
**Journal of Macromolecular Science, Part A**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

# Chemistry of Synthetic Bilayer Membranes

Toyoki Kunitake[a]
[a] Department of Organic Synthesis Faculty of Engineering, Kyushu University, Fukuoka, Japan

**To cite this Article** Kunitake, Toyoki(1979) 'Chemistry of Synthetic Bilayer Membranes', Journal of Macromolecular Science, Part A, 13: 5, 587 — 602
**To link to this Article: DOI:** 10.1080/00222337908056676
**URL:** http://dx.doi.org/10.1080/00222337908056676

J. MACROMOL. SCI.–CHEM., A13(5), pp. 587-602 (1979)

# Chemistry of Synthetic Bilayer Membranes

TOYOKI KUNITAKE

Department of Organic Synthesis
Faculty of Engineering
Kyushu University
Fukuoka 812 Japan



ABSTRACT

The preparation of bilayer membranes from synthetic dialkyl amphiphiles is described. According to the electron microscopic observation, the bilayer structure similar to that of biomembranes is formed spontaneously when dialkyldimethylammonium bromides with $C_{10}$-$C_{20}$ alkyl groups are dispersed in water by sonication. The line broadening in the NMR spectrum strongly suggests that these synthetic bilayers constitute liquid crystalline or solid phases. The hydrophilic head group may be sulfonium and modified ammonium moieties. The bilayer assembly is also formed from dialkyl amphiphiles with anionic head groups: (phosphate, sulfonate, and carboxylate), from nonionic dialkyl amphiphiles (oligomeric ethylene oxide) moiety) and from zwitterionic dialkyl amphiphiles. These bilayers incorporate equimolar cholesterol fairly readily. The redistribution of catalyst (a cholesterol derivative) and substrate (p-nitrophenyl palmitate) molecules is very slow when they are tightly bound to the aggregate. The intravesicle reaction is much faster than the intervesicle counterpart in the case of the didodecyldimethylammonium bromide vesicle.

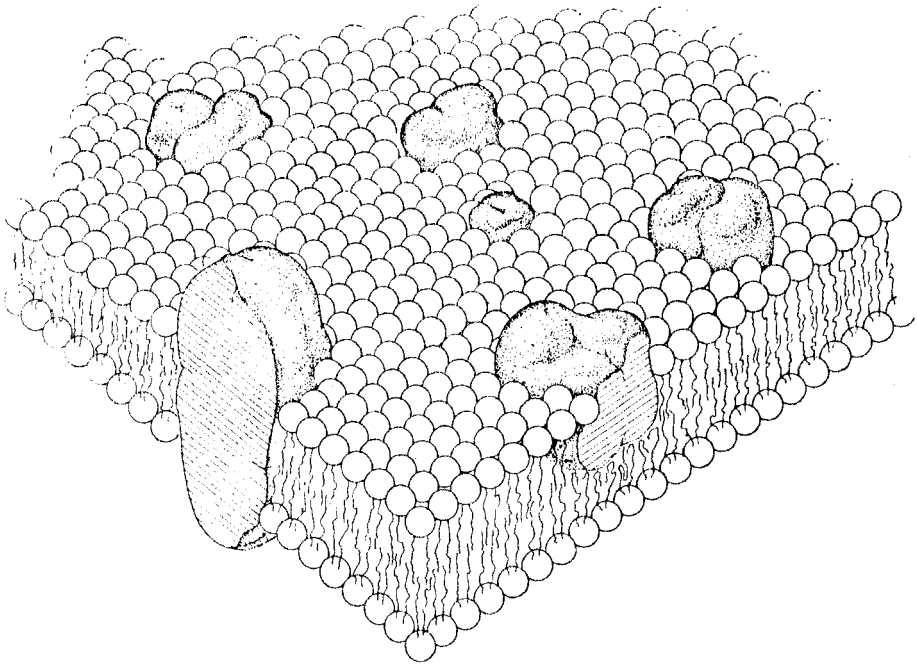


FIG. 1. Fluid mosaic model of biomembranes [1].



