Synthetic Bilayer Membranes with Anionic Head Groups*

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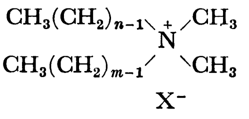
Anionic amphiphiles which possess two normal alkyl groups as the hydrophobic residue and sulfonate, phosphate or carboxylate groups as the hydrophilic residue were prepared. Most of these compounds aggregate extensively in water to form vesicles and lamellae which are probably formed by the molecular bilayer. These aggregates are quite similar to the lecithin liposome with respect to the restricted molecular motion and cholesterol binding.

The bilayer structure similar to that of biomembranes has been prepared mostly from biolipid molecules and related compounds (lecithins and modified lecithins). Although molecular bilayers can be prepared also from other simpler compounds, they are usually unstable and/or formed under rather exotic conditions.[1-3]

A series of dimethyldialkylammonium salts (C_nC_m-N^+2C_1) aggregate extensively in water to form stable structures similar to that of the biomembrane.[4,5] The aggregate structure (lamella or vesicle) depends on the length of the alkyl group, and no structure was found when one of the alkyl group was octyl or shorter. The results suggest that the formation of the stable molecular bilayer is a general physicochemical phenomenon and that particular molecular structure of biolipids is determined by biological necessity.

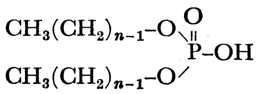
In order to establish the generality of bilayer formation, we prepared some amphiphilic compounds which possess anionic hydrophilic groups (phosphate, carboxylate and sulfonate) and two alkyl chains. The aggregation behavior of these compounds would be of interest since biolipid molecules contain phosphate, carboxylate and sulfonate groups in the hydrophilic head group. The chemical structures of the compounds used in this study are shown below.



$CH_3(CH_2)_{n-1}(CH_3(CH_2)_{m-1})N^+(CH_3)_2\ X^-$

$m, n \geq 10$

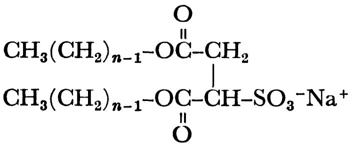
$C_mC_nN^+2C_1$



$(CH_3(CH_2)_{n-1}O)_2P(=O)OH$

$n = 10, 12, 16$

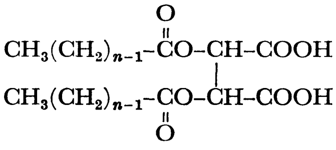
$2C_nPOH$



$CH_3(CH_2)_{n-1}OC(=O)CH_2CH(SO_3^-Na^+)C(=O)O(CH_2)_{n-1}CH_3$

$n = 10, 12, 16$

$2C_nSO_3^-Na^+$



$CH_3(CH_2)_{n-1}C(=O)CH(COOH)CH(COOH)C(=O)(CH_2)_{n-1}CH_3$

$n = 13$

$2C_n2COOH$

Experimental

Preparation of $2C_nSO_3Na$. This type of compound is known as a lipophilic surfactant such as Aerosol OT. Maleic anhydride and twice molar alkyl bromides were heated in toluene in the presence of a few drops of concentrated sulfuric acid, and azeotropic water was removed for 3 h. The solvent was then removed and the residue was recrystallized twice: didecyl maleate, mp 40—42 °C (colorless flakes from hexane); didodecyl maleate, mp 53—55 °C (colorless flakes from hexane); dihexadecyl maleate, mp 64—65 °C (colorless flakes from toluene). These dialkyl maleates and equimolar amounts of $NaHSO_3$ were dissolved in a small amount of water, and allowed to react with stirring at 100 °C for 2—3 h[6] Water was removed *in vacuo* and the residue was dissolved in a large amount of hot methanol in order to separate inorganic materials. Colorless powder was recovered by removal of methanol and dried. NMR spectra are in line with the expected structures[7] The following characteristic bands were observed in the IR spectrum: 1725 cm^{-1} ($\nu_{C=O}$), 1250 cm^{-1} (ν_{SO_3}, ν_{C-O-C}) and 1050 cm^{-1} (ν_{SO_3}). The elemental analyses are summarized in Table 1.

