Deuterium NMR Spectroscopy of Biosynthetically Deuterated Mammalian Tissues†

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ABSTRACT: The choline-containing phospholipids of mammalian membranes have been biosynthetically deuterated by raising rats on a diet supplemented with $[HOCH_2CH_2N(CD_3)_3]^+Cl^-$ or $[HOCD_2CH_2N(CH_3)_3]^+Cl^-$. Deuterium NMR spectra have been obtained from excised deuterated brain, sciatic nerve, heart, and lung, from isolated brain myelin and brain microsomes, and from aqueous dispersions of lipid extracts. Measurements of residual quadrupole splittings for excised deuterated neural tissues demonstrate that the orientational order of the choline head group is similar to that observed in model membranes. The spin–lattice relaxation time of the choline head group in deuterated neural tissue is indistinguishable from that observed in model membranes. These results support the proposal that the conformation and motional dynamics of the choline head groups of the bulk choline-containing lipids of neural tissue are similar to those in model membranes. Spectra of biosynthetically deuterated brain myelin and brain microsomes exhibit similar quadrupole splittings. Since these membranes have significantly different protein contents, these results indicate that no strong polar interactions exist between membrane proteins and the choline head groups of choline-containing membrane lipids. Spectra of intact deuterated heart and lung exhibit broad lines and a range of quadrupole splittings. Due to the heterogeneous nature of these tissues, interpretation is difficult. However, no strong ordering of the lipid head group by protein is indicated.

Extensive studies by a variety of techniques have been carried out in an effort to define the conformation of the zwitterionic choline head group of phosphatidylcholine (PC)[1] in model membrane bilayers. X-ray diffraction studies of single crystals of dimyristoylphosphatidylcholine and neutron diffraction studies of multilamellar aqueous dispersions of deuterated dipalmitoylphosphatidylcholine have demonstrated that the choline head group is oriented approximately parallel to the bilayer surface (Pearson & Pascher, 1979; Buldt et al., 1978). Similar studies have shown that the polar head group of phosphatidylethanolamine also lies parallel to the bilayer plane in crystals and single-component aqueous dispersions (Hitchcock et al., 1974; Buldt & Seelig, 1980).

The ultimate goal of such physical studies of model membranes is an understanding of the structure and function of natural membranes. This jump from the study of synthetic systems to complex natural systems has remained particularly elusive in the field of membrane biophysics. Deuterium nuclear magnetic resonance (^2H NMR) is a promising approach that uses the deuterium atom as a probe of local order. The deuterium atom exerts little steric or electronic perturbation upon the system under study and, by virtue of its quadrupole moment, exhibits a spectral doublet when in an ordered environment. The splitting $\Delta\nu_Q$ of this doublet is directly related by theory to an order parameter for the C–D bond, S_{CD} [for a review, see Seelig (1977)]. Furthermore, the dynamic behavior of the deuterium probe can be monitored by observation of its spin–lattice relaxation (Brown et al., 1979; Stark et al., 1983). Extensive ^2H NMR studies of model membranes composed of synthetically deuterated phospholipids have provided useful information on the gradient in order along phospholipid acyl chains, on head-group orientation, and on lipid–protein interactions (Seelig & Seelig, 1974; Seelig & Gally, 1976; Seelig et al., 1977, 1981; Skarjune & Oldfield, 1979; Oldfield et al., 1978a; Paddy et al., 1981; Bienvenue et al., 1982; Tamm & Seelig, 1983). Studies with bacteria and mycoplasma have revealed the in vivo physical behavior of phospholipid acyl chains (Kang et al., 1979; Gally et al., 1979; Stockton et al., 1977). However, extension of studies of this type to mammalian systems is complicated by metabolic problems associated with incorporation of exogenous deuterated fatty acids.

In the present study, we have labeled mammalian phospholipids in vivo with deuterated choline and have obtained deuterium NMR spectra of (1) excised brain, sciatic nerve, heart, and lung, (2) isolated brain myelin and brain microsomes, and (3) aqueous dispersions of extracted lipids. These deuterium NMR spectra provide information about the conformation (via the quadrupole splitting) and dynamics (via the spin–lattice relaxation time) of the choline head group of PC and sphingomyelin in intact mammalian membranes.

