data points, $t_1 = t_2 =$ 40 μs, 6 μs 90° pulse widths, 5,000 to 25,000 scans, and 150 Hz line broadening. Sample volume was 200 μl. Quantitative analyses of the fluid percentage composition of all spectra are given in Table I of the text.

[1] C. Reiner, R. Jacobs, and E. Oldfield, to be published.

TABLE I

Percentage of phospholipids and glycolipids in a fluid, disordered state in Acholeplasma laidlawii B (PG9) membranes enriched with [14-$^2H_3$]tetradecanoic acid, as a function of temperature, and a comparison with results obtained on lyophilized membranes, and their lipid extracts

Temperature	Plasma membrane	Lyophilized membrane	Lipid extract
°C[a]		% fluid[b]	
50	100	100	100
46	45	40	25
42	20	20	10
37	10	5	5
31	5	NM[c]	5

[a] The accuracy of the temperature is ±1°C over the whole sample.
[b] Obtained from computer simulations of the data shown in Fig. 1. The accuracy is ~ ±5%.
[c] NM, not measured.

isolated plasma membranes, lyophilized and rehydrated plasma membranes, and a hydrated lipid extract of the plasma membranes, as a function of temperature. The spectra of Fig. 1 (and fluid percentages of Table I) were obtained on heating runs, and the low temperature spectra were reproducible even after the membranes had been heated to 50°C. This result implies that any denaturation of protein that occurs is either reversible or has no effect on ^{2}H-NMR spectra at low temperatures. Scanning calorimetric results (2, 4) have previously indicated that protein denaturation is only significant at temperatures (>60°C) considerably higher than those used in this study, so we believe that the results of Fig. 1 and Table I do not originate from protein denaturation.

At least four conclusions may be drawn from the results shown in Table I. First, it may be seen that lyophilization of the *Acholeplasma* plasma membrane, followed by rehydration and suspension in 50 mM pH 7.4 phosphate buffer, causes no change in the ^{2}H-NMR spectra between 37° and 50°C. This observation is perhaps not too surprising since this simple microorganism is routinely stored in a lyophilized state. The conclusion that lyophilization causes no change in membrane structure detectable by ^{2}H-NMR is also true for *E. coli* (22) but may not be applicable to more complex biological membranes.

Second, a comparison of the results for lipid extract and intact plasma membrane (Table I) reveals that between the growth temperature (37°C) and 46°C the lipid extract contains only about one-half of the fluid component signal intensity observed for the intact plasma membrane. This result indicates that crystallization of lipid hydrocarbon chain into a rigid gel-like state may be inhibited by the presence of protein, a result we have obtained previously for *E. coli* L48-2 enriched with [16-$^2H_3$]palmitate (23) and for several model systems (15, 16, 31).

The third point of interest about the results of Fig. 1 and Table I is that at their growth temperature of 37°C, only ~10% of the membrane lipids are in a fluid, disordered, liquid crystalline state. Nevertheless, growth yields of 60 to 70% of the maximum ("normal") values are obtained. These results are quite different from those obtained with *E. coli* by Jackson and Cronan (32) who have suggested that while *E. coli* can grow normally with as much as 20% of its membrane in the ordered state, if more than ~55% of the lipids are in an ordered state then growth ceases.

The fourth point that may be seen from the results of Fig. 1 and Table I is that the quadrupole splittings of the fluid phase component in the intact plasma membrane spectra (Fig. 1*A*) and in the isolated lipid extract (Fig. 1*C*) are very similar (~3.5 kHz), only differing by ~0.1 kHz, essentially within our experimental error. This might at first be thought to be in contradiction to our previous observation that protein causes a disordering of hydrocarbon chain organization, especially toward the methyl terminus of a chain (15, 16, 31). However, it must be remembered that the *Acholeplasma* have very low protein-lipid ratios for biological membranes (7). At the ~1:1 protein-lipid ratio present in the plasma membranes, assuming quadrupole splittings similar to those found previously for a free and protein-associated lipid (16) then only about a 0.1 kHz decrease in $\Delta\nu_Q$ should be observed at 50 weight% protein (16).

