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Polyion Complexation of Black Lipid Membranes (BLM) and Transport of a Hydrophobic Ion 1)

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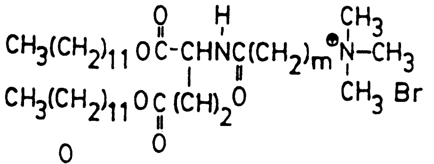
Black lipid membranes were obtained from synthetic ammonium amphiphiles and their stabilities were enhanced by complexation with an anionic polymer. The permeation behavior of dipicrylamine anion was examined by the charge-pulse relaxation technique.

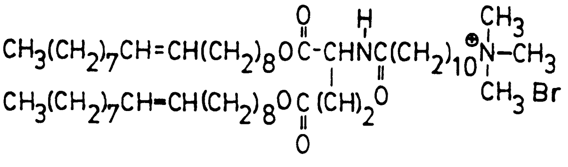
Aqueous dispersions of charged bilayer membranes can be immobilized without losing the bilayer characteristics by polyion-complex formation with oppositely-charged linear polymers.3,4) Cast films of these polyion complexes have been used for dye binding3) and for controlling ion permeation.5,6) Self-oscillation of electric potential was observed across a porous membrane impregnated with polyion-complexed bilayers.7) The complexation approach was later applied to monolayers at the air-water interface, and polyion-complexed monolayers were found to be readily deposited onto solid substrates.8,9) In this paper, we report application of the polyion complex concept to the black lipid membrane(BLM).10)

The electrochemical property of BLM of biolipids has been investigated extensively since the inception of BLM.11) Among others, Benz et al. devised an improved version of the charge-pulse relaxation technique and conducted a detailed examination of the permeation mechanism of hydrophobic organic ions (tetraphenylborate, dipicrylamine, etc.).12) Möllerfeld et al. recently used this technique for permeation experiments with BLM's coated with polysaccharide derivatives and found significant stabilization of BLM due to anchoring of the hydrophobic side chains of the polysaccharide.13)



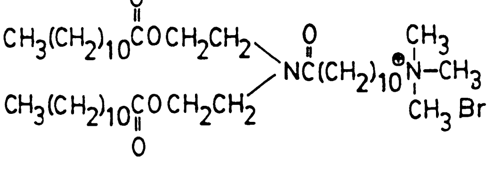
$$\begin{array}{l} CH_3(CH_2)_{11}O\overset{\overset{O}{\|}}{C}\text{-}\underset{|}{C}H\overset{H}{N}\underset{\|}{C}(CH_2)_m\overset{\oplus}{N}(CH_3)_2\text{-}CH_3\ \ Br \\ CH_3(CH_2)_{11}O\underset{\underset{O}{\|}}{C}(CH)_2O \end{array}$$

1



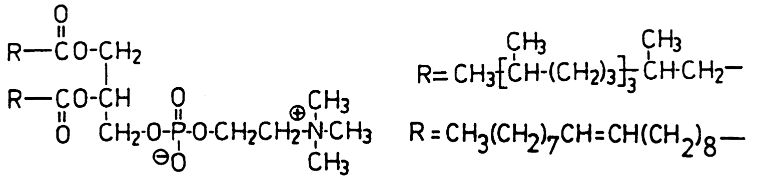
$$\begin{array}{l} CH_3(CH_2)_7CH{=}CH(CH_2)_8O\overset{\overset{O}{\|}}{C}\text{-}\underset{|}{C}H\overset{H}{N}\underset{\|}{C}(CH_2)_{10}\overset{\oplus}{N}(CH_3)_2\text{-}CH_3\ \ Br \\ CH_3(CH_2)_7CH{=}CH(CH_2)_8O\underset{\underset{O}{\|}}{C}(CH)_2O \end{array}$$

3



$$\begin{array}{l} CH_3(CH_2)_{10}\overset{\overset{O}{\|}}{C}OCH_2CH_2\diagdown \\ \qquad\qquad\qquad\qquad\qquad N\overset{\overset{O}{\|}}{C}(CH_2)_{10}\overset{\oplus}{N}(CH_3)_2\text{-}CH_3\ \ Br \\ CH_3(CH_2)_{10}\underset{\underset{O}{\|}}{C}OCH_2CH_2\diagup \end{array}$$

