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Molecular Design of Black Lipid Membranes (BLM) by Polymerized Double-Chain Ammonium Amphiphiles[1)]

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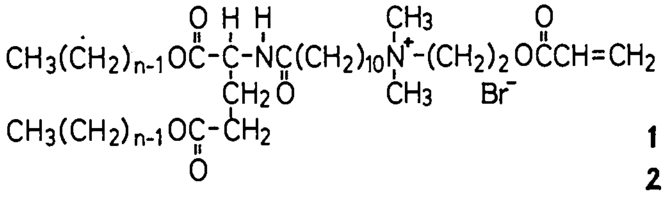
Planar bilayer membranes were obtained from polymerized double-chain ammonium amphiphiles. Their properties were closely related to those of the corresponding aqueous bilayers, and a life time longer than one day was observed for a well-organized polymer sample.

Black lipid membranes (BLM) possess single-walled, planar bilayer structures. They are commonly prepared by painting a small hole in a plate with membrane components dissolved in hydrocarbons.[2)] Although BLM's have attracted much interest due to their macroscopically bilayer nature, their limited stabilities make it difficult to adopt conventional membrane techniques.

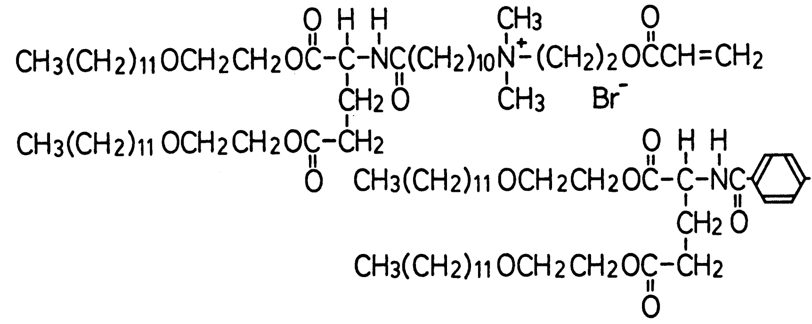
The stability may be improved by using polymeric components. Benz et al.[3),4)] and Rolandi et al.[5)] polymerized BLM's by photoirradiation, and observed improved stabilities and changes in membrane characteristics. Improvement of BLM stability has been realized also by anchoring of modified polysaccharides on a BLM of glyceryl monooleate,[6)] and by ion complexation of cationic BLM's with anionic polymers.[7)] In spite of these efforts, systematic design of stable BLM's has not been accomplished, due to the fact that BLM formation has been an art rather than science. We therefore decided to look for the structure-stability relationship of BLM's. The present communication describes that the stability of polymerized BLM's is related to their formation of aqueous bilayer membranes. This relation provides a basis for rational molecular design of BLM's.



$$\mathrm{CH_3(CH_2)_{n-1}O\overset{O}{\overset{\|}{C}}-\underset{CH_2}{\overset{H}{C}}-\overset{H}{N}\underset{O}{\underset{\|}{C}}(CH_2)_{10}\overset{CH_3}{\underset{CH_3}{N^+}}-(CH_2)_2O\overset{O}{\overset{\|}{C}}CH=CH_2\quad Br^-}$$
$$\mathrm{CH_3(CH_2)_{n-1}O\underset{O}{\underset{\|}{C}}-CH_2}$$

1 $n = 12$ $2C_{12}$-L-Glu$C_{11}N^{+}$Ac

2 14 $2C_{14}$-L-Glu$C_{11}N^{+}$Ac



3 $2(C_{12}OC_2)$-L-$GluC_{11}N^+Ac$

4 $2(C_{12}OC_2)$-L-$GluPhC_6N^+Ac$

Polymerization of ammonium amphiphiles **1**—**4** was conducted by photoirradiation (high-pressure Hg lamp) of their aqueous bilayer dispersions.[8,9] The polymerizable acryl unit is introduced at their head group portions, since polymerization of alkyl tails interferes with formation of BLM's.[3] The amphiphiles have been shown to give stable bilayer membranes with well aligned alkyl chains, when polymerized under appropriate conditions.[8] In particular, the ether linkage can effectively insulate the steric restriction of the polymer chain and the regular alignment of alkyl side chains.[9]

BLM's were prepared by brushing a hole (diameter 0.85 mm) in a Teflon plate that was immersed in 1 M aqueous KCl. The amphiphiles were dissolved in mixtures of decane and $CHCl_3$ (4 : 1 by volume for unpolymerized amphiphiles and 1 : 1 for polymerized amphiphiles). The electrical properties of BLM's were measured as described previously with a newly developed system that is essentially identical to the previous instrument[7]: see Fig. 1.

