

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

メタデータ	言語: en 出版者: Shizuoka University 公開日: 2017-06-07 キーワード (Ja): キーワード (En): 作成者: Sayed, UI Alam Shibly メールアドレス: 所属:
URL	https://doi.org/10.14945/00024340

学 位 論 文 要 約

Summary of Doctoral Thesis

専 攻 : Bioscience
Course :

氏 名 : Sayed Ul Alam Shibly
Name :

論文題目 : 浸透圧が誘起する膜の張力と水の膜透過係数の増加の直接的な評価

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

論文要約 :

Summary :

Osmotic pressure (Π) induces the stretching of plasma membranes of cells or lipid membranes of vesicles, which plays various roles in physiological functions. However, there have been no experimental estimations of the membrane tension of vesicles upon exposure to Π . It is important to measure the quantitative values of lateral tension due to osmotic pressure in order to understand the effect of osmotic pressure on the activities of membrane proteins and membrane active peptides such as antimicrobial peptides, and on the physical and chemical properties of lipid membranes. In this thesis, I tried to estimate experimentally the lateral tension on the membranes of giant unilamellar vesicles (GUVs) following transfer to a hypotonic solution. It turned out during this project that it is important to elucidate water permeability across lipid bilayers to understand the response of vesicles to osmotic pressure. Therefore, I also investigated water permeability in lipid bilayers.

(Chapter 1)

I summarized the effects of osmotic pressure on the physical properties and

functions of lipid membranes and cells, and also the effects of membrane tension due to external forces on the physical properties and functions of lipid membranes and cells. Then, I described the purpose of the Ph.D. thesis.

(Chapter 2)

In this chapter, I estimated experimentally the lateral tension of the membrane of GUVs after transfer to a hypotonic solution. For this purpose a following new idea was used. If an external force is applied to a GUV to induce a constant tension, σ_{ex} , in the presence of Π , the total tension, σ , on the GUV membrane is increased by the lateral tension due to Π , and thus the rate constant of σ_{ex} -induced rupture of a GUV, k_p , increases. Therefore, if the σ_{ex} dependence of k_p in GUVs under Π is compared with that in the absence of Π to obtain the difference of the values of σ_{ex} to induce the same k_p value, one can estimate the membrane tension of the GUV due to Π . Based on this idea, several kinds of experiments were performed.

First, I investigated the effect of Π on the rate constant, k_p , of σ_{ex} -induced rupture of dioleoylphosphatidylcholine (DOPC)-GUVs using the method developed by Yamazaki and colleagues recently (Levadny et al., *Langmuir*, 2013). I examined the effect of Π due to the initial difference in solute concentration between the inside and the outside of the GUV, ΔC^0 , (i.e., 2.8 mM). The external, constant tension, σ_{ex} , of the GUV membrane due to aspiration was 3.8 mN/m. For this purpose, GUVs containing 98.0 mM sucrose solution were transferred into a chamber containing 95.2 mM glucose solution and incubated the suspension for more than 5 min so that the swelling of the GUV attained equilibrium against Π . Next, a single GUV was held at the tip of a micropipette for 2 min using only slight aspiration pressure to provide a tension of 0.5

mN/m on the bilayer to eliminate the problems of the hidden area. Then the GUV was rapidly (~ 10 s) aspirated to obtain a tension of 3.8 mN/m. After a period of time, the GUV was suddenly aspirated into the micropipette. This sudden aspiration is due to a pore forming in the GUV membrane, causing rupture of the GUV and the GUV being completely aspirated into the micropipette due to the pressure difference between the inside and the outside of the micropipette. When I repeated the same experiment with 19 single GUVs ($n = 19$), I found that rupture of a GUV occurred stochastically at different times. The time-course of the fraction of intact (un-ruptured) GUVs among all the GUVs, $P_{\text{intact}}(t)$, was fit to a single exponential decay function, and this fitting provided a value of k_p . I made this experiment using different external, constant tension, σ_{ex} , and obtained the σ_{ex} dependence of k_p in GUVs under Π . By comparing this result with that in the absence of Π , I estimated the membrane tension due to Π at the swelling equilibrium, $\sigma_{\text{osm}}^{\text{eq}}$. I also developed a theory on the membrane tension of a GUV induced by Π , which provides the theoretical values of membrane tension at equilibrium quantitatively. The experimentally obtained values of $\sigma_{\text{osm}}^{\text{eq}}$ agreed with their theoretical values within the limits of the experimental errors.

