Translocation of a vesicle through a narrow pore

著者	KHUNPETCH Petch
number	82
学位授与機関	Tohoku University
学位授与番号	理博第3153号
URL	http://hdl.handle.net/10097/00123956

（別紙様式５）

論 文 内 容 要 旨

(NO. 1)

氏 名	Petch Khunpetch	提出年	平成 30 年
学位論文の題目	Translocation of a vesicle through a narrow pore （ベシクルの細孔透過過程）		

論 文 目 次

page

（別紙様式５）

(NO. 2)

Abstract

Introduction

Vesicles are considered as one of the main components of life. Vesicles consist of a bilayer membrane of amphiphilic molecules that are composed of a “water-loving (polar)” head-group and a “water-avoiding” hydrocarbon chains. Amphiphilic lipid molecules play a role in the evolution from molecular assembly to cellular life. Soap and detergent are common amphiphilic substances that we can see in everyday life. Due to hydrophobic interactions of the hydrocarbon chains, the amphiphilic molecules start to assemble spontaneously into two layers held together by non-covalent forces when the concentration in aqueous solution is high enough. In order to avoid the interaction with water at the edge of the membrane, the bilayer forms a closed surface called a vesicle. Even though the membrane of vesicle has a thickness of typically a few nanometers, the size of vesicle can be in the order of up to 100 micrometers. Due to external conditions lipid bilayer vesicles show a wide variety of shape deformation. Their remarkably flexible surfaces attract researchers for many decades.

By using laser tweezers, the dynamics of lipid bilayer vesicles induced by changing external parameters becomes accessible. Considering bilayer membranes as two-dimensional surfaces embedded in three-dimensional space, the theoretical description on this mesoscopic length scales is introduced

independently more than forty years ago in three seminal papers [1], [2], [3]. The bilayer configurations are fundamentally determined by bending elasticity. This fundamental property is the reason why there is a wide variety of non-spherical shapes of vesicles. Bending elasticity not only causes a large variety of vesicle shapes, but also leads to various dynamical properties. The dynamical aspect of vesicles, especially, the translocation through a small hole due to external driving forces is an interesting problem. The mechanism is relevant to, for example, many transdermal applications [4], [5], transendothelial migration in immune system [6] and drug delivery in pharmacological research [7], [8], [9]. The translocation phenomena of a vesicle through a small hole are the subject matter of this research. The interesting question is that how the effectiveness of such filtration process depends on various parameters of the system such as the driving forces, bending and stretching moduli of the vesicle, the initial size of the vesicle, and geometry and size of the pore.

In this research, we use Onsager variational principle to study translocation phenomena of a vesicle through a pore. We are interested in two problems of such a kind of this translocation process. First, we investigate translocation process of a vesicle through a hole in a solid membrane separating two chambers, where we focus on the kinetic pathway of the translocation. Onsager principle can give us the kinetic pathways of the state changes. On the other hand, the minimum energy paths or the reaction paths can be obtained by employing the string method. Our main purpose is to discuss the paths obtained from these two different methods when the friction parameter changes.

Recently, Sakuma and Imai [10] established a temperature-controlled cyclic self-reproducing vesicle system without feeding. The characteristic feature of their system is that the vesicle composed of cylinder-shaped lipids [1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)] and inverse-cone-shaped lipids [1, 2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE)]. The vesicle forms an inclusion vesicle called daughter vesicle inside the mother vesicle and then the daughter vesicle is expelled through the small pore on the mother vesicle. In this second problem, we present theoretical model on the birthing process of a single, rigid daughter vesicle through a pore. By using a simple geometric picture, we derive the free energy constituting the material properties of the mother vesicle, i.e., bending, stretching and line tension moduli, as functions of the distance between centers of the daughter and mother vesicles. We see clearly the disappearance of the energy barrier by selecting appropriate moduli. The dynamics of the system is studied by employing the Onsager principle. The results indicate that translocation time decreases as the friction parameter decreases, or the initial size of the daughter vesicle decreases.

