Water Content of Phospholipid Bilayers

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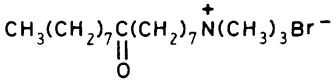
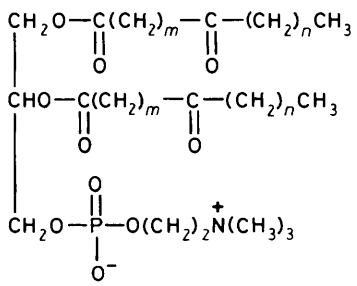
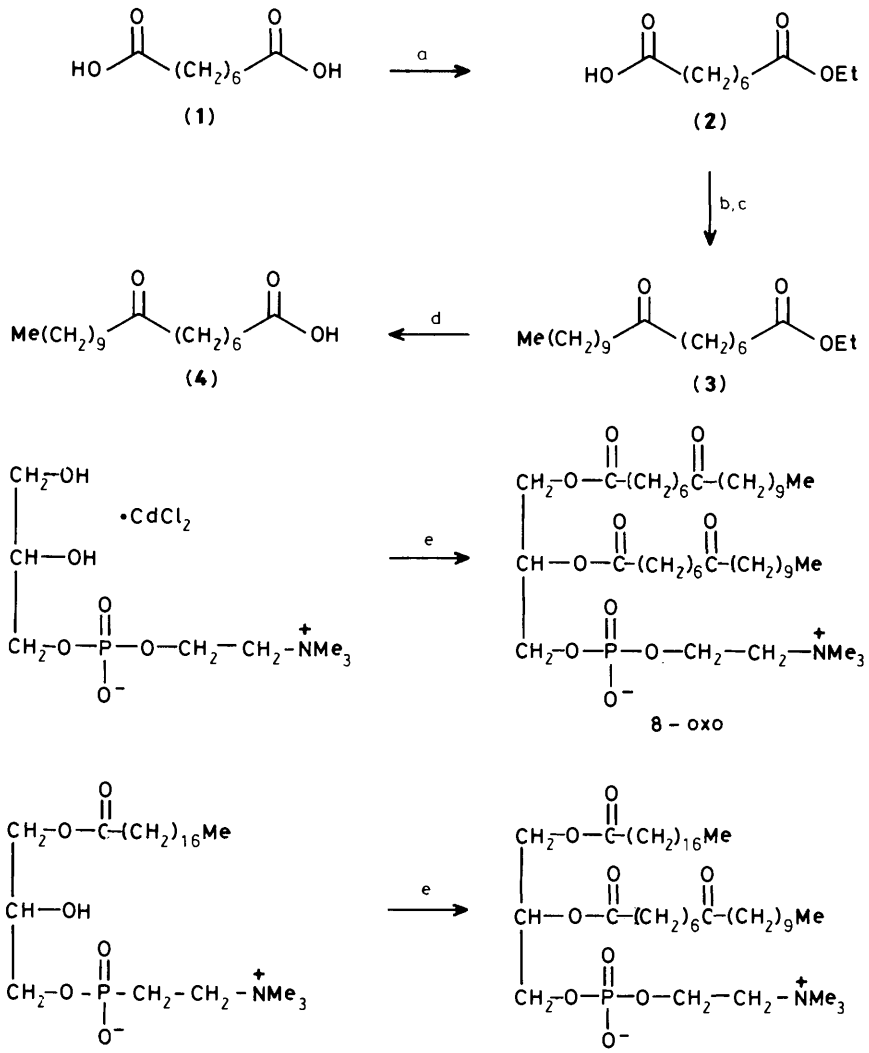
^{13}C N.m.r. data show that static water is absent at five locations along bilayer chains, in sharp contrast to the situation with micelles.

Almost a decade has passed since it was first shown that ^{13}C n.m.r. spectroscopic shifts of carbonyl groups (δ_{CO}) can be used for probing micellar structure.[1] The method was based on the sensitivity of δ_{CO} to solvent polarity.[2] Thus, δ_{CO} data revealed that the carbonyl in a micelle of 8-oxohexadecyltrimethylammonium bromide (8-OHTAB) is embedded in a polar microenvironment (equivalent to that of an alcohol solvent). The carbonyl probe provided two key advantages: (a) since the group was small, it would perturb the micelle structure less than fluorescent compounds, spin labels, and other polarity probes commonly used with micelles and related assemblages; (b) the 'primary' location of the reporting unit was known.



8-OHTAB





Scheme 1. Synthesis of oxo-lipids; *Reagents:* a, $EtOH/H_2SO_4$, cyclohexane; b, $SOCl_2$; c, $[CH_3(CH_2)_9]_2Cd$; d, KOH/H_2O; e, (**4**)/dicyclohexylcarbodiimide/4-*N*,*N*,-dimethylaminopyridine/$CHCl_3$.

