

Ultrathin Films as Biomimetic Membranes

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(Received November 26, 1990)

ABSTRACT: Synthetic bilayer membranes possess physicochemical characteristics that are fundamentally the same as those of biolipid bilayer membranes. Superior self-assembling properties of the component amphiphiles are advantageous for preparing other molecular membranes such as cast multilayers, surface monolayers, Langmuir-Blodgett multilayers, and planar lipid membranes (BLM). The ultrathin nature of bilayer membranes can be transplanted in other molecular systems by using cast multilayer films as molecular templates. This is achieved by incorporation of monomers and polymers in the cast film, accompanied by crosslinking and removal of the matrix multilayers.

KEY WORDS Ultrathin Film / Bilayer Membrane / Surface Monolayer / BLM / Molecular Template / Ammonium Amphiphile

1. Common Molecular Design for Different Forms of Self-Assembling Membranes

Spontaneous formation of bilayer membranes is based on superior self-assembling properties of component amphi-

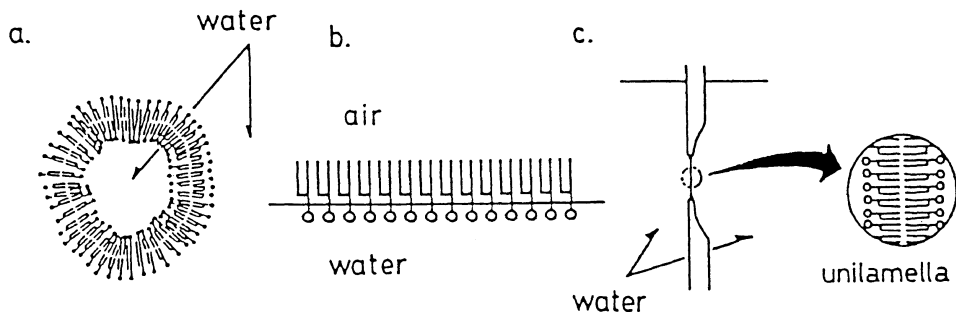


Fig. 1. Schematic illustrations of aqueous bilayer vesicle (a), surface monolayer (b), and planar bilayer membrane (c).

philes. Therefore, these amphiphiles are suitable as components of related self-assembling membranes. Fig. 1 shows schematic illustrations of aqueous bilayer vesicle, surface monomolecular film on water and planar lipid membrane. The modes of molecular organization in these systems are intrinsically identical. We describe that here are common features of molecular design by using polymerized double chain ammonium amphiphiles as typical examples.

Photopolymerization was conducted for aqueous bilayer membranes of glutamate-based double-chain ammonium amphiphiles **1**, **2** which have the ether linkage in the alkyl tail portion and the acrylate moiety attached to the ammonium head. The polymerization readily proceeded to completion (M_w ca. 10^7), in contrast to that of related bilayer **3** which lack the ether linkage. The reactivity difference was particularly large in the polymerization in the crystalline state. According to DSC and CD measurements and fluorescence spectroscopies, the bilayer polymerized in the liquid crystalline state (50 °C) showed lessened side chain alignment, whereas regular packing was maintained in the polymerization in the crystalline state (20 °C). Re-dispersion in water was not possible for the former polymer, though possible for the latter. These different properties may be attributed to the steric difference in the main chain portion which, however, could not be detected by 400 MHz $^1\text{H-NMR}$ spectroscopy.



