Direct Estimation of Osmotic Pressure-Induced Membrane Tension and Enhanced Water Permeability

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

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Abstract：

Osmotic pressure (Π) induces the stretching of plasma membranes of cells or lipid membranes of vesicles, which plays various roles in physiological functions. To understand the response of cell to Π, suspensions of large unilamellar vesicles (LUVs) with a diameter 100-400 nm have been used for measurement of Π-induced volume change of vesicles and leakage of internal contents from vesicles. However, in the LUV suspension method, the average values of physical parameters of many LUVs have been obtained, and therefore, much information has been lost. On the other hand, giant unilamellar vesicles (GUVs) with a diameter ≥ 10 μm can be observed using microscopes, and hence GUVs of any specific size and shape can be chosen based on the purpose of experiments and changes of structures and shapes of GUVs can be observed in real time. Using GUVs, Π-induced volume decrease and shape change has been observed. However, there have been no experimental estimations of the membrane tension of vesicles upon exposure to Π. On the other hand, recently there have been methodological progress in the research on the effects of mechanical tension on vesicles. In this thesis, using this new methodology, I tried to estimate experimentally the membrane tension of vesicles under Π. During this experiment, I found that water permeability in lipid membrane increased with Π. Therefore I also investigated the enhanced water permeability induced by stretching of lipid bilayers.

[Chap. 2] I estimated experimentally the lateral tension of the membranes of GUVs when they were transferred into a hypotonic solution. First, I investigated the effect of Π on the rate constant, k_p, of constant tension (σ_{ex})-induced rupture of dioleoylphosphatidylcholine (DOPC)-GUVs using the method developed by Yamazaki and colleagues recently. When constant tension was applied on a GUV under Π using the micropipette aspiration method, rupture of GUV occurred. If the same experiments were performed using many GUVs, it turned out that GUV rupture occurred stochastically. By analysis of the time course of fraction of intact GUVs among all examined

GUVs, k_p of rupture GUVs were obtained. k_p increased with applied tension (σ_{ex}) under the same Π. By comparing the σ_{ex} dependence of k_p in GUVs under Π with that in the absence of Π, the tension of the GUV membrane due to Π at the swelling equilibrium, σ_{osm}^{eq} was estimated. Next, I investigated the volume change of DOPC-GUVs under small Π by measuring the decrease of the projection length of a GUV after transferring it in to a hypotonic solution. The volume of the GUVs rapidly decreased with time, and reached its equilibrium value. I made a theory on the membrane tension σ_{osm}^{eq}, of a GUV induced by Π. The experimentally obtained values of σ_{osm}^{eq} and the volume change agreed with their theoretical values within the limits of the experimental errors. Finally I investigated the characteristics of the Π-induced pore formation in GUVs. When values of Π were large, rapid transient leakage of sucrose from GUVs were observed. This leakage is due to Π-induced pore formation in the GUV membrane. The fraction of GUV where a transient leakage occurred increased with Π. The σ_{osm}^{eq} corresponding to the threshold Π to induce pore formation is similar to the threshold tension of the σ_{ex}-induced rupture. The time course of the radius change of GUVs in the Π-induced pore formation depends on the total membrane tension, σ_t; for small σ_t the radius increased with time to an equilibrium one, which remained constant for a long time until pore formation, but for large σ_t the radius increased with time and pore formation occurred before reaching the swelling equilibrium. Generalized emission polarization (GP) values of Laurdan in vesicles decreased with Π supported the theory on the dependence of σ_{osm}^{eq} on the radius of vesicles which indicates σ_{osm}^{eq} increased the membrane fluidity of GUVs. Based on these results, we discussed the σ_{osm}^{eq} and the Π-induced pore formation in lipid membranes.

[Chap. 3] The effect of tension on water permeability of lipid bilayer was investigated in order to understand the effect of Π on water permeability. After a constant tension was applied on a DOPC–GUV using the micropipette aspiration method, the GUV was transferred to a hypotonic solution and then the volume of the GUV increased with time. By analyzing the time course of the volume change, membrane permeability of water (i.e., water permeability) was obtained. Water permeability in DOPC membrane greatly increased with tension without leakage of a water–soluble fluorescent probe Alexa Fluor 647, i.e., without pore formation in the membranes. In another lipid (i.e., dilinoleoyl phosphatydilcholine), water permeability increased with tension. The present theories on water permeability cannot explain this enhanced water permeability. I proposed a new theory (i.e., prepore model); water can translocate across bilayers through transient hydrophilic prepores and the enhanced water permeability in stretched bilayers can be explained by an increase in the frequency of hydrophilic prepore formation and the size of prepores in the bilayer with increasing tension.

These results revealed new aspects of Π-induced membrane tensions and its effects on pore formation and water permeability.