Despite the relatively innocuous nature of the carbonyl probe, one cannot definitely exclude two possible explanations for the polar environment observed in the 8-OHTAB experiment: (a) that the carbonyl groups 'drag' water into a normally dry region; (b) anomalous chain-folding positions the carbonyl groups at the aqueous micelle surface. These possibilities have prompted us to examine a series of phospholipids each containing a ketone group at known positions along the chain.

The oxo-lipids, whose synthesis is depicted in Scheme 1,† form vesicles in water as demonstrated by dynamic light scattering. If the carbonyl groups tended to 'drag' water into hydrophobic regions, then we should also have been able to detect a 'polar' δ_{CO} in the spectra of our phospholipid bilayers. If the carbonyl groups within the bilayers manifested a non-polar environment, then this would indicate the absence of both static water and chain-looping. Concurrently, the 'wet' micelle model[3,4] as supported by the ^{13}C n.m.r. data[1] would receive added credibility.

† Structures were substantiated by ^1H and ^{13}C n.m.r., i.r., fast atom bombardment mass spectrometry, and elemental analyses.

An oxo-phosphatidylcholine (70—180 mg) was dissolved in 30—40 ml of h.p.l.c. grade chloroform in a 100 ml round-bottom flask. The solvent was removed under reduced pressure (40—50 °C, 6 h) to form a lipid film on the glass. Ultrapure water (3 ml) was pipetted into the flask which was then placed in a bath-type sonicator (30—40 min). Finally, the dispersion was subjected to probe-type sonication (Branson 185, 60 °C, 20—30 min). Dynamic light scattering measurements, taken on each sample, indicated a high population of small unilamellar vesicles (200—500 Å diameter), but we were not able to rule out the presence of larger species as well. ^{13}C N.m.r. spectra were secured immediately after sample preparation (WP 200SY spectrometer, SW = 242, PW = 8). N.m.r. tubes (8 mm) were fitted with a coaxial insert containing 10% dioxane in D_2O for locking and reference purposes. The temperature of the n.m.r. probe was maintained at 65 °C (above the transition temperatures of the lipids).

Table 1 provides δ_{CO} data on the phospholipids and, for calibration, the carbonyl shifts of dihexyl ketone in various solvents. The low δ_{CO} for the 4-oxo lipid, relative to its 6-, 8-, 10-, and 12-isomers, is merely a substituent effect (as shown by a similar shift for the 4-oxo compound in $CDCl_3$ where vesicles are not formed). Clearly, the δ_{CO} values show that the

Table 1. ^{13}C N.m.r. chemical shifts of carbonyl carbons in vesicular oxo-phosphatidylcholines.[a] Shifts for di-n-hexyl ketone in various solvents are provided for comparison.

Lipid	δ_{CO}	Solvent	δ_{CO}
4-Oxo	140.2 (142.4)[b]	MeOH	146.9
6-Oxo	143.2	EtOH	145.5
8-Oxo	142.6	MeCN	145.2
10-Oxo	143.0	$CHCl_3$	145.1
12-Oxo	142.1	Dioxane	142.1
		Benzene	141.7
		Heptane	139.5

[a] In water at 65 °C relative to external dioxane. [b] Corrected for substituent effects as judged from relative chemical shifts in chloroform.

carbonyl group, no matter what its location along the chain, resides in a non-polar region of the bilayer. Thus, the lipid shifts correspond closely to that of dioxane (well out of range of the protic solvents). Note that the observed micropolarity should not equal that of heptane even if the membrane interiors were perfectly dry; local polarity is enhanced because the carbonyl groups lie side-by-side within the bilayer. The small effect could not be eliminated by providing the lipids with only a single carbonyl-bearing chain (δ_{CO} 142.7 p.p.m. for the lipid at the bottom of Scheme 1). Presumably, one could achieve a heptane-like polarity by mixing small amounts of oxo-lipid with an excess of conventional lipid, but sensitivity problems precluded such an experiment.

Importantly, we could find no evidence for non-transient water either near the head-groups (C-4) or deep within the bilayer (C-12). This is consistent with current thought,[5] although definitive data on membrane hydration are not available. The striking differences between micelle and membrane are undoubtedly related to packing order. Micelles are comprised of 50—100 disorganized molecules (a 'brush-heap') replete with hydrocarbon–water contact.[3,4] Membranes above their phase transition temperature possess a liquid-crystalline order in which, apparently, the chains resist extensive occupation by water. Below the phase transition temperature, of course, water should be even less prevalent.

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5 For a particularly readable discussion of membrane structure, see R. N. Robertson, 'The Lively Membranes,' Cambridge University Press, 1983, pp. 63—67.