is also unlikely since the same reaction occurs, and at a similar rate, with less than 1 equiv of LiMe. On the other hand, if the breaking of the P–C bond were the first step of the process, one would expect the benzyl group to migrate too, and even faster, in the benzyl-P analogue of **9**; this was not observed. So far the only rearrangement reported for a (σ-allyl)iron derivative, as in η^5-CpFe(CO)(PPh_3)(σ-allyl), is its thermal σ/π rearrangement, leading to the stable η^5-CpFe(CO)(π-allyl), which implies the dissociation of the phosphane complex.[11]

Thus the mechanism of the process reported here most likely consists in the insertion of iron into an allylic or a terminal vinylic C–H bond followed by a 1,3-proton shift to the terminal olefinic or allylic carbon atom, respectively, with concomitant P–C bond cleavage. This reactivity pattern differs both from the previously found phenyl group migration between phosphorus and iron, which implies only the breaking of a P–C bond, and from an ortho-metalation reaction, which implies only the insertion of a metal into a C–H bond. The basicity of the metal is known to play a determining role in the activation of C–H bonds; low oxidation states are usually required. In the present case, the anionic phosphoranide ligand in **9** is likely to increase the charge density on iron, and hence its basicity.

This unprecedented phenomenon is a further indication that the recently discovered phosphoranide ligands employed here promise to lead to new reactivity patterns.

Orientational Order in Phospholipid Bilayers. ^{2}H NMR Study of Selectively Deuterated Palmitic Acids in Unilamellar Vesicles

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We have addressed the current controversy on whether the orientational order of acyl chains in unilamellar vesicles, whose surface is highly curved, is the same or different from acyl chains in multilamellar systems (where the surface has a much lower curvature). Finer,[1] Stockton et al.,[2] and Bloom et al.[3] have stated that the order is essentially the same in both systems, whereas Petersen and Chan[4] and, more recently, Fuson and Prestegard[5] suggest that the order is lower in the vesicle system. We present evidence which shows that the C–D order parameter of ≈5 mol % selectively deuterated palmitic acids incorporated into unilamellar vesicles composed of 15% w/v egg phosphatidylcholine/bovine brain sphingomyelin (85:15 w/w) in deuterium-depleted water are *significantly lower* than the values[2] found in multilamellar liposomes at comparable reduced temperatures.

Fatty acids are considered to be reliable probes of the phospholipid acyl chain in model membranes.[2,6] In fact, Pauls et al.[7] examined the fidelity of deuterated fatty acids used as probes of dipalmitoylphosphatidylcholine bilayers and concluded that, even at 20 mol % incorporation, the acids reflect the order of the membrane to within 10%.

The unilamellar vesicles were prepared by codissolving 300 mg of egg phosphatidylcholine (isolated from fresh eggs[8,9]), 52 mg of bovine brain sphingomyelin (Sigma Chemical Co.), and 6 mg of the selectively deuterated palmitic acid in chloroform. The solvent was removed in a stream of dry nitrogen followed by vacuum pumping overnight to leave a thin lipid film. Deuterium-depleted water (2.5 mL) was added and the mixture rapidly agitated on a vortex mixer until the solution appeared homogeneous (approximately 5–10 min). Vesicles were prepared by sonication for 15 min at ~4 °C under a stream of nitrogen using a Biosonic III probe-type sonicator. The sonicated dispersion was centrifuged 20–25 min on a Clay–Adams clinical centrifuge to remove titanium fragments and undispersed lipid. The vesicles were then filtered through glass wool into an NMR sample tube, and ^{2}H NMR spectra were determined immediately on a