Incorporation of [16-$^2H_3$]palmitic acid into the *A. laidlawii* B (PG9) plasma membrane, in the presence of avidin, again results in a highly enriched labeled membrane preparation, with some 92 ± 2% of the fatty acid chains being palmitate. The phase transition temperature of the membrane lipids increases considerably due to the increase in chain length (2), and cell growth is slow with only fair yields (27). At their growth temperature of 37°C there is no evidence for *any* fluid phase (from spectral simulations).[2]

Profiles of Chain Ordering—The above results and conclusions are based on observation of only one chain label, a terminal methyl group, and we have shown previously that the effect of protein is largest at this position (16). It is, therefore, of some interest to investigate the effect of protein on lipid order as a function of the position of the ^{2}H-label in the fatty acid hydrocarbon chain. Our previous results (16, 30, 31) would suggest that there should be essentially no change in $\Delta\nu_Q$, but perhaps a change in line width parameter (δ, HWHH).

We show in Fig. 2 spectra of *A. laidlawii* plasma membranes and of their lipid extracts, obtained from cells grown in the presence of avidin, and myristic acid labeled as CD_2 at one of positions 4, 6, or 8. Visual inspection of the results of Fig. 2 indicates clearly that within our experimental error (~ ±2%) the quadrupole splitting ($\Delta\nu_Q$) of each plasma membrane spectrum is the same as that of its lipid extract. These observations are confirmed by more accurate spectral simulation results (data not shown) which give the following $\Delta\nu_Q$ parameters: 4-label, $\Delta\nu_Q = 31 \pm 0.6$ kHz; 6-label, $\Delta\nu_Q = 33 \pm 0.7$ kHz; 8-label, $\Delta\nu_Q = 29 \pm 0.6$ kHz. These results are within experimental error the same as those seen in a 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine bilayer at 25 ± 2°C (27). There are no large line broadening effects seen in the intact plasma membrane spectra of Fig. 2, such as we have observed in model systems (16, 30), although as explained previously the relatively low protein-lipid ratio in the *Acholeplasma* membranes compared to the model systems investigated would make any differences difficult to detect. Similar results were obtained at the high temperature end of the phase transition (50°C, data not shown). A similar lack of any significant line broadening at 1:1 protein-lipid ratios but large broadening at ~4:1 ratios is seen in the ^{31}P-NMR spectra of DMPC[3]·cytochrome oxidase complexes shown in the accompanying publication (25). Such line broadening effects, if they exist in intact biomembranes, may only be easy to demonstrate in systems containing high protein to lipid ratios.

The results at high temperature strongly suggest that fast exchange occurs between the various fluid lipid classes. Since the overall transition width in the case of myristate-labeled membranes is 15 to 20°C, we may assume that the difference in transition temperatures between the main lipid components

[2] S. Y. Kang, R. Kinsey, S. Rajan, H. S. Gutowsky, M. G. Gabridge, and E. Oldfield, *unpublished results*.
[3] The abbreviation used is: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine.

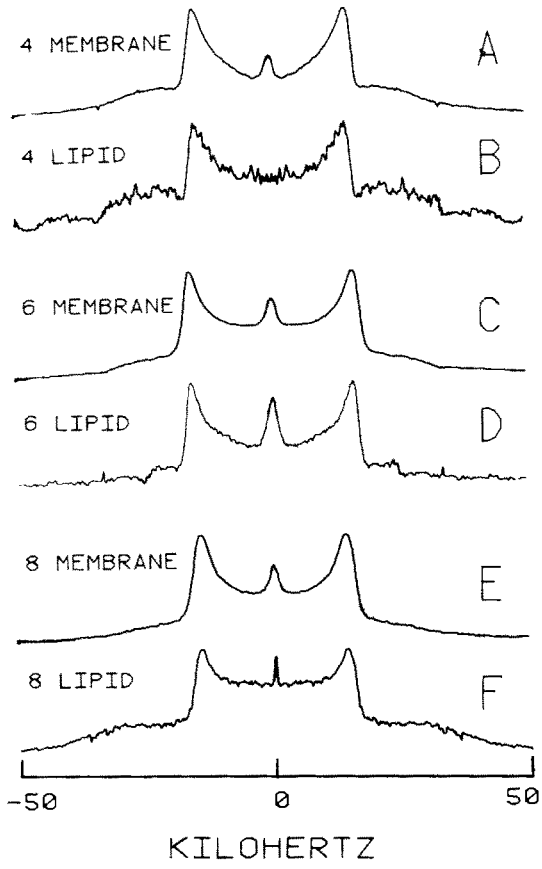

FIG. 2. **Deuterium NMR spectra of plasma membranes and lipid extracts of *A. laidlawii* B (PG9) grown in the presence of one of [4-$^2H_2$]-, [6-$^2H_2$]-, or [8-$^2H_2$]tetradecanoic acids and avidin.** Sample temperature was 46°C. Spectra were recorded at 55 MHz using basically the spectrometer conditions described in Fig. 1. The central narrow features are due primarily to residual HO^{2}H.

