Experimental study of lateral diffusion on the bilayer membrane

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2分子膜ベシクルの構成成分が相分離して形成されたマイクロドメインは、液体膜の性質を持つベシクルの球面に沿ってブラウン運動する。過去の幾つかの研究と同様に、通常の相分離と同じくドメインが融合合体成長して最終的に大きく二相分離するベシクルと、ベシクル上の相分離ドメインが小さなドメインのままで長時間維持されるベシクルの、2種類が観察された。本実験では、クエンチからの時間をさほど考慮しなくても良いと思われる、マイクロドメインが安定に分散された状態のベシクルを対象として観察を行った。このときの画像を取得する事でドメインの拡散係数を求めると共に、ドメイン間の距離を測定する事で実効的なドメイン間相互作用を直接求めた。

1 Experimental outline

In order to prepare multicomponent membrane vesicle, the phospholipids solution, e.g., containing DOPC/SM/Chol/Rho-DHPE at 4.5/4.5/1.0/0.020 in mM concentration, was briefly dried under nitrogen gas flow and then incubated under a vacuum condition at about 300 K in several hours. We applied the enhanced natural swelling method developed by K. Tsumoto for preparing supersized giant unilamellar vesicles (SGUVs) [1]. The method enables to yield giant unilamellar vesicles efficiently. After waiting 2 or 3 hours for the spontaneous natural swelling at approximately 325 K, giant vesicles were achieved as a polydisperse solution of lipid vesicles. The sample solution was pipetted and enclosed in an approximately 50 μm spaced glass chamber constructed from cover slips for microscopy (MATSUNAMI, Japan).

Figure 1 shows a typical image of the lateral phase separation on a giant vesicle. The dark circles, which seem like holes on the vesicle, are micro-domains that contain rich SM and Chol (poor DOPC and Rho-DHPE) as compared to the bright part [2]. In this condition, the bright part exhibits a fluidic property, and the dark domains are fluidic or solid like phase. Though several to dozens μm-sized vesicles as in Fig. 1 are frequently yielded in the normal natural swelling method, it is difficult to yield enough number of data to calculate lateral diffusion or pair interaction because the spherical surface makes difficult to achieve an omnifocal observation

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at same time and motion analyses of the domains. The more important problem is that such cell-sized vesicles usually include not so many domains. In order to avoid such problems, we applied the SGUV method to obtain small curvature vesicles accompanied with a lot of micro domains.

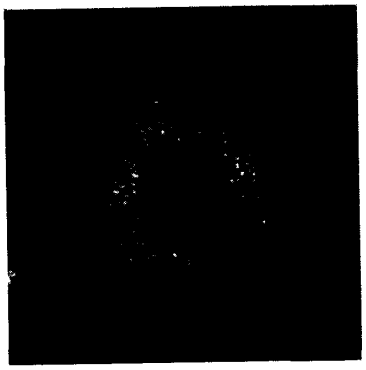

Figure 1: Snapshot of lateral phase separation on a giant vesicle.

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References

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