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Complexation of Synthetic Bilayers with Water-Soluble Polymers

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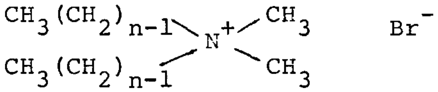
The lipid bilayer of the biomembrane can be reconstructed from biolipids and their derivatives, and numerous investigations have been carried out using these semi-artificial membranes(TIEN, 1974). Recently, we found that the bilayer structure similar to that of biomembranes is readily formed in aqueous dispersions of ammonium salts with two long alkyl chains(KUNITAKE et al. 1977a; KUNITAKE and OKAHATA, 1977a, 1977b). It is shown more recently that the hydrophilic group may be anionic(KUNITAKE and OKAHATA, 1978), nonionic or zwitterionic(KUNITAKE et al., 1978). These bilayers aggregate further to form lamellae or closed vesicles, according to the electron microscopic observation. Some physicochemical characteristics of these stable aggregates are analogous to those of the biolipid bilayer. Most notably, the synthetic bilayer undergoes a transition between the solid and liquid crystalline phases and can incorporate appreciable amounts of cholesterol.

The biomembrane is basically composed of the lipid bilayer and protein molecules. In this respect, it should be extremely interesting to study complexation of the synthetic bilayers with synthetic polymers. In this communication, we report the influence of some water-soluble polymers on the aggregate structure of dialkyldimethylammonium salts.



$n = 12, 18$

MATERIALS AND METHODS

Dimethyldioctadecylammonium bromide($2C_{18}N^{+}2C_{1}Br^{-}$) mp 90

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- 93°C and dimethyldidodecylammonium bromide($2C_{12}N^+2C_1Br^-$) mp 55 - 56°C were prepared by successive alkylation of dimethylamine. Homopolymers are commercially available. The polyethylenimine derivatives were prepared by us previously. Acrylamide was polymerized under several conditions either with azobisisobutyronitrile or with potassium persulfate, and the resulting polymers were subjected to alkaline hydrolysis at 100°C. The copolymers of varying compositions or of varying molecular weight were thus obtained.

The ammonium salts were dispersed in water by sonication (Branson sonifier 185) for 15 min to give clear to slightly turbid solutions(20 mM ammonium). Aqueous solutions of the polymers were added and the mixture was sonicated for another 15 min. One ml of 2 % aqueous solution of uranyl acetate was added to 1 ml of the above solution, and the mixture was sonicated for 15 sec and kept for 30 min in an ice bath. This solution was placed on a carbon grid for electron microscopic observation with a Hitachi H-500 instrument.

RESULTS AND DISCUSSION

As the first step it was studied how various water-soluble polymers affect the aggregate structure of $2C_{12}N^+2C_1Br^-$ and $2C_{18}N^+2C_1Br^-$. The didodecyl compound usually yields more vesicles than lamellae. Addition of sodium polyacrylate(20 mole% for the ammonium salt) causes partial destruction of the vesicle structure of $2C_{12}N^+2C_1Br^-$. No structure could be found when 80 mole% of the polymer was added. Other anionic homopolymers such as polyvinylsulfonate and poly-L-glutamate showed similar effects. Equimolar amounts(in unit moles) of positively-charged alkylpolyethylenimines completely destroyed the bilayer structure. Among the uncharged polymers, polyacrylamide and polyvinylpyrrolidone did not produce detectable morphological changes. In contrast, no structure was observed when 20 mole% of poly(vinyl alcohol) was added.

The effect of these polymers was studied also for the aqueous aggregate of $2C_{18}N^+2C_1Br^-$. However, morphological changes were difficult to detect in the case of the lamella, and further studies were performed only for $2C_{12}N^+2C_1Br^-$.

Polyanions and the ammonium salt readily form polyion complexes and precipitates are formed from water. The influence of the polyanion becomes moderate as the charge density along the polymer chain decreases. Figure 1 illustrates the morphological