is at least this large. At 50°C, when chain melting is complete then some lipid components should be close to their T_c value, while others should be ~15–20° above T_c. Such a large difference in "reduced" temperature would result in a ~4 kHz difference in $\Delta\nu_Q$ of the 4, 6, or 8 position for DMPC (27). Since we have already indicated that the absolute values of $\Delta\nu_Q$ for the *Acholeplasma* lipids at ~50°C are essentially the same as those of DMPC at (T_c + 2°C), and since the quadrupole splittings of a 6-labeled glycolipid (*N*-palmitoylgalactosylceramide) immediately above its T_c (82°C) are also essentially the same as DMPC immediately above its T_c (33) it would be reasonable to expect a ~4 kHz range of quadrupole splittings for the *A. laidlawii* individual lipid fractions if lipid classes were segregated for times longer than ~1 ms. Since only one narrow component is seen in the ^{2}H-NMR spectrum at 46° and 50°C, then fast exchange must occur both between "free" lipid and protein-associated lipid and between different lipid classes, in both "intact" plasma membranes and their lipid extracts. Above the high temperature end of the solid to fluid phase transition there are, therefore, unlikely to be any long-lived (>10^{-3} s) lipid clusters.

Below the high temperature end of the solid-fluid phase transition both gel and liquid-crystalline regions co-exist and when using 333 kHz spectral widths, an additional broad component may be discerned in spectra of intact lipids labeled at other than the terminal methyl[2] (see also Ref. 14). The observation of both broad and narrow components requires that at these temperatures the average lifetime of a given lipid molecule in either fluid or solid state must be longer than ~10^{-4} s. However, since the nature of the diffusion between lipid domains, the domain sizes, etc., are unknown, it is possible that diffusion is the rate-determining step for exchange between the fluid and solid phases, rather than the actual exchange of individual molecules between these states (14).

The Cytochrome Oxidase-DMPC System—We reported previously (15, 16) spectra of DMPC labeled at either the 14 or 6 positions, in the presence and absence of the membrane protein cytochrome *c* oxidase (EC 1.9.3.1). We have now carried out additional experiments with DMPC's labeled on the 2-chain at one of positions 2, 10, or 12 (data not shown). We find that the effects of protein on $\Delta\nu_Q$ are relatively small for all positions except those near the methyl terminus. At ~70 weight% protein the quadrupole splitting decreases in the order C-14 (30%), C-12 (15%), C-10 (2%), C-6 (0%), and C-2 (−5%) with an error of ~ ±5% for each decrease. These results support the idea that in many biological membranes having relatively low protein to lipid ratios (1:1 to 2:1 protein to lipid weight ratio), there will be only minor effects of protein on the average lipid hydrocarbon chain organization. By contrast, the sterol cholesterol has a dramatic effect, even at lower weight ratios (28), increasing $\Delta\nu_Q$ by about a factor of 2 at 30 weight% (28).

Our results with cytochrome oxidase also show that, even in a model protein-lipid complex, the nonequivalence in ^{2}H-quadrupole splittings of the 2-chain 2-position is preserved (as in the case of the interaction with cholesterol, Ref. 34) and that the inner and outer resonances of the 2-chain α-methylene doublet signal (28) retain their ~1:1 intensity ratios. Similar results have been reported for *A. laidlawii* (13) suggesting that the two signals arise from the nonequivalent deuterons at the 2-position and that this nonequivalence is maintained even in protein-lipid complexes.

Escherichia coli L48-2—We present in Fig. 3 our ^{2}H-NMR results obtained with *E. coli* L48-2 membranes (and lipid extracts) containing biosynthetically incorporated [6-$^2H_2$]- and [10-$^2H_2$]palmitic acids. Although the lipid extract and intact cell membrane spectra have approximately the same quadrupole splittings (or order parameters) as judged from spectral simulation (results not shown), there are clearly very

