

Sealed or Not Sealed? Interaction of Lipid Bilayers With Nanoporous Substrate

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Recently we described a novel method for studying membrane proteins in a native lipid bilayer environment by solid-state NMR spectroscopy [1-2]. The method is based on self-assembling phospholipids into nanotubular bilayers inside macroscopically aligned nanochannels of anodic aluminum oxide membranes (AAO). We have shown that these nanotubular bilayers retain many biophysical properties of unsupported bilayers and suitable for aligning membrane proteins for high resolution solid state NMR studies [1-4].

Properties of nanotubular bilayers were also examined by MAS NMR in an independent study [5]. It was suggested that nanotubes formed from 1-*sn*-3-phosphatidylcholine (POPC) form a tight seal with the pore surface making it impermeable to metal ions such as shift reagent Pr³⁺ [*ibid*]. Here we examined accessibility of both leaflets nanotubular bilayers formed from POPC, DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine) to divalent and trivalent ions by solid-state NMR and EPR at ambient temperatures. We have found that, in accord to previously reported MAS NMR results [5], the inner leaflets of POPC are inaccessible to metal ions. In contrast to POPC, nanotubular bilayers formed from DMPC and DPPC exhibited full ion accessibility. We relate this difference in accessibility properties to phase characteristics of the lipids. Full accessibility of nanotubular bilayers formed from DMPC, DPPC, and several other lipids make such structures suitable for studying reversible effects of binding of mono- and divalent ions to ion channels using physically the same sample.

Ion accessibility NMR and EPR experiments were complimented by parallel differential scanning calorimetry (DSC) studies of effects of mono-, di-, and trivalent metal ions on phase properties of multilamellar and nanopore-supported DMPC bilayers.

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