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^{19}F -Nuclear magnetic resonance of fluorine-labeled fatty acids in phospholipid bilayer vesicles

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^{19}F -labeled fatty acids, labeled at the 4, 6, 9, 12 and terminal positions, and intercalated into dimyristoylphosphatidylcholine (DMPC) small unilamellar vesicles were studied using ^{19}F -NMR as a function of temperature and perturbant incorporation. The perturbants studied were cholesterol, gramicidin A and polylysine. It was found that the fluorines give rise to a single resonance whose line width decreases substantially with increasing temperature and increases as the fluorine is positioned from the chain terminus toward the polar head group, peaking at the 6 position. When cholesterol is incorporated into the phospholipid vesicles, the fluorine resonance line widths increase dramatically and uniformly along the chain at all temperatures. Gramicidin A incorporation leads to a similar though smaller increase in line widths which, in contrast, diminish with increasing temperatures. On the other hand polylysine interactions lead to little change in the line width of the fluorine-substituted fatty acids. Incorporation of cholesterol increases the size of the phospholipid vesicles but the other perturbants do not, as determined by gel-permeation chromatography. These results are consistent with what is known about the changes in phospholipid bilayer structure brought about by the incorporation of these substances and indicate that ^{19}F -NMR of fluorine-substituted fatty acids intercalated in lipid bilayers can extend our knowledge of model membrane systems.

Keywords: cholesterol; gramicidin; polylysine; intercalation; liposomes; phosphatidylcholine.

Introduction

Fluorine-labeled fatty acids and phospholipids have been successfully used as probes of lipid structure in a wide variety of applications [1]. These include the imbedding of labeled phospholipids in model membrane systems in both multilamellar liposomes [2-4] and unilamellar vesicles [4-7], the imbedding of labeled fatty acids in multilamellar systems [8-11], as well as the biochemical incorporation of labeled fatty acids in biological membranes [8,9,11,12-15]. Fluorine-labeled fatty acids have not as yet been studied in phospholipid unilamellar vesicles. The

small size of the fluorine atom makes it especially suitable as a relatively non-perturbing replacement probe for the hydrogen atom. Moreover, since fluorine can be substituted at various positions along the fatty acid chain, these positional isomers can test various portions of the lipid structure.

Some caution has been raised recently regarding the use of fluorine-labeled fatty acids as lipid probes. For example, the presence of geminal difluoromethylene groups substantially alters the thermotropic phase behavior of synthetic model membranes [16,17]. Moreover, it has been suggested [8] that monofluoro-substituted fatty acids are far less perturbing than the geminal difluoro-substituted ones. Nevertheless, deuterium NMR of specifically deuterated fluorine probes [18] has

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shown that despite the somewhat perturbing effect of these probes, they can provide useful and reliable results on other perturbants such as cholesterol on lipid systems. Since it is in this application we intend to use the fluorine-substituted fatty acids, we feel confident in proceeding with this study.

We have therefore incorporated geminal difluoro-substituted fatty acids labeled at the 4, 6, 9 and 12 positions and a monofluoro-substituted palmitic acid with fluorine in the terminal position into unilamellar phospholipid vesicles and observed changes in the fluorine spectra as a function of their interaction with cholesterol, gramicidin A and polylysine at various temperatures. The results are interpreted in terms of changes incurred in the lipid bilayer structure as a consequence of the incorporation of the perturbant into the lipid vesicles.

Experimental

Materials

Preparation of the keto esters (6-ketomethylpalmitate)

Adipic acid was converted into its half acid ester in 60% yield by the procedure of Swann et al. [19]. The corresponding acid chloride was then prepared with phosphorus pentachloride in 75% yield as described by Cason [20]. Reaction of this half ester-acid chloride (ω -carbomethoxy pentanoyl chloride) with dodecylcadmium prepared from decylbromide Grignard reagent and cadmium chloride in ethyl ether gave a 42% yield of 6-ketomethylpalmitate [20] (m.p. 34–35°C, recrystallized from hexane).