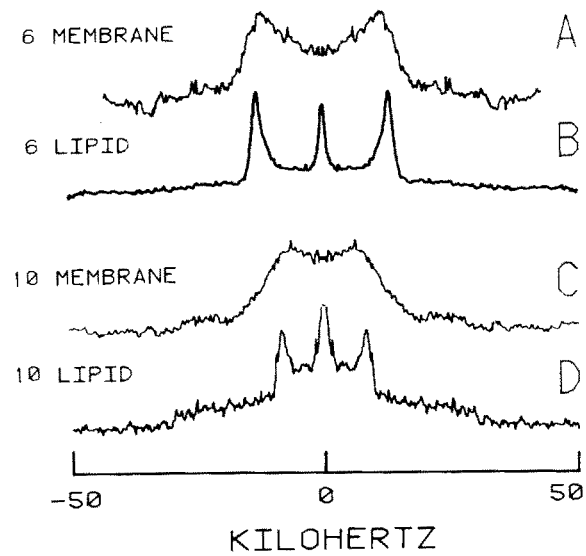

FIG. 3. **Deuterium NMR spectra of cell membranes and their lipid extracts of *Escherichia coli* L48-2 grown in the presence of either [6-$^2H_2$] or [10-$^2H_2$]hexadecanoic acid.** Spectra were recorded at 55 MHz using basically the instrument conditions of Fig. 1, except that the sample volume was 700 μl. Sample temperature was 37 ± 2°C. Lipid extracts were hand dispersions in H_2O.

considerable differences in line shapes between the intact cell membrane and lipid extract spectra, the cell membrane spectra closely resembling those obtained previously (35) with the model system gramicidin A′-DMPC.

The spectra of Fig. 3 do show a very small decrease in $\Delta\nu_Q$ on going from lipid extract to intact cell membrane, $\Delta\nu_Q$ (simulated) decreasing from 27 to 25.8 kHz (Fig. 3, *A* and *B*) for the C-6 label, and from 17.5 to 16.5 kHz (Fig. 3, *C* and *D*) for the C-10 label. In principle, the decrease in order could be apparent rather than real. Such broad lines could arise from fast isotropic rigid body rotation of entire lipid molecules, in which case complete line shape calculations would be necessary to obtain reliable $\Delta\nu_Q$'s and order parameters. However, other results favor the interpretation that follows.

As mentioned above, the cell membrane spectra of Fig. 3 closely resemble those obtained previously for the system DMPC-gramicidin A′ (35), which we show in the accompanying publication to be characterized (at high polypeptide to lipid ratios) by isotropic ^{31}P-NMR line shapes (25). Similar isotropic phospholipid line shapes have recently been reported by deKruijff *et al.* (36) who proposed that complete motional narrowing of the ^{31}P-chemical shift anisotropy could be accounted for by fast exchange between "normal" bilayer regions and regions containing isotropic "lipidic particles" (36) which were visualized in freeze-fracture electron microscopy experiments (36). Such a model would be very attractive for the gramicidin A′-DMPC system since it could account for our observation of considerable normal bilayer x-ray scattering.[4] Also, it is consistent with the observation that while the terminal methyl ($\Delta\nu_Q$ ~3 kHz in pure bilayer) and ^{31}P resonances ($\Delta\nu$ ~48 ppm or 3 kHz at the fields employed) were collapsed to isotropic line shapes with $W \approx 100$ Hz, the line shapes for the other ^{2}H resonances ($\Delta\nu_Q$ ~25 kHz in the pure bilayer) were very broad and appeared to originate in some type of isotropic methylene segment reorientation (35). Moreover, these authors have also very recently reported that *E. coli* membranes and lipid extracts may also show the presence of considerable fractions of phospholipids undergoing almost isotropic motion (37), so it seems likely that such exchange could occur in the case of intact *E. coli* cell membranes, causing the collapsed terminal Me $\Delta\nu_Q$ splittings (23) and broad line shapes (Fig. 3, *A* and *C*).

[4] G. G. Shipley and E. Oldfield, unpublished results.