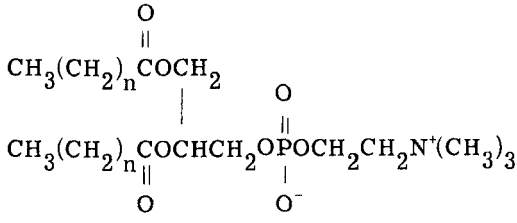
## INTRODUCTION

Biomembranes are directly involved in most of the fundamental biological function of cells such as compartmentalization, energy transduction and information transfer. The major structural components of the biomembrane are lipid bilayers and proteins. Although there have been proposed several models for the structure of biomembranes, the fluid mosaic model of Singer and Nicolson [1] is most widely accepted at the present time (see Fig. 1). In this model, protein molecules are wholly or partially immersed in the hydrophobic core of the lipid bilayer. Some of the proteins (peripheral proteins) are placed at the hydrophilic membrane surface. The bilayer structure similar to that of biomembranes can be reconstructed from biolipid molecules and related compounds (lecithins and modified lecithins) [2]. In a few cases, molecular bilayers are prepared from other, simpler compounds, but they are usually unstable and/or formed under rather exotic conditions [3, 4].

We found recently that the stable bilayer assembly is formed spontaneously from simple dialkyl amphiphiles. The structural characteristics of these bilayers are intrinsically the same as those of the biolipid bilayer. This article is a progress report of our recent research efforts on the chemistry of synthetic bilayer membranes.

## FORMATION OF THE MOLECULAR BILAYER FROM POSITIVELY CHARGED AMPHIPHILES

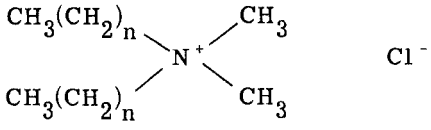
The lecithin (phospholipid) molecule (I) possesses two long acyl chains as the hydrophobic group and the zwitterionic hydrophilic group.



Lecithin

I

It is conceived that the bilayer assembly may be formed from amphiphiles with two long alkyl groups. The amphiphilic behavior of quaternary ammonium chlorides containing two long-chain alkyl groups has been studied by Ralston et al. [5]. They found that the conductivity behavior of these dialkylammonium salts were quite different from those of alkyltrimethylammonium surfactants. More recently, Kunieda studied the dissolution mechanism of dialkylammonium chlorides (II) and discussed the results in terms of the hydrophilic-lipophilic balance [6].



II

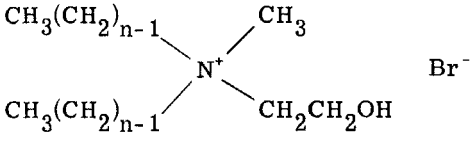
Didodecyldimethylammonium bromide can give a clear aqueous

solution when dispersed in water by sonication [7]. A few drops of this solution was applied to a 150-mesh copper grid coated with a carbon film, which was then dried in a dessicator. A 2% aqueous solution of uranyl acetate was similarly applied. Spherical objects with diameters of 300-500 Å were clearly seen in an electron micrograph of this sample. This picture was indistinguishable from that of dipalmitoyllecithin vesicles reported by Sheetz and Chan [8]. When the ammonium solution was sonicated in the presence of uranyl acetate, multiwalled vesicles and lamellae were observed. The layer thickness was 30-40 Å. Therefore, it is concluded that didodecyldimethylammonium bromide aggregates extensively in aqueous solutions into stable bilayers which further form vesicles and lamellae.

Subsequently, the investigation was extended systematically to other dialkylammonium salts [9]. Table 1 summarizes the electron microscopic observation. Didococyldimethylammonium bromide ($2C_{22}N^{+}2C_1$) is not soluble in water. Other ammonium salts produce clear or slightly turbid solutions which contain well-defined molecular aggregates. The dioctadecyl compound ($2C_{18}N^{+}2C_1$) yields lamellar structures, and the aggregate structure tends to change from lamellae to vesicles as the alkyl chain length is lessened. When the chain length of one of the alkyl group is varied from $C_{18}$ to $C_{10}$ (the other chain fixed at $C_{18}$), a similar trend is observed. Aqueous solutions of octadecyloctyldimethylammonium bromide ($C_{18}C_8N^{+}2C_1$) and hexadecyltrimethylammonium bromide (CTAB) did not provide any indication of the structure formation. Thus, the alkyl chain length of $C_{10}$ to $C_{20}$ is most suitable for the bilayer formation as in the case of the lipid bilayer.

