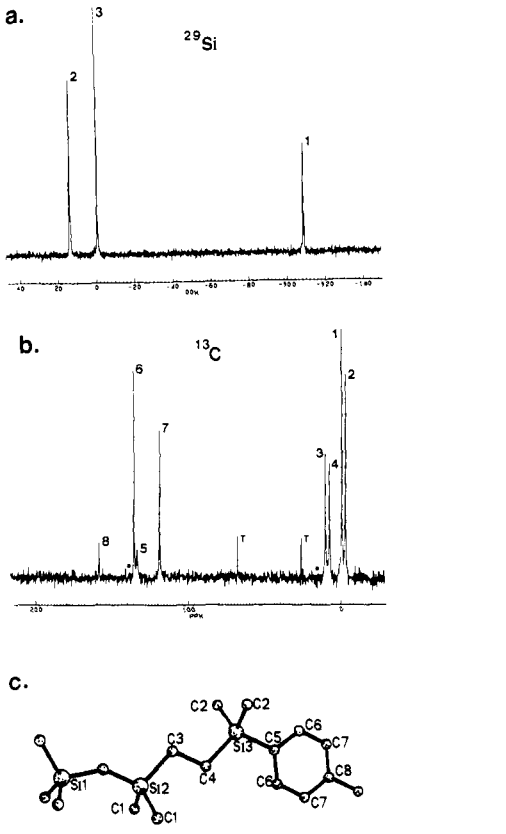

Figure 2. (a) ^{29}Si CP/MAS 59.6-MHz NMR spectrum of the OMM. (b) ^{13}C CP/MAS 75.5-MHz NMR spectrum of the OMM. The peaks labeled T are assigned to tetrahydrofuran. The stars are directly over the positions of the missing peaks mentioned in notes 15 and 17. (c) Ball-and-stick drawing of the repeating unit of this OMM. The atom numbers correspond to peak numbers in a and b. Unlabeled atoms are oxygen atoms.

indicate that the cross-linking reaction has gone almost to completion[15] and the spherosilicate cores have retained their structural integrity.[16] The addition of the ≡SiH groups in **3** across the ≡SiCH=CH_2 groups in **2** seems to have exclusively generated ≡SiCH_2CH_2Si≡linkages.[17] These two spectra are shown in parts a and b of Figure 2, respectively. They seem to suggest that the OMM is crystalline; however, XRD traces show that it is amorphous.

The ^{13}C CP/MAS NMR spectrum of the OMM recorded at room temperature after most of the THF has evaporated shows only broad resonances, but at 100 °C the ones assigned to the methyl and methylene carbons are considerably narrower. This behavior is consistent with the fact that the material is not microporous, which was determined experimentally by low-temperature argon adsorption measurements.[18] This result could be explained as being due to the interpenetration of two or more porous networks, as is observed in crystalline adamantane-1,3,5,7-tetracarboxylic acid.[19]

We are currently engaged in a search for a way to control the secondary structure of the OMM by the use of templating additives and by varying the structure of the cross-linking groups, so that the potential of permanent microporosity is realized.

Acknowledgment. This work was supported by the Department of Chemistry at Virginia Polytechnic Institute and State University.

Supplementary Material Available: Spectral and analytical data for **2** and IR and NMR spectra, TGA thermograms, and Ar adsorption isotherm of the OMM (16 pages). Ordering information is given on any current masthead page.

(14) ^{13}C CP/MAS (external standard = glycine; 75.5 MHz) δ −3.4[2], −1.0[1], 7.1[4], 9.8[3], 25.6[T], 67.4[T], 118.4[7], 133.1[5], 135.2[6], 158.0[8]. ^{29}Si CP/MAS (external standard = M_8Q_8, 59.6 MHz) δ −109.1[1], −0.9[3], 13.5[2]. Superscripts on chemical shift values refer to peak identifiers in Figure 2.

(15) >95% based on absence of a peak at ca. 138 ppm from unreacted ≡SiCH=CH_2 groups.

(16) Only one sharp line can be seen in the Q^4 region.

(17) The alternative mode of addition generates a ≡Si$CH(CH_3)$Si≡ linkage. The CH_3 carbon in this group should appear in the 10–15 ppm region of the ^{13}C NMR spectrum.

(18) Ar adsorption at 87 K, total uptake 0.0033 mL/g. Measurements were made on an Omnisorb 100 dynamic flow adsorption apparatus.