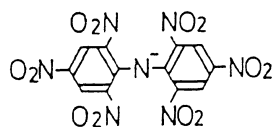
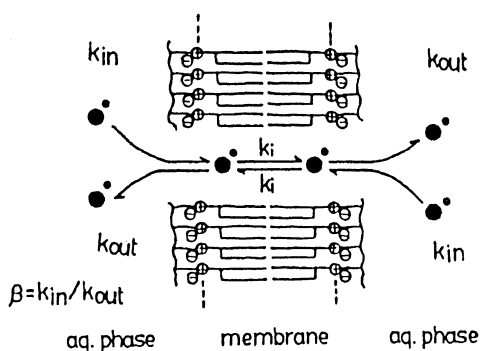
These behaviors are reflected in the formation of BLM's.² Very stable BLM's were obtainable from poly **1** (20 °C) due to facile alkyl chain packing. BLM's of poly **2** and **1** (50 °C) were fragile, probably because the alkyl chain alignment was not sufficient. The rate of ion transport was as small as those of monomer BLM's in the case of well aligned BLM of poly **1** (20 °C), but was much larger for less

ordered BLM's of poly 2 and 1 (50 °C). The formation and transport property of BLM were closely related to the extent of alkyl chain alignment.

Table 1. Stability and electrical parameters of polymerized BLM's at 25°C

amphiphile ^{a)} (polymn. temp)	life time	$C_M/\mu F \cdot cm^{-2}$	transport parameter	
			k_i/s^{-1}	$\beta/10^{-3}cm$
poly 2 (50°C)	within 1 h	0.83 ± 0.05	99 ± 11	20 ± 2
poly 1 (50°C)	within 1 h	1.0 ± 0.1	110 ± 20	18 ± 1
poly 1 (20°C)	over 1 d	1.2 ± 0.0	12 ± 2	27 ± 3

a) poly 2(50°C): \bar{M}_w , 1.6×10^7 ; \bar{M}_w/\bar{M}_n , 1.38.
 poly 1(50°C): \bar{M}_w , 1.3×10^7 ; \bar{M}_w/\bar{M}_n , 1.29.
 poly 1(20°C): \bar{M}_w , 1.5×10^7 ; \bar{M}_w/\bar{M}_n , 1.26.



dipicrylamine (DPA^-)

Fig. 2. Schematic illustration for transport of dipicrylamine anion across cationic polymerized BLM.

A related polymer amphiphile 4 was obtained by photoirradiation of an aqueous dispersion at 35 °C for 60 min. A solution of 4 in a mixture of $C_6H_6:CH_2Cl_2:EtOH$ (8:1:1) was spread on water and the surface pressure-area (π -A) isotherm was examined. A stable monolayer was formed as shown in Fig. 3. The pressure increased steeply at a molecular area of about $0.4 \text{ nm}^2 \text{ molecule}^{-1}$, which corresponded to the molecular cross section of two normal alkyl chains and indicated close packing of long hydrocarbon chains.

When direct force measurement was made,³ two parallel

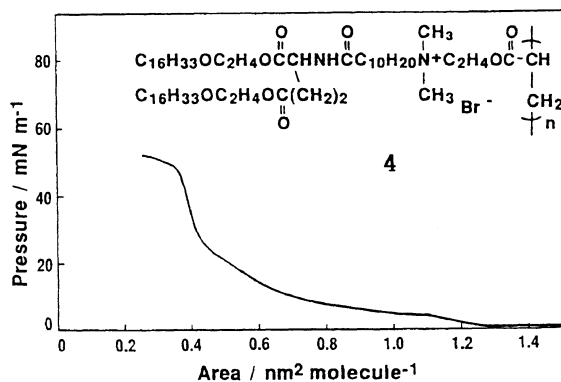


Fig. 3. The π -A isotherm of monolayer 4 on pure water at 20.0 ± 0.1 °C.

mica surfaces in pure water were repulsive in the whole separation range down to 3 nm where the surfaces jump together to contact (the largest repulsion of 3 mN m^{-1} at this separation). The repulsion was ascribed to a long-range repulsive double-layer force, since mica surfaces in pure water possess weak negative charges due to dissociation of potassium ion from the cleavage plane.