The aggregate weight of these assemblies is larger than one million daltons. The extent of aggregation increases with increasing alkyl chain lengths, the largest aggregation weights being 10-20 million daltons.

The positively-charged hydrophilic moiety need not be limited to the simple ammonium group. Most of amphiphiles such as III-VII form lamellar or vesicle structures [10].

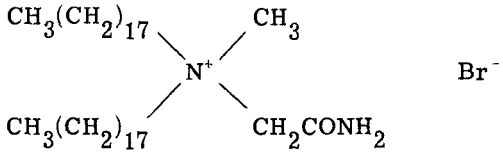



n = 12, and 18

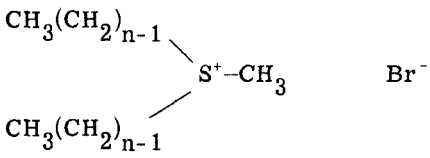
III

TABLE 1. Electron Microscopy of Ammonium Salts

| Ammonium salts | Appearance of stock solution | Electron micrograph | Aggregate weight $\times 10^{-4}$ |
|---|---|---|---|
| $2C_{22}N^{+}2C_1$ | Insoluble | - | - |
| $2C_{18}N^{+}2C_1$ | Slightly turbid | Lamella | 1000 |
| $2C_{14}N^{+}2C_1$ | Slightly turbid | Vesicle, lamella | 500 |
| $2C_{12}N^{+}2C_1$ | Clear | Vesicle | 100 |
| $C_{18}C_{16}N^{+}2C_1$ | Slightly turbid | Lamella | 2000 |
| $C_{18}C_{14}N^{+}2C_1$ | Slightly turbid | Vesicle, lamella | 2000 |
| $C_{18}C_{12}N^{+}2C_1$ | Clear | Vesicle | 800 |
| $C_{18}C_{10}N^{+}2C_1$ | Clear | Vesicle | 300 |
| $C_{18}C_8N^{+}2C_1$ | Clear | No structure | 30 |
| $C_{16}N^{+}3C_1$(CTAB) | Clear | No structure | 4 |
| $2C_{12}N^{+}CC_2OH$ | Slightly turbid | Vesicle | 400 |

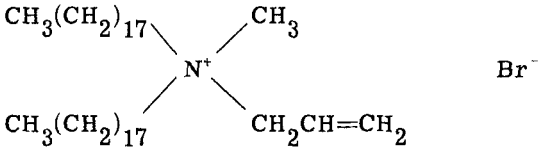



IV

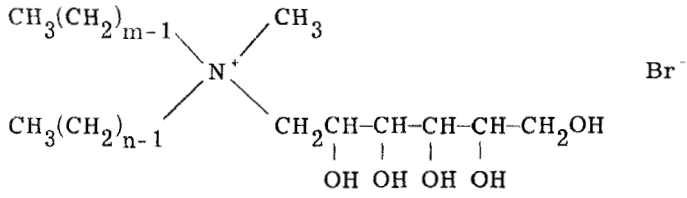



V

n = 12, 14, 18




VI



VII

m = n = 18, m = 18 and n = 12

An electron micrograph of a clear solution of $2C_{12}N^{+}C_{1}\cdot C_{2}OH$ is shown as an example in Fig. 2. Multi-walled vesicles of various sizes are observed very clearly. Some of the vesicles appear completely filled with lamellae of ca. 50 Å layer width.