(12-Ketomethylstearate)

12-Hydroxystearic acid was converted to 12-ketostearic acid (m.p. = 82–83°C, 75% ethanol-water) via the CrO_3 /pyridine oxidation [21] in 62% yield. This compound was evaluated for positional purity by converting it to the corresponding keto methylester. The mass spectral analysis of this derivative showed that the original 12-hydroxystearic acid was >92% isomerically pure. Major ions were observed at 242 and 128

characteristic of fragments corresponding to $\text{C}_{14}\text{H}_{26}\text{O}_3^+$ and $\text{C}_8\text{H}_{16}\text{O}^+$, respectively.

Diethylaminosulfur trifluoride (DAST) was prepared by the procedure of Middleton and Bingham [22].

Preparation of the gem-difluoro fatty acids-general procedure

Approximately 6 mmol of keto ester was added to a Teflon screw top bottle (previously dried and flushed with nitrogen) containing 5 ml (38 mmol) of DAST and 5 ml of Freon 11 which was previously cooled to 0°C. The reaction mixture was slowly warmed to room temperature, stirred, and allowed to react for 9 days. The reaction was quenched by slowly adding the reaction mixture to 250 ml of an ice-water slurry. Caution! This reaction is highly exothermic and can be dangerous if addition is too rapid. Hydrolysis of the final product was effected by the addition of 3 ml of 50% NaOH and 100 ml of methanol and heating at 65°C for 5 h. The reaction mixture was then acidified with 20% HCl, extracted three times with 60-ml portions of methylene chloride, and dried over anhydrous Na_2SO_4 . Evaporation of the solvent on a rotary film evaporator gave a 90–95% crude yield of gem difluoro fatty acid. Subsequent recrystallization of these products from acetone gave materials with a purity >95% by GLC. The melting points of the derived fatty acids are: 6,6-difluoropalmitic, 76–77°C and 12,12-difluorostearic, 70.5–72.0°C.

Preparation of 16-fluoropalmitic acid (ω -F palmitic acid)

(16-hydroxymethylpalmitate). To a solution of 30 ml of anhydrous methanol containing 5% sulfuric acid (w/w) was added 8 g of 16-hexadecanolide (Farchan Division; Storey Chemical Corp.). The reaction mixture was refluxed for 2 h, solvent removed, and the residue crystallized from methanol, m.p. = 93–94°C.

(16-Fluoropalmitic acid). 16-Hydroxymethylpalmitate was fluorinated with DAST except that its fluorination was complete within 2 h. Workup was the same as described above for the keto acid derivatives. The melting point of the final product was 73.5–74°C (90% hexane-acetone).

DMPC was obtained from the Sigma Chemical Co. as the L- α form, synthetic, approx. 99% pure; poly-L-lysine, as the hydrobromide, molecular weight range 15,000—30,000; and gramicidin A from Nutritional Biochemicals. All were used without further purification. All other chemicals were of the highest purity obtainable from commercial sources.

Preparation of the phospholipid vesicles

The vesicles were prepared as follows. (1) 50 mg DMPC, 2.5 mg of the fluorinated fatty acid, and varying amounts of cholesterol or gramicidin A as appropriate were added to a round bottom flask and dissolved in a small amount of CHCl_3 . (In experiments with polylysine, appropriate amounts were added at stage (2) below). A thin film was cast on the interior surface of the flask by evaporating the chloroform in a stream of dry N_2 gas while rapidly rotating the flask. The flask was then evacuated for 2 h to remove residual traces of CHCl_3 . (2) The lipid film was then emulsified with 1 ml D_2O containing 0.05 M Tris (pH 7.5) (no correction for deuterium was made), and 0.1 M KCl. (3) The emulsion was then sonicated using a Branson Model 180 sonicator with microprobe tip at a temperature of 50°C.

Sonication was carried out until the emulsion mixture was translucent, roughly 30—60 min. (4) The NMR spectra were obtained on the samples at room temperature or above.

