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# BILAYER MEMBRANES PREPARED FROM MODIFIED DIALKYLAMMONIUM SALTS AND METHYLDIALKYLSULFONIUM SALTS

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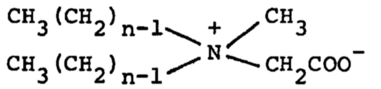
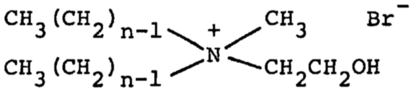
Electron microscopy showed that modified dialkylammonium salts and methyldialkylsulfonium salts formed bilayers in dilute aqueous solutions. The effect of polar head groups on the aggregation properties of the amphiphiles was discussed.

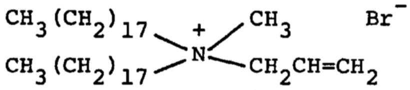
In the previous papers, the authors showed that a series of dimethyldialkylammonium salts($C_nC_mN^+2C_1$) form bilayers in aqueous solutions.[1,2] The bilayer structure observed was quite similar to that of dipalmitoyllecithin vesicles. The length of the alkyl chain was directly related to the aggregation properties of the compounds. The dimethyldialkylammonium bromide with alkyl groups shorter than decyl did not provide a well-defined structure in electron micrographs. The chemical structure of the hydrophilic head group was kept the same in these instances.

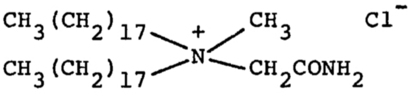
The bilayer structure as well as its ease of formation will be governed by the nature of both hydrophobic and hydrophilic groups. Thus, in this study, the hydrophilic head group was variously modified and electron microscopic examination was performed.

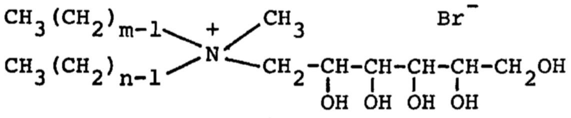
The structure of the ammonium and sulfonium salts employed in this study is as follows. Abbreviated formulae are given in the bracket.

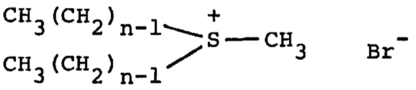
A:

[n=18; $2C_{18}N^+C_1\cdot COOH(Cl^-)$
n=12; $2C_{12}N^+C_1\cdot COOH(Br^-)$]

B:

[n=18; $2C_{18}N^+C_1\cdot C_2OH$
n=12; $2C_{12}N^+C_1\cdot C_2OH$]

C:

[$2C_{18}N^+C_1\cdot C{=}C$]

D:

[$2C_{18}N^+C_1\cdot CONH_2$]

E:

(m=n=18; $2C_{18}N^+C_1\cdot Glu$
m=18, n=12; $C_{18}C_{12}N^+C_1\cdot Glu$)

F:

(n=18; $2C_{18}S^+C_1$
n=14; $2C_{14}S^+C_1$
n=12; $2C_{12}S^+C_1$)

Ammonium salts A, B, C, D, were prepared by the reaction of dioctadecylmethylamine(mp 54 - 56°C) and didodecylmethylamine(bp 172 - 176°C(0.03 mmHg)) with two equivalents of bromoacetic acid, bromohydrine or chloroacetamide in dimethylformamide at 50 - 60°C for 50 - 70 hrs. The products were recrystallized from ethyl acetate more than twice. Ammonium salt E was obtained by refluxing an ethanol solution of N-methyl-D-glucamine and octadecyl bromide in the presence of sodium carbonate for 80 hr, followed by quaternization(dimethylformamide, 50°C, 50 hr) with octadecyl bromide or dodecyl bromide. The hygroscopic products were recrystallized from ethyl acetate. The sulfonium salts F were prepared by reaction of dialkyl sulfide and methyl bromide in ethyl acetate.

The purity of these dialkyl compounds was confirmed by Iatron Thinchrograph(TLC apparatus with a flame ionization detector, Iatron Laboratories, Model TFG-10), elemental analysis and PMR and IR spectroscopies.