The animal plasma membrane usually contains large amounts of



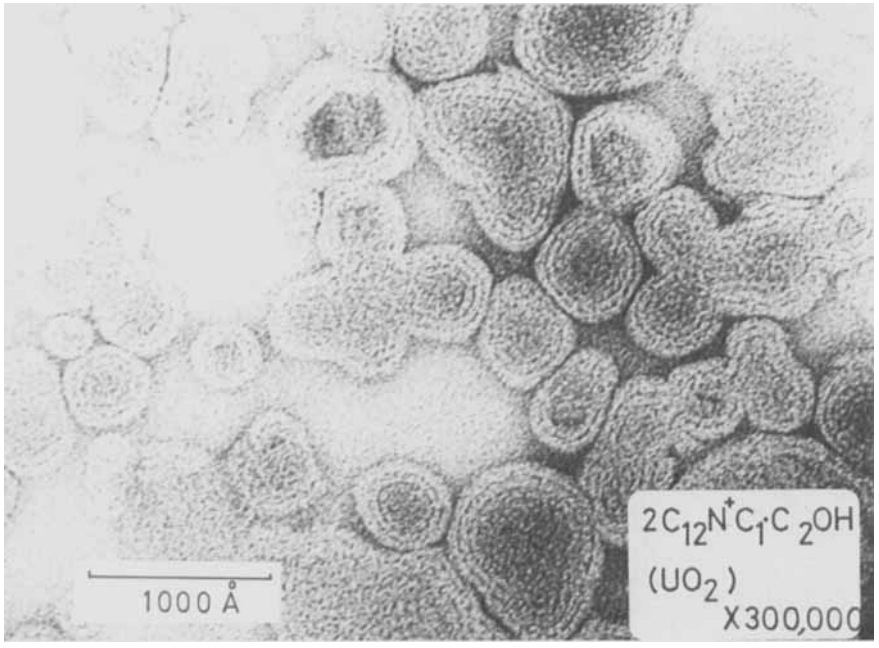
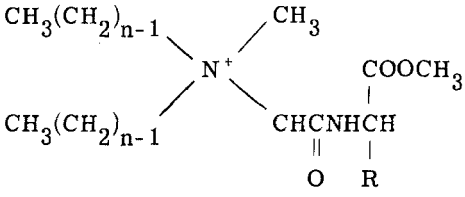


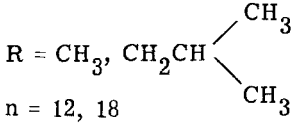

FIG. 2. Electron micrograph; sample: $2C_{12}N^{+}C_{1}\cdot C_{2}OH$, 10 mM. Negatively stained by uranyl acetate.

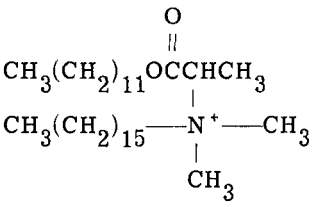
cholesterol, and liposomes of the biolipid can take up cholesterol well [2]. The synthetic bilayer can also incorporate cholesterol. For instance, when equimolar amounts of cholesterol were added to 10 mM aqueous solutions of $2C_{12}N^{+}2C_1$ or $2C_{18}N^{+}2C_1$ and sonicated, slightly turbid solutions were obtained. Electron micrographs of these solutions show the formation of lamellae and vesicles.

Chiral bilayers may be formed by using amino acids in the hydrophilic head group. The structures VIII-X represent the chiral dialkyl amphiphiles [11].



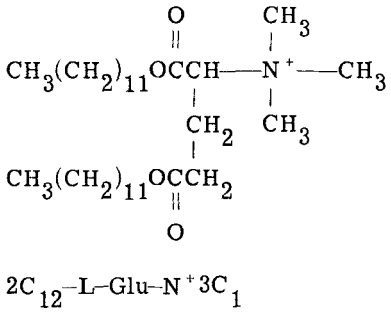
VIII





$C_{16}C_{12}$–L–Ala–$N^{+}2C_1$

IX



$2C_{12}$–L–Glu–$N^{+}3C_1$

X

The bilayer structure was observed very clearly except for the first group of the chiral amphiphiles in which R was isobutyl. When the leucine moiety is used, the head group becomes less hydrophilic and the bilayer structure would be destabilized. Figures 3 and 4 show two examples of the formation of well-developed bilayers from the dialkyl amino acid derivative.