Second, I measured the volume change of DOPC-GUVs under small Π . According to my theory on the membrane tension of a GUV induced by Π , if the change in the GUV radius induced by Π at equilibrium can be determined experimentally, the value of $\sigma_{\text{osm}}^{\text{eq}}$ can be estimated indirectly. For this purpose, I measured the volume change of a single DOPC-GUV upon exposure to Π using the method developed by Evans and colleagues. A single GUV containing 98.0 mM sucrose solution was held at the tip of a micropipette for a few minutes using only slight aspiration pressure in chamber A containing 98.0 mM glucose solution (the tension applied to the bilayer was 0.5 mN/m).

The GUV was then transferred to chamber B containing 96.0 mM glucose solution (i.e., $\Delta C^0 = 2.0$ mM). The projection length, ΔL , of the GUV inside the micropipette decreased with time and attained an equilibrium value, ΔL_{eq} , after 70 s. Using the theoretical equation, the volume change of the GUV, ΔV , was calculated to obtain the ratio of ΔV to its initial volume, V_0 , i.e., $\Delta V/V_0$. The experimental data indicate that $\Delta V/V_0$ increased with time to an equilibrium value, $\Delta V_{eq}/V_0$. The values of $\Delta V_{eq}/V_0$ allow us to experimentally estimate the membrane tension due to Π at swelling equilibrium of the GUV, $(\sigma_{osm}^{eq})^{ex}$, which agreed with their theoretical values within the limits of the experimental errors.

Third, I investigated Π -induced pore formation in a GUV to compare it with the constant tension-induced pore formation where the tension in the GUV membrane is produced by an external force. For this purpose, I initially held a single GUV containing 98.0 mM sucrose solution in chamber A containing 98.0 mM glucose solution at the tip of a micropipette for a few minutes using only slight aspiration pressure. The tension on the bilayer was 0.5 mN/m. The GUV was then transferred into chamber B containing 90.0 mM glucose solution ($\Delta C^0 = 8.0$ mM), the suction pressure was decreased, and the GUV was released into the hypotonic solution in chamber B. The GUV was continuously observed after release. Phase contrast images of the response of a GUV showed that the diameter of the GUV in chamber B at 31.033 s after transfer into chamber B was larger than its diameter in chamber A in the absence of Π . Rapid leakage of sucrose solution was observed at 31.066 s and consequently the diameter of the GUV rapidly decreased in 33 ms (i.e., from 31.033 s to 31.066 s), and then after 31.099 s it gradually increased, indicating that the leakage of sucrose stopped at 31.099 s. The phase contrast of the GUV did not decrease significantly due to this leakage.

