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Effect of hydroxybutyric-acid on lipid bilayers with respect to layer phase

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요 약 : 형광 강도 변화를 이용하여 세포막의 위상에 따른 하이드록시부티르산 혼입이 생체막에 미치는 영향을 조사하였다. 구형 인지질 이중층인 소포(vesicle)가 이중 에멀젼 기술을 통해 각 층 단위로 제조되었다. 소포 내부의 수성에는 아미노나프탈렌트리술폰산디소듐(ANTS)이 캡슐화되었으며, 소광제(quencher)로 자일렌비스피리디늄브로마이드(DPX)를 소포가 분산된 버퍼에 포함시켰다. 형광 등급은 100% 형광으로서 DPX가 포함된 완충액의 소포와 0% 형광으로서 ANTS와 DPX의 혼합물이 포함된 완충액의 소포를 고려하여 조정되었다. 소포 용액에 하이드록시부티르산 혼입은 막 구조의 변화를 유도하였으며, 이러한 변화는 하이드록시부티르산 대 지질의 비율에서 소포의 각 층의 상과 관련이 있는 것으로 관찰된다. 관찰된 결과는 머리 그룹과 꼬리 그룹 모두의 패킹 붕괴에 대한 삼투압 및 체적 효과로 인해 소포의 안정성에 의존하는 것으로 보인다.

주제어 : Hydroxybutyric-acid, Vesicles, Lipid layer, Phase, Fluorescence

Abstract : The behavior changes of the lipid bilayer, induced by the hydroxybutyric-acid incorporation, were investigated with respect to each phase of the layer using fluorescence intensity change. Spherical phospholipid bilayers, called vesicles, were prepared using an emulsion technique. Only in the aqueous inside of the vesicles was encapsulated 8-Aminonaphthalene-1,3,6-trisulfonic-acid-disodium-salt(ANTS). *p*-Xylene-bis-*N*-pyridinium-bromide(DPX) was included as a quencher only outside of the vesicles. The fluorescence scale was calibrated with the ANTS-encapsulated vesicles in DPX-dispersed-buffer taken as 100% and the mixture of ANTS and DPX in the buffer as 0%. Hydroxybutyric-acid addition into the vesicle solution led the change in the bilayer. The change was found to be related to the phase of each layer according to the ratio of hydroxybutyric-acid to lipid. These results seem to depend on the stability of the vesicles, due to the osmotic and volumetric effects on the arrangement in both head-group and tail-group.

Keywords : Hydroxybutyric-acid, Vesicles, Lipid layer, Phase, Fluorescence

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1. Introduction

Hydroxybutyric-acid (synonym, β-hydroxybutyric-acid) is known to be relevant to diabetes and cranial nerve symptoms [1,2]. This compound is critical as an alternative if the level of glucose is scarce in a body, because hydroxybutyric-acid is synthesized from acetyl-CoA in the liver [3]. And since the compound is able to inhibit a histone deacetylase, it induces the increase in brain-derived neurotrophic factor (BDNF) [2]. This knowledge may be clinically associated with the treatment of psychological symptoms. Furthermore, hydroxybutyric-acid has been reported to be an anesthetic in animal and to modulate the glycine-receptor function [4]. The anesthetic action was caused by the compound to modify mechanical behaviors of cell membranes to modulate the membrane protein function [5].

Since lipid bilayers are a barrier between extracellular and intracellular compartments of a biological cell, the layers have been widely utilized to investigate the biological processes. The structural changes of the layers, caused by the reagents, were studied monitoring the release of the intra-contents [6]. The lipid mixing assay is able to provide the information for the change of the layers with and without the reagents. Furthermore, hydroxybutyric-acid has been reported to be an anesthetic in animal and to modulate the glycine-receptor function [7,8].

Although hydroxybutyric-acid is associated with the biological membranes, the effect of hydroxybutyric-acid on the membranes is little understood [9,10]. Lipid monolayers are widely used to gain insight into how a compound behaves in cell membranes [11-13]. These results rely on the equivalence of the two systems in their response to hydroxybutyric-acid. Although the headgroup/water interfaces in the two systems may be similar, a bilayer is complicated rather than just a simple assembly of two monolayers [14,15]. In this study, the effect of hydroxybutyric-acid was investigated on the bilayers composed of lipids systematically in each phase.

