FORMATION AND PROPERTY OF MIXED VESICLES OF PHOSPHATIDYLCHOLINE AND MDP DERIVATIVES

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The aggregation form of mixtures of phosphatidylcholine and MDP derivatives was studied. The mixed vesicles containing less than 40% of B30-MDP were unilamellar, and over 40% B30-MDP aggregates of mixed vesicles or oligolamellar vesicles appeared. The mixed vesicles containing less than 20% of DV-7401 were unilamellar, and over 20% of it fibriform structures appeared.

KEYWORDS vesicle; muramyl dipeptide; MDP derivative; vaccine; membrane

Muramyl dipeptide (MDP) is the smallest unit of peptide glycan which possesses cell-mediated immunity and adjuvant activity. Recently, construction of artificial membrane vaccine or liposome vaccine which was composed of antigen, phospholipid and MDP derivatives has been tried.1) For this purpose, amphiphilic MDP derivatives were useful because of their ability to form mixed vesicles with phospholipid. As a link in the basic study for the preparation of artificial membrane vaccine, we started a study on formation and property of mixed vesicles constituted of amphiphilic MDP derivatives and phospholipid.2) MDP derivatives used were 6-O-(2-tetradecylhexadecanoyl)-n-acetylmuramyl-L-alanyl-D-glutamic acid-1 amide (B30-MDP) and 6-O-(2-tetradecylhexadecanoyl)-n-acetylmuramyl-L-alanyl-D-isoglutamin-5 amide (DV-7401), which were gifts from Daiichi Pharmaceutical Co. Ltd.

Mixed vesicles were prepared by the detergent-removal method3); egg yolk phosphatidylcholine and MDP derivatives were mixed, so that their total amount was 10 mg; the mixtures were solubilized by a nonionic detergent, octyl glucoside (175 mM, 1 ml); mixed vesicles were formed by removing octyl glucoside by dialysis. Particle size was estimated from quasi-elastic light scattering (QELS) and calcein-trapped volume.4) The shape and morphology of the vesicles were detected by electron microscopic observation. Membrane barrier efficiency was estimated by Cl− ion permeability, which was

Fig. 1. Mean Diameter of Phosphatidylcholine-
B30-MDP Mixed Vesicles
○: QELS, ●: calcein-trapped volume.

Fig. 2. Rate Constant of Cl− Ion Efflux
through the Vesicle Membrane

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determined by electrometric measurement of Cl\(^-\) ion efflux using Cl\(^-\) selective electrode.\(^5\)\)

Figure 1 shows the diameter of the mixed vesicles composed of B30-MDP and phosphatidylcholine. Apparent diameter obtained from QELS measurement (D\(_{\text{QELS}}\)) was about 220 nm and agreed with that obtained from trapped volume of the vesicles (D\(_{\text{trap}}\)) up to 40% of B30-MDP. This observation suggests that the mixed vesicles are basically unilamellar up to 40% of B30-MDP.\(^6\) Over 40% of B30-MDP, D\(_{\text{QELS}}\) became much larger than D\(_{\text{trap}}\), which suggests the formation of aggregates among vesicles or another association form of lipid except for unilamellar vesicles, such as multilamellar vesicles. In the electron microscopic view, aggregates of the vesicles like bunches of grapes were observed at 60% of B30-MDP, and giant oligolamellar vesicles were observed at 80-100% of B30-MDP (Fig. 5-a and -b). Rate constants of Cl\(^-\) ion efflux through the vesicle membrane composed of various mixed ratios of phospholipid and B30-MDP are shown in Fig. 2. Up to 40% of B30-MDP permeability of Cl\(^-\) ion lowered with increasing B30-MDP content. Over 40% of B30-MDP, on the contrary, the permeability rose with increasing B30-MDP. The negative charge of B30-MDP suppressed Cl\(^-\) ion permeability at low contents of B30-MDP, but over 40% of B30-MDP it might perturb the vesicle membrane or change the vesicle form. Figure 3 shows the diameter of mixed vesicles composed of phosphatidylcholine and DV-7401. Up to 20% of DV-7401, no notable change in vesicle size was observed. Over 20% of DV-7401, vesicle size became larger with increasing DV-7401 content, and obvious phase separation was observed over 60% of DV-7401. The plots at 60% and 80% of DV-7401 in Fig. 3 are those of supernatants. In Fig. 4, the ratio of trapped volume calculated from vesicle diameter (V\(_{\text{dia}}\)) and trapped volume obtained from calcein entrapment (V\(_{\text{cal}}\)) was shown. Up to 20% of DV-7401 the ratio was almost unitary, showing that the vesicles were basically unilamellar. Over 20%, the ratio became much lower than unitary, showing the appearance of another aggregate form except unilamellar vesicle. Electron microscopic observation showed the appearance of fibriform aggregate, which was in contrast with the aggregate form of phosphatidylcholine-B30-MDP mixture. In the supernatant at 60% of DV-7401, almost all were in the vesicle form (Fig. 5-D); in the precipitate, vesicles and fibriforms coexisted (Fig. 5-E). At 100% of DV-7401, all was in the fibriform (Fig. 5-F). These observations suggest that up to 20% DV-7401 was soluble in membrane phase and forms mixed vesicles with phospholipid. Over 20%, DV-7401, being insoluble in vesicle membrane, forms another

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**Fig. 3.** Mean Diameter of Phosphatidylcholine-DV-7401 Mixed Vesicles from QELS

**Fig. 4.** Ratio of Trapped Volume V\(_{\text{dia}}\) Calculated from Vesicle Diameter and V\(_{\text{cal}}\) from Calcein-Trapped Volume
aggregate, fibriform. The fiber was constructed by bundles of countless filaments. Judging from the fact that the diameter of the filament is about 5 nm, the filament can be seen as an infinitely extended rod-like micelle, and the fiber can be regarded as a variation of hexagonal assembly of the rod-like micelle. It is very interesting that only substitution of the carboxyl group in the glutamine residue with the amide group entirely changed the hypermorphosis of aggregation form of MDP derivatives. In conclusion, the unilamellar limit of the mixed vesicles composed of phosphatidylcholine and MDP derivatives is below 40 % for B30-MDP and below 20 % for DV-7401.

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