REFERENCES

1. Razin, S. (1975) *Prog. Surf. Membr. Sci.* **9,** 257–312
2. Steim, J. M., Tourtelotte, M. E., Reinert, J. C., McElhaney, R. N., and Rader, R. L. (1969) *Proc. Natl. Acad. Sci. U. S. A.* **63,** 104–109
3. Melchior, D. L., Morowitz, H. J., Sturtevant, J. M., and Tsong, T. Y. (1970) *Biochim. Biophys. Acta* **219,** 114–122
4. Steim, J. M. (1970) in *Liquid Crystals and Ordered Fluids* (Johnson, J. F., and Porter, R. A., eds) pp. 1–11, Plenum Publishing Corp., New York
5. Reinert, J. C., and Steim, J. M. (1970) *Science* **168,** 1580–1582
6. Danielli, J. F., and Davson, H. (1934) *J. Cell. Comp. Physiol.* **5,** 495–508
7. Chapman, D., and Urbina, J. (1971) *FEBS Lett.* **12,** 169–172
8. Engleman, D. M. (1970) *J. Mol. Biol.* **47,** 115–117
9. Esfahani, M., Limbrick, A. R., Knutton, S., Oka, T., and Wakil, S. J. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68,** 3180–3184
10. Metcalfe, J. C., Birdsall, N. J. M., and Lee, A. G. (1972) *FEBS Lett.* **21,** 335–340
11. Oldfield, E., Chapman, D., and Derbyshire, W. (1972) *Chem. Phys. Lipids* **9,** 69–81
12. Stockton, G. W., Johnson, K. G., Butler, K. W., Polnaszek, C. F., Cyr, R., and Smith, I. C. P. (1975) *Biochim. Biophys. Acta* **401,** 535–539
13. Stockton, G. W., Johnson, K. G., Butler, K. W., Tulloch, A. P., Boulanger, Y., Smith, I. C. P., Davis, J. H., and Bloom, M. (1977) *Nature* **269,** 267–268
14. Smith, I. C. P., Butler, K. W., Tulloch, A. P., Davis, J. H., and Bloom, M. (1979) *FEBS Lett.* **100,** 57–61
15. Oldfield, E., Gilmore, R., Glaser, M., Gutowsky, H. S., Hsung, J. C., Kang, S. Y., King, T. E., Meadows, M., and Rice, D. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75,** 4657–4660
16. Kang, S. Y., Gutowsky, H. S., Hsung, J. C., Jacobs, R., King, T. E., Rice, D., and Oldfield, E. (1979) *Biochemistry* **18,** 3257–3267
17. Seelig, J., and Browning, J. L. (1978) *FEBS Lett.* **92,** 41–44
18. Wieslander, Å., Ulmius, J., Lindblom, G., and Fontell, K. (1978) *Biochim. Biophys. Acta* **512,** 241–253
19. Birdsall, N. J. M., Ellar, D. J., Lee, A. G., Metcalfe, J. C., and Warren, G. B. (1975) *Biochim. Biophys. Acta* **380,** 344–354
20. Urbina, J., and Waugh, J. S. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71,** 5062–5067
21. Urbina, J. (1975) Ph.D. thesis, Massachusetts Institute of Technology
22. Davis, J. H., Nichol, C. P., Weeks, G., and Bloom, M. (1979) *Biochemistry* **18,** 2103–2112
23. Kang, S. Y., Gutowsky, H. S., and Oldfield, E. (1979) *Biochemistry* **18,** 3268–3271
24. Davis, J. H., Jeffrey, K. R., Bloom, M., Valic, M. I., and Higgs, T. P. (1976) *Chem. Phys. Lett.* **42,** 390–394
25. Rajan, S., Kang, S.-Y., Gutowsky, H. S., and Oldfield, E. (1981) *J. Biol. Chem.* **256,** 1160–1166
26. Silvius, J. R., and McElhaney, R. N. (1978) *Nature* **272,** 645–647
27. Silvius, J. R., and McElhaney, R. N. (1978) *Can. J. Biochem.* **56,** 462–469
28. Oldfield, E., Meadows, M., Rice, D., and Jacobs, R. (1978) *Biochemistry* **17,** 2727–2740
29. Razin, S., Tourtellotte, M. E., McElhaney, R. N., and Pollack, J. D. (1966) *J. Bacteriol.* **91,** 609–616
30. Rice, D., Hsung, J. C., King, T. E., and Oldfield, E. (1979) *Biochemistry* **18,** 5885–5892
31. Rice, D. M., Meadows, M. D., Scheinman, A. O., Goñi, F. M., Gomez, J. C., Moscarello, M. A., Chapman, D., and Oldfield, E. (1979) *Biochemistry* **18,** 5893–5903
32. Jackson, M. B., and Cronan, J. E., Jr. (1978) *Biochim. Biophys. Acta* **512,** 472–479
33. Skarjune, R., and Oldfield, E. (1979) *Biochemistry* **18,** 5903–5909
34. Haberkorn, R. A., Griffin, R. G., Meadows, M. D., and Oldfield, E. (1977) *J. Am. Chem. Soc.* **99,** 7353–7355
35. Rice, D., and Oldfield, E. (1979) *Biochemistry* **18,** 3272–3279
36. De Kruijff, B., Verkley, A. J., Van Echteld, C. J. A., Gerritsen, W. J., Mombers, C., Noordam, P. C., and De Gier, J. (1979) *Biochim. Biophys. Acta* **556,** 200–209
37. Burnell, E., Van Alphen, L., Verkleij, A., and De Kruijff, B. (1980) *Biochim. Biophys. Acta* **597,** 492–501