Translocation of a vesicle through a narrow hole across a membrane [11]

We have used Onsager principle to investigate the translocation process of a vesicle through a narrow hole across a membrane. The equations of motion for the translocating vesicle are derived by choosing the appropriate slow variables. The potential energy is calculated by considering the stretching energy of the translocating vesicle and the pressure difference across the membrane. There is a free energy barrier for translocation process of a vesicle through a narrow hole due to stretching of the vesicle. In the absence of the pressure difference, the free energy landscape is symmetric around the translocation coordinate and its magnitude depends on the initial size of the vesicle. The height of the barrier can be decreased by applying the pressure gradient across the membrane. By increasing the pressure difference or decreasing the initial size of the vesicle, the translocation time becomes shorter.

The kinetic paths obtained from the Onsager principle are independent of the friction coefficients when the pressure difference is small and those paths are close to the reaction paths (the minimum energy paths) obtained from the string method. On the other hand, they are significantly different from each other if the pressure difference is large. Because the pressure exerted on the liquid flow induces a quick flow of the liquid compared to the diffusion of the surfactant molecules, the diffusion flow of the liquid volume inside the vesicle is much faster than the migration of the number of the surfactant molecules. This is the reason why we have the difference between the result from the string method and the kinetic paths obtained from the Onsager principle. This means that we have already shown that Onsager principle is an extension of the conventional string method when the external driving force is large.

In our model, we have considered the hole on a rigid membrane wall as a circular hole with fixed radius. However, in real physical system e.g. translocation of a white blood cell through a hole on blood vessel wall (transcellular diapedesis), pore can change its size during the translocation process. In such a case, our model needs to be extended by introducing one more variable i.e. size of the pore. In the present work, however, we neglected such a degree of freedom, and predicted the dependencies of translocation kinetics of a single vesicle on its stretching property and size and on the applied pressure difference. We hope that our simple model would stimulate experiments on the study of translocation of vesicle through a narrow hole.

Birthing of a Daughter Vesicle in Self-reproducing Vesicle System

We have used a simple geometric ansatz to study the birthing of the daughter vesicle in self-reproducing vesicle system. Onsager principle is used to derive the equation of motion for the birthing vesicle in terms of the bending, stretching and line tension moduli of the mother vesicle, and the size of the daughter vesicle, and the distance between the centers of the daughter and mother vesicles. The deformation of the mother vesicle is treated within the Helfrich free energy formalism and the stretching energy plays a role as a driving force for the system.

The derived free energy suggested that changing the stretching and line tension moduli affects the free energy landscapes clearly, while changing the bending modulus does not affect much the energy landscapes. This suggested that the system is mainly governed by the competition between the stretching and line tension energies. Adding more water into the mother vesicle will give an increase in the free energy and a decrease in the energy barrier. Because of the competition between the stretching and line tension energies, adding water up to some certain amount causes the dip in the free energy landscape. Further adding more water, the metastable state occurs which means the daughter vesicle is trapped and the birthing process is not completed. The translocation time decreases when the friction caused by the hole against the motion of the daughter vesicle decreases. Our results also suggest that when the daughter vesicle is large, the mother vesicle feels more uncomfortable. Consequently, the large daughter vesicle is then expelled with higher speed compared to the case of small daughter vesicle. However, the high speed will be decreased by the effect of barrier from the line tension energy.

In our model, the specific details regarding the chemical properties of the vesicle are parametrized in terms of the material parameters such as bending, stretching, and line tension moduli for the purpose of investigating the large scale properties of the birthing process. From the experimental point of view, measuring the bending, stretching, and line tension moduli of the membrane directly is rather difficult. However, experimentalists could compare their experimental data to our predicted results to estimate the value of the moduli.

Conclusions

In this research, we study translocation phenomena of a vesicle through a narrow pore by using the Onsager principle. The first problem is the translocation of a vesicle through a hole in a solid membrane separating two chambers. We found that the translocation time decreases as the pressure difference across the membrane increases, or the initial size of the vesicle decreases as expected. The reaction paths obtained from the string method and the actual kinetic paths obtained from the Onsager principle are significantly different from each other if the external driving force is large. This suggests that the Onsager principle is an extension of the string method at large external driving force. For the second problem, we investigate the birthing process of a daughter vesicle in self-reproducing vesicle system. The equation of motion for the daughter vesicle is derived by using the Onsager principle. We found that the translocation time decreases when the friction caused by the hole against the motion of the daughter vesicle decreases. When the daughter vesicle is large, the mother vesicle feels more uncomfortable. Consequently, the large daughter vesicle is then expelled with higher speed compared to the case of small daughter vesicle.