These results can be considered as follows. First, sudden pore formation occurred in the GUV membrane due to the swelling of GUV due to Π , inducing a transient leakage of a small amount of sucrose solution. Thus, the diameter of the GUV rapidly decreased, then the leakage stopped within 99 ms after pore formation, indicating the resealing of the GUV membrane. Then the GUV volume increased again due to Π . The time required for closure of the pore (here 99 ms) was determined by observation of visible sucrose leakage. I investigated the effect of ΔC^0 on the rate of Π -induced pore formation in lipid membranes. I could not obtain the rate constant of Π -induced pore formation, which is different from the σ_{ex} -induced rupture of GUVs. However, I was able to use one measure of this rate, namely, the fraction of GUVs in which transient leakage occurred during the first 6 min among all the examined GUVs, P_{Leak} (360 s). P_{Leak} (360 s) was negligible (≤ 0.12) at $\Delta C^\bullet \leq 3.7$ mM, but at $\Delta C^\bullet \geq 4.2$ mM, P_{Leak} (360 s) increased with increasing ΔC^\bullet and reached 1.0 at $\Delta C^\bullet = 6.5$ mM. This result indicates that the rate of Π -induced pore formation increased with an increase in ΔC^\bullet , i.e., Π . After conversion of ΔC^\bullet to $(\sigma_{\text{osm}}^{\text{eq}})^{\text{th}}$ using my theory, I obtained the dependence of P_{Leak} (360 s) on $(\sigma_{\text{osm}}^{\text{eq}})^{\text{th}}$. At $(\sigma_{\text{osm}}^{\text{eq}})^{\text{th}} \leq 5.3$ mN/m, P_{Leak} (360 s) was negligible (≤ 0.12). At $(\sigma_{\text{osm}}^{\text{eq}})^{\text{th}} = 6.0$ mN/m, P_{Leak} (360 s) became significant ($= 0.31$), and at $(\sigma_{\text{osm}}^{\text{eq}})^{\text{th}} \geq 6.0$ mN/m, P_{Leak} (360 s) increased with $(\sigma_{\text{osm}}^{\text{eq}})^{\text{th}}$. This behavior is similar to the σ_{ex} -induced rupture of DOPC-GUVs. The $\sigma_{\text{osm}}^{\text{eq}}$ corresponding to the threshold Π to induce pore formation is similar to the threshold tension of the σ_{ex} -induced rupture.

Next, I measured quantitatively the time courses of the volume change (or radius change) of GUVs in Π -induced pore formation using the micropipette method, and observed two different time courses of the volume change. In these experiments, there

are two origins inducing membrane tension (σ_{ex} and $\sigma_{\text{osm}}^{\text{eq}}$); consequently, $\sigma_{\text{t}} = \sigma_{\text{ex}} + \sigma_{\text{osm}}^{\text{eq}}$. The time course of the radius change of GUVs in the Π -induced pore formation depends on the total membrane tension, σ_{t} . For relatively small σ_{t} , the radius of the GUV increased to an equilibrium value, r_{eq} , which remained constant for a long time, indicating that σ_{t} also remained constant for a long time after the swelling equilibrium, and then suddenly the GUV ruptured. When I observed many GUVs under the same conditions, it is clear that the rupture of GUVs occurred stochastically. These experimental results are very similar to the phenomenon observed in the σ_{ex} -induced rupture of a GUV. In contrast, for relatively large σ_{t} , the radius of a GUV increased with time and the rupture of the GUV occurred at various values of $\Delta r/r_0$ (0.0043–0.010) before reaching the swelling equilibrium. In this case, $(\sigma_{\text{osm}}^{\text{eq}})^{\text{th}} = 5.8$ mN/m, and hence the theoretical value of $\sigma_{\text{t}} = \sigma_{\text{ex}} + \sigma_{\text{osm}}^{\text{eq}} = 5.0 + 5.8 = 10.8$ mN/m at swelling equilibrium, which is much larger than the σ_{ex} values in the σ_{ex} -induced rupture of DOPC-GUVs. Therefore, rupture occurred before the membrane tension of a GUV reached equilibrium. In this situation, the membrane tension increased with time because the radius of the GUV increased with time and then pore formation occurred suddenly. Membrane tension at the time of rupture of the GUV varied widely, similar to tension-induced rupture when tension changed with time.

It is noteworthy to consider the dependence of Π -induced tension, $\sigma_{\text{osm}}^{\text{eq}}$, on the radius of the vesicles. If the values of the parameters other than the radius are the same, $\sigma_{\text{osm}}^{\text{eq}}$ is proportional to the radius of the vesicles when ΔC^{eq} is the same. For example, $\sigma_{\text{osm}}^{\text{eq}}$ for GUVs with a radius of 10 μm is 100 times larger than $\sigma_{\text{osm}}^{\text{eq}}$ for LUVs with a radius of 100 nm. However, for GUVs, ΔC^{eq} is much smaller than that ΔC^0 , in contrast