Chromatography

The vesicle preparations were characterized using gel permeation chromatography with Sepharose 4B (cross-linked) as the support material. With Sepharose 2B (cross-linked), the vesicle fraction eluted very close to the total volume and with Sepharose 6B (cross-linked), very close to the void volume. The column used was approx. 30 cm high with an inside diameter of 1.5 cm. Total volume of the packed column was approx. 18 ml with a void volume of approx. 7 ml as determined by indigo blue and blue dextran, respectively. The column was loaded with 0.5 ml of the sonicated emulsion and the sample eluted with H_2O buffer of the same pH, buffering capacity, and ionic strength as the sample D_2O

buffer. The effluent was monitored with a refractive index detector in a flow through cell. All measurements were made at room temperature.

NMR measurements

All NMR measurements were carried out on a Bruker WH-90 spectrometer operating at a frequency of 84.7 MHz at variable temperatures on approx. 0.5 ml of sample in D_2O buffer in a 5-mm sample tube. The D_2O provided the lock signal. A narrow capillary containing 1% by volume of trifluoroacetic acid in H_2O was inserted into the sample tube and was used as reference signal. Accumulations (5000—10,000) of 90° pulses with a repetition rate of 1—2 s were sufficient for each measurement depending on the line width of the fluorinated fatty acids to produce spectra with good signal-to-noise ratios. Sweep widths were generally 5000—10,000 Hz using 8 K data points of memory. Broad band proton decoupling with maximum power (~2 W) was used throughout.

Results and discussions

Chromatography

Chromatographs of vesicle preparations showed in virtually all cases a broad featureless peak with K_{av} values in the range ~0.40—~0.70. Averages for specific vesicle compositions are given in Table I. Some chromatograms showed a small peak at the void volume indicative of multilamellar material. In those cases in which the chromatography indicated large amounts of the multilamellar material or more than a single eluent peak, the samples were discarded.

K_{av} values, as seen in Table I, for the DMPC control as well as for vesicles containing ^{19}F fatty acids, gramicidin A and polylysine, fell in a very narrow range around 0.64. Only those vesicles containing cholesterol showed a change in K_{av} , with values dropping to ~0.45. These results indicate that the phospholipid preparations yielded very uniformly-sized vesicles even when combined with the fluorine-labeled fatty acids, gramicidin, and polylysine. Vesicles containing cholesterol, on the other hand, show a significant

TABLE I

Gel permeation chromatographic data on DMPC vesicles.

Vesicle composition ^a	K_{av} ^b
Control ^c	0.64
20% Cholesterol	0.43
40% Cholesterol	0.46
3% Gramicidin	0.64
3% Gramicidin/5% fatty acid	0.63
20% Gramicidin	0.64
20% Gramicidin/5% fatty acid	0.60
25% Poly-L-lysine	0.66
50% Poly-L-lysine	0.66

^aWeight% relative to the weight of DMPC.^b $K_{av} = (V_e - V_0)/(V_t - V_0)$, where V_e , V_0 , and V_t are the peak elution volume, void volume and total volume, respectively.^cDMPC.

increase in size. Because of the lack of size calibration, this size increase could not be quantitated.

NMR

The NMR results are displayed in Figs. 1 through 3 for vesicles containing cholesterol, gramicidin and polylysine, respectively. Reported in each figure are the data for the control experiments for easy comparison. Values shown in the bar graphs are the line widths (full widths at half-height) of the fluorine resonances of the fluorine-substituted fatty acids intercalated into the DMPC unilamellar vesicles at probe temperatures of ambient, 50°, and 70°. Ambient probe temperatures were 28–30°C. Fluorine was incorporated at various positions along the fatty acid chain from the 4th carbon atom down to the terminal carbon and thus provides a measure of the interaction of the lipid bilayer with guest molecules at various points along the lipid chain.

