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EFFECT OF PH ON THE FLOCCULATION OF PHOSPHATIDYLCHOLINE VESICLE WITH TRIMETHYLAMMONIUM GLYCOL CHITOSAN IODIDE

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Effect of pH on the flocculation of phosphatidylcholine vesicle with trimethylammonium glycol chitosan iodide (MGCH) was investigated over the pH region of 3 - 12. The flocculation based on the salt-linkage formation between phosphate group on the vesicle surface and trimethylammonium group in MGCH is affected by pH of bulk-phase.

Most of the previous studies on the interactions of phospholipid vesicle and lamella with metal ions,[1] acids and bases,[2] and proteins[3] gave important informations about the physicochemical and functional properties of polar groups on the biological membranes. However, little attention has been paid to the interactions with polyelectrolyte ions. Recently we found that phosphatidylcholine (PC) vesicle is quantitatively flocculated with trimethylammonium glycol chitosan iodide (MGCH).[4] The flocculation was understood by the explanation that the salt-linkage of polar groups (phosphate and choline groups) on the PC vesicle surface is destroyed by the presence of MGCH ion in the bulk-phase, and then new salt-linkage is formed between phosphate group on the vesicle surface and trimethylammonium group in MGCH. Now we wish to report the significant effect of pH on the flocculation mentioned above.

PC (egg yolk) was obtained from Wako Pure Chemical Industries, Ltd. and the purity was confirmed by TLC[5] and UV spectrum.[6] The PC vesicle was prepared in the same manner as described previously.[4] The PC concentration in the dispersion was determined by the dry weight method. The physical properties of MGCH was described in previous papers.[7] The volume of MGCH solution to flocculate the PC vesicle at various pH was determined by means of turbidimetric titration method. The 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. The pH of the titration system was adjusted with 0.1 - 1M HCl and KOH. The change in pH during the titration process was measured by using a Hitachi-Horiba pH meter (model F-5) equipped with a Horiba pH electrode (model 6028-10T).

A typical turbidimetric titration curve is shown in Fig. 1 together with the change in pH caused by the addition of MGCH. The result indicates that the PC vesicle is flocculated rapidly by MGCH. On the other hand, it is observed that the pH of the titration system decreases around the minimum point of titration curve.

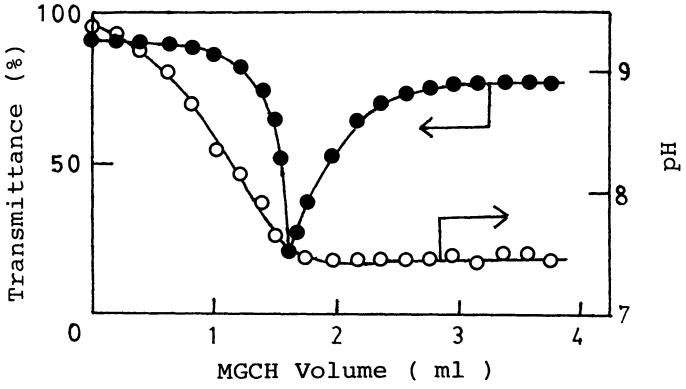

Fig. 1 Turbidimetric titration curve of PC vesicle with MGCH and the pH change in the course of the titration. The vesicle dispersion (40ml) containing 9 mg of PC was titrated with 5.15×10^{-4}M MGCH solution.

In order to investigate the effect of pH on the flocculation of the vesicle, the MGCH volume (V_m) at the minimum point of titration curve was plotted against the pH at the minimum point (see Fig. 2). The value of M_S in Fig. 2 represents the number of moles of trimethylammonium group in MGCH which is salt-linked with phosphate group on the vesicle surface. The studies[7] on the electrophoresis and colloid titration for MGCH showed that the dissociation is constant in the pH region below 5 and above 7, but varies slightly over the pH region from 5 to 7 because of the effect of lower amino groups (5.8%) partially remaining in MGCH. To calculate the M_S value, the effect of the dissociation of the lower amino groups on the M_S value was corrected by the degree of dissociation (α) of MGCH ($M_S = 5.15 \times 10^{-7} V_m \alpha$); the curve of M_S vs. pH is not affected by the dissociation of MGCH. From the curve of M_S vs. pH it was found that the pH of bulk-phase plays an important role in the flocculation of the PC vesicle with MGCH. In contrast to the conventional view for the electrochemical property[2,8] of the polar groups on the PC vesicle surface, the result obtained here suggests that the phosphate group, which is salt-linked with the nearest neighbouring choline group, behaves like a liberating group when new salt-linkage is formed between the phosphate group on the vesicle surface and trimethylammonium group in MGCH.