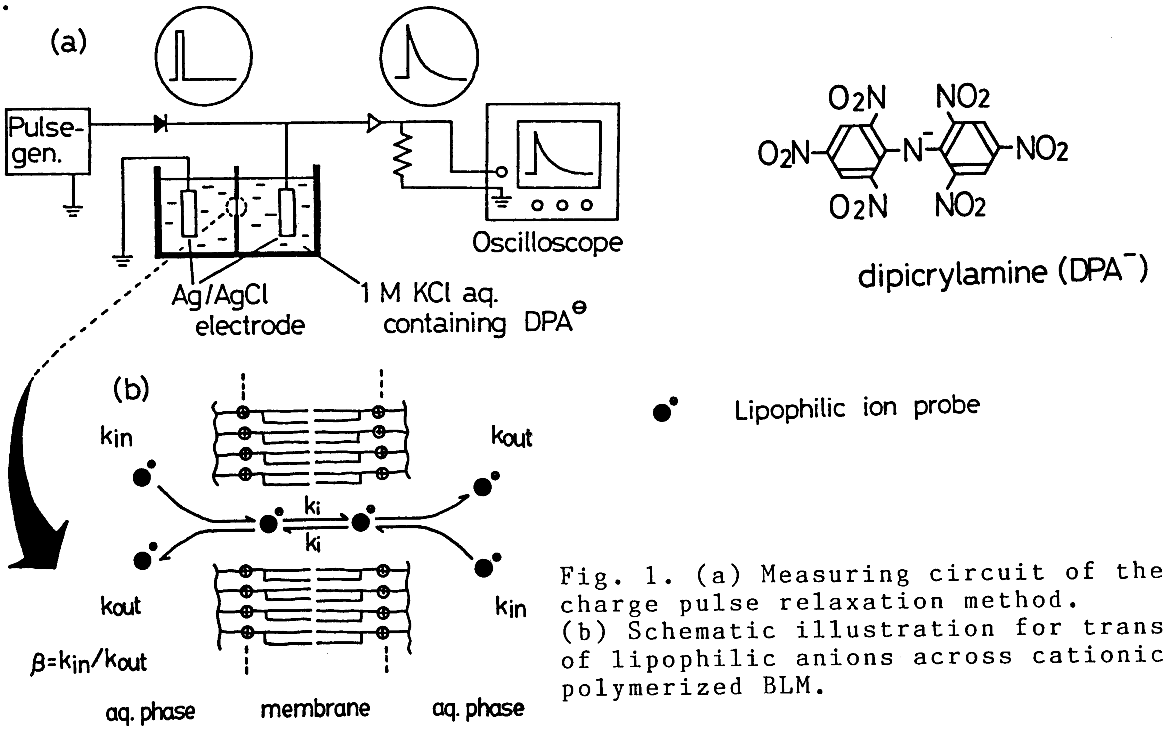


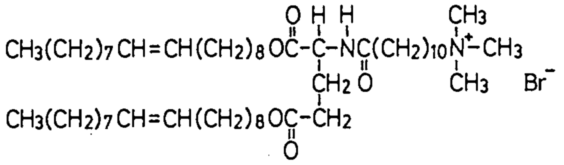
Fig. 1. (a) Measuring circuit of the charge pulse relaxation method.
(b) Schematic illustration for transport of lipophilic anions across cationic polymerized BLM.

Table 1 summarizes formation and transport characteristics of BLM's of unpolymerized amphiphiles. The BLM formation as inferred from optical

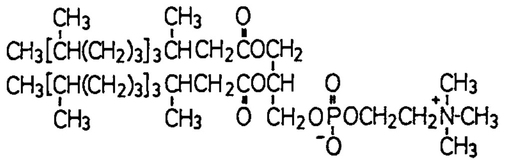
Table 1. Formation, stability and electrical parameters of unpolymerized BLM's at 25 °C

Amphiphile	Formation[a)]	Life time	$C_M/\mu F \cdot cm^{-2}$	Transport parameter	
				k_i/s^{-1}	$\beta/10^{-3}cm$
1	15-30 min	over 1 d	1.2 ±0.1	8.3± 0.8	23 ±3
2	15-30 min	over 1 d	1.1 ±0.0	6.2± 0.3	18 ±2
3	3-5 min	5-10 h	0.92±0.03	16 ± 1	40 ±7
4	3-5 min	5-10 h	1.2 ±0.0	10 ± 1	27 ±2
5	less than 30 s	1 h	0.54±0.05	66 ± 8	16 ±2
5[7)]	—	3 h	0.52±0.02	90 ±15	12 ±2
DPh-PC[7)]	—	—	0.37±0.02	670 ±20	3.0±0.5

a) Time required between appearance of interference colors and BLM formation.