It is worth noting that in all cases, only a single ¹⁹F resonance was observed. When the phospholipid itself is fluorine-labeled, two resonances appear in the NMR spectrum of the vesicle [9–11]. The dual resonance is thought to arise

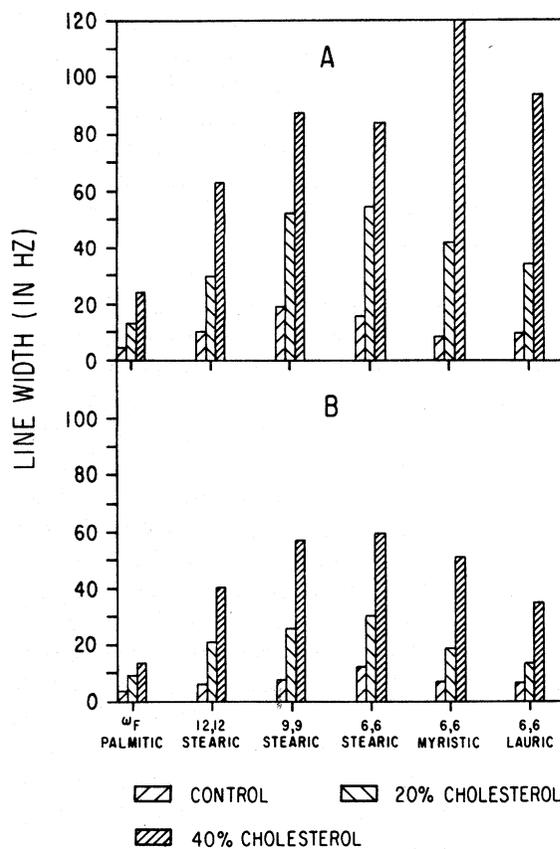


Fig. 1. ¹⁹F-NMR line widths (full width at half height) of fluorine-labeled fatty acids incorporated into DMPC unilamellar vesicles with 0, 20% and 40% cholesterol added. (A) 50°C, (B) 70°C.

from the bilayer asymmetry, the two resonances coming from labeled phospholipid molecules in the inner and outer halves of the phospholipid bilayer, respectively. This asymmetry is also evident in the fluorine NMR spectrum of fluorine-labeled C terminal groups of Gramicidin A, incorporated into phospholipid vesicles. In that case, the fluorine resonances from C terminal groups at opposite ends of the bilayer have different chemical shifts [23]. Since this asymmetry is expected to persist in the vesicles studied here, it appears that the fatty acid molecule is rapidly equilibrating between different halves of the bilayer and only a single resonance with an

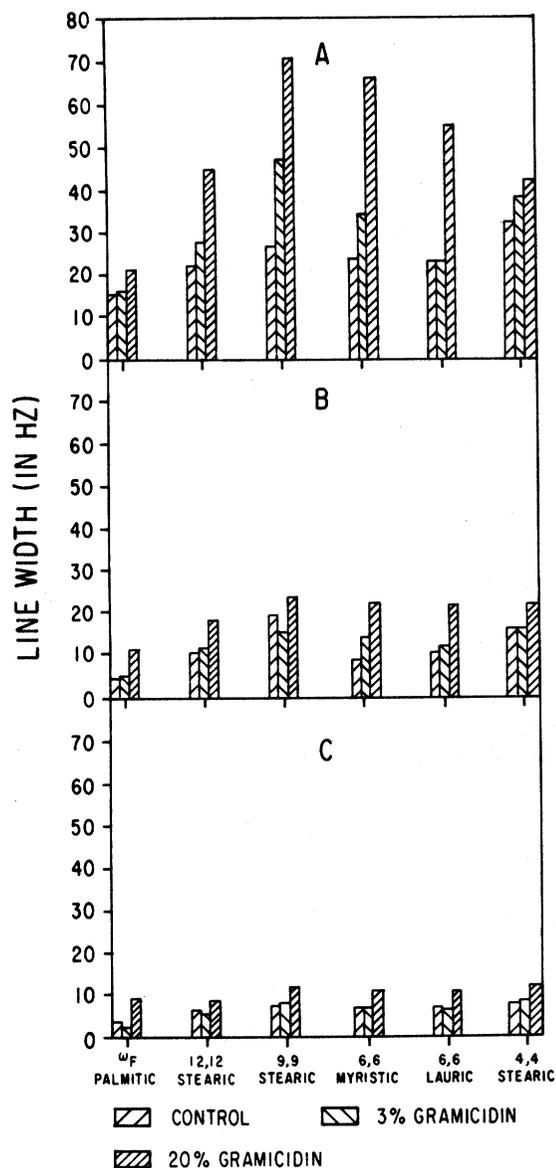


Fig. 2. ^{19}F -NMR line widths (full width at half height) of fluorine-labeled fatty acids incorporated into DMPC unilamellar vesicles with 0, 3% and 20% gramicidin A added. (A) Room temperature, (B) 50°C, (C) 70°C.