Materials and Methods

$[HOCH_2CH_2N(CD_3)_3]^+I^-$ was synthesized by the method of Brulet & McConnell (1976). $[HOCD_2CH_2N(CH_3)_3]^+I^-$ was synthesized according to Gally et al. (1975). These

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[1] Abbreviations: PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleylphosphatidylcholine; γ-d_9 tissue, $N(CD_3)_3$ choline-labeled tissue; α-d_2 tissue, $-CD_2CH_2N(CH_3)_3$ choline-labeled tissue; BovCER, bovine cerebroside; BovSULF, bovine sulfatide; BovPE, bovine phosphatidylethanolamine; BovPS, bovine phosphatidylserine; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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compounds were converted to the chloride or acetate form with Dowex resins. Dipalmitoylphosphatidylcholine was synthesized by acylation of glycerylphosphocholine with the acylimidazole of palmitic acid (Boss et al., 1975) and was converted to the phosphatidic acid with phospholipase D (Dawson & Hemington, 1967). Deuterated choline acetate was coupled to dipalmitoylphosphatidic acid as previously described (Sears et al., 1976). Deuterated 1-palmitoyl-2-oleylphosphatidylcholine (POPC) was synthesized by phospholipase A_2 digestion of deuterated dipalmitoylphosphatidylcholine, followed by reacylation with oleic anhydride (Gupta et al., 1977). The N-$(CD_3)_3$ POPC (γ-d_9 POPC) was purified by silicic acid chromatography and exhibited only one spot on thin-layer chromatography in CH_2Cl_2–CH_3OH–H_2O (65:25:4).

Bovine cerebrosides (BovCER) and bovine sulfatides (BovSULF) were isolated as previously described (Curatolo, 1982). Bovine phosphatidylethanolamine (BovPE), bovine phosphatidylserine (BovPS), and cholesterol were purchased from Sigma (St. Louis, MO) and were shown to be pure by thin-layer chromatography. Phospholipase A_2 was purchased from Sigma, and phospholipase D was prepared from savoy cabbage (Yang, 1969).

Female Sprague-Dawley rats were fed a choline-deficient diet for 3–4 weeks to deplete endogenous stores, followed by 15 days on the same diet supplemented with deuterated choline chloride (approximately 8 mg/day). The rats were impregnated and continued on the deuteriocholine-supplemented diet through birth of a litter and weaning at 15 days. The litter was maintained on the same deuteriocholine-supplemented diet until sacrifice between day 45 and day 60. Various organs and tissues were excised, washed in 10 mM Tris-HCl, pH 7.4–154 mM NaCl in deuterium-depleted water at 4 °C, and were studied by ^2H NMR as quickly as possible (generally 2–3 h after sacrifice). Spectra were also obtained of deuterated tissues that were frozen and thawed, and no differences were ever noted compared to fresh tissue. Organs and tissues studied by ^2H NMR were not segmented or homogenized. The metabolic state of the excised tissues was not monitored; however, the reproducibility of the ^2H NMR spectra indicates that the deuterated choline containing lipids (PC and sphingomyelin) were not degraded significantly with time.

Lipid extracts, brain myelin, and brain microsomes were prepared by standard procedures (Folch et al., 1957; Smith & Curtis, 1979; Koul et al., 1980). The hydrophobic proteolipid apoprotein was removed from neural lipid extracts by precipitation via repeated solvent evaporation (Folch & Lees, 1951). Membranes were prepared for deuterium NMR spectroscopy by sequential bathings in deuterium-depleted buffer at 4 °C. Lipid extracts were lyophilized twice from deuterium-depleted water and finally suspended in deuterium-depleted buffer.

Control-feeding experiments were also carried out with $HOC^3H_2C^3H_2N^+(^{14}CH_3)_3$. The $^{14}C/^3H$ ratio in excised brain did not differ significantly from the ratio in the initial fed choline. Extraction of brain tissue resulted in complete partitioning of the radioactivity in the nonpolar lipid containing phase (Folch et al., 1957).

