ordered, separated threads (e.g., B in Figure 1d) are followed by irregular regions with partial fusions of the threads (e.g., A in Figure 1d).

The primary molecular binding forces between two threads are presumably hydrogen-bond chains between carboxyl protons and carboxylate anions of neighboring fibers (Figure 2). A similar situation has been encountered in crystals of tartaric acid salts: they are most stable if hydrogen-bonded water molecules and counterions (H^+, NH_4^+) are bound together with one alkali-metal ion.[8,9] Pasteur's sodium ammonium tartrate hydrate being the most famous example.[8]

Separated threads or sheets of bilayers have also been observed in cubic and hexagonal phases,[10] myelin figures,[11,12] or multiwalled vesicles[12] consisting of double-chain amphiphiles in aqueous emulsions. None of these materials form complex shapes and isolated bodies such as shown in Figure 1a. It is presumably the relatively good water solubility of the amphiphiles **1a,b** in micellar form that allows rapid interfiber associations.[2]

As usual with ultrathin micellar fibers, only the pure enantiomers of **1a,b** produce such aggregates with large surfaces. The corresponding racemate rather forms smooth planar bilayer platelets under the same conditions[7] and precipitates from aqueous solution ("chiral bilayer effect" [1]).

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Supplementary Material Available: Electron micrographs of different cloth structures made of micellar fiber aggregates of potassium salt **1b** and sodium salt **1a** (6 pages). Ordering information is given on any current masthead page.

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Polymerized Liposomes Designed To Probe and Exploit Ligand-Receptor Recognition at the Supramolecular Level[1]

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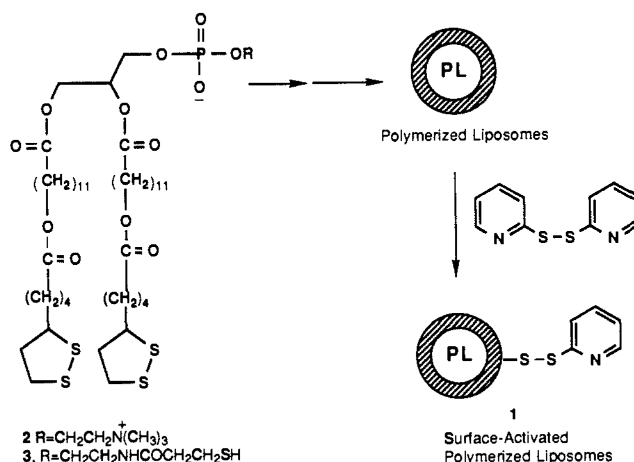
This paper describes the synthesis of surface-activated polymerized liposomes (**1**) derived from 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (**2**) and 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phospho-*N*-(3-mercaptopropionyl)ethanolamine (**3**). Such liposomes, which bear activated sites that are locked into a random distribution on the membrane surface, have a surface density that can be controlled by adjusting the molar ratio of **2**/**3** that is used, and reversibly bind organic thiols, should provide a unique opportunity for probing and exploiting ligand-receptor recognition at the supramolecular level.

Understanding how biological membranes recognize and respond to extracellular signals, and learning how to control such



recognition, represent two of the most important challenges presently facing chemists and biologists. At the supramolecular level, the key issues extend beyond the specific chemistries involved; they relate to questions of *valency, proximity, and cooperativity among multiple pairs of ligands and receptors*. One may ask, for example, how many ligand-receptor bonds are necessary to "switch on" a specific membrane function? What are the spatial requirements that must be met? How does ligand-receptor cooperativity affect the overall "supramolecular recognition" process (Figure 1)?