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Quinone-Functionalized Liposomes. Biomimetic Assemblies from a Phosphatidylcholine Anthraquinone

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Respiratory energy transduction involves the transport of electrons and protons across the inner mitochondrial membrane by the lipophilic molecule ubiquinone.[1] Questions concerning the motion and mode of action of ubiquinone[2] within this phospholipid bilayer membrane have prompted us to initiate a study of the redox and transport properties of quinone-functionalized liposomes (vesicles).[3-5] We report herein that we have synthesized the first agent necessary to prepare these biomembrane models. DPPC-AQ, an anthraquinone-containing dipalmitoylphosphatidylcholine (DPPC), is the first example of a quinone-functionalized phospholipid. Quinone-functionalized liposomes can be prepared by sonic dispersion of mixtures of DPPC-AQ and simple phospholipids (e.g., DPPC). Spectrophotometric titrations and kinetics experiments indicate that the DPPC-AQ amphiphiles can be reduced upon addition of external, aqueous $S_2O_4^{2-}$ or BH_4^-.

DPPC-AQ was prepared from L-α-dipalmitoylphosphatidyl-*N*,*N*-dimethylethanolamine (DPP–DMEA)[6] and 2-(bromomethyl)anthraquinone[7] as shown in Scheme I. After 16 h at 60 °C the reaction mixture was filtered and separated by flash chromatography on SiO_2 with $CHCl_3$/MeOH/H_2O (79:20:1, then 65:25:4) as the eluent. Extraction of a $CHCl_3$ solution of the phospholipid with aqueous EDTA to remove metal ions and precipitation with cold acetone[8] yielded pure material in 40–50% yield. The 300 MHz ^{1}H NMR spectrum of DPPC–AQ in $CDCl_3$ (Figure 1) reveals the expected aromatic resonances from 7.3 to 8.4 ppm, the glycerol and choline resonances between 3.4 and 5.2 ppm, and the acyl chain resonances from 0.8 to 2.2 ppm.

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(3) Functionalized liposomes have been prepared previously with various spin labels[4] and photoactive groups.[5]

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(6) DPP–DMEA was obtained from Sigma Chemical Company; DPPC was obtained from Avanti Lipids. Their purity (99%) was verified by NMR and thin-layer chromatography.

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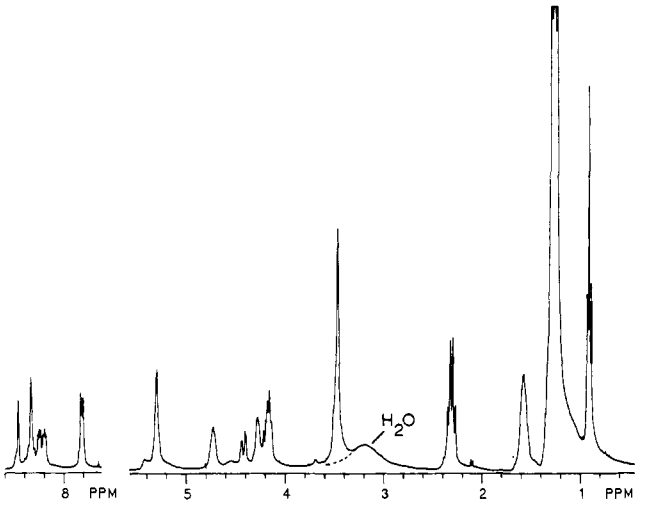
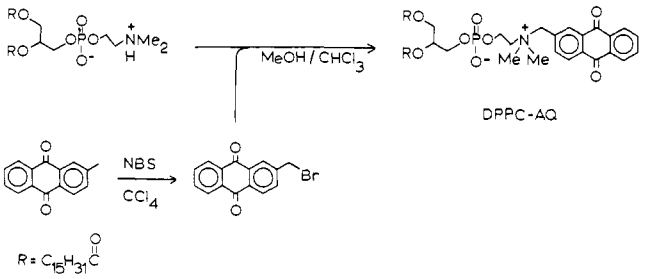


Figure 1. The 300 MHz ^1H NMR spectrum of DPPC-AQ in $CDCl_3$. The H_2O peak at 3.2 ppm corresponds to 2.4 H_2O/DPPC-AQ for this particular sample; CHN analysis confirms this quantity of H_2O in the sample.

Scheme I



DPPC-AQ is hygroscopic as evidenced by the H_2O peak observed in small amounts between 1.5 and 3.5 ppm and the CHN analysis which typically reveals 2–4 H_2O molecules/DPPC-AQ.

