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FLOCCULATION OF PHOSPHATIDYLCHOLINE VESICLE WITH TRIMETHYLAMMONIUM GLYCOL CHITOSAN IODIDE

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Phosphatidylcholine (PC) vesicle was quantitatively flocculated with trimethylammonium glycol chitosan iodide (MGCH) over the pH region of 3 - 12. The results obtained by IR analysis of the aggregate and by turbidity titration in the presence of metal cations, suggested that the flocculation would be based on the salt-linkage formation between phosphate group of PC and trimethylammonium group in MGCH.

Phospholipid vesicle has been known to serve as a simplified model for biological membranes. To obtain an information about the functions of polar groups on the surface of the membranes, the interactions of phospholipid vesicle and lamella with metal cations,[1] acids and bases,[2] and proteins[3] have been studied. However, little attention has been paid to the interaction between phospholipid vesicle and polyelectrolyte ion.

In this communication, we report the interaction of phosphatidylcholine (PC) vesicle with trimethylammonium glycol chitosan iodide (MGCH) which is frequently used as the standard titrant in colloid titration. The results obtained were discussed in terms of the proposed two models[4,5] for PC membrane structure.

PC was obtained from Wako Pure Chemical Industries,Ltd. and the purity was confirmed by TLC[6] and UV spectrum.[7] PC vesicle was prepared by the usual sonication method; the aqueous suspension (ca. 3 %) of PC was ultrasonically irradiated at 4°C for 1 hr under a nitrogen atmosphere by using an ultrasonic disintegrators (Ultrasonics Ltd.). After removal of the undispersed matter by centrifugation at 28000g for 1hr, the dispersion was filtered through a membrane filter (0.1 μ pore size). The final dispersion was stable and showed some fluorescent color. The PC concentration (C_{pc}) in the dispersion was determined by the dry weight method. On the other hand, the physical properties of polyelectrolytes used here were described in previous papers.[8,9]

Turbidity titration was carried out at 25°C under a nitrogen atmosphere using a spectrophotometer (Atago model 440-S). The transmittance was measured at the wavelength of 500 nm. As the titration proceeds, the sample dispersion begins to become turbid, and the turbidity reaches a maximum at a certain titrant volume. In the dispersion at the maximum turbidity, large aggregates were observed by an electron microscope (Hitachi model H-500).

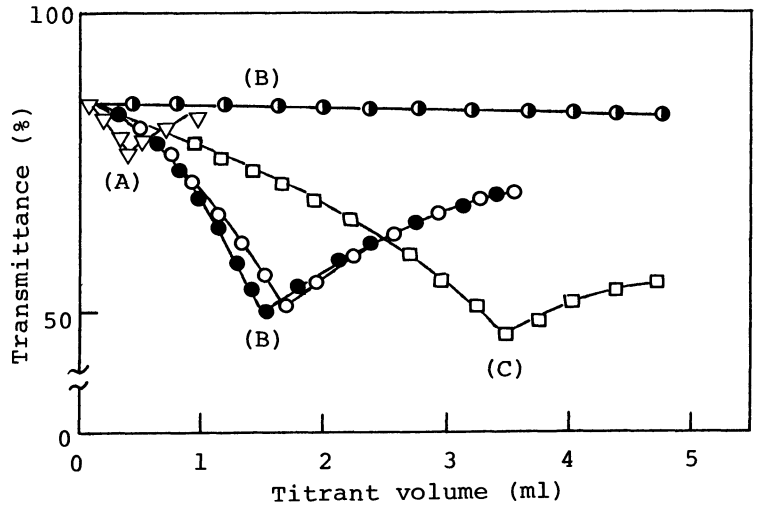

Fig. 1. Turbidity titration curves of PC vesicle with 0.0005N MGCH (▽, ○, □, pH 5.6) and 0.0005N GCH (◑, pH 10.2; ●, pH 6.1). C_{pc} (g/dl): A, 0.0128; B, 0.0320; C, 0.0640

Typical turbidity titration curves with MGCH are shown in Fig. 1. In the same figure, the curves obtained by glycol chitosan (GCH) are also shown. Each turbidity titration curve with MGCH shows a minimum point at a certain titrant volume over the pH region from 3 to 12. In the case of GCH, however, the minimum point was not observed in the pH region above 8 where protonated amino groups of GCH dissociate completely.[8] This behavior suggests that trimethylammonium group or protonated amino group attached to the polymer chain plays an important role in the flocculation of PC vesicle. On the other hand, the plots of the MGCH volume at the minimum point against C_{pc} show a straight line passing through the origin (see Fig. 2). This indicates that the PC vesicle is quantitatively flocculated by MGCH.