to LUVs, where $\Delta C^{\text{eq}} \approx \Delta C^0$. For example, at $C_{\text{in}}^0 = 98.0 \text{ mM}$ and $C_{\text{out}} = 96.0 \text{ mM}$ (hence $\Delta C^0 = 2.0 \text{ mM}$), ΔC^{eq} for GUVs with a radius of $10 \text{ }\mu\text{m}$ is 0.2 mM , but ΔC^{eq} for LUVs with a radius of 100 nm is 1.9 mM (as estimated theoretically). The final size dependence of $\sigma_{\text{osm}}^{\text{eq}}$ is determined by $\Delta r_{\text{eq}}/r_0$ according to the theory. Under the above concentration conditions, $\Delta r_{\text{eq}}/r_0$ for GUVs with a radius of $10 \text{ }\mu\text{m}$ is 6.2×10^{-3} , which is 13 times larger than that for LUVs with a radius of 100 nm ($= 4.9 \times 10^{-4}$), and hence $\sigma_{\text{osm}}^{\text{eq}}$ for the GUV is 13 times larger than $\sigma_{\text{osm}}^{\text{eq}}$ for the LUV. To validate this theory on the radius dependence of $\sigma_{\text{osm}}^{\text{eq}}$, I measured a change in a physical property of lipid membranes induced by Π . It is recently reported that the membrane stretching due to lateral tension increases the fluidity of lipid membranes and diffusion coefficient of lipid molecules. To monitor the fluidity of the membranes, I used a generalized emission polarization value (GP) of Laurdan in membranes because it is generally considered that with an increase in fluidity of lipid membranes the interaction of water molecules with a Laurdan molecule in the membrane interface increases, which decreases the GP value. Both GP values for DOPC-LUVs and DOPC-GUVs decreased with an increase in ΔC^0 , indicating that the fluidity of the membranes of both vesicles increased with Π , but to induce similar decrements of the GP values ~ 10 times higher ΔC^0 were required for DOPC-LUVs compared with DOPC-GUVs. These results suggest that the stretching of the membranes due to lateral tension increased with an increase in Π , but to induce similar amounts of stretching, ~ 10 times higher ΔC^0 were required for DOPC-LUVs compared with DOPC-GUVs. After converting ΔC^0 to $\sigma_{\text{osm}}^{\text{eq}}$, I found that the GP values were essentially the same in DOPC-GUVs and DOPC-LUVs at the same $\sigma_{\text{osm}}^{\text{eq}}$. This result supports the theory on the radius dependence of $\sigma_{\text{osm}}^{\text{eq}}$.

In this chapter, I succeeded in determining the membrane tension, $\sigma_{\text{osm}}^{\text{eq}}$, of

DOPC-GUVs under osmotic pressure (Π) experimentally for the first time by analyzing the effects of Π on the constant tension-induced rupture of GUVs. I also estimated $\sigma_{\text{osm}}^{\text{eq}}$ by the analysis of the volume change of the GUVs under small Π . These experimentally estimated values of $\sigma_{\text{osm}}^{\text{eq}}$ agreed with their theoretical values within the limits of their experimental errors. The total tension due to $\sigma_{\text{osm}}^{\text{eq}}$ and the external tension determine the response of GUVs: at lower total tensions, the radius of the GUVs increased to an equilibrium value, r_{eq} , which remained constant for a long time, and then stochastic rupture of the GUV occurred, as is the case for the constant tension-induced rupture of GUVs. These results provide quantitative information on membrane tension due to Π , which is valuable for research on the effects of Π on the activities of membrane proteins and membrane active peptides.

(Chapter 3) I investigated the effect of tension on the water permeability in lipid bilayer to understand the effect of osmotic pressure on water permeability. In the experiment of osmotic pressure-induced volume change of a GUV, the membrane tension of the GUV continuously changes because the volume of a GUV continuously increases. Hence, water permeability may change continuously. To overcome the problem of change in tension, a very small Π was used, so that the contribution of tension due to Π becomes small in the total tension. Various constant tensions were applied on the membrane of the DOPC-GUV by the micropipette aspiration method in the presence of small Π , then the volume change of the GUV was measured in the presence of small Π . By analyzing the time course of the volume change, the water permeability in the GUV membrane was obtained.

(Chapter 4) The overall conclusions in the total thesis were discussed.