Coating of the surfaces with LB films of **4** changed the interaction drastically. Fig. 4 shows the forces between mica surfaces modified by LB deposition of **4**. Surprisingly, a measurable attraction at distances as long as 300 nm was observed in pure water. The surfaces jumped into contact from a separation of 76 nm. An attraction extending nearly to 300 nm has never been demonstrated.⁴ It is difficult to explain this (apparently hydrophobic) interaction only in terms of the conventional van der Waals interaction whose maximum measurable range is 20–30 nm. Other forces like structural (hydration) forces and cavitation need to be considered in order to understand the observed attractive interaction at both of macroscopic and molecular levels. Stable monolayers of polymerized surfactants provide an ideal system for further investigation of this interaction.

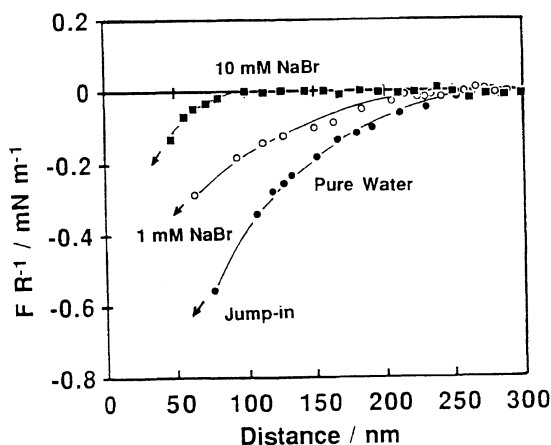


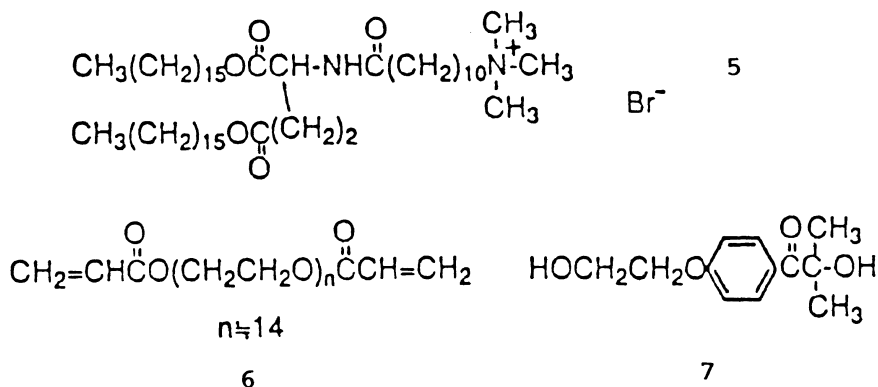
Fig. 4. Attractive forces between monolayers of **4** in water.

2. Cast Multi-bilayer Films as Molecular Template

It has been shown that self-supporting, multilayered films are obtainable by casting of aqueous synthetic bilayer dispersions on solid substrates.⁵ The highly-regular structure of these cast films is useful for producing macroscopic anisotropy of functional units. We used this structural characteristics to prepare novel two-dimensionally cross-linked polymers.⁶

Bilayer-forming ammonium amphiphile **5** was dispersed in water by sonication (15 mM), and an equimolar amount of monomer **6** and photoinitiator **7** (2 mol% of the monomer) were added. The mixture was cast on a fluorocarbon membrane filter for 48 h at 25 °C, and the resulting transparent film was subjected to polymerization by photoirradiation.

X-ray diffraction patterns of a single-component cast film of **5** and a composite cast film of the equimolar mixture indicated that highly regular structures were maintained.



In particular, the equatorial diffraction patterns in the small angle region indicated the presence of regular multilayers parallel to the film plane, as have been observed in other cast films. The reflection diffraction patterns of cast films showed the presence of a long spacing of 6.0 nm up to the 10th order (Fig. 5a). When the cast film contained an equimolar amount of the photoirradiated monomer, a long spacing of 7.4 nm was observed up to the 15th order (Fig. 5b). The intensity and sharpness of the diffraction peaks were very close between the two cast films. Therefore, the regular multibilayer structure is maintained even after polymerization.