averaged chemical shift is observed. It is possible that contributions to the line width might arise from the exchange mechanism, however they would be expected to be small and not materially affect the result obtained.

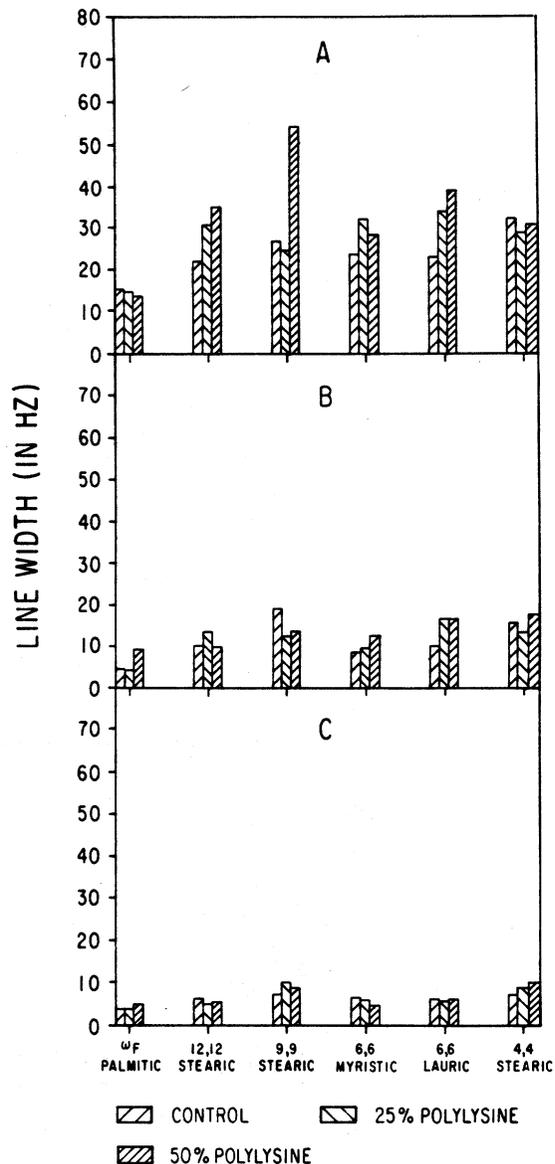


Fig. 3. ^{19}F -NMR line widths (full width at half height) of fluorine-labeled fatty acids incorporated into DMPC unilamellar vesicles with 0, 25% and 50% polylysine added. (A) Room temperature, (B) 50°C, (C) 70°C.

Controls

Turning first to the data of the DMPC vesicles without additional perturbants (Table II, under control), we see that the data display a number of

TABLE II

Line widths^a of ¹⁹F resonances from substituted fatty acids intercalated in DMPC vesicles.