5 $2C_{18}^{\Delta 9}$-L-GluC$_{11}$N$^+$



diphytanoyl-lecithin (DPh-PC)

interference colors and capacitance measurement was faster for amphiphiles **3** and **4** than for **1** and **2**. The ether linkage in the alkyl tail must be responsible for this difference. It is important that the effect of the ether linkage observed in the aqueous bilayer is also found in the BLM formation. These BLM's are stable compared with those reported by other workers.[3,4)] The stabilities of **1** and **2** are particularly noteworthy. The conventional BLM's are short-lived.[2)] Reinforcement by polymers prolongs the life time up to 6 h.[7)] A related amphiphile **5** did not give stable BLM's in our previous attempts.[7)]

The membrane capacitance, C_M, and the transport property of dipicrylamine anion (DPA$^-$) were subsequently measured. All the C_M values of those polymerizable amphiphiles were larger than those of diphytanoyl phosphatidylcholine and dioleyl ammonium amphiphiles.[7)] These difference (2-3 fold) may be related to better alignment of the alkyl chain in the present system. In the corresponding transport parameters, k_i values of amphiphiles **1**—**4** were much smaller than those obtained in the previous study.[7)] We believe that better chain alignment suppressed transport of DPA$^-$ in the BLM. The β values are mainly influenced by the surface charge, and the ammonium BLM's give much larger β values relative to the phosphatidylcholine BLM.

Subsequently, BLM's of polymerized amphiphiles **3** and **4** were examined, as summarized in Table 2. Since polymerization of amphiphiles with saturated aliphatic tails (**1** and **2**) did not proceed smoothly,[9)] they were not used for this BLM study. On the other hand, the introduction of the ether linkage resulted in smooth, quantitative polymerization.[9)] Interestingly, the nature of the resulting polymeric bilayers were strongly influenced by

Table 2. 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 **3** (50 °C)	within 1 h	0.83±0.05	99±11	20±2
poly **4** (50 °C)	within 1 h	1.0 ±0.1	110±20	18±1
poly **4** (20 °C)	over 1 d	1.2 ±0.0	12± 2	27±3

a) poly **3**(50 °C): $\bar{M}_w$, 1.6×10^7; $\bar{M}_w/\bar{M}_n$, 1.38.
poly **4**(50 °C): $\bar{M}_w$, 1.3×10^7; $\bar{M}_w/\bar{M}_n$, 1.29.
poly **4**(20 °C): $\bar{M}_w$, 1.5×10^7; $\bar{M}_w/\bar{M}_n$, 1.26.

temperature of polymerization. When polymerized in the liquid crystalline state (at 50°C), aqueous bilayer **4** showed lessened side chain alignment, whereas regular side chain packing was maintained in the polymerization in the crystalline state (at 20°C).$^{9)}$ These polymers were equally soluble in $CHCl_3$. However, when $CHCl_3$ was removed, redispersion in water was not possible for the former polymer, though possible for the latter due to facile realignment of the alkyl tails.

These contrasting behavior of the aqueous bilayers was reflected in the observed BLM behavior. Very stable BLM's were obtainable from poly **4**(20°C) due to facile alkyl chain packing. BLM's of poly **3** and **4** (50°C) were fragile, probably because the alkyl chain alignment was not sufficient. The k_i values were consistent with this supposition. It was as small as those of monomer BLM's in the case of well aligned BLM of poly **4**(20°C), but was much larger for less ordered BLM's of poly **3** and **4** (50°C).

In conclusion, we demonstrated in this study that formation and transport property of BLM were closely related to the extent of alkyl chain alignment. There exist common structural features between the aggregation behavior of BLM and aqueous bilayer. Ammonium BLM's can be very stable, if component molecules are appropriately designed. Improved stability would broaden the range of BLM studies.

References

1) Contribution No. 932 from Department of Organic Synthesis.
2) H. Ti Tien, "Bilayer Lipid Membranes (BLM). Theory and Practice," Marcel Dekker Inc., New York (1974).
3) R. Benz, W. Prass, and H. Ringsdorf, Angew. Chem. Suppl., **94**, 869 (1982).
4) R. Benz, R. Elbert, W. Prass, and H. Ringsdorf, Eur. Biophys. J., **14**, 83 (1986).
5) R. Rolandi, S. R. Flom, I. Dillon, and J. H. Fendler, Prog. Colloid Polym. Sci., **73**, 134 (1987).
6) J. Moellerfeld, W. Prass, H. Ringsdorf, H. Hamazaki, and J. Sunamoto, Biochim. Biophys. Acta, **857**, 265 (1986).
7) N. Higashi, T. Kunitake, J. Moellerfeld, and H. Ringsdorf, Chem. Lett., **1988**, 13.
8) T. Kunitake, N. Nakashima, and M. Kunitake, Macromolecules, **22**, 3544 (1989).
9) S. Kato and T. Kunitake, Polym. J., submitted.

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