Conceptually, *cross-linked polymerized liposomes*, possessing uniform, highly stable and "biomembrane-like" surfaces, represent attractive probes for investigating the above questions.[3] In particular, the ability to control the *spatial availability* of pendant molecules, by adjusting their surface density, and by altering the curvature of the liposomal surface to which they are attached (i.e., by changing the vesicle's size), should allow one to study multivalent binding in a controlled manner. This paper reports the synthesis of a unique class of polymerized liposomes that have been specifically designed for this purpose. Work that will be reported elsewhere will describe the use of such liposomes in defining the supramolecular recognition features of the Arg-Gly-Asp (RGD) moiety toward cell surface receptors.[4,5]

Using methods similar to those previously described, **2** was converted into its corresponding ethanolamine via phospholipase D catalyzed exchange.[6,7] Subsequent treatment with 1.5 equiv of *N*-succinimidyl 3-(2-pyridyldithio)propionate in chloroform and deprotection with 20 equiv of dithiothreitol in methanol afforded **3**.[8]

Surface pressure-area isotherm analysis of monolayers produced from **2** and **3**, at the air-water interface, establish their miscibility.[9] For an ideally miscible or completely immiscible monolayer, the mean area per molecule, A_m (at a specific surface pressure), is defined by the mole fraction of lipid employed, X_1, and by the partial molar areas of each lipid (A_1 and A_2, respectively), according to eq 1. Any deviation from linearity establishes that

$$A_m = X_1A_1 + (1 - X_1)A_2 \quad (1)$$

the pair of surfactants is nonideally miscible. By use of the phase rule of Defay and Crisp, it is also possible to distinguish between

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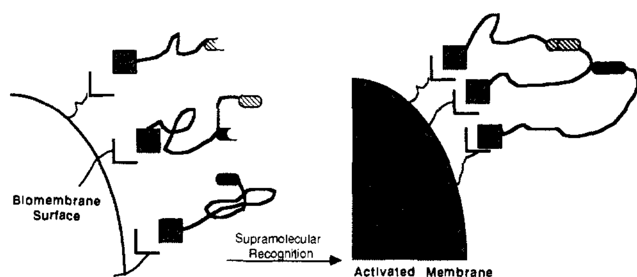

Figure 1. Stylized illustration of a supramolecular recognition event; i.e., the formation of a trivalent ligand-receptor complex at a biomembrane surface, held together via hydrogen bonding, ion pairing, and/or hydrophobic interactions.

ideal miscibility and complete immiscibility.[10,11] Specifically, for a completely immiscible two-component monolayer, the collapse points are equal to those of the pure components; for a completely miscible mixture, the collapse point is linearly related to the mole fraction of lipid used (eq 2). Here, P_m equals the collapse pressure

$$P_m = X_1P_1 + (1 - X_1)P_2 \quad (2)$$

of the mixed monolayer, and P_1 and P_2 represent the collapse pressures for the pure lipids. On the basis of the A_m values observed at 20 dyn/cm and the variation in the collapse pressures for these mixed monolayers (Figure 2), it is evident that **2** and **3** are nonideally miscible. Since both lipids bear the *same* polymerizable moiety, mixtures of **2** and **3** must lead to copolymerized membranes having a random distribution.

Large unilamellar vesicles (ca. 1000-Å diameter; 4.8 mM) were prepared by using a molar ratio of **2**/**3** of 9/1 via standard extrusion procedures (0.1 μm Nucleopore membranes)[12] in 10 mM borate buffer (140 mM NaCl, 2 mM NaN_3, pH 6.4). Polymerization was initiated by raising the pH to 8.4 and was complete after 6 h, as judged by the loss of lipid monomer (TLC, UV).[13] After polymerization, the pH was returned to 6.4 for subsequent reactions. Mean external diameters that were observed for the polymerized liposomes were similar to their nonpolymerized precursors (dynamic light scattering, Nicomp 270). Reaction with 5 equiv of 2,2′-dithiodipyridine afforded a surface-activated polymerized liposomal dispersion, **1**, containing 0.84 ± 0.06 mol of 2-pyridyldithio groups/mol of **3**.[14] Similar to homopolymerized liposomes of **2**, but unlike homopolymerized liposomes produced from 1-palmitoyl-2-[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine,[6] **1** is completely insoluble in $CHCl_3$ and $CHCl_3/CH_3OH$ (1/1, v/v). These solubility properties infer that **1** is a *cross-linked* liposome.