Neat suspensions of DPPC-AQ do not form liposomes efficiently; however, a 1:1 mixture of DPPC and DPPC-AQ forms liposomes that are similar to those prepared from DPPC.[2d,9] Apparently substitution of the methylanthraquinone group for a methyl group has some effect on amphiphile assembly,[10] but dilution of the functionalized phospholipid rectifies this situation. Unilamellar liposomes with 5–15 mol % DPPC-AQ can be prepared routinely and are used as "typical" functionalized liposomes.[9] These quinone-functionalized liposomes are redox-active as evidenced by the addition of external, aqueous $S_2O_4^{2-}$ or BH_4^-. Figure 2 illustrates that the liposome-bound quinones (λ_{max} = 322 nm) are completely reduced to the hydroquinone form (λ_{max} = 384 nm) by $S_2O_4^{2-}$. The spectrum reverts to the original upon exposing the solution to oxygen. The inset in Figure 2 shows that the hydroquinone peak is formed nearly instantaneously (within ca. 3 s) upon addition of $S_2O_4^{2-}$. Since $S_2O_4^{2-}$ is known[2d] to penetrate into the bilayer, we have also employed BH_4^- as a reductant. BH_4^- does not penetrate the liposome surface[2d] and is also transparent between 200 and 800 nm. ($S_2O_4^{2-}$ absorbs at 315 nm and is responsible for the shoulder at 315 nm in Figure 2 (--).) BH_4^- typically reduces 85–90% of the DPPC-AQ amphiphiles; these are presumably located within the outer monolayer of the liposome. $S_2O_4^{2-}$ apparently penetrates far enough into the liposome to reduce all of the quinones. Statistical considerations[10,11] alone predict that 70% of the DPPC-AQ amphiphiles should reside in the outer monolayer of the liposomes, so DPPC-AQ must preferentially assemble on the outer surface. Considering the large head group of DPPC-AQ, the outer monolayer should be the most energetically favorable location.[10,11] Our continuing work[12] with spectrophotometric, high field NMR, and membrane electron transport measurements should provide a more complete picture of these interesting biomimetic assemblies.

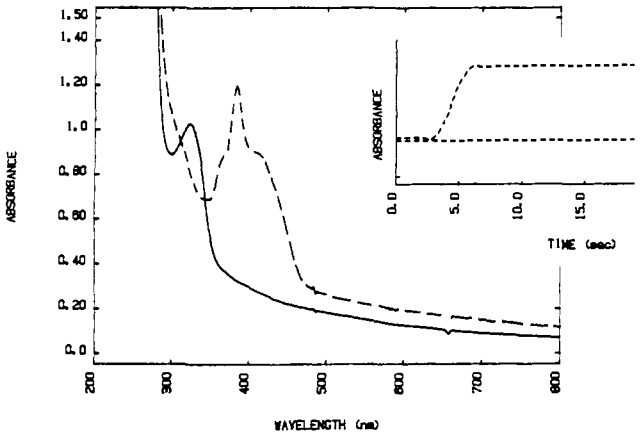


Figure 2. Spectrophotometric response of a 10% DPPC-AQ/DPPC liposome solution to addition of excess, external $S_2O_4^{2-}$: (—) before addition and (--) after addition of $S_2O_4^{2-}$. The small absorbance peak at 315 nm after addition is due to the ca. 5% excess of $S_2O_4^{2-}$. The inset is time dependence of absorbance at 384 nm following addition of $S_2O_4^{2-}$ at t = 3 s; lower trace is background absorbance before addition of $S_2O_4^{2-}$.

It is important to recognize that DPPC-AQ closely resembles phosphatidylcholine (lecithin), a major component of mammalian mitochondrial membranes.[13] This strong structural similarity between the endogenous phospholipids and our biomimetic agent is crucial in assuring relevance of our planned biophysical study of membrane electron transport. We should emphasize that DPPC-AQ is the first member of what we view as an extensive family of phospholipid quinones from which quinone-functionalized liposomes may be prepared. Efforts are currently underway[14] to employ both chemical modification of phospholipids and multistep chemical syntheses to prepare analogous phospholipid quinones with varying alkyl and acyl chains and with internal quinone groups that should more closely model the present views[2] of ubiquinone within the inner mitochondrial membrane. Combining these chemical routes to functionalized phospholipids with the diverse methods for preparing controllable sizes of liposomes[10] should provide the ability to engineer liposomes with controllable chemical and physical properties. This is one of the tools that we have at our disposal in preparing simple, yet varied, chemical models for mitochondrial energy transduction.

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(9) Liposomes were prepared by sonic dispersion at 52 °C of DPPC and DPPC-AQ in N_2-blanketed pH 8 (Tricine) 0.1 M KCl solutions with a Sonic Materials VC40 processor. The liposomes were fractionated on Sephadex G50 at 52 °C. The 5–15% DPPC-AQ/DPPC liposomes possess optical and size exclusion chromatographic characteristics identical with DPPC liposomes,[2d] so the functionalized liposomes should possess an average diameter of ca. 25 nm.[10] Liposome suspensions were maintained at 52 °C at all times to maintain the DPPC liposomes in the liquid crystalline phase (T_c = 42 °C[2d]).

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