2



$R = CH_3[CH(CH_3)(CH_2)_3]_3CH(CH_3)CH_2-$ 4

$R = CH_3(CH_2)_7CH{=}CH(CH_2)_8-$ 5

A large variety of synthetic bilayer membranes that became available recently are good candidates for new BLM's, and we made a brief survey in the case of double-chain ammonium amphiphiles. A series of bilayer-forming compounds 1 did not give stable BLM's. This situation did not change when the glutamate unit was replaced with the diethanolamine unit as in 2. In contrast, replacement of the dodecyl group in 1 with the oleyl group as in 3 led to very stable BLM's. Therefore, the subsequent experiments were conducted with this compound,[14] and permeation of dipicrylamine anion(DPA$^-$) was examined by the charge-pulse relaxation technique. Diphytanoylphosphatidylcholine 4 was used for comparison. The experimental procedures are the same as those described by Möllerfeld et al.[13] BLM's obtained from 3 in a mixed solvent of n-decane/$CHCl_3$/ethanol (18:1:1 in vol) were stable for 3 h at 25 °C. When potassium poly(styrene-p-sulfonate) (PSS^-K^+, DP=12600, 10 mg/l) was dissolved in the aqueous phases, the BLM became stable for more than 6 h.

The membrane capacitance (C_M) is given by[13,15]

$$C_M = \frac{I_0 \cdot T}{V_0 \cdot A} \tag{1}$$

where I_0 is the current extrapolated to zero time, T is the exponential decay time, V_0 is the applied voltage, and A is the effective membrane area. The results are summarized in Table 1. The C_M value for 3 decreased by addition of PSS^-K^+, whereas that for 4 remained unchanged. This indicates that there exists strong coulombic interaction (polyion complexation) between BLM of the double-chain ammonium amphiphile and added polyion. The zwitterionic BLM of 4 does not show detectable interaction with PSS^-K^+. The calculated membrane thickness increased as a result of polyion complexation.

Table 1. Electrical Property of BLM with and without PSS^-K^+ (1 M KCL, 25 °C)

Membrane	PSS^-K^+	C_M	Membrane thickness,d[a]
		μFcm^{-2}	nm
3	none	0.52	4.0
	10 mg/l	0.31	5.8
4	none	0.37	4.8
	10 mg/l	0.38	4.9

a) $d = \varepsilon_0 \cdot \varepsilon_M / C_M$.

ε_0 : permittivity of the vacuum ($=8.854 \times 10^{-12} F\ m^{-1}$).

ε_M : dielectric constant of the membrane (=2.0).

Table 2. Permeation characteristics of dipicrylamine

Membrane	PSS^-K^+	Temp	k_i	β
		°C	s^{-1}	$x10^{-3}cm$
3	none	25	90	12
	none	35	360	11
	10 mg/l	25	900	25
4	none	25	670	3.0
	10 mg/l	25	690	2.5
5	none	25	431[a]	——

a) from Ref. 12.

Subsequently, the permeation behavior of dipicrylamine was examined. As shown in Fig. 1, permeation of hydrophobic ions proceeds via the following three steps[16)] : (1) adsorption of the ion from the aqueous phase to the membrane-solution interface; (2) translocation to the opposite interface ; and (3) desorption into the aqueous solution. The rate constant of translocation, k_i, and the relative rate constant of adsorption and desorption, $\beta = k_{AM}/k_{MA}$, can be evaluated directly by the charge-pulse technique.

Figure 2 displays the experimental set-up and the equivalent circuit diagram of this technique. An example of a charge pulse and the subsequent decay of membrane voltage is shown in Fig. 3. The decay curves were analyzed according to the procedure of Benz et al. to obtain k_i and β. The results are given in Table 2. The data for dioleoylphosphatidylcholine 5 is included for comparison.

In the absence of the polymer anion, k_i for 3 is enhanced four times with increasing temperature from 25 to 35 °C. The difference can be attributed to the fluidity change of the BLM, as a very broad peak (20-40 °C, peak top 32 °C) was observed in DSC and a gradual depolarization was noted at 10 to 50 °C in fluorescence of 1,6-diphenylhexatriene in the case of the aqueous bilayer.[14)] The k_i values for 4 and 5 (at 25 °C) are in the same range as that for 3 at 35 °C. In