Deuterium NMR spectroscopy was carried out with a Bruker HX-270 spectrometer at 41.4 MHz, with single 90° pulses of 25-μs duration. Temperature was controlled at ±0.5 °C, and the samples were not spun. T_1 relaxation times were determined with a 180–τ–90 pulse sequence.

Results

Neural Tissues, Membranes, and Extracts. Typical ^2H NMR spectra of excised N$(CD_3)_3$ choline-labeled brain and sciatic nerve are presented in Figure 1. (These deuterated tissues will be referred to as γ-d_9 brain and γ-d_9 nerve.) Intact γ-d_9 brain and γ-d_9 nerve each exhibit a quadrupole powder pattern typical of PC in the liquid-crystalline state and clearly unlike gel-state PC (Gally et al., 1975). The major lipid pool in these tissues derives from the multilamellar myelin sheath, and the ^2H NMR spectrum of myelin isolated from γ-d_9 brain is similar to that of the intact tissue (Figure 1). The cholesterol content of myelin is quite high (approximately 30%) (Smith, 1968); thus, the lipid acyl chains in the intact neural tissues (and myelin) may be quite ordered, even though the headgroup ^2H NMR spectra are typical of liquid-crystalline PC. This is consistent with the observation that addition of cholesterol to model membrane bilayers composed of γ-d_9 dimyristoyl-PC or γ-d_9 dipalmitoyl-PC has very little effect on the ^2H NMR spectrum, although acyl chain order is significantly increased (Brown & Seelig, 1978; Oldfield et al., 1978b). γ-d_9 microsomes exhibit a similar spectrum, characteristic of PC in the liquid-crystalline state (Figure 1).

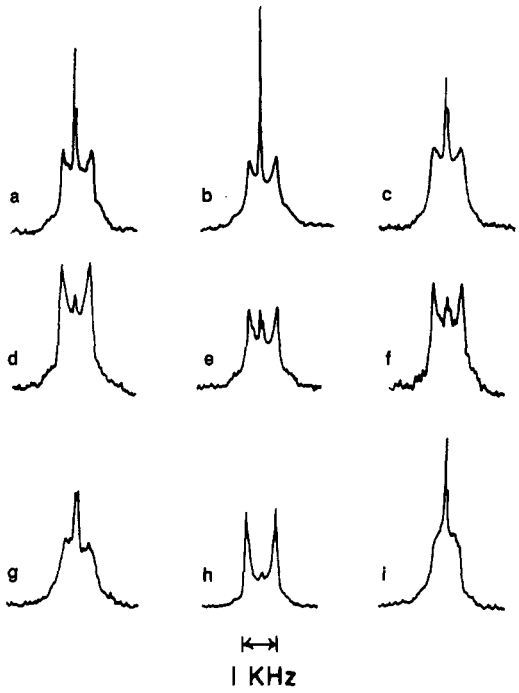

FIGURE 1: ^2H NMR spectra of (a) intact γ-d_9 brain, (b) isolated γ-d_9 myelin, (c) γ-d_9 brain microsomes, (d) γ-d_9 brain lipid dispersion, (e) intact γ-d_9 sciatic nerves, (f) γ-d_9 sciatic nerve lipid dispersion, (g) intact γ-d_9 lung, (h) γ-d_9 lung lipid dispersion, and (i) intact γ-d_9 heart. All spectra were obtained at 25 °C, at a sweep width of ±6 kHz, with 12000 acquisitions, with the exception of (b) and (c), which required 200000 acquisitions. An exponential line broadening of 30 Hz was applied to the spectra.

^2H NMR spectra of aqueous dispersions of lipids extracted from γ-d_9 neural tissues are also presented in Figure 1. Quadrupole powder patterns are observed for each of the extracts.