In an effort to distinquish between activated sites present on the inner- and outer-monolayer leaflet, a dispersion of **1** was treated with 5 equiv of the polymeric reducing agent, dihydrolipoamide–dextran (DHLA-dextran).[15,16] A maximum of ca. 45% of the liposomal-bound 2-mercaptopyridine was released after a total of 1.5 h. Similar cleavage experiments carried out with DTT released ca. 80% of the bound 2-mercaptopyridine. If it is assumed that DHLA–dextran cannot "reach across" a lipid bilayer,[15] these

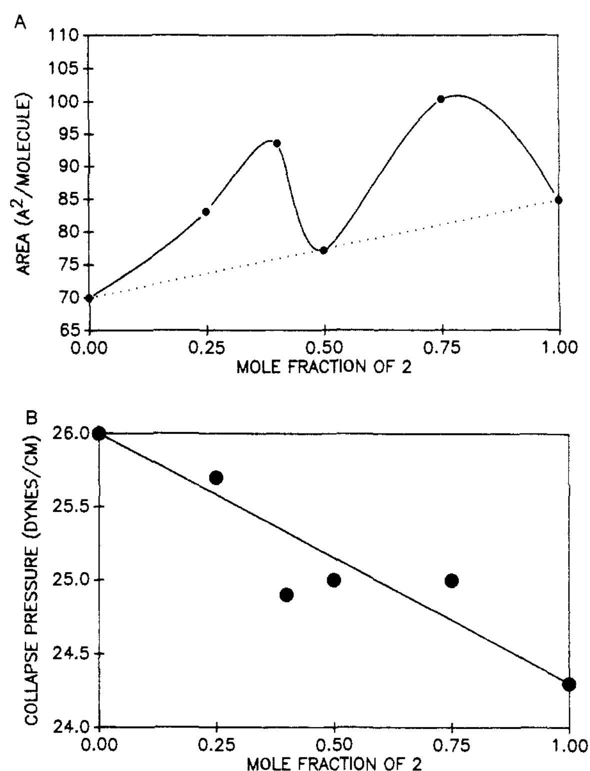

Figure 2. (A) Plot of mean area per molecule (20 dyn/cm, 25 °C) as a function of the mole fraction of **2** in mixed monolayers of **2** plus **3**. A theoretical plot expected for a completely immiscible or ideally miscible monolayer (···) is included. (B) Plot of collapse pressure as a function of lipid composition. Isotherms were recorded at 25 °C over a subphase whose pH was 8.5 (10 mM borate buffer).

results infer that the mercaptopyridine groups are distributed nearly evenly between the inner and outer monolayers.

In model studies, reaction of **1** with 1.1 equiv of 1-thio-β-D-galactopyranose (48 h, 23 °C, pH 5.5) resulted in ca. 50% substitution for 2-mercaptopyridine (analyzed by released 2-mercaptopyridine). Subsequent treatment with 5 equiv of DTT (pH 8.0) for 8 h released 100% of the bound sugar.[17] These results demonstrate that the covalent attachment of this sugar to **1** is completely reversible and occurs exclusively and quantitatively at the activated sites. The ability to bind and detach ligands or receptors from **1** should make it particularly well-suited for mechanistic studies, e.g., for use in affinity labeling experiments.

Polymerized liposomes, of the type reported herein, should prove valuable in defining the recognizability of pendant molecules at the supramolecular level and may also provide a means for modulating their bioactivity. The fact that closely related, homopolymerized liposomes of **2** have been shown to be biocompatible, as judged by their lack of thrombogenicity[18] and toxicity,[19] together with their potential for being fully biodegraded, suggests that **1** may find immediate practical use, e.g., as a novel carrier of appended drugs and antigens. Studies that are now in progress are being directed toward biomechanistic and medical applications of these and related phospholipid surfaces.

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