2. Experiments

2.1. Reagents

Dioleoylphosphatidic acid(DOPA), dipalmitoylphosphatidic acid(DPPA), dioleoylphosphatidylcholine(DOPC), dipalmitoylphosphatidylcholine(DPPC), Tris-HCl, *tert*-butyl methyl ether, hydroxybutyric-acid, 8-Aminonaphthalene-1,3,6-trisulfonic- acid-disodium-salt(ANTS), and *p*-Xylene-bis-*N*-pyridinium-bromide(DPX) were purchased from Sigma Aldrich (St. Louis, MO), respectively. PTFE membranes was purchased from Hyundai micro (Seoul, Republic of Korea).

2.2. Vesicle preparation

The lipids of either DOPA or DPPA were dissolved in 2 ml of *tert*-butyl methyl ether at 5 mg/ml, followed by adding 100 μl distilled water of 25 mM ANTS, 10 mM Tris-HCl at pH 7.4. To form the inverse micelles, this solution was extruded through the 50 nm pores of 78 mm diameter PTFE membranes above the transition temperature of the desired lipid. Several drops (less than 10 μl) of the micelle solution was continuously added with tert-butyl methyl ether solution of 5 mg/ml either DOPC or DPPC into the 10 ml aqueous solutions of 90 mM DPX, 10 mM Tris-HCl at pH 7.4, respectively. The final lipid concentration of the aqueous solution was 1 mg/ml. The vesicle solution was acquired from the supernatant of the solution that underwent through the centrifugation (3700 × g). These procedures are well established for vesicle preparation [16].

2.3. Vesicle characterization

The diameters of the micelles and the vesicles were measured, respectively, using

ELS-8000 (Otsuka Electronics Co. Ltd, Osaka, Japan) so that the formations of both micelles and vesicles could be confirmed. Their diameters were 75 ± 10 nm and 80 ± 10 nm (Fig. 1). The viscosity and the refractive index of the tert-butyl methyl ether are 0.23 cP and 1.3686, respectively [17]. Furthermore, no leakage of the ANTS molecules inside of both micelles and vesicles indicated that the structure of each layer was little disturbed. Otherwise, the fluorescence intensity at 530 nm would be dropped tremendously with the addition of several drops of aqueous solutions of 10 mM Tris-HCl at pH 3.0 into the vesicle solution (excitation at 384 nm and emission at 530 nm).

2.4. Fluorescence measurements

The amount of hydroxybutyric-acid was determined with the desired ratio of hydroxybutyric-acid to PC (0, 0.1, 0.3, 0.5, 0.7, and 1.0). The fluorescence intensity was monitored in real time with a Wallac Victor3 multiwell fluorometer (Perkin-Elmer, Waltham, MA). Since the fluorescence intensity of the ANTS is different only if the ANTS is mixed with the DPX, the intensity difference of the vesicle solution between with the hydroxybutyric-acid-solution addition and with buffer-only solution addition means that the structural change occurs in the layers. The fluorescence scale was calibrated with the ANTS vesicles, made with the liquid-condensed phase of the layers in DPX-included-buffer, taken as 100%. For 0% of the scale, the well-mixed solution of ANTS and DPX was considered. Therefore, the intensity was quantitatively estimated to investigate the effect of the hydroxybutyric-acid.

3. Results and Discussion

All experiments were performed at room temperature, unless otherwise specified. Dioleoyl lipids were used in the liquid expanded phase at room temperature because its transition temperature was much lower than room temperature, and dipalmitoyl lipids were in the liquid condensed [18]. Therefore, four types of vesicles were shown in Fig. 2. And the successful encapsulation was confirmed identically with the previous research [19].

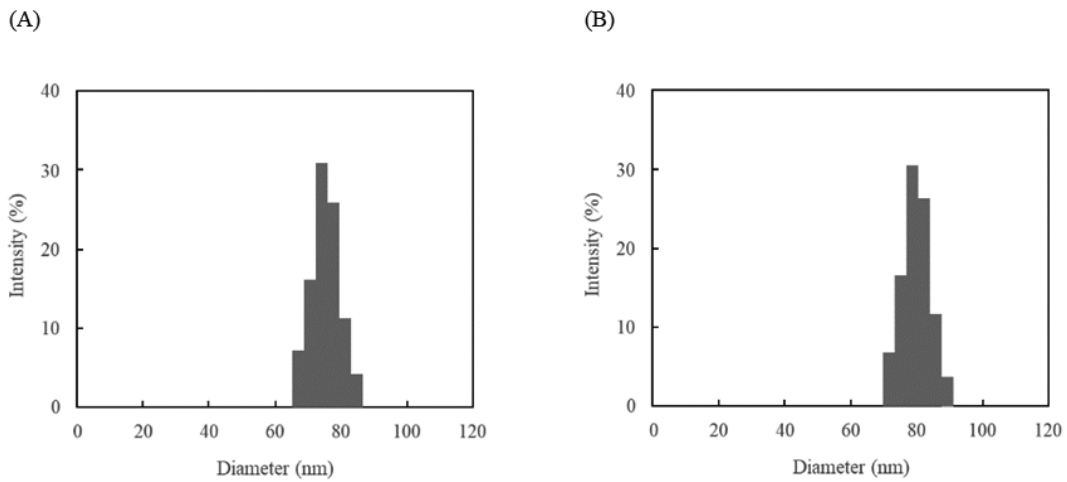

Fig. 1. Diameter distributions of (A) micelles and (B) vesicles.