Quadrupole splittings ($\Delta\nu_Q$) are presented in Table I for γ-d_9 brain, γ-d_9 nerve, γ-d_9 myelin, γ-d_9 microsomes, and γ-d_9 lipid extracts. All exhibit a $\Delta\nu_Q$ value of approximately 0.9 kHz at 25 °C. For comparison, $\Delta\nu_Q$ was determined for model membrane multilayers composed of synthetic γ-d_9 POPC in the presence and absence of cholesterol (Table I). At 25 °C, γ-d_9 POPC and γ-d_9 POPC/30% cholesterol do not differ significantly in $\Delta\nu_Q$ and exhibit $\Delta\nu_Q$'s that are 18–27% larger than the $\Delta\nu_Q$ values observed for γ-d_9 neural tissues and extracts. A multilamellar aqueous dispersion of bovine brain total lipids doped with 10% γ-d_9 POPC was also studied. This dispersion exhibited a ν_Q value of 0.95 kHz, which is close to

Table I: Quadrupole Splittings $\Delta\nu_Q$ for Deuterated Tissues, Membranes, and Extracted Lipid Dispersions at 25 °C[a]

source	$\Delta\nu_Q$ (kHz) whole tissue	isolated membrane	lipid dispersion
γ-d_9 brain	0.85		0.94
γ-d_9 brain myelin		0.84	
γ-d_9 brain microsomes		0.83	
γ-d_9 sciatic nerve	0.90		0.90
γ-d_9 POPC			1.18
γ-d_9 POPC/30% cholesterol			1.15
bovine brain lipids doped with 10% γ-d_9 POPC			0.95
γ-d_9 lung	0.69		0.89
γ-d_9 heart	0.70		
α-d_2 brain[b]	6.76		6.54
α-d_2 lung[b]			8.61

[a] Spectra for γ-d_9 samples were obtained as in Figure 1. [b] Spectra were obtained at a sweep width of ±20 kHz. Spectra of α-d_2 brain, α-d_2 brain lipids, and α-d_2 lung lipids required 500K, 200K, and 150K acquisitions, respectively. No spectrum was observed for intact α-d_2 lung after 450K acquisitions.

the values observed for the biosynthetically labeled γ-d_9 neural tissues and extracts (Table I).

Since the γ-d_9 neural tissues, membranes, and extracts all exhibit the same $\Delta\nu_Q$, it appears that membrane proteins do not exert a large effect on choline head group orientational order in neural membranes. In order to attempt to account for the difference between the synthetic γ-d_9 POPC/cholesterol bilayers and the biosynthetically labeled materials, multilamellar dispersions were studied consisting of γ-d_9 POPC and a variety of bovine brain lipids. These dispersions contained phosphatidylethanolamine, phosphatidylserine, cerebroside, and sulfatide in amounts based upon their abundance relative to PC in myelin. [We have previously shown sphingomyelin to be an essentially isomorphous replacement for PC in model membranes (Sears et al., 1980).] Cerebroside and sulfatide appear to have the largest effect on the $\Delta\nu_Q$ of γ-d_9 POPC, and a system composed of all the lipids approximates the $\Delta\nu_Q$ of biosynthetically labeled γ-d_9 tissues, membranes, and extracts (Table II).

The γ-methyl groups of the choline head group in these systems exhibit low $\Delta\nu_Q$ values and therefore are not very ordered. Regardless of their intrinsically small degree of order, deuterated methyl groups are sensitive to their environment. γ-d_9 POPC/30% cholesterol bilayers, for example, exhibit $\Delta\nu_Q$'s that vary from 1.13 kHz at 37 °C to 1.40 kHz at 4 °C (not shown). Deuteriomethyl groups at the terminal (16-) position of the acyl chains of dipalmitoyl-PC exhibit $\Delta\nu_Q$'s that range from 2.5 kHz in the liquid-crystalline state to 16 kHz in the gel state (Mendelsohn et al., 1982). However, in order to verify the results obtained with γ-d_9 neural tissues, rats were also biosynthetically labeled with $HOCD_2CH_2N^+(CH_3)_3$ (α-d_2 choline), and ^2H NMR spectra were obtained from α-d_2 brain and α-d_2 brain lipids. The $\Delta\nu_Q$ values for α-d_2 brain and α-d_2 brain lipids are similar to each other (Table I) and to the value reported for simple α-d_2 POPC dispersions at 25 °C (Tamm & Seelig, 1983).