Stock solutions for electron microscopy were prepared by sonication(Branson Sonifier 185) of the salts suspended in deionized water for 5 - 15 min. Of the amphiphilic compounds employed, $2C_{12}N^+C\cdot COOH\ Br^-$ and $2C_{18}S^+C\ Br^-$ did not give stable dispersions in water even by extended sonication. The others gave clear to turbid solutions. One ml of the stock solution(10 mM) thus obtained was mixed with 1 ml of a 2 % uranyl acetate solution, sonicated for 15 sec and kept in an ice-water bath for 30 - 60 min. Drops of this solution were applied to a carbon grid, which was then evacuated in a desiccator.

Figure 1a is an electron micrograph of a clear solution of $2C_{12}N^+C_1\cdot C_2OH$. Multiwalled vesicles of various sizes are observed. The vesicles are mostly filled with lamellae of ca. 50 Å width. This width is consistent with the bilayer structure, as mentioned previously.[1] The molecular weight of the vesicle was 3.5 million daltons as measured by the light scattering method. In the case of $C_mC_nN^+C_1\cdot Glu$, fragmentary lamellae are observed as shown in Figure 1b. Lamellar structures were observed for $2C_{18}N^+C_1\cdot CONH_2$ and $2C_{18}N^+C_1\cdot C{=}C$.

Sulfonium salts provided lamellar structures. An example is shown in Figure 1c for $2C_{14}S^+C_1$. The average molecular weight of the lamellar aggragate was 22 million daltons for $2C_{12}S^+C_1$ and 63 million daltons for $2C_{14}S^+C_1$.

These results are summarized in Table I. It is evident that these onium salts (ammonium and sulfonium) form bilayer structures when dispersed in water. The head group structure exerts strong influences on the ease of the bilayer formation. For example, dimethyldialkylammonium bromides form lamellae or vesicles composed of bilayers. When one of the methyl groups is converted to a hydroxyethyl group, the bilayer structure shows up more clearly. However, the glucamine residue seems to lower the regularity of the bilayer.

The animal plasma membrane usually contains large amounts of cholesterol, and liposomes of the biolipid can take up cholesterol well.[3] Similarly, the synthetic bilayer solubilizes 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. An electron micrograph of these solutions showed the presence of lamellae and vesicles(see Figure 1d).