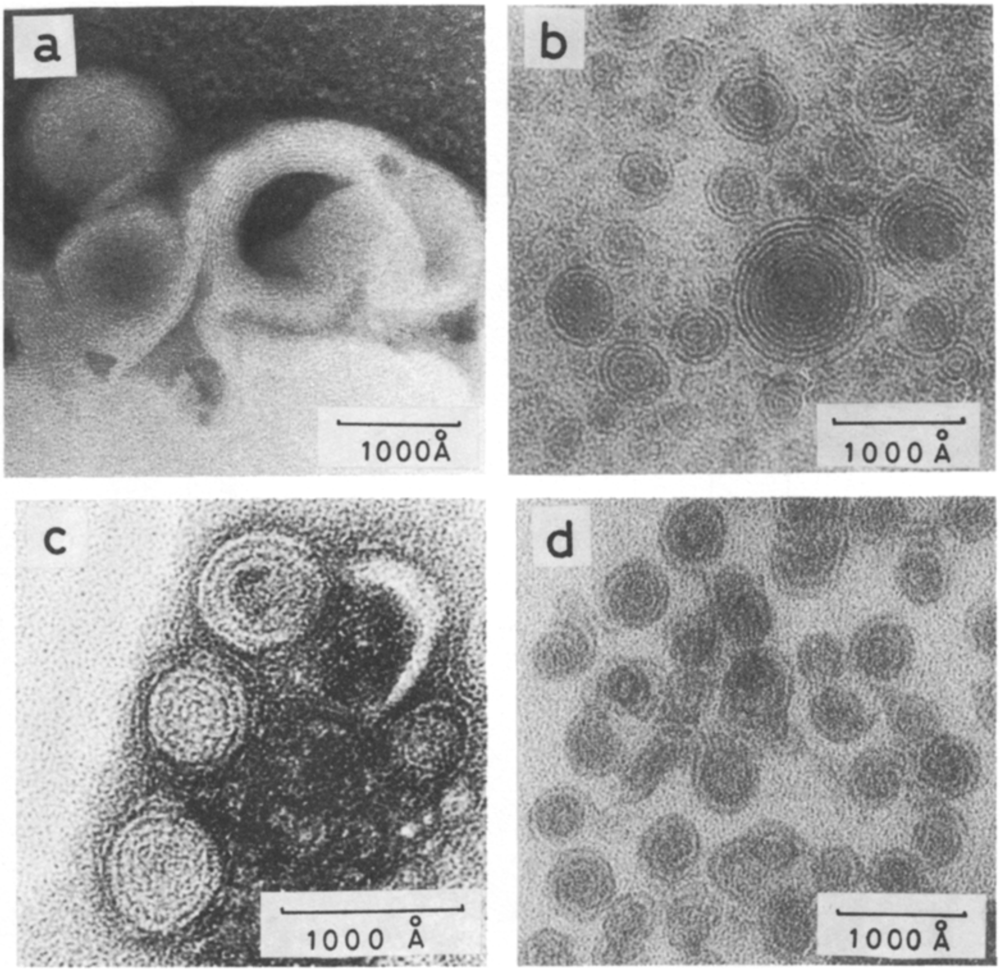

Figure 1 Electron Micrograph

a: $2C_{12}N^{+}2C_{1}Br^{-}$(10 mM) + 100 mole% of polyacrylamide. ×160,000

b: $2C_{12}N^{+}2C_{1}Br^{-}$(10 mM) + M-27(20 mole% in the acrylate unit). ×170,000

c: $2C_{12}N^{+}2C_{1}Br^{-}$(10 mM) + M-22(20 mole% in the acrylate unit). ×240,000

d: $2C_{12}N^{+}2C_{1}Br^{-}$(10 mM) + M-13(20 mole% in the acrylate unit). ×170,000

TABLE 1

Morphology of Polymer-Ammonium Complexes

copolymer[a]	aqueous mixture of polymer and $2C_{12}N^{+}2C_{1}Br^{-}$		electron micrograph		
	prior to sonication	after sonication	no. of layers in vesicles	vesicle diameter (Å)	separation of bilayers (Å)
L-11	turbid	turbid	3 - 5[b]		100 - 120
L-19	"	"	3 - 5	600 - 1000	ca. 80
L-24	"	"	4 - 5	1500	ca. 80
M-13	turbid	turbid	2 - 3	600	
M-22	"	"	ca.5	500 - 1000	70
M-27	"	"	multi	600 - 1100	60
H-8	precipitation	turbid	2 - 3	300 - 500	80 - 100
H-14	"	"	3 - 5	1000	70 - 80
H-27	"	"	multi	1200 - 1700	60

[a] Molecular weight of the unhydrolyzed polyacrylamide: L, 6000; M, 50000; H, 200000. The number indicates the content of the acrylate unit.

[b] The vesicle is not completely closed.

change of $2C_{12}N^{+}2C_1$ vesicles produced by addition of partially hydrolyzed polyacrylamide. The aggregate structure is not detectably altered by acrylamide homopolymer. In the case of the copolymer, the vesicle becomes smaller with decreasing acrylate units(i.e. with increasing total unit of the copolymer), and the bilayers become more separated. Table 1 is a summary of the morphological change. Without addition of the copolymer $2C_{12}N^{+}2C_1Br^{-}$ forms multi-walled vesicles and lamellae. The smallest vesicles formed by addition of the least charged copolymers are double or triple-walled, and the bilayer separation is ca. 100 Å. Apparently, the copolymer is present in the hydrophilic phase between the bilayers, with concomitant bilayer separation. The coulombic interaction of the copolymer and the bilayer is not large enough to destroy the vesicle structure. These changes appear not particularly dependent of the molecular weight of the copolymer.

SUMMARY

The morphological influence of water-soluble polymers on the aqueous bilayer aggregate(vesicle and/or lamella) of didodecyldimethylammonium bromide was investigated by electron microscopy. Positively charged polymers and uncharged polymers either destroyed the aggregate or was ineffective. On the other hand, the negatively-charged copolymer of acrylamide and acrylate separated the bilayer and produced smaller vesicles.

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