Deuterium spin–lattice relaxation times (T_1) were determined at 25 °C for γ-d_9 brain, γ-d_9 brain lipids, γ-d_9 nerve, and γ-d_9 nerve lipids (Table III). The observed T_1 values were similar for all the labeled neural tissues and extracts. For comparison, T_1 was determined for a multilamellar dispersion of γ-d_9 POPC at various temperatures (Table III). The spin–lattice relaxation time for γ-d_9 POPC dispersions at 25 °C was similar to that of the biosynthetically deuterated γ-d_9 neural tissues and extracts at the same temperature. Thus, the dynamic behavior of the choline head group in intact neural membranes (monitored at the γ-d_9 position) is similar to that in simple POPC model membranes.

Heart and Lung. The ^2H NMR spectra of intact γ-d_9 lung and γ-d_9 heart are also presented in Figure 1. The line shapes and $\Delta\nu_Q$ values of these spectra are significantly different than those of the γ-d_9 neural tissues and membranes. The intact heart and lung spectra have rounded features (see Discussion) and exhibit $\Delta\nu_Q$ values of approximately 0.70 kHz (Table I). An aqueous dispersion of γ-d_9 lung lipids exhibits a sharp quadrupole powder pattern with a $\Delta\nu_Q$ value of 0.89 kHz (Figure 1, Table I). Attempts to obtain spectra of α-d_2 lung were unsuccessful, probably because of the lower deuterium content (compared to γ-d_9 lung) and the increased spectral width, coupled with the fact that lung has a lower lipid content than brain. Spectra were obtained from an α-d_2 lung lipid dispersion, which exhibited a $\Delta\nu_Q$ value of 8.61 kHz (Table I). This value is significantly higher than that observed for α-d_2 brain lipids (6.54 kHz) and may reflect the high content of dipalmitoyl-PC in the lung.

Discussion

X-ray and neutron diffraction studies of single crystals and multilamellar aqueous dispersions of phosphatidylcholine have indicated that the zwitterionic choline head group lies parallel to the bilayer surface (Pearson & Pascher, 1979; Buldt et al., 1978). Deuterium NMR studies of deuterated PC dispersions have previously been interpreted to support this general orientation of the PC head group (Seelig et al., 1977). However, it has also been argued that ^2H NMR spectra of PC choline head groups are consistent with a range of orientations and are not sufficient to define a PC head-group conformation (Skarjune & Oldfield, 1979). In the current study, we have shown that the quadrupole splittings and T_1 relaxation times of the choline head groups in neural tissues are similar to those observed for pure lipid bilayers. These observations support (but do not rigorously prove) the proposal that the head groups of the bulk choline-containing lipids in neural membranes have a conformation that is similar to that observed in model membranes. The observed ^2H NMR spectra of biosynthetically deuterated tissues and extracts are presumably composed of contributions from both PC and sphingomyelin. We have observed no evidence that would suggest the presence of overlapping PC and sphingomyelin powder patterns in neural

Table II: Effect of Bovine Brain Lipids on the Quadrupole Splitting $\Delta\nu_Q$ of γ-d_9 POPC in Aqueous Dispersions

bilayer composition (wt %)					$\Delta\nu_Q$ (kHz)	
γ-d_9 POPC	BovPE	BovPS	BovCER	BovSULF	−cholesterol	+30% cholesterol
100	0	0	0	0	1.18	1.15
65	35	0	0	0	1.18	1.02
68	0	32	0	0	1.12	1.00
41	0	0	59	0	*a*	0.92
53	0	26	0	21	0.93	0.91
22	28	10	32	8	*a*	1.00

[a] Broad singlet.

