Short communication

Effect of mucin on lipid bilayers by phase of layer

Abstract: Changes in the behavior of the prepared lipid bilayer according to each phase of the layer, induced by mucin incorporation, were investigated. 8-Aminonaphtalene-1,3,6-trisulfonic acid disodium salt (ANTS) was encapsulated only in the water-soluble interior of the vesicle. *p*-xylene-bis(*N*-pyridinium bromide) (DPX) settled only outside of the vesicles to quench the ANTS. The calibration was established for the fluorescence with the encapsulated ANTS separated from the DPX (100%) and the ANTS mixed with DPX (0%). The changes were found due to the layer phase over the ratio of mucin to lipid. The changes are determined by the stability of the vesicles.

Keywords: Mucin, Lipid bilalyer, Vesicle, Fluorescence, Layer phase

1. Introduction

Mucin is commonly observed on luminal surface of animals [1]. This group consists of a linear peptide with oligosaccharide [2,3]. Additionally, a hydrophobic transmembrane stretch of the mucin is used to its anchorage to the plasma membrane and signaling [1]. The mucin has been recognized as being involved in the development of cancer, especially adenocarcinomas [4]. Furthermore, its association with tumor has been identified [5].

The mucin has been believed to limit the lateral movement of the lipids, because the mucin was distributed at the interface of the lipid layer [6,7]. The mucin became more stable due to the presence of lipid [8]. Particularly, the phosphatidylcholine was found to have an affinity for mucin [9]. Additionally, the phosphatidylcholine was observed to penetrate more easily into the mucin compared to other nanocarriers [10].

Little has been found for the effect of mucin on the layers. Although the behaviors of lipid monolayers were investigated with the addition of a compound, a bilayer is more complicated than an assembly of two monolayers [11-15]. In this study, it is aimed to investigate the systematic effect of mucin on the bilayer by each phase of each layer.

2. Materials and methods

2.1 Vesicle preparation

Ten mg of dipalmitoylphsphatidic acid(DPPA) or dioleoylphosphatidic acid(DOPA) from Merck (Rahway, NJ) was dissolved in 2 ml of tert-butyl methyl ether. To this solution was added 100 μl DI water of 25 mM 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt(ANTS), 10 mM Tris-HCl at pH 7.4. For the formation of the desired inverse-micelles, the extrusion of the above solution was performed through the 50 nm pores of 78 mm diameter PTFE membranes at a temperature higher than the transition of the chosen lipid. To the 10 ml Tris-HCl buffer of 90 mM p-Xylene-bis(N-pyridinium bromide)(DPX), either dioleoylphosphatidylcholine(DOPC) or dipalmitoylphosphatidylcholine(DPPC) was added

continuously in a drop way less than 10 μl micelle-solution. This solution underwent through the centrifugation (3700 × g), and its supernatant only was the vesicle solution [16].

2.2 Vesicle confirmation

From the diameters, both micelles and vesicles were confirmed. As shown in Fig. 1, each diameter was 75 ± 10 and 80 ± 10 nm. The refractive index and the viscosity of the solvent are 1.3686 and 0.23 cP [17]. In addition, little change in the 530 nm fluorescence intensity with pH change outside vesicle indicated that each layer was secured.

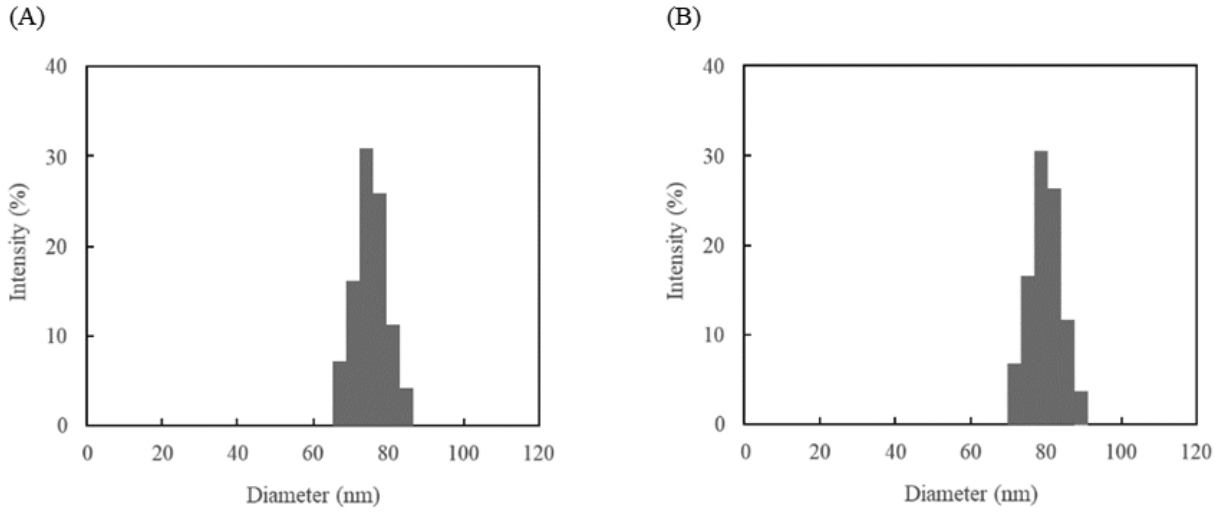

Figure 1. Diameter data of (A) micelles and (B) vesicles

2.3 Fluorescence measurement

The fluorescence intensity was monitored at the desired ratio of mucin to PC using a fluorometer (Perkin-Elmer, Waltham, MA). The intensity change generated by the mucin means that the the layer arrangement was disturbed because the change in the ANTS fluorescence intensity is caused only due to the mixing of ANTS and DPX. The calibration was established for the fluorescence with the encapsulated ANTS separated from the DPX (100%) and the ANTS mixed with DPX (0%) [18]. The quantitative intensity was found to understand the mucin effect.