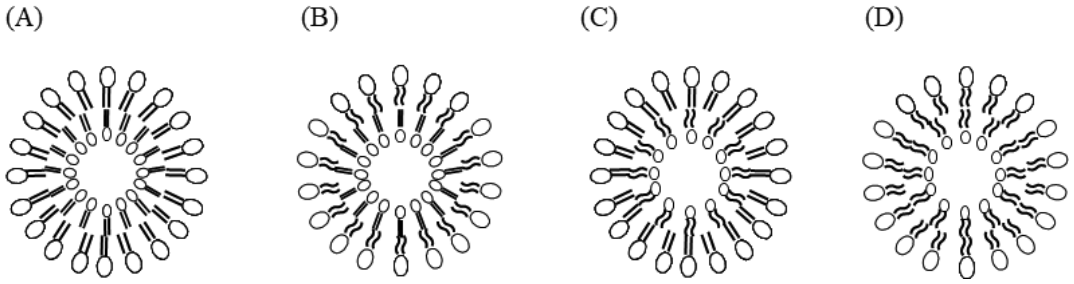

Fig. 2. Types of vesicles. Straight line corresponds to liquid-condensed phase of the lipid layer, and curved line to its liquid-expanded phase. (A) Liquid-condensed phases for both layers. (B) Liquid-expanded phase for outer layer and liquid-condensed phase for inner layer. (C) Liquid-condensed phase for outer layer and liquid-expanded phase for inner layer. (D) Liquid-condensed phase for both layers.

Table 1. Fluorescence intensity for each phase of the vesicle layer after the hydroxybutyric-acid addition into the vesicle solution

	Liquid-condensed Outer-layer											
	Liquid-condensed Inner-layer						Liquid-expanded Inner-layer					
	Ratio of Hydroxybutyric-acid to Lipid						Ratio of Hydroxybutyric-acid to Lipid					
	0	0.1	0.3	0.5	0.7	1.0	0	0.1	0.3	0.5	0.7	1.0
Fluorescence Intensity (%)	100	90	70	50	50	50	70	60	45	30	30	30
	Liquid-expanded Outer-layer											
	Liquid-condensed Inner-layer						Liquid-expanded Inner-layer					
	Ratio of Hydroxybutyric-acid to Lipid						Ratio of Hydroxybutyric-acid to Lipid					
	0	0.1	0.3	0.5	0.7	1.0	0	0.1	0.3	0.5	0.7	1.0
Fluorescence Intensity (%)	70	70	70	70	70	70	30	30	30	30	30	30

3.1. Vesicle fusion phenomena induced by hydroxybutyric-acid

The fluorescence intensity is listed for each phase of the layer according to the hydroxybutyric-acid ratio (Table 1). The intensity was different at each phase. In the liquid-condensed phase of the outer layer, the intensity clearly decreased to about 0.5 hydroxybutyric-acid molecules per lipid. Furthermore, it was also suggested that the cosmotropic effect of hydroxybutyric-acid on the water surrounding the headgroup resulted in the change in the lipid-layer phase, because most of the lipid was associated with the hydroxybutyric-acid at the ratio. The saturation of the changes was found at the hydroxybutyric-acid ratio of 0.5.

However, in the liquid-expanded phase of the outer layer, the intensity was different. The intensities were little changed for both phases of the inner layer when the outer layer was at liquid-expanded phase. Therefore, the vesicle fusion was induced by hydroxybutyric-acid only at the liquid-condensed phase of the outer layer. In other words, the intensity change was influenced mainly by the phase of the outer layer due to the exposure of the layer to the hydroxybutyric-acid. For further

analysis, the different phase of the outer layer was considered for the identical phase of the inner layer. Interestingly, it was found that the fluorescence intensity behavior was not consistent each other (Fig. 3, on next page). When the values of the intensity were compared for the different-phase outer-layers of the liquid-condensed inner-layers, the intensity of the liquid-condensed outer-layer changed from higher to lower compared to that of the liquid-expanded outer-layer (Fig. 3 (A)). However, for the different-phase outer-layers of the liquid-expanded inner-layers, the lowest intensity of the liquid-condensed outer-layer was identical with that of the liquid-expanded outer-layer (Fig. 3 (B)). These results seemed to suggest that hydroxybutyric-acid, even distributed only outside the vesicle, made an effect on not only the liquid-condensed phase of the outer layer but also the liquid-condensed phase of the inner layer. In other words, the hydroxybutyric-acid made little effect on the stability of both layers for the liquid-expanded phase of the membrane. The previous investigation was supportive for this interpretation [20].