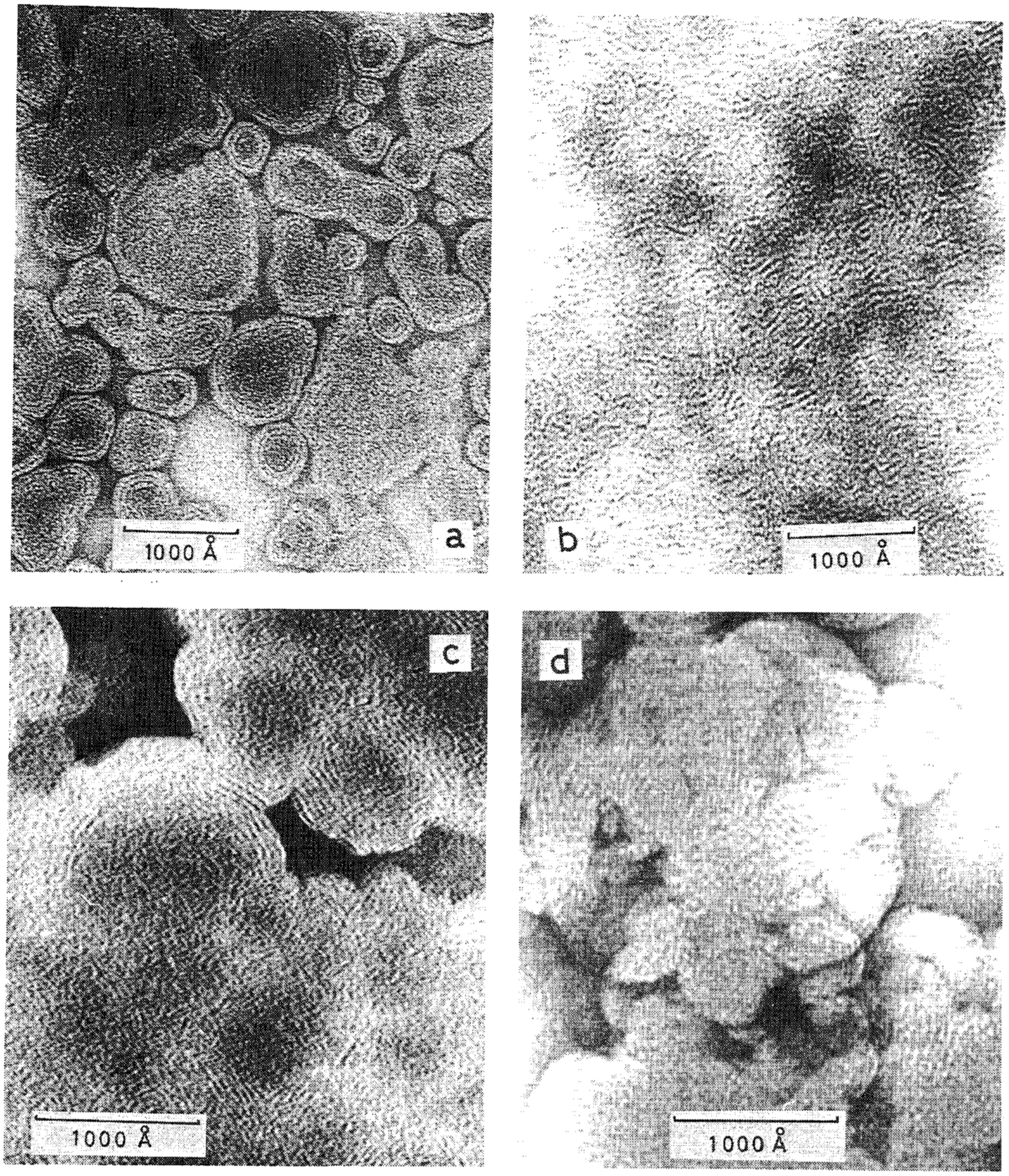


Fig. 1. Examples of the electron micrographs (stained by uranyl acetate)

a. $2C_{12}N^{+}C_{1}\cdot C_{2}OH$(10 mM), magnification × 220,000

b. $C_{18}C_{12}N^{+}C_{1}\cdot$Glu(10 mM), magnification × 240,000

c. $2C_{14}S^{+}C_{1}$(10 mM), magnification × 320,000

d. $2C_{12}N^{+}2C_{1}$(10 mM) + cholesterol(10 mM), magnification × 320,000

Table I Aggregate Structure

| Compound[a] | | | Stock solution | Electron micrographic | Aggregate weight[b] |
|---|---|---|---|---|---|
| Group | Abbreviation | mp (°C) | (10 mM) | appearance | (million dalton) |
| A | $2C_{18}N^+C_1\cdot COOH^*$ | 43-45 | turbid | lamella | 10 |
| | $2C_{12}N^+C_1\cdot COOH$ | 70-71 | turbid (insoluble) | — | |
| B | $2C_{18}N^+C_1\cdot C_2OH$ | 72-73 | slightly turbid | lamella | |
| | $2C_{12}N^+C_1\cdot C_2OH$ | 66-67 | clear | vesicle | 3.5 |
| C | $2C_{18}N^+C_1\cdot C{=}C$ | 62-64 | slightly turbid | lamella | |
| D | $2C_{18}N^+C_1\cdot Glu$ | syrop | slightly turbid | fragmentary lamella | |
| | $C_{18}C_{12}N^+C_1\cdot Glu$ | syrop | slightly turbid | fragmentary lamella | |
| E | $2C_{18}N^+C_1\cdot CONH_2^*$ | 48-50 | slightly turbid | lamella | |
| F | $2C_{12}S^+C_1$ | syrop | slightly turbid | lamella | 22 |
| | $2C_{14}S^+C_1$ | 46-47 | slightly turbid | lamella | 63 |
| | $2C_{18}S^+C_1$ | 65-66 | insoluble | — | |

[a] Bromide salts except for those indicated by the asterisk(chloride).
[b] Determined by the light scattering method: Union Giken Co., Model LS-600.

We conclude from the above results that aqueous amphiphiles which possess two long alkyl groups and positively-charged head groups can form bilayers which further aggregate into lamellae and vesicles. Although the bilayer is observed in all cases, the aggregate structure varies to some extents depending on the nature of the hydrophilic head group. In a separate publication,[4)] we examined electron micrographs of aqueous solutions of amphiphiles that possess two long alkyl chains and anionic head groups(carboxylate, sulfonate and phosphate). The bilayer structure was not developed particularly well for these anionic amphiphiles. The electrostatic repulsion among the negatively-charged head groups may interfere with the formation of the regular structure more effectively than that among the onium head groups.

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## References

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