The polymer/multibilayer composite film was subsequently immersed in methanol for 3 h in order to remove the amphiphile component. The remaining clear film was self-supporting and showed an IR spectrum that was identical with a bulk-polymerized sample of 6. Its scanning electron micrograph proved the presence of a multilayered structure with one layer thickness of 50–100 nm. These values are much larger than the thickness of the interbilayer space (ca. 2 nm) which is estimated from the X-ray diffraction data. The limited reso-

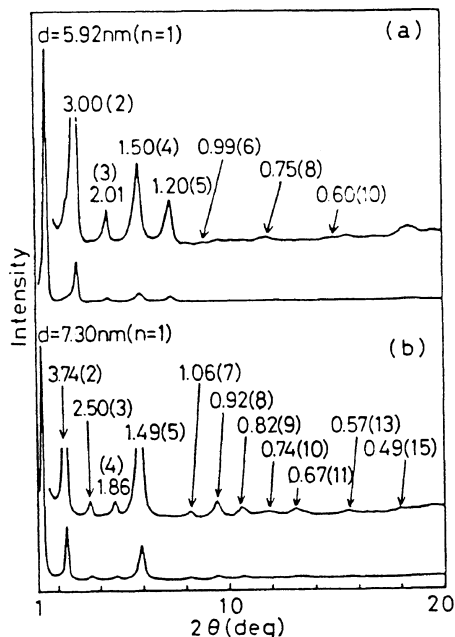


Fig. 5. X-Ray diffraction patterns obtained by the reflection method. (a) cast film of 5; (b) composite cast film (after polymerization). The observed spacings and their orders of diffraction are given.

lution of the SEM instrument made it difficult to observe the individual layer. Since the vinyl group was completely consumed in the polymerization, a two-dimensionally crosslinked polymer must have been formed. A schematic illustration of the preparative process of a laminated 2D polymer network is given in Fig. 6.

Two dimensionally cross-linked polymers have been obtained by polymerization of aqueous bilayer vesicles, polymerization of adsorbed monolayers at the air-water interface and at the oil-water interface, and polymerization of monomers intercalated into montmorillonite. In particular, two-dimensional graphite was produced from the latter system. The present approach is further applicable to cross-linking of incorporated linear polymers and immobilization of alkoxy-silanes.⁷

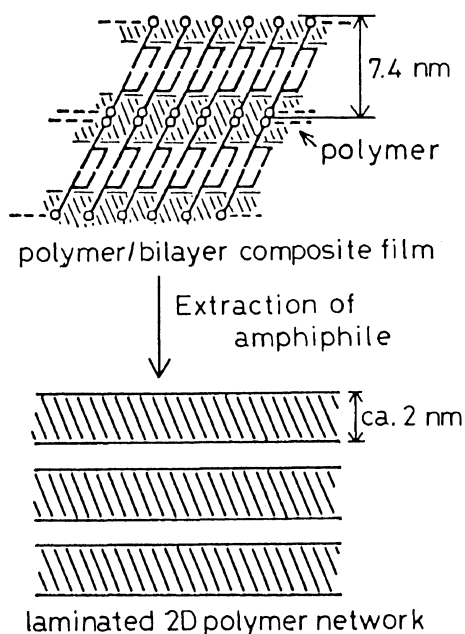


Fig. 6. Schematic illustration of preparation of a laminated 2D polymer network.

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1. S. Kato and T. Kunitake, Polymer J., in press.
 2. S. Kato and T. Kunitake, submitted to Chem. Lett.
 3. K. Kurihara, S. Kato, and T. Kunitake, Chem. Lett., **1990**, 1555.
 4. P.M. Claesson and H.K. Christenson, J. Phys. Chem., **92**, 1650 (1988).
 5. N. Nakashima, R. Ando, and T. Kunitake, Chem. Lett., **1983**, 1577.
 6. S. Asakuma and T. Kunitake, Chem. Lett., **1989**, 2059.
 7. K. Sakata and T. Kunitake, J. Chem. Soc. Chem. Commun., **1990**, 504.