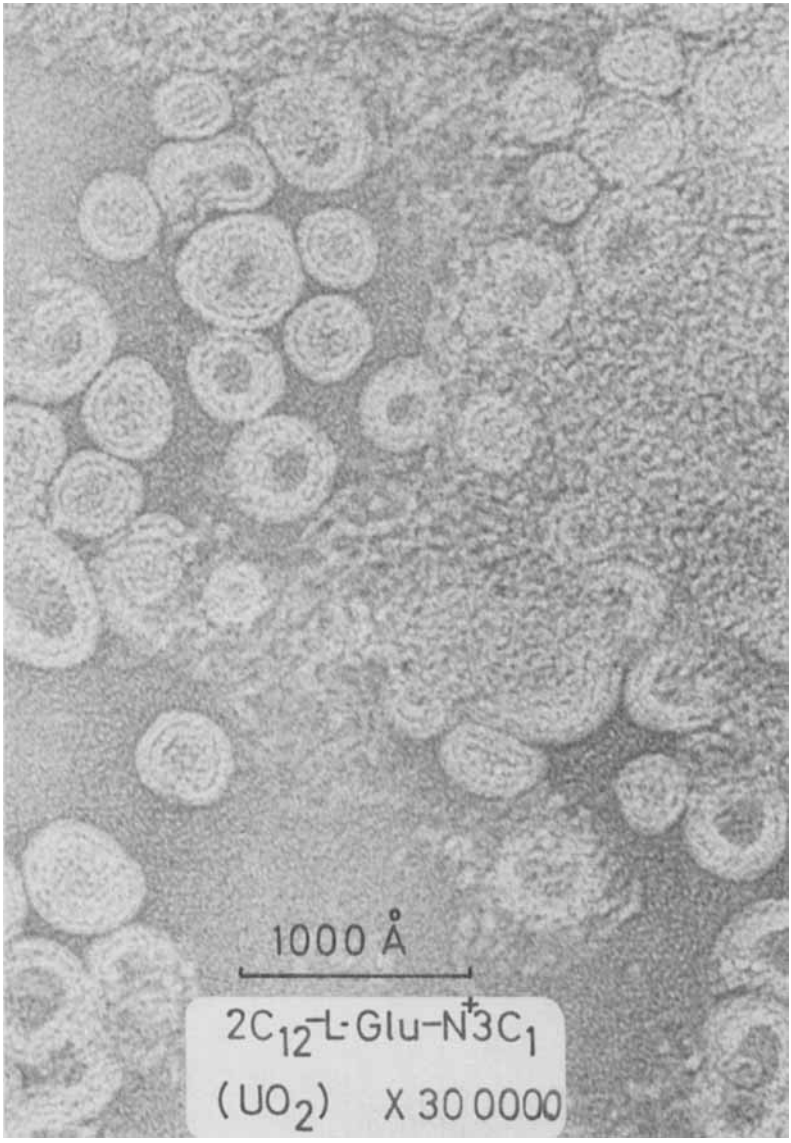



FIG. 3. Electron micrograph; sample: $2C_{12}$–L–Glu–$N^{+}3C_1$. Negatively stained by uranyl acetate.

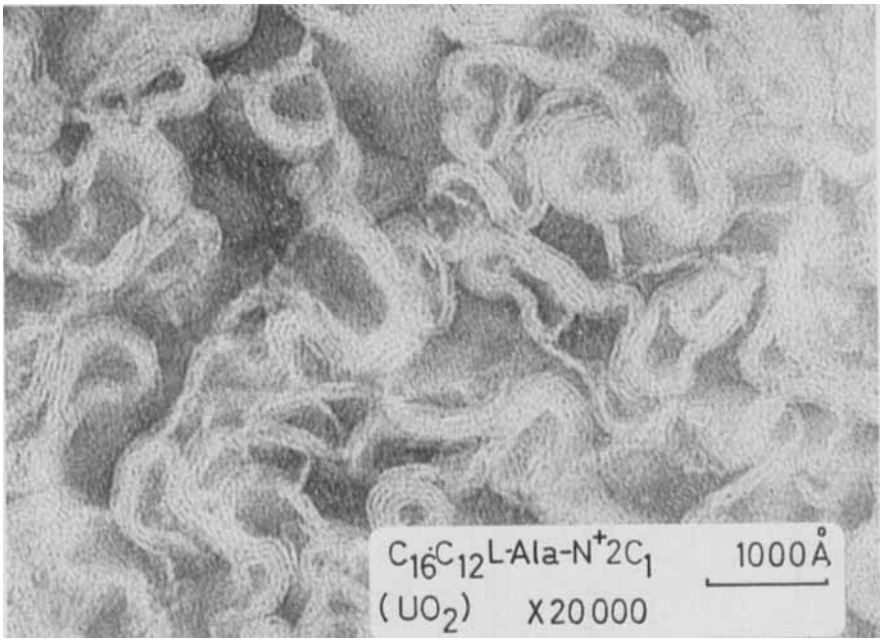
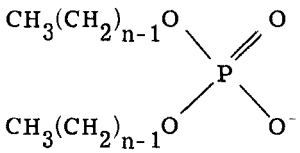



FIG. 4. Electron micrograph; sample: $C_{12}C_{12}$–L–Ala–$N^{+}2C_1$. Negatively stained by uranyl acetate.