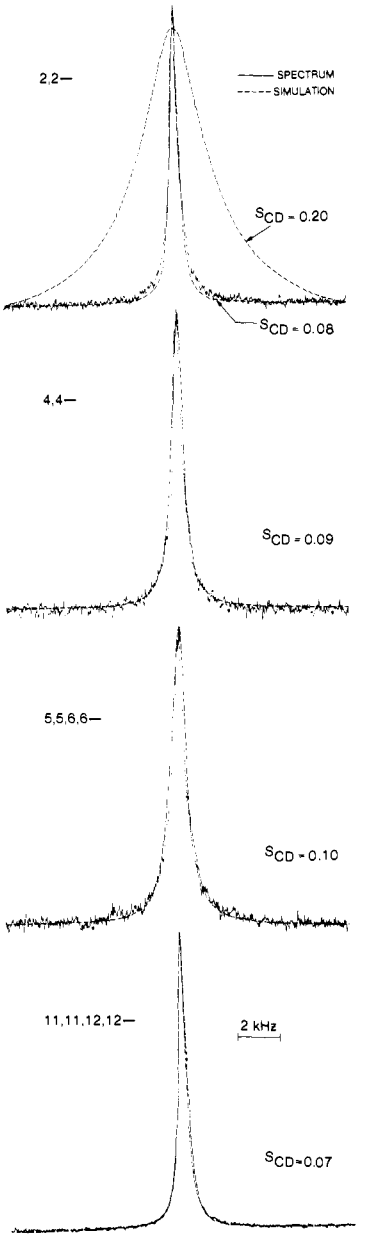

Figure 1. Deuterium NMR spectra (solid lines) of approximately 5 mol % selectively deuterated palmitic acids in unilamellar vesicles of 15% w/v egg phosphatidylcholine/beef brain sphingomyelin (85:15 w/w) in deuterium-depleted water. Position of selective deuteration is indicated to the left of each spectrum. Spectral parameters: sweep width = 50 kHz (16 kHz plotted), pulse width = 8 μs (90° flip angle), data set = 8K, delay before acquisition = 10 μs, line broadening = 20 Hz. The dotted lines are the best fit superposition of Lorentzian lines simulating the spectrum by using the order parameter S_{CD} indicated (see text for details).

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home-built spectrometer[10] at 38.8 MHz. Electron micrographs were also determined immediately by negative staining with 2% ammonium molybdate on 200 mesh Formvar carbon-coated grids in a Philips 300 electron microscope at 80 kV.

Figure 1 contains ^2H NMR spectra of various selectively deuterated palmitic acids in egg phosphatidylcholine/sphingomyelin vesicles. The widths of the peaks at half height $\Delta\nu_{1/2}$ were (top to bottom) [2,2-$^2H_2$] 386, [4,4-$^2H_2$] 446, [5,5,6,6-$^2H_4$] 612, and [11,11,12,12-$^2H_4$] 365 Hz. These widths, of course, would be heavily weighted to reflect the contributions due to the smaller vesicles. Lest the addition of 15% sphingomyelin be suspected of drastically decreasing the bilayer order, the spectrum of ≈5 mol % [5,5,6,6-$^2H_4$]palmitic acid in egg phosphatidylcholine vesicles has been determined and gave a signal with a $\Delta\nu_{1/2}$ = 520 Hz.

Vesicles are heterogeneous particles with diameters from <250 to >600 Å, hence, the ^2H NMR line shapes in Figure 1 are the superposition of resonances of large to small sizes. We have determined a statistical weighting for six size categories on the basis of vesicle sizes determined from electron micrographs.[11] A theoretical line shape $S(\nu)$ was calculated on the basis of a sum of Lorenztian lines

$$S(\nu) = A\sum_{i=1}^{n} \frac{F_i\bar{R}_i^2}{W_i} \frac{1}{1 + (4\nu^2/W_i^2)} \quad (1)$$

where F_i = fraction of vesicles in size category i,[11] and where $W_i \approx (9\pi/20)(e^2qQ/h)^2S_{CD}^2\tau_{v_i}$, (e^2qQ/h) is the static quadrupolar coupling constant (≈ 168 kHz[12]), and τ_{v_i} is the effective correlation time for the i^{th} set of vesicle reorientations given by