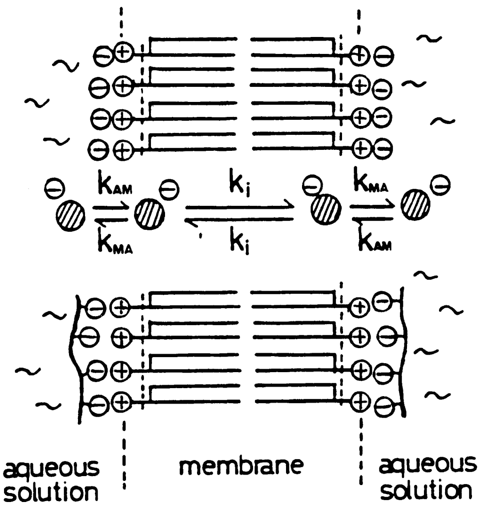


Fig. 1. Transport scheme of hydrophobic ions(▨$^{\ominus}$) through BLM. Upper half, uncomplexed BLM: lower half, polyion-complexed BLM.

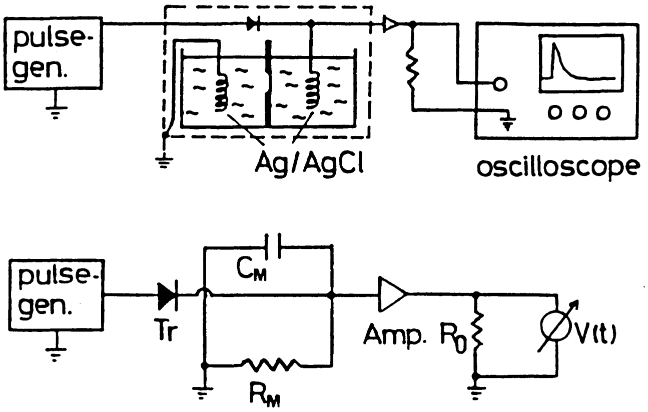


Fig. 2. Measuring circuit for the charge-pulse relaxation method. Upper part: experimental setup; lower part: equivalent circuit.

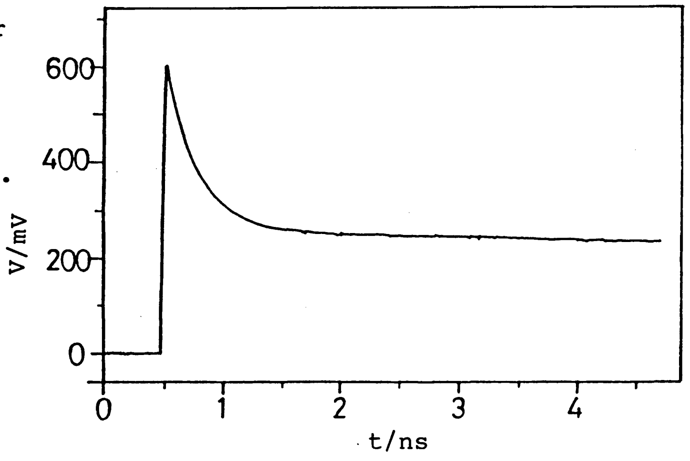


Fig. 3. Decay of the membrane voltage(V_m) after a charge pulse. Membrane, 4; 1 M KCl; DPA$^-$, 1 x 10^{-7}M; 25 °C.

fact, **4** and **5** are in the liquid crystalline state at 25 °C, and the translocation rate is enhanced with increasing membrane fluidity. The β value is much larger for **3** than for **4**. This is easily understandable since anionic dipicrylamine would be incorporated more readily to the positively-charged BLM of **3** than to the zwitterionic BLM of **4**. Addition of PSS^-K^+ does not alter the permeation characteristics of **4**, as also the case with C_M. Polyion-complex formation in the case of **3** raises k_i and β values simultaneously.

The phase transition of the bilayer of **3** is not much affected by polyion complex formation. Therefore, the 10-times enhancement of k_i upon polyion complexation suggests that the translocation rate become larger due to changes in molecular orientation and/or formation of defects rather than due to increased fluidity. The enhanced β value upon complexation is also not readily explicable if the coulombic interaction is most important. The hydrophobic interaction between BLM and dipicrylamine may play a greater role in this case.

In conclusion, we have shown that some of the bilayer-forming amphiphiles are useful for BLM formation. The amphiphiles can be used by themselves or as polyion complexes (Fig. 1). Since they have been obtained in a great variety, tailor-made BLM's will become available. Stabilization in the form of polyion complexes should also facilitate their uses as BLM's.

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