References

[1] P. B. Canham, J. theoret. Biol. **26**, 61 (1970).
[2] W. Helfrich, Z. Naturforsch. C **28**, 693 (1973).
[3] E. Evans, Biophys. J. **14**, 923 (1974).

[4] M. M. Mezei and V. Gulasekharam, Life Sci. **26**, 1473 (1980).

[5] C. Wachter, U. Vierl and G. Cevc, J. Drug. Targeting **16**, 611 (2008).

[6] D. Vestweber, Nat. Rev. Immunol. **15**, 692 (2015).

[7] M. R. Prausnitz, S. Mitragotri, and R. Langer, Nat. Rev. Drug Discov. **3**, 115 (2004).

[8] T. Gratieri, I. Alberti, M. Lapteva, and Y. N. Kalia, Eur. J. Pharm. Sci. **50**, 609 (2013).

[9] X. Gao and F. C. Szoka, Acc. Chem. Res. **36**, 335 (2003).

[10] Y. Sakuma and M. Imai, Phys. Rev. Lett. **107**, 198101 (2011).

[11] P. Khunpetch, X. Man, T. Kawakatsu, and M. Doi, J. Chem. Phys. **148**, 134901 (2018).

別　紙

論文審査の結果の要旨

細胞膜は、細胞内の環境を一定に保つ容器としての機能のみならず、細胞の移動のための機能も併せ持っている。細胞の移動が関与する現象の例として、腎臓における血液の濾過過程や、細菌の感染部位における白血球の血管内から血管外への移動、細胞分裂の際の細胞の分離過程など、空間的に制限された領域内で細胞が移動する現象が挙げられる。このような生体膜の変形や流動を伴う力学的な現象は、生物物理学の研究対象として関心を集めている。

Khunpetch 氏提出の博士論文では、ベシクルと呼ばれる閉じた膜構造が小さな穴を通って移動する過程を、統計物理学の立場から調べた。粗視化された理論モデルとして、膜の表面張力および曲げ弾性エネルギーにより膜の変形を表す Helfrich の弾性膜モデルと、Onsager の変分原理による散逸動力学を組み合わせたモデルを構築した。解析の対象とした現象は (1) 平板状の隔壁に開けられた小孔をベシクルが透過する過程および (2) 球状のベシクルの内部から表面の穴を通して別の球状のベシクルが排出される過程の２つである。

論文の第１部では (1) の過程について解析した。圧力差によって隔壁の一方から他方に透過するベシクルの動力学を、膜を構成するリン脂質分子の透過量とベシクルに内包される水の透過量の２つを独立変数とする相空間において記述した。その結果、圧力差が大きい場合にはリン脂質と水の粘性の差によって、自由エネルギー曲面の最急勾配に従う経路から遠く離れた経路を通ることが示された。これは、従来の自由エネルギー曲面を用いた静的な運動経路の記述が必ずしも正しくなく、運動方程式を用いた動的な記述が必要であることを示す。

論文の第２部では (2) の過程を考察した。この過程は細胞の自己増殖のモデルとして注目されている。膜の表面張力エネルギーと穴の周囲の線張力エネルギーの競合によって、ベシクルの透過の動力学が準安定状態に捕らわれるなどの非単調なふるまいを示すことが示された。また、モデル計算と実験結果を比較することで直接測定が困難なベシクルの物質定数を推定するための処方箋が示された。

これらの成果は、細胞の機能を物理学の観点から解析した研究として先駆的なものであり、生物物理学における基礎研究として有用かつ価値あるものであると認められる。これは申請者が自立して研究活動を行うに必要な高度の研究能力と学識を有していることを示しており、Khunpetch 氏提出の博士論文は合格であると認める。