Vesicle composition ^b	Temp.	Substituted fatty acids									
		ω F Palmitic	12.12 Stearic	9.9 Stearic	6.6 Stearic	6.6 Myristic	6.6 Lauric	4.4 Steric			
Control ^c	RT ^d	15.0	21.9	26.7	34.5	23.6	22.8	32.0			
	50	4.1	10.3	19.2	16.4	8.6	10.3	15.8			
	70	3.5	6.0	7.5	12.1	6.8	6.3	7.5			
3% Gramicidin	RT	15.9	27.5	46.7		34.0	22.8	37.9			
	50	4.7	11.3	15.1		14.0	11.8	15.8			
	70	2.4	5.6	7.8		6.7	5.8	8.1			
20% Gramicidin	RT	20.9	44.6	70.7		65.8	54.4	41.9			
	50	11.1	17.9	23.4		22.0	21.3	21.7			
	70	9.3	8.7	12.0		10.9	10.4	11.8			
20% Cholesterol	50	13.4	29.8	52.5	55.1	42.2	35.0				
	70	8.8	20.8	25.5	30.0	18.4	13.3				
40% Cholesterol	50	24.1	63.1	88.0	84.1	123.7	94.4				
	70	13.2	40.2	57.4	59.5	51.3	35.2				
25% Poly-L-lysine	RT	14.6	30.6	24.3		31.9	33.8	28.5			
	50	3.8	13.7	12.5		9.5	16.6	13.6			
	70	3.5	4.9	10.3		6.0	5.9	8.9			
50% Poly-L-lysine	RT	13.7	34.9	54.1		28.3	38.6	30.7			
	50	9.1	9.7	13.9		12.5	16.6	17.9			
	70	4.9	5.6	9.0		4.8	6.5	10.3			

^aFull width at half height. Values are accurate to $\pm 10\%$.^bWeight% relative to the weight of DMPC.^cDMPC with 5% fluorine-substituted fatty acid.^d28–30°C

interesting features. Firstly, the line widths uniformly decrease substantially with increasing temperature, a result well established for proton resonances [24], and fluorine resonances [4,6] in unilamellar lipid bilayer vesicles. The relationship of the ^{19}F line widths (and the corresponding spin-spin relaxation times) to the motion of the hydrocarbon chain in unilamellar vesicles, however, is not a simple one. It has been shown [4] that in addition to contributions to the spin-spin relaxation arising from F-F dipole-dipole interactions (which are related to motions of the fatty acid chain characterized by a correlation time τ_c), there are substantive contributions from the chemical shift anisotropy in these systems. Since it is difficult to separate out the relative magnitudes of these two contributions in any specific case, the interpretation of the line width changes is not altogether straightforward. Nevertheless, it has generally been assumed [6] that the more constrained the hydrocarbon chain-motions, the broader will be the observed NMR resonances.

The variations of the fluorine resonance line widths with position along the hydrocarbon chain bear out this suggestion. In the stearic acid series, as the fluorine is positioned from the region closer to the end of the chain down toward the polar head group, the line width increases at all temperatures, reaching a plateau or maximum about the 6 position. Moreover, for the series of 6,6 disubstituted fatty acids, there is a decrease in the line widths as the fatty acid chain becomes shorter (stearic to myristic or lauric). The variations in line widths appear to reflect the decreasing mobility of the intercalated fatty acid chain the closer that portion is to the carboxylate end of the molecule. For the substituted stearic acids, the line widths peak or plateau at the 6 position indicating maximum motional restriction at that point in the chain. Previous studies [25] using deuterium-labeled stearic acid probes intercalated in unilamellar egg lecithin vesicles showed a similar variation in line widths. In that case, the line widths correlated very well with the measured order parameters which peaked at about the 8th position. This corroboration of the variation of the line widths lends support to the idea that the ^{19}F

spin-spin relaxation times are reflecting local motions of the hydrocarbon chain.

Cholesterol interactions

Figures 1A and B display the variations of the ^{19}F -NMR line widths for the substituted fatty acids in vesicles containing 0%, 20% and 40% cholesterol at 50° and 70°, respectively. The increase in line widths with cholesterol concentration is very apparent and represent the greatest changes seen in these studies. The widths at room temperature were so broad that meaningful results could not be obtained and therefore no data are shown for room temperature.

Despite the significant increase in line width, the variations noted in the controls with fluorine position and fatty acid chain length are generally maintained in the DMPC/cholesterol vesicles. The line widths increase as the fluorine is positioned from the end of the chain to the 9th or the 6th carbon position and decrease generally with the decreased chain length for the 6 carbon atom position, 6,6 stearic acid at 50°C and 40% cholesterol being a notable exception with a resonance line width much narrower than expected.