3.2. Effect of hydroxybutyric-acid on vesicle inner-layer

Since the more hydroxybutyric-acid molecules on the layer of a liquid-condensed phase induced the higher-fluidized layer, it was expected for the liquid-condensed inner layer that the intensity of the liquid-condensed outer-layer would be close to that of the liquid-expanded outer-layer. However, the clear difference between them was observed. This unexpected result seemed to be interpreted as interference due to the mismatch between the tail-groups of each layer. Due to the cosmotropic effect, the distance between the lipid head-groups of the liquid-condensed outer-layer was increased. Although the lipids of the liquid-condensed outer-layer became far each other, still the tail-groups had no fluidity. Therefore, the hydroxybutyric-acid incorporation apparently disturbed the arrangement of the tail-groups of the layers. If the tail-group was liquid-expanded, the layer seemed more flexible to overcome the disturbance. Otherwise, perhaps the vice versa effect would be possible. This interpretation was for the intensity of the liquid-condensed outer-layer unexpectedly lower than that of

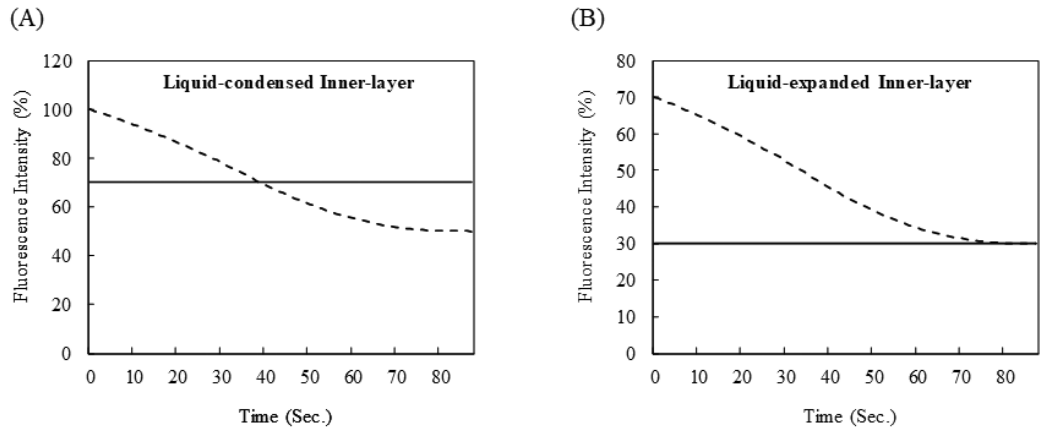

Fig. 3. Fluorescence intensity of vesicles with liquid-condensed outer-layer (- - -) and with liquid-expanded outer-layer (--) for 0.5 ratio of hydroxybutyric-acid to lipid. (A) liquid-condensed inner-layer and (B) liquid-expanded inner-layer.

the liquid-expanded outer-layer in the case of the liquid-condensed inner-layer.

The fluorescence intensity of 20% below indicated that most of the vesicles lost their initial structure. All of these results described above seem attributable to the cosmotropic and volumetric effects on not only the head-group packing disruption but also the tail-group arranging disturbance. Since the functions can be performed at the liquid-expanded phases of both layers, the liquefaction of the layers, led by the hydroxybutyric-acid incorporation, may be relevant to the functions.

4. Conclusion

In this study, the fluorescence intensity of vesicles was monitored at each phase of the layer. The intensity in the liquid-condensed phase of the outer layer decreased proportionally to the increase to 0.5 of hydroxybutyric-acid to lipid. In the liquid-expanded phase, the relative change of the intensity was opposite compared to the liquid-condensed phase. These results seem attributed to the osmotic and volumetric effect on the arrangement of both head-group and tail-group. The present study may provide a platform to control biological functions related to cellular processes. Therefore, it would be interesting to investigate the hydroxybutyric-acid effect on the mechanism of the reagent-triggered cells, such as anesthetic action and BNDF release.

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