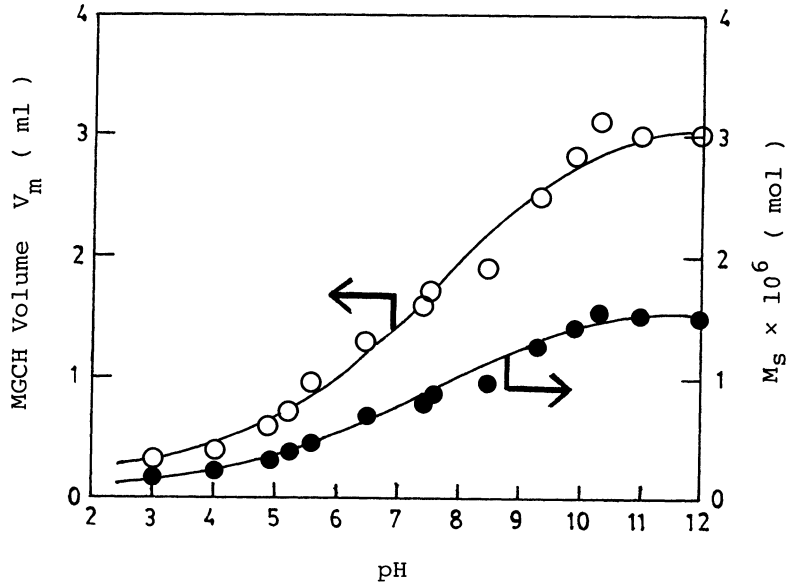

Fig. 2 Plots of V_m and M_s against pH.

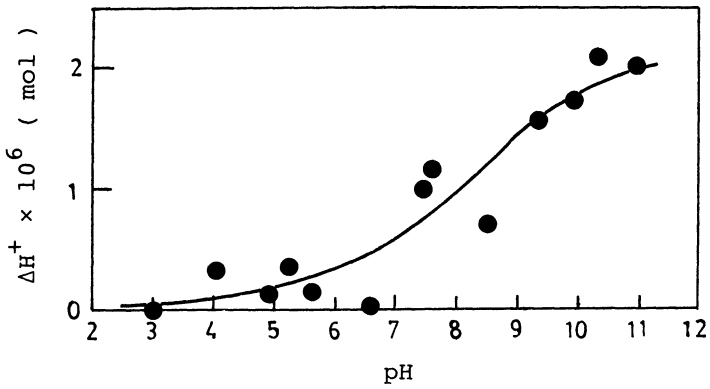

Fig. 3 Evolution of proton caused by the flocculation of the vesicle with MGCH.

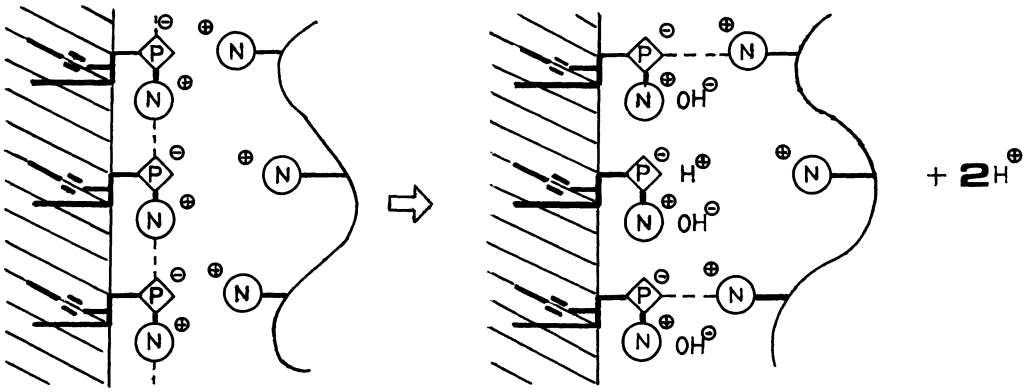

Fig. 4 Schematic representation for the mechanism of the salt-linkage formation between phosphate group on the vesicle surface and trimethylammonium group in MGCH.

The effect of pH on the salt-linkage formation mentioned above was also investigated by the change in pH during the titration process. The increase of proton (ΔH^+) in the vesicle dispersion with the addition of MGCH solution is shown in Fig. 3. The value of ΔH^+ was estimated by means of the difference in maximum and minimum pH values of the curve of pH vs. MGCH volume (see Fig. 1) and was plotted against the pH at the minimum point of the turbidimetric titration curve. The ΔH^+ value is enhanced by the increase in the salt-linkage between the phosphate group on the vesicle surface and the trimethylammonium ion in MGCH, indicating that the evolution of proton is caused by the salt-linkage formation (see Fig. 2). This might be interpreted by the schematic representation shown in Fig. 4: The salt-linkage between the phosphate and choline groups on the PC vesicle surface is destroyed by the electrostatic effect of MGCH ion, and then the phosphate group is affected by the pH of the bulk-phase and is partially protonated. Moreover, the choline groups attract hydroxyl ions from the bulk-phase to keep "the electroneutrality". Therefore, protons equimolar with the phosphate groups which are not protonated, remain in the bulk-phase so that the decrease in pH is apparently observed during the titration process. This interpretation seems to be supported by the fact that the result shown in Fig. 3 approximately agrees with the curve of M_S vs. pH obtained by the turbidimetric titration.

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