3. Results and discussion

3.1 Vesicle fusion induced by mucin

The results for each phase of the layer are summarized with respect to the mucin ratio (Table 1). Each phase corresponded to the intensity. For the solid outer, the intensity dropped to about 0.5 ratio. In addition, the change in the lipid phase was generated by the cosmotropic effect of mucin on the water around the headgroup, because most lipids were associated with the mucin at the ratio. The change was saturated at 0.5 ratio.

Table 1. Fluorescence intensity(FI) for each layer phase after the mucin addition

	Solid Outer											
	Solid Inner						Liquid Inner					
	Ratio of Mucin to Lipid						Ratio of Mucin to Lipid					
	0	0.1	0.3	0.5	0.7	1.0	0	0.1	0.3	0.5	0.7	1.0
FI (%)	100	90	70	50	50	50	70	60	45	30	30	30
	Liquid Outer											

	Solid Inner						Liquid Inner					
	Ratio of Mucin to Lipid						Ratio of Mucin to Lipid					
	0	0.1	0.3	0.5	0.7	1.0	0	0.1	0.3	0.5	0.7	1.0
FI (%)	70	70	70	70	70	70	30	30	30	30	30	30

However, for the liquid outer, the difference in the intensity rose. Little change was observed for any phase of the inner in the case where the outer was liquid. In other words, the mucin-caused-induction of the vesicle fusion was generated only at the solid outer. Therefore, the intensity change was determined dominantly by the outer phase due to the layer exposure to the mucin. In addition, the influence of difference in the outer phase was considered under the identical phase of the inner. The behavior of the intensity was not identical each other (Fig. 2). In the case of the liquid inner, the lowest intensity was identical for any phase of the outer (Fig. 2 (A)). However, in the solid inner, the intensity of the solid outer became from higher to lower rather than the liquid outer (Fig. 2 (B)). These results indicated that mucin, only outside the vesicle, disturbed both outer solid phase and inner solid phase. In other words, the mucin little disturbed both liquid layers. The previous investigation was consistent with this interpretation [19].

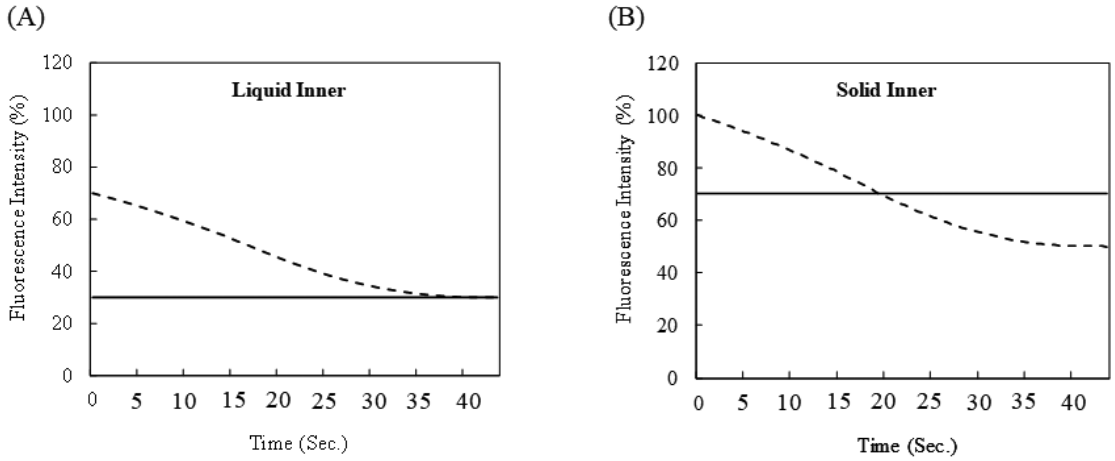

Figure 2. Fluorescence intensity of vesicles with solid outer (- - -) and with liquid outer (——) at 0.5 ratio. (A) liquid inner and (B) solid inner.

3.2 Effect of mucin on vesicle inner

The intensity of the solid outer was expected similar with that of the liquid outer because the mucin induced the fluidity on the solid layer. However, the observation was different. The discrepancy from the expectation seemed to be caused by the tail-group mismatch between each layer. The cosmotropic effect increased the distance between the head-groups of the solid outer. Although the lipids of the solid outer were far from the others, little fluidity was in the tail-groups. Therefore, the mucin disrupted the arrangement of the layer tail-groups. If the tail-group was liquid, the layer was less rigid over the disruption. This interpretation corresponded to the lower intensity of the solid outer compared to the liquid outer in the case of the solid inner.

From about 20% reduction in the fluorescence intensity, it has been inferred that the change in the structure occurred to most of the vesicles. All results found out in this study may attribute to the cosmotropic and volumetric effects, which disrupt the head-group packing and the tail-group arranging. Since the liquid of layers is critical for the functions, the liquefaction by the mucin may be related to the mechanism.

4. Conclusion

The mucin-induced vesicle instability was monitored according to layer phase using the fluorescence intensity. The intensity in the solid outer was inversely proportional to the mucin ratio up to 0.5. In the liquid, the behavior of the intensity was distinguished from the solid. These results may attribute to the cosmotropic and volumetric effects, which disrupt the head-group packing and the tail-group arranging. The mucin study may be related to the biological mechanism connected to cellular processes.

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