$$\tau_{v_i}^{-1} = \tau_{t_i}^{-1} + \tau_{d_i}^{-1} \quad (2)$$

$\tau_{t_i} = 4\pi\eta\bar{R}_i^3/(3kT)$ is the Stokes–Einstein term with η = solvent viscosity, $\bar{R}_i$ = mean radius of i^{th} vesicle set, k = Boltzmann's constant, and T = absolute temperature. The correlation time τ_{d_i} is given by $\tau_{d_i} = \bar{R}_i^2/(6D)$ where D is the diffusion coefficient of the palmitic acid in the bilayer plane. The diffusion coefficient has been measured for [$^2H_{31}$]palmitic acid in egg phosphatidylcholine vesicles, from the ^2H NMR, by the method outlined by Cullis.[13] The value we measured was $D \approx (7 \pm 1) \times 10^{-8}$ cm^2 s^{-1}.[14] The term $\bar{R}_i^2$ in eq 1 compensates for the fact that larger vesicles have greater numbers of palmitic acid molecules presumably proportional to the surface area of the vesicle, $4\pi R^2/S$, where S is the cross sectional area of a palmitic acid molecule. For convenience, S, along with other constants, is incorporated into the scaling factor A. Finally, the quantity S_{CD} is the C–D order parameter $S_{CD} = \langle(3\cos^2\theta) - 1\rangle/2$ where θ is the angle between the C–D bond and the normal to the bilayer surface and the angular brackets represent the average over all orientations of the lipid molecule.[15] (We have also assumed that the order is the same throughout the range of vesicle sizes.) Equation 1 will give an upper limit only to $|S_{CD}|$ since no contribution from local segmental motions of the chains is included.

We have used the solvent viscosity in our calculations, although, for a 15% solution, solution viscosity η = 1.85 cP might be more appropriate.[16] The resulting visual best fit simulated $S(\nu)$ is also shown in Figure 1 (dotted lines) for each chain segment and the calculated best fit order parameters (to the nearest 0.01) were S_{CD} = 0.08 for [2,2-$^2H_2$], 0.09 for [4,4-$^2H_2$], 0.10 for [5,5,6,6-$^2H_4$], and 0.07 for [11,11,12,12-$^2H_4$]. The spectrum for the terminal [16,16,16-$^2H_3$]palmitic acid, not shown in Figure 1, gave S_{CD} = 0.015. The value of S_{CD} from positions 2 to 12 of selectively deuterated stearic acid in egg phosphatidylcholine is 0.205 ± 0.035 at 30 °C,[2] similar to the values found in perdeuterated chains of dipalmitoylphosphatidylcholine.[17,18] A simulated spectrum with S_{CD} = 0.20 is also shown in Figure 1 for the 2-position and is clearly much too broad for the spectral line.

Our results with selectively deuterated palmitic acids are most directly compared with the pioneering work of Stockton et al.,[2] who studied selectively deuterated stearic acids in egg phosphatidylcholine vesicles at 30 °C. Those authors report ^2H NMR line widths of 1.2–1.3 kHz (giving S_{CD} = 0.23–0.24) for positions 2–10 and 800 Hz for position 12 (S_{CD} = 0.17), significantly larger than the line widths reported in this communication. We believe a substantial part of the disparity may lie in the values of τ_{v_i} in eq 1. Stockton et al.[2] prepared their vesicles in a bath-type sonicator for ≈1 h. This could lead to vesicles with substantially greater radii than used in their calculations, and, indeed, the authors admit to a possible disparity in their t_v of 40% (ref 2, p 964).

We have considered the possibility that structures smaller than vesicles (e.g., micelles containing palmitic acid) were formed and would contribute to the narrow portion of the NMR signals in Figure 1. First, [^{14}C]palmitic acid was included in an NMR run with [5,5,6,6-$^2H_4$]palmitic acid. The sample was subsequently chromatographed on Sepharose 4B at 4 °C. All of the radioactivity (92.5% of the initial counts) was eluted coincident with the OD 300-nm peak. No other radioactive peak was subsequently eluted, indicating the absence of smaller structures. Second, a synthetic phosphatidylcholine containing [4,4-$^2H_2$]palmitic acid at the *sn*-2 position was run in dipalmitoylphosphatidylcholine vesicles at 48 °C and gave a ^2H NMR signal whose width at half-height $\Delta\nu_{1/2}$ was 548 Hz.

We therefore conclude that acyl chain order in unilamellar vesicles is at least 2 times lower than in multilamellar liposomes.

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Registry No. Palmitic acid, 57-10-3; [2,2-$^2H_2$]palmitic acid, 62689-96-7; [4,4-$^2H_2$]palmitic acid, 30719-28-9; [5,5,6,6-$^2H_4$]palmitic acid, 75736-47-9; [11,11,12,12-$^2H_4$]palmitic acid, 75736-57-1.

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On the Binding Site of Bacteriorhodopsin. A Study with Artificial Pigments

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The molecular mechanism of the photocycle of bacteriorhodopsin (bR), the protein pigment in the purple membrane of *Halobacterium halobium*, has been the subject of considerable interest.[1] Artificial pigments, in which the native retinal moiety

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