According to the electrophoretic study[2] of PC vesicle in aqueous medium, the

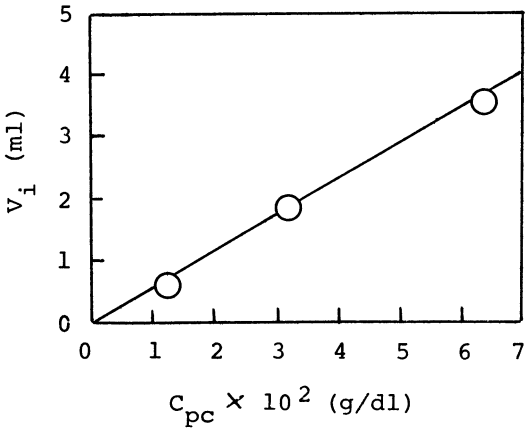

Fig. 2. Linear relationship between C_{pc} and MGCH volume (V_i) at the minimum point of turbidity titration curve.

zeta potential of the vesicle is zero in the pH region of 3 - 11. This result was understood by the explanation that the polar groups (trimethylammonium and phosphate groups) of PC form the inter- and/or intramolecular salt-linkage on the vesicle surface.[2] Nevertheless, the present experiment shows that the PC vesicle is flocculated by MGCH. This contradiction might be avoided if we assume that the salt-linkage of polar groups on the vesicle surface were destroyed by the presence of polycation in the bulk-phase, and new salt-linkage were formed between phosphate group on the vesicle surface and trimethylammonium group in MGCH.

In order to confirm this assumption, IR spectra were measured for PC molecule and the aggregate of PC vesicles. The results are shown in Fig. 3. The absorbance of PC molecule at 1170 cm^{-1}, which is assigned to P-O stretching vibration, is different from that of the aggregate, indicating an interaction between phosphate group of PC vesicle and MGCH ion. The turbidity titrations with MGCH were also carried out in the presence of KCl and $CaCl_2$ at ionic strength 0.001. The titration curves are shown in Fig. 4. It is observed that the MGCH volume at the minimum point decreases with the increase in the valency of metal cation. This suggests that the salt-linkage formation between phosphate group on the vesicle surface and trimethylammonium group in MGCH could be hindered by the binding effect[2] of the metal cation on the phosphate group.

As to PC membrane structure, two models have been proposed; one in which the polar groups of PC are approximately parallel to the fatty acyl chains and perpendicular to the interface,[4] and the other in which the polar groups are coplanar and the plane is perpendicular to the fatty acyl chains.[5] The finding reported here suggests that the latter model is more reasonable since the exposed phosphate group on the

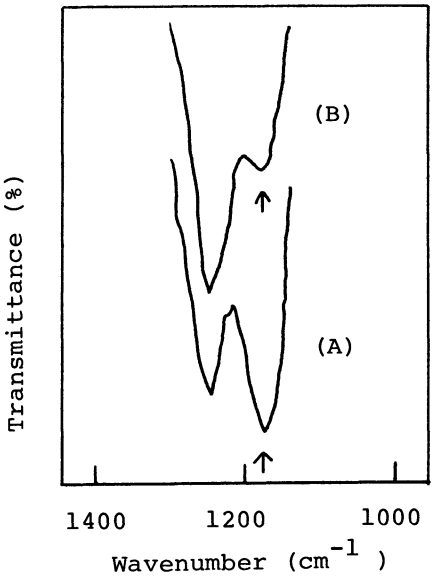

Fig. 3. IR spectra of PC molecule (A) and the aggregate (B) of PC vesicles in KBr disk.

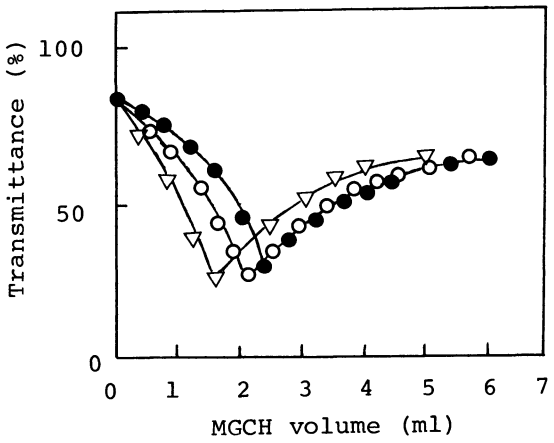

Fig. 4. Effects of metal cations on the flocculation of PC vesicle (C_{pc} 0.042 g/dl) with 0.0005N MGCH at ionic stength 0.001. Added salts: ▽, $CaCl_2$; ○, KCl; ●, non

vesicle surface may interact only with the polycation. It is difficult for trimethylammonium cation attached to MGCH to penetrate the narrow space of trimethylammonium groups of PC molecules on the vesicle surface as required in the former model.

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