Table III: Deuterium Spin–Lattice Relaxation Times for Biosynthetically Deuterated γ-d_9 Tissues and Lipid Extracts and for γ-d_9 POPC

tissue/extract/lipid	temperature (°C)	T_1 (ms)
γ-d_9 brain	25	38.9
γ-d_9 brain lipids	25	40.3
γ-d_9 nerve	25	37.9
γ-d_9 nerve lipids	25	37.3
γ-d_9 POPC	5	18.0
γ-d_9 POPC	15	27.5
γ-d_9 POPC	25	37.1
γ-d_9 POPC	35	55.4
γ-d_9 POPC	45	72.7

membranes or extracts, indicating that the quadrupole splittings for biosynthetically deuterated PC and sphingomyelin are similar or identical. Small differences would be difficult to detect in rat CNS tissue where the PC/sphingomyelin ratio is 3.3 and would be easier to detect in rat PNS tissue where the ratio is 1.1 (Smith & Curtis, 1979).

Attempts to assess the effects of membrane proteins on lipid behavior by comparison of intact tissue with lipid extracts can be fraught with potential artifacts. The NMR signal of an intact tissue (or cell) derives from a variety of membrane pools, primarily the plasma membrane, the endoplasmic reticulum, and the mitochondria. (This problem exists, of course, for any physical or chemical comparison of whole tissues and extracts.) Each of these membrane pools has a unique structure and a unique lipid and protein composition. A total lipid extract from an intact tissue (or cell preparation) derives from all these pools, and study of such an extract may provide little relevant information. Finally, the asymmetric transbilayer distribution of lipids is lost in a lipid extract. All comparisons of tissues, membranes, and extracts should be made with these problems in mind. In the present work, intact deuterated neural tissues, membranes, and extracts exhibit similar spectral behavior. It is unlikely that this situation would result from some unique combination of these experimental complications. The spectra of γ-d_9 heart and γ-d_9 lung, however, are quite different and cannot be interpreted in an unequivocal manner. The rounded features and lower $\Delta\nu_Q$ values of these spectra may derive from a variety of causes: (1) line broadening due to lipid–protein interactions, (2) overlap of spectra from a variety of subcellular organelles, and (3) spectral averaging in membranes with a high radius of curvature. Both of these tissues possess large quantities of intracellular organelles; lung has intracellular surfactant storage bodies, and heart has large quantities of mitochondria in which a portion of the polar lipid is located in structures with high radii of curvature. It is likely that the observed rounded spectral features and the spectral components with low $\Delta\nu_Q$ values derive from overlapping spectra from these subcellular organelles. A previous ^{2}H NMR study of rat liver mitochondria presented spectra with similar rounded features (Oldfield et al., 1976).

Direct comparison of isolated membrane fractions possessing different protein contents is likely to be the best approach that can be used to assess the effect of membrane proteins on lipids in a mammalian membrane. In the present work, comparison of $\Delta\nu_Q$ for γ-d_9 myelin and γ-d_9 microsomes indicates no difference in choline head group orientational order (Table I). The protein content of rat brain myelin is approximately 30%, while that of rat brain microsomes is approximately 55% (Toews et al., 1976). We conclude that, as monitored by ^{2}H NMR, no strong interactions occur between membrane proteins and the head group of choline-containing lipids in these intact neural membranes. ^{2}H and ^{31}P NMR studies of model membranes composed of PC and a single membrane protein are in agreement with this conclusion (Seelig et al., 1981; Tamm & Seelig, 1983). Whether membrane proteins interact strongly with anionic phospholipids or with glycolipids will be difficult to assess in native membranes because of difficulties involved in biosynthetically labeling such lipids.

The spectrum of γ-d_9 microsomes exhibits a broadened line shape compared to that of γ-d_9 myelin. Similar line broadening has also been observed in spectra of model membranes composed of deuterated PC and a single membrane protein, and a variety of possible explanations have been proposed related to slow lipid motions and to the time scale of exchange of lipid molecules between sites adjacent to protein and sites in the bulk lipid (Paddy et al., 1981; Bienvenue et al., 1982; Tamm & Seelig, 1983). However, at present, there is no single consistent explanation for the observed line broadening.