## FORMATION OF THE MOLECULAR BILAYER FROM OTHER DIALKYL AMPHIPHILES

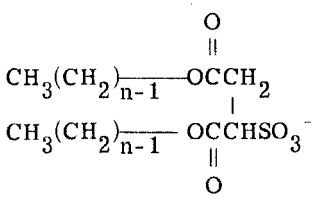
Further modifications of the hydrophilic moiety are possible [12]. The dialkyl compounds with the anionic head group have the structures XI-XIII.



$2C_nPO^{-}$

n = 10, 12, 16

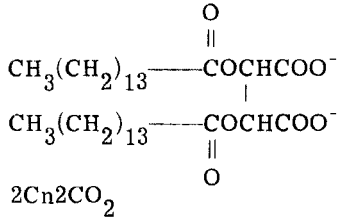
XI



$2CnSO_3^-$

n = 10, 12, 16

XII



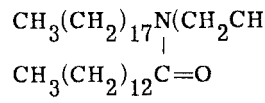
$2Cn2CO_2$

XIII

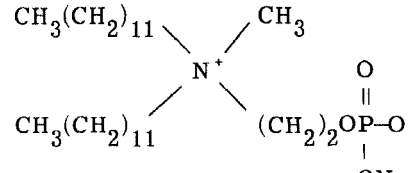


These amphiphiles form lamellar or vesicle structures (layer width 30 - 50 Å) in water. As noted in Table 2, the lamellar structure was formed more readily with increasing chain lengths. The aggregate weight determined by means of the laser light scattering method is in the range of 2-20 million daltons. These aggregates can solubilize at least one third molar cholesterol; however, the electron microscopic examination does not indicate definite structure formation. The bilayer structure is less developed in the anionic aggregate compared with those of the ammonium bilayer, and the bound cholesterol may deteriorate the regular aggregate structure.

The list of bilayer-forming amphiphiles can be extended to include nonionic and zwitterionic varieties [13] (XIV-XVI).



XIV

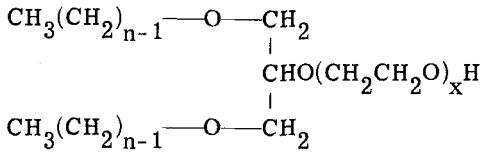


XV

TABLE 2. Electron Microscopy of Anionic Amphiphile

| Compound[a] | Stock solution (10 mM) | Electron micrograph | Aggregate weight × $10^6$ (dalton) |
|---|---|---|---|
| $2C_{16}SO_3Na$ | Slightly turbid | Lamella | - |
| $2C_{12}SO_3Na$ | Clear to slightly turbid | Large vesicle | 22[b] |
| $2C_{10}SO_3Na$ | Clear | Large vesicle | 1.9 |
| $2C_{16}POH$ | Slightly turbid | Lamella | 12 |
| $2C_{12}POH$ | Slightly turbid | Vesicle | 20 |
| $2C_{10}POH$ | Clear | Vesicle | 4.4 |
| $2C_{13}2COOH$ | Slightly turbid[c] | Vesicle | - |

[a]All compounds are in the anionic form in the stock solution.
[b]Light scattering instrument, Union Giken Co. (Japan), Model LS-600; light source, He-Cd laser.
[c]Tends to precipitate upon cooling.



$2C_n$–xG

n = 8, 12, 16, x = 5 - 30.

XVI

When the lipophilic-hydrophilic balance of these dialkyl compounds is adequate, the bilayer structure is invariably formed. Table 3 summarizes the electron microscopic observation for nonionic dialkyl amphiphiles. No well-defined structure was observed with the dioctyl compounds with various ethylene oxide chains. This is consistent with the previous results of the dialkylammonium amphiphile. In all other cases the lamellar or vesicle structures are formed.