Notwithstanding this last result, there is a remarkable consistency in the data shown for cholesterol. Moreover, the general increase in line widths fully agrees with the results of many experiments involved with phospholipid/cholesterol interactions. The nature of cholesterol interactions in aqueous phospholipid dispersions has been extensively studied and details of the structure of the complexes have been elucidated for multilamellar systems [26,27]. Cholesterol is known to induce motion (disorder) in phospholipid bilayers at temperatures below the gel-to-liquid crystal phase transitions and to restrict motion (induce order) in the bilayer at temperatures above the transition point as shown by deuterium NMR [28—30] and by Raman spectroscopy [31] in multilamellar systems. Details concerning the structure of cholesterol/phospholipid complexes in small unilamellar vesicles are less well known but fluorescence polarization measurements on these systems have shown an

increase in microviscosity with cholesterol content at temperatures above the thermal transition point [32–35]. ESR studies of spin probes imbedded in phospholipid/cholesterol vesicles also show an increase in the order parameters with increasing cholesterol concentration [14].

Qualitatively, at least, the increase in microviscosity or restricted motion ought to be reflected in an increase in line widths for the fluorine-substituted fatty acid probes. This is precisely what is observed. Furthermore, the relative ordering of the line widths for the various positions along the fatty acid chain as well as for the chain lengths for the 6 substituted acids is generally maintained with regard to the controls. This implies that the effect of the motional restrictions of the cholesterol molecule is uniform along the length of the fatty acid chain.

The increase in the size of the vesicle with cholesterol incorporation is also in accord with previous results [36,37]. Cholesterol, interestingly enough, is the only one of the three perturbants to cause a change in vesicle size. At the same time, cholesterol brings about the greatest changes in line widths of the fluorine probes. While both effects are undoubtedly a result of the significant interaction the cholesterol molecule has with the lipid portion of the phospholipid molecules, the connection between the two effects is not immediately obvious.

Interactions with gramicidin A

Gramicidin A, the linear decapeptide, is known to dimerize in artificial and natural membranes to form an ion-transporting transmembranous channel [38–42]. ^{19}F -NMR of fluorine-labeled gramicidin A has been used [43,44] to determine details of the dimer conformation, but to date there have been no ^{19}F -NMR studies of fluorine-labeled lipids in the gramicidin/phospholipid complex. Our results for ^{19}F -labeled fatty acids intercalated in DMPC/gramicidin vesicles are shown in Fig. 2.

The line widths as displayed for the various positional isomers as a function of temperature and gramicidin content show substantial increases with gramicidin concentration. This effect is most

pronounced for the results obtained at ambient probe temperature and diminishes appreciably at higher temperatures. At 70°C, there is only a small increase over the controls. This latter result contrasts sharply with those obtained with DMPC/cholesterol vesicles which show that the increases in line width as a function of cholesterol concentration do not diminish at the highest temperature studied (70°C).

The changes in line width with gramicidin content while not as large as seen with cholesterol are nonetheless appreciable. Even with 3% gramicidin (mole ratio of 1:87.3 gramicidin/DMPC), there are small increases in the widths at ambient probe temperature ranging up to 20 Hz for intercalated 9,9 difluorostearic acid. At 3% gramicidin, changes in width essentially disappear at 70°C. With 20% gramicidin (mole ratio of 1:10.8) increases in line width occur at all temperatures but at 70° they amount to only 4–5 Hz.

While most of the studies of gramicidin A/phospholipid complexes have concentrated on the determination of the structure and conformations of the gramicidin channel, a number of studies have also looked at the effects of gramicidin incorporation on the lipid portion of the system. It has been found that gramicidin A increases the lipid fluidity of phosphatidylcholine liposomes in the gel state but decreases the fluidity in the liquid crystalline state [45–47]. However, in a ^{13}C -NMR study of DMPC/gramicidin multilamellar emulsions [48], no change in the carbonyl region of the phospholipid was detected up to 40% by weight incorporation of gramicidin. Nevertheless, in another ^{13}C -NMR study of gramicidin/DMPC unilamellar vesicles [49], while no change in T_1 or T_2 of the fatty acid chain carbons was observed at low gramicidin incorporation (mole ratio of 30:1, lipid/gramicidin), there were substantial perturbations of carbon T_1 s and T_2 s along the entire length of the fatty acid chain with higher gramicidin content (mole ratio of 10:1). These changes in relaxation times also indicated immobilization of the lipid chain by gramicidin as observed in the present study of the ^{19}F -substituted fatty acids.