A quantitative comparison of the orientational order of γ-d_9 POPC/cholesterol bilayers and biosynthetically deuterated γ-d_9 tissues and extracts reveals small but significant differences (Table I). Thus, it appears that lipids other than cholesterol exert a small disordering effect on the head groups of choline-containing lipids in neural membranes. A likely candidate for the source of this minor head group disordering is galactocerebroside, which makes up approximately 20% of myelin lipid. Table II demonstrates that galactocerebroside does, in fact, affect the head-group order of γ-d_9 POPC.

The spin–lattice relaxation time (T_1) is a sensitive motional probe, as evidenced by the significant temperature dependence observed for pure γ-d_9 POPC bilayers (Table III). Comparison of the T_1 values of γ-d_9 neural tissues with that of γ-d_9 POPC at 25 °C indicates that the dynamic behavior of the choline head group is indistinguishable from that observed in pure POPC bilayers. Thus, neither membrane protein nor other neural lipids have any significant effect on the dynamic behavior of the choline head groups of the bulk of the choline-containing lipids, as measured by this parameter.

In summary, we have successfully obtained ^{2}H NMR spectra of biosynthetically labeled choline head groups in intact neural tissue and have shown that the orientational order and dynamics of the choline head group are similar to those in model membranes. The capability of observing ^{2}H NMR spectra of intact neural tissue with high sensitivity provides the opportunity to approach membrane-associated neurophysiological phenomena at the molecular level. Deuteration of the choline moieties of neural phospholipids places a probe at the membrane–environment interface and should allow study of the interactions of physiologically significant agents, such as divalent cations and local anesthetics, with intact neural membranes.

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Second-Derivative Infrared Spectroscopic Studies of the Secondary Structures of Bacteriorhodopsin and Ca^{2+}-ATPase†

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ABSTRACT: The resolution of minor amide components in the infrared spectra of membrane proteins has, in the past, been limited by the small differences in frequency compared to the large half-widths of the bands that are assigned to different secondary conformations. Here, second-derivative calculations are used to resolve the relatively weak bands that are associated with the β-sheet conformation and the vibrations of some amino acid side chains in the infrared spectra of bacteriorhodopsin and Ca^{2+}-activated adenosine-5′-triphosphatase (Ca^{2+}-ATPase). The spectra presented indicate that bacteriorhodopsin in the purple membrane contains an appreciable amount of β structure in addition to the predominant α_{II}-helical structure. Both sarcoplasmic reticulum and purified Ca^{2+}-ATPase in native lipids contain α-helical and random coil conformations together with a small amount of β structure. In 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) Ca^{2+}-ATPase adopts a secondary conformation similar to that in the sarcoplasmic reticulum, and this structure is unaffected by the phospholipid phase transition. A shift to a predominantly random coil conformation is associated with solubilization of both bacteriorhodopsin and Ca^{2+}-ATPase in 20% Triton X-100. Second-derivative analysis of the carbonyl stretching vibrations of DMPC bilayers indicates that below the phase-transition temperature (T_m) both bacteriorhodopsin and Ca^{2+}-ATPase perturb the interface region such that the *sn*-2 carbonyls adopt a conformation similar to the *sn*-1 carbonyls. Above T_m, these integral proteins have no effect on the static order of the interface region, and the conformational inequivalence of the *sn*-1 and *sn*-2 carbonyls is similar to that found in a pure lipid bilayer.

Infrared (IR)[1] spectroscopy is an established technique for the study of the structures of polypeptides and proteins. The IR-active amide bands are associated with the –CONH– grouping that these molecules have in common. Initial qualitative studies related the frequencies of the relatively

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[1] Abbreviations: ATP, adenosine 5′-triphosphate; Ca^{2+}-ATPase, Ca^{2+}-activated adenosine-5′-triphosphatase; CD, circular dichroism; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DTT, dithiothreitol; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; IR, infrared; M_r, relative molecular weight; SR, sarcoplasmic reticulum; T_m, midpoint temperature of the gel to liquid-crystalline phase transition of a hydrated phospholipid or phospholipid/protein recombinant; Tris, tris(hydroxymethyl)aminomethane.

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