TABLE 3. Electron Microscopy of Nonionic Dialkyl Amphiphiles

| Compound | Character | 10 mM solution | Electron micrograph |
|---|---|---|---|
| $C_{18}C_{13}$-10G | Waxy | Clear | Lamella |
| $2C_8$-6G | Liquid | Clear | No structure |
| $2C_8$-10G | | | |
| $2C_8$-12G | | | |
| $2C_{12}$-10G | Waxy | Turbid | Vesicle |
| $2C_{12}$-14G | Waxy | Translucent | Lamella |
| $2C_{12}$-28G | Waxy | Clear | Lamella |
| $2C_{16}$-12G | Waxy | Turbid | Lamella |
| $2C_{16}$-15G | Waxy | Turbid | Lamella |
| $2C_{16}$-32G | Waxy | Clear | Lamella |



## CATALYTIC FUNCTIONS OF AMMONIUM BILAYERS

The molecular motion in the synthetic bilayer membrane is considerably restricted because of its ordered nature. This property is quite different from that of the fluid hydrophobic core of the conventional surfactant micelles, and it is possible to control the rate of various aqueous reactions by using the bilayer system.

The cationic micelle and polysoap can enhance the reactivity of anionic nucleophiles through the formation of hydrophobic ion pairs [14, 15].

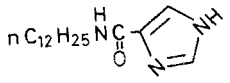
The ammonium bilayer produces similar rate enhancement [16]. As shown in Table 4, the reactivity of a hydrophobic imidazole nucleophile, LImAm, toward p-nitrophenyl acetate is somewhat greater in the presence of the $2C_{12}N^+2C_1$ bilayer than in the presence of the CTAB micelle. A much greater rate enhancement was obtained with the aggregate of trioctylmethylammonium chloride (a triple alkyl chain amphiphile), TMAC. On the other hand, the reactivity of cholest-Im, the cholesteryl ester of imidazolecarboxylic acid, in the ammonium bilayer is much greater than those in CTAB and TMAC aggregates. These results cannot be explained in terms of the hydrophobic microenvironment alone. The cholesteryl catalyst may be bound to the bilayer in a specific manner.

In a subsequent experiment [17], the intra- and intervesicle

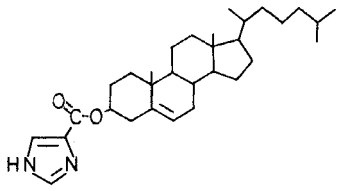
TABLE 4. Reaction of Imidazole Nucleophiles with PNPA in the Presence of Various Ammonium Salts[a]

| | $k_{a,obsd}$ (liter/mole-sec) | | | | |
|---|---|---|---|---|---|
| Nucleophile | None | CTAB ($1 \times 10^{-3}$ M) | TMAC ($1 \times 10^{-4}$ M) | $2C_{12}N^{+}2C_1$ ($1 \times 10^{-3}$ M) | $2C_{18}N^{+}2C_1$ ($1 \times 10^{-3}$ M) |
| LImAm | 0.09 | 105 | 1200 | 155 | 810 |
| Cholest-Im | 0.09 | 61 | 650 | 2570 | - |

[a]Conditions: 30°C, pH 8.90 ± 0.05, $\mu$ = 0.01 (KCl), 0.01 M $H_3BO_4$; [PNPA] = $3.79 \times 10^{-6}$ M, [nucleophile] = $(4.18\text{-}6.72) \times 10^{-5}$ M.



LImAM



Cholest-Im



reactions were differentiated. Table 5 summarizes two series of the experiment. The catalyst and substrate were solubilized in aqueous solutions of didodecyldimethylammonium bromide separately. In series A, the two stock solutions were mixed without sonication with the buffer for initiation. Therefore, catalyst and substrate are contained in different vesicles, and the intervesicle reaction is observed. In the second series, the stock solutions were mixed, sonicated, and added to the buffer solution. Each vesicle contains both catalyst and substrate, so that the intravesicle reaction is observed. It is clear from Table 5 that the pseudo first-order rate constant $k_{obsd}$ for the hydrolysis of PNPA in the presence of the dialkylammonium salt is fairly constant, regardless of whether or not a mixture of the stock solutions is sonicated. In sharp contrast, $k_{obsd}$ for PNPP is enhanced by a factor of over 200 by sonication. Thus the intravesicle reaction is much faster than the intervesicle counterpart, suggesting that transfer of catalyst and/or substrate molecules among vesicles is rate-limiting.

The corresponding rate difference was not found when the CTAB micelle was substituted for the dialkylammonium aggregate. It is concluded that the rate difference between inter- and intravesicle reactions can be made very large by selecting appropriate reactants which tightly bind to vesicles. The conventional micellar system is too soft for this purpose.