TABLE 1. ELEMENTAL ANALYSIS

Compound		C	H
$2C_{10}SO_3Na$	Calcd for $C_{24}H_{45}SO_7Na \cdot 6H_2O$	47.35	9.10
	Found	46.68	9.06
$2C_{12}SO_3Na$	Calcd for $C_{28}H_{53}SO_7Na \cdot 4H_2O$	53.48	9.78
	Found	54.83	8.48
$2C_{16}SO_3Na$	Calcd for $C_{36}H_{69}SO_7Na \cdot 4H_2O$	58.35	10.47
	Found	58.48	9.29
$2C_{16}POH$	Calcd for $C_{32}H_{67}PO_4$	70.33	12.27
	Found	70.70	12.81
$2C_{12}POH$	Calcd for $C_{24}H_{51}PO_4$	66.32	11.83
	Found	66.43	12.01
$2C_{10}POH$	Calcd for $C_{20}H_{43}PO_4$	63.46	11.46
	Found	64.39	11.69
$2C_{13}COOH$	Calcd for $C_{32}H_{58}O_8$	73.03	12.19
	Found[a]	67.34	10.24

a) About 4 mol% of myristic acid is contained.

Preparation of $2C_nPOH$.[8] $POCl_3$ and three equivalents of the respective alcohols were refluxed in benzene for 18—20 h. The solvent was removed and the residue recrystallized twice or more: didecyl phosphate, mp 33—34 °C (colorless granules from hexane); didodecyl phosphate, mp 51—52 °C (colorless granules from hexane); dihexadecyl phosphate, mp 66—68 °C (colorless granules from benzene). The elemental analyses are given in Table 1. IR and NMR spectral data are in line with the expected structures: IR, $\nu_{P=O}$ 1230 cm^{-1}, ν_{P-O-C} 1060 cm^{-1}.

Preparation of $2C_{13}2COOH$.[9] Myristoyl chloride (24.7 g,

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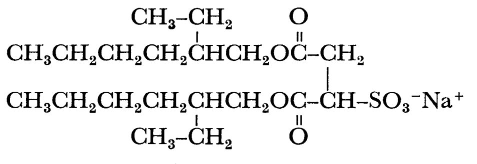
* Contribution No. 477 from this department.

0.1 mol) in toluene was added to a pyridine solution of 7.5 g (0.05 mol) of *dl*-tartaric acid at 10 °C over a period of 30 min, the mixture being then stirred at room temperature for 1 h. Precipitates formed were separated and the solution was concentrated. The solid product was recrystallized twice from hexane, mp 49—50 °C. IR and NMR spectra are consistent with the expected structure but the elemental analysis shows some deviations from the calculated values. (Table 1, footnote).

Electron Microscopy. Weighed amounts of $2C_nSO_3Na$ were suspended in water (1—40 ml) and sonicated for 5—15 min using a Branson cell disruptor. $2C_nPOH$ and $2C_{13}$-2COOH were dissolved in water of pH 9 and sonicated. The final pH of the solutions was 8—9. All of these stock solutions were 10 mM. The stock solution (1 ml) was mixed with 1 ml of appropriate staining reagents (2% aqueous uranyl acetate, 2% aqueous phosphotungstic acid (pH 7), or 2% aqueous ammonium molybdate), sonicated for 15 s, kept for 30—60 min in an ice-water bath, and applied to carbon grids. A Hitachi electron microscope, model H-500 was used.