The temperature variations of the line widths

indicate that the immobilizing effect of gramicidin incorporation on the fatty acid chain decreases with increasing temperature. It is interesting to note that at 45°C, gramicidin in a 1:4 mixture with selectively deuterated 1-palmitoyl-lysophosphatidylcholine induced no change in the deuterium quadrupole splitting [50].

We also saw no increase in vesicle size with gramicidin content (Table I). Since the gel-permeation chromatography measurements were carried out at room temperature (above the thermal transition temperature for DMPC), gramicidin interactions with the phospholipid in the liquid crystal state do not lead to an increase in vesicle size. However, substantial increases in vesicle size were observed with gramicidin incorporated into DMPC vesicles at temperatures below the thermal transition [51] but not at temperatures above. Whatever temperature-dependent interactions are occurring, they appear to lead to very strong interactions at lower temperatures to affect vesicle size and to much weaker interactions at high temperatures reflected in the decreased immobilization in the fatty acid portions of the lipid bilayer.

Polylysine incorporation

Polylysine has been used as a model for an extrinsic protein in phospholipid bilayer systems, interacting primarily with the polar end groups [47]. In fact, vibrational spectroscopy of the fatty acid chains in DMPC/polylysine liposomes showed no effect on the hydrocarbon structure by the presence of the polypeptide chain, above 25°C [47]. We were interested in seeing if an effect of the presumably extrinsic interaction would be seen by the intercalated ¹⁹F-substituted fatty acids in DMPC/polylysine vesicles. The results are shown in Fig. 3.

At 50°C and 70°C, the line widths are essentially the same with and without polylysine incorporation. While there are some small differences, (in some positional isomers, the line widths even decrease slightly with increasing polylysine content), there are no systematic variations along the chain. At room temperature, the differences are somewhat more pronounced, most

notably for the intercalated 9,9 difluoro steric acid, but they are still generally small and the smallest variations seen in this study.

These results are consistent with the results of other experiments with phospholipid/polylysine interactions in unilamellar vesicles. Polylysine is known to induce aggregation and subsequent fusion of unilamellar vesicles which are negatively charged [52—55]. However, with uncharged vesicles such as DMPC, aggregation does not occur [55] as confirmed by our result obtained at room temperature that the vesicle size is independent of polylysine incorporation. Moreover, electron spin resonance of spin labels trapped in the lipid bilayer of egg yolk phosphatidylcholine vesicles [55] showed no change with polylysine incorporation. The ¹⁹F-substituted intercalated fatty acids also show little change in the NMR line widths except when the temperatures drop close to the thermal transition temperature. These small variations principally at positions near the middle of the fatty acid chains may be indicative of incipient changes taking place near the gel-to-liquid crystal transition.

Conclusions

The line widths seen in ¹⁹F-NMR of fluorine-substituted fatty acids intercalated in DMPC unilamellar vesicles appear to reflect the nature of the phospholipid acyl chain environment. When cholesterol is incorporated into the phospholipid vesicles, significant immobilization of the fatty acid chains takes place which seems to be uniform along the chain and is not diminished at higher temperatures. These strong interactions also lead to an increase in vesicle size. When gramicidin A is incorporated into the bilayer vesicles, a similar immobilization of the fatty acid chain takes place but it is much weaker than that seen for cholesterol, diminishes with increasing temperature, and causes no change in the size of the vesicles. When polylysine is incorporated into these systems the presumably extrinsic interactions seem to have no effect on the hydrophobic lipid portion of the bilayer. Thus, fluorine-labeled fatty acids have been found to be very useful probes of phospholipid bilayer vesicle structure and can poten-

tially offer a wide application to future studies of the effects of perturbants on model membrane systems.

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