## CONCLUSION

Prior to the present study, stable molecular bilayers had been formed only from biolipids and their derivatives. However, the stable bilayer can now be prepared from amphiphilic molecules if they

TABLE 5. Effect of Sonication of Mixed Stock Solutions on $k_{obs}$ [a]

| | $k_{obs}$ $sec^{-1}$ | | | |
|---|---|---|---|---|
| | $2C_{12}N^{+}2C_1Br^{-}$ | | CTAB | |
| | PNPA | PNPP | PNPA | PNPP |
| Series A | | | | |
| simple mixing of stock solutions | 0.53 | 0.032 | 0.066 | 0.12 |
| Series B | | | | |
| Sonication of mixed stock solutions | 0.45 | 7.8 | 0.065 | 0.19 |

[a] Substrate, $1.2 - 1.5 \times 10^{-5}$ M; catalyst, $(1.1 - 1.2) \times 10^{-4}$ M except for $1.1 \times 10^{-5}$ M in the hydrolysis of PNPP in series B. The observed rate constants were corrected to the values for [catalyst] = $1.0 \times 10^{-4}$ M. $2C_{12}N^{+}2C_1Br^{-}$, $1.0 \times 10^{-3}$ M; CTAB, $2.0 \times 10^{-3}$ M. Conditions; 30°C, pH 9.5 ± 0.1, 0.01 M borate buffer; $\mu$ = 0.01 (KCl).

contain two linear alkyl groups of appropriate chain lengths ($C_{10}$ - $C_{20}$). The hydrophilic head group may be cationic, anionic, nonionic, or zwitterionic and need not be as complex as those of biolipid molecules. These synthetic bilayers possess characteristics common to those of the biolipid bilayer. They can readily solubilize up to 40 mole % cholesterol. Furthermore, the molecular motion in the synthetic bilayer is severely restricted and the phase transition is observed between solids and liquid crystals. This phenomenon can be used for controlling the reaction rate as described above. Many other interesting applications would be possible.

## ACKNOWLEDGMENT

The author appreciates the effort of his co-workers, especially that of Dr. Okahata. He is also very grateful to Prof. Takayanagi for the use of an electron microscope.

This paper is contribution No. 489 from the Department of Organic Synthesis.

## REFERENCES

[1] S. J. Singer and G. L. Nicolson, Science, 175, 720 (1972).
[2] H. T. Tien, Ed., Bilayer Lipid Membranes, Marcel Dekker, New York, 1974, Chapt. 1.
[3] J. M. Gebicki and M. Hicks, Nature, 243, 232 (1973).
[4] M. Okuyama, T. Yoshida, T. Tanii, M. Kunugi, and I. Ishizaki, Bull. Chem. Soc. Japan, 48, 191 (1975).
[5] A. W. Ralston, D. N. Eggenberger, and P. L. DuBrow, J. Am. Chem. Soc., 70, 977 (1948).
[6] H. Kunieda, Nippon Kagaku Kaishi, 1977, 151, 1428.
[7] T. Kunitake and Y. Okahata, J. Am. Chem. Soc., 99, 3860 (1977).
[8] M. P. Sheetz and S. I. Chan, Biochemistry, 11, 4573 (1972).
[9] T. Kunitake, Y. Okahata, K. Tamaki, F. Kumamaru, and M. Takayanagi, Chem. Letters, 1977, 387.
[10] T. Kunitake and Y. Okahata, Chem. Letters, 1977, 1337.
[11] N. Nakashima, S. Hayashida, and T. Kunitake, paper presented at the Annual Meeting of the Chemical Society of Japan, Tokyo, April 1978.
[12] T. Kunitake and Y. Okahata, Bull. Chem. Soc. Japan, 51, 1877 (1978).
[13] Y. Okahata, M. Nagai, and T. Kunitake, Polym. Preprint Japan, 27, 231 (1978).
[14] T. Kunitake, S. Shinkai, and Y. Okahata, Bull. Chem. Soc. Japan, 49, 540 (1976).
[15] T. Kunitake, S. Shinkai, and S. Hirotsu, J. Org. Chem., 42, 306 (1977).
[16] Y. Okahata, R. Ando, and T. Kunitake, Bull. Chem. Soc. Japan, submitted.
[17] T. Kunitake and T. Sakamoto, J. Am. Chem. Soc., 100, 4615 (1978).