Results and Discussion

Electron Micrograph. All the dialkyl compounds were soluble under the conditions employed, giving clear to slightly turbid aqueous solutions. Multi-walled vesicles are observed in the electron micrograph of aqueous $2C_{12}SO_3Na$ (Fig. 1a). In contrast, no structure was detected for a 10 mM solution of Aerosol OT which posesses 2-ethylhexyl groups in place of dodecyl group.



$$\begin{array}{l} \qquad\qquad\quad CH_3\text{-}CH_2 \qquad\quad O \\ \qquad\qquad\qquad\qquad | \qquad\qquad\ \ \| \\ CH_3CH_2CH_2CH_2CHCH_2OC\text{-}CH_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ CH_3CH_2CH_2CH_2CHCH_2OC\text{-}CH\text{-}SO_3^-Na^+ \\ \qquad\qquad\qquad\qquad | \qquad\qquad\ \ \| \\ \qquad\qquad\quad CH_3\text{-}CH_2 \qquad\quad O \end{array}$$

Aerosol–OT

Lamellar structures are observed in the electron micrograph of aqueous $2C_{16}SO_3Na$ (Fig. 1b). However, vesicle structure is observed for $2C_{12}POH$ and $2C_{10}POH$ (Fig. 1c).

The layer width of these aggregates is 3—5 nm. This suggests that the molecular bilayer as in biomembranes is formed as the basic structural unit. The electron microscopic observation is summarized in Table 2. The aggregate weight determined by means of the light scattering method is also given. They are in the range of 2—20 million daltons, and much larger than those observed for the conventional anionic micelles: 10^5 daltons for sodium dodecyl sulfate.[10]

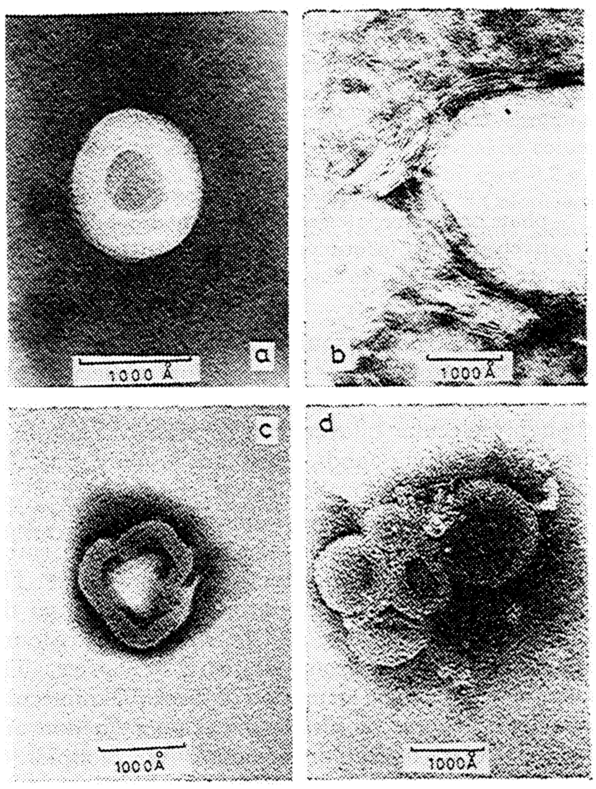


Fig. 1. Electron micrograph. a: $2C_{12}SO_3Na$, stained by phosphotungstic acid, ×300000. b: $2C_{16}SO_3Na$, stained by phosphotungstic acid, ×200000. c: $2C_{10}PONa$, stained by phosphotungstic acid, ×230000. d: $C_{18}C_{12}N^+2C_1$, stained by uranyl acetate, ×186000.

TABLE 2. ELECTRON MICROSCOPY

Compound[a)]	Stock solution 10 mM	Electron micrograph	Aggregate weight[b)] 10^{-6}·dalton
$2C_{16}SO_3Na$	slightly turbid	lamella	—
$2C_{12}SO_3Na$	clear to slightly turbid	large vesicle	22
$2C_{10}SO_3Na$	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 anionic form in the stock solution. b) Light scattering instrument: Union Giken Co. (Japan) Model LS-600. Light source. He–Cd laser. c) Prone to precipitate upon cooling.

The present results obtained for the anionic dialkyl compound are similar to those obtained for dialkylammonium salts. Figure 1d shows an example of the aggregate structure of the latter salts. Both types of the amphiphile form molecular bilayers when dispersed in water. The aggregate structure (vesicle or lamella) commonly depends on the length of the alkyl chain. In the series of dialkyldimethylammonium bromides,[5] the didodecyl compound tends to form vesicles, whereas lamellar structures are produced readily with higher dialkyl compounds. A similar situation holds in the present study between $2C_{12}SO_3Na$ (Fig. 1a) and $2C_{16}SO_3Na$ (Fig. 1b). The difference in the nature of the hydrophilic head group may not change the aggregate structure drastically.

Cholesterol Binding. Cholesterol can be readily solubilized by these aggregates. Slightly turbid solutions were obtained when $2C_{12}SO_3Na$ or $2C_{12}POH$ and about one third molar cholesterol were suspended in deionized water (pH 8—9) and sonicated. Electron microscopic examination of these solutions indicates no definite structure formation. Bound cholesterol may destroy the regular aggregate structure. Only a turbid dispersion was obtained from $2C_{13}COOH$ and one third molar cholesterol.

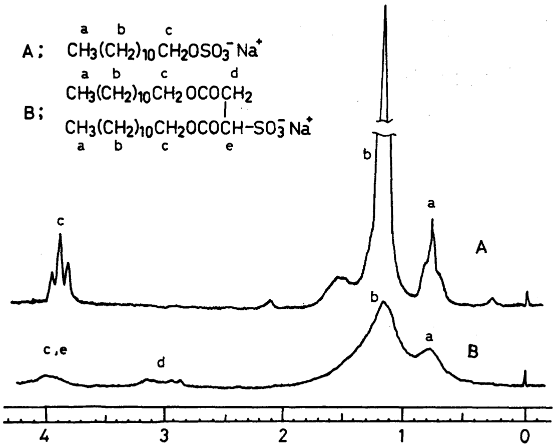


Fig. 2. ^{1}H-NMR spectra. 10 mM in D_2O. Internal reference, DSS.

NMR Spectroscopy. Figure 2 shows an ^{1}H-NMR spectrum of $2C_{12}SO_3Na$ (D_2O solution) obtained at an ambient temperature, in comparison with that of sodium dodecyl sulfate, the assignments being given. The methyl and methylene protons of the alkyl chain of the former compound are much broader than those of the latter. A similar broadening is seen for other protons. The extent of broadening is similar to that of the alkyl proton in the dipalmitoyllecithin vesicle at around the phase transition temperature (≈40 °C).[11] It is suggested that the molecular motion of the aggregated $2C_{12}SO_3Na$ is as restricted as that of the lecithin bilayer.

Comparisons of Lipid and Synthetic Bilayers. The major lipid constituent of biomembranes belongs to phospholipids. The hydrophilic head group of the phospholipid may bear a net negative charge or may be zwitterionic at neutral pH. In a very rare case, the head group is positively charged, since two positive groups (lysyl) and one negative group (phosphate) are present.[12]

The thermodynamic justification for the presence of two alkyl groups in the bilayer formation has been discussed.[13] However, how the head group structure is related to the bilayer formation was not explicitly stated, apart from the importance of its hydrophilic property.

Previous studies[4,5,14] have suggested that simple and modified ammonium groups can be the hydrophilic head group. The present study indicates that simple anionic groups such as phosphate, sulfonate and carboxylate can also be used as the head group. Thus, we conclude that the stable molecular bilayer can be produced from amphiphilic molecules if they contain two normal alkyl groups of appropriate chain length (C_{10}–C_{20}). The hydrophilic head group need not be as complex as those of biolipid molecules.

These synthetic bilayers possess characteristics very similar to that of biomembrane. In the first, they solubilize cholesterol of up to 40 mol% readily. Similar solubilization was observed for the dialkylammonium aggregate.[14] Secondly, considerable broadening of the proton NMR peak of the aggregate is observed, the extent of broadening being similar to that of the lecithin bilayer. This is also the case for the dialkylammonium bilayer.[4] The molecular motion of the synthetic bilayer might thus be as restricted as that of lipid bilayers.

In conclusion, some simple, synthetic amphiphiles, both cationic and anionic, can produce molecular bilayers in aqueous solutions. The structure and some properties of these bilayer aggregates are intrinsically the same as those of the biomembrane.

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