PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Physical and Mathematical Sciences 2009, № 1, p. 49–53

Physics

THE KINETICS OF VESICLES SWELLING IN PRESENCE OF TRANSMEMBRANE DIFFERENCE OF POTENTIALS

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It is shown, that the transmembrane difference of potentials applied to vesicles causes the decrease of the swelling time. Vesicle swelling time exponentially decreases with the increase of the potential. The swelling time of vesicle is greatly increased with the increase of work for the porous perimeter unit formation.

***Keywords*:** vesicles, kinetic swelling, transmembrane potentials

Introduction. Osmotic phenomena play an essential role in many cell processes. For instance, osmosis is directly linked with hormone excretion and secretion [1, 2]. Proteins eject from the cell limits as a result of swelling and lysis of chromaffin granules [3]. It should be noted too that serotonin secretion from human thrombocytes occurs as a result of osmotic lysis of thrombocytes [4]. Despite the fact that osmosis underlies a whole range of physiological processes, the phenomenon itself has not exhaustively been studied yet, and especially in presence of transmembrane potential. Works covering the physical mechanism of cell lysis are still scarce [5–8]. However, it is quite obvious that the lack of a detailed analysis of such a mechanism hampers understanding of normal and pathological running regimes of the noted processes. An essential stage of the cell lysis is its swelling. In a living cell swelling is rather a complex process, so it makes it reasonable to study the process, using a model system a lipid vesicle. Wholly, in broader terms the obtained results can be extrapolated to real cell systems. The work [7] highlights a study of vesicle swelling process, narrowing however to a case with lacking transmembrane potential difference. As some transmembrane potential difference is common to the cell membrane, so it poses the interest to reveal the way such potential difference impacts the lipid vesicle swelling. It is the issue, which this work focuses on.

Theoretical part. Similarly to [6, 9] let's consider a vesicle, inside of which the concentration of osmotically active substance (OAS) c_{in} is higher than outside

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c_{out}. Osmotic pressure is equal to $\Delta\pi_{osm} = \alpha\Delta c$, where $\alpha = RT$ is the product of gas constant and absolute temperature, and Δc is the transmembrane potential difference of OAS concentrations. Under the impact of osmotic pressure the water pumps into the vesicle. This will lead to expansion of the membrane. As a result, in the membrane the mechanic pressure ΔP will be brought about, inducing in turns in vesicle pressure excessive vs. the outside medium and thus preventing the osmotic water flow movement into the vesicle. As soon as the ΔP pressure reaches the osmotic pressure, the water flow will stop and the membrane will expand, i.e. the membrane surface area will increase by ΔS vs. its equilibrium area S_0. As proved by works [6, 9], in the case the extension of the area oversteps a critical value ΔS^*, quasi-equilibrium pores can originate on the membrane. Such a fact supports a necessity to consider 2 swelling regimes. Let's begin with a variant providing a transmembrane potential difference of OAS concentrations is low, and osmotic water flow stops moving into the vesicle before elongation reaches the critical value. In such a case, a temporal change in the volume of the vesicle resulted from the osmotic water flow penetration into the vesicle, is preassigned by equation [10]

$$\frac{d\Delta V}{dt} = L_p S(\sigma\Delta\pi_{osm} - \Delta P), \qquad (1)$$

where L_p is a membrane permeability factor for water, S is the vesicle area, σ is a reflection factor. Equation (1) allows to easily determine the relationship between the change in the volume and the time. Considering that $\Delta P = 4Y_{||}h\Delta S/(R_0 S_0)$ ($Y_{||}$ is Young's modulus, h is the membrane thickness, R_0 is the radius of the equilibrium vesicle) and expressing a relative change in the area by a relative change in the volume, one can derive $\Delta P = 8Y_{||}h\Delta V/(3R_0 V_0)$. Then substituting the expanded values $\Delta\pi_{osm}$ and ΔP into (1) and considering that at the initial moment $\Delta V = 0$ at $\sigma = 1$, one can derive the vesicle swelling kinetics [6]:

$$\Delta V = \Delta V_{\max}\left(1 - \exp\left(-\frac{t}{\tau_0}\right)\right), \qquad (2)$$

where $\Delta V_{\max} = \pi R_0^4 \alpha\Delta c/(2Y_{||}h)$ is the maximal excessive volume, $\tau_0 = \dfrac{R_0^2}{8L_p Y_{||}h}$ is a defined swelling time. Let's consider a variant providing a transmembrane potential difference of OAS is high and the excessive volume ΔV oversteps a critical value ΔV^*, at which a pore can originate on the membrane. For further analysis let's divide a time interval between the start of swelling and the pore origination moment into 2 stages. The first stage covers swelling up to a moment τ_1, when the excessive pressure value is low vs. a critical value $\Delta V < \Delta V^*$. The second stage lasts until prevalence of the excessive volume over the critical one is reached to the moment of a real pore origination on the membrane. In the case the rupture occurs prior to a defined vesicle swelling time, then τ_1 can be determined from the equation (1) substituting it into a right side of critical value ΔV^***:**

$$\tau_1 = -\tau_0 \ln\left(1 - \frac{\Delta V^*}{\Delta V_{\max}}\right). \tag{3}$$

To determine ΔV^* we use the result of the work [9], which proves that

$$\frac{\Delta S^*}{S_0} = \frac{3}{4}\left(\frac{\gamma}{Y_{\|} h R_0}\right)^{1/3} - \frac{C\varphi^2}{4Y_{\|}h}, \tag{4}$$

$$C = C_0\left(\frac{\varepsilon_s}{\varepsilon_m} - 1\right),$$

where $\gamma = \sigma_p h$ is a linear pore tension coefficient, σ_p is the energy, needed for the cylindrical pore area unit formation, $\tilde{N}_0$ is the electrical capacity of the membrane area unit, ε_s and ε_m are dielectric permittivity of the solution and the membrane respectively. Expressing a relative change in the volume by a relative change in the area, and considering that $\Delta V^* < \Delta V_{\max}$, we derive the ultimate expression from (3) to evaluate τ_1 as

$$\tau_1 = \frac{3}{8L_p\alpha\Delta c}\left(\frac{\sigma_p^2 R_0}{Y_{\|}^2}\right)^{1/3} - \frac{R_0 C\varphi^2}{8Y_{\|}hL_p\alpha\Delta c}. \tag{5}$$

To determine the average time for the second stage of τ_2 let's at first define the energetic barrier of pore formation. Using the result of the work [9], which gives calculation of free energy of the elongated vesicle membrane (Φ), on which membrane one detects a transmembrane potential difference and a transverse cylindrical pore of radius r. From equation (4) of the work [9] for major elongation cases derived can be

$$\Phi = 2\pi r h\sigma_p - \pi r^2\left(\frac{C\varphi^2}{2} + 2Y_{\|}h\frac{\Delta S}{S_0}\right) + \frac{Y_{\|}h(\Delta S)^2}{S_0}. \tag{6}$$

Considering that the membrane elongation is equal to $\sigma = 2Y_{\|}h\dfrac{\Delta S}{S_0}$.

Let's re-write the expression (6) as

$$\Phi = 2\pi\gamma r - \pi r^2\left(\frac{C\varphi^2}{2} + \sigma\right) + \frac{Y_{\|}h(\Delta S)^2}{S_0}, \tag{6a}$$

where $\sigma_p h = \gamma$. The analysis of (6a) indicates, that the swelling time at the 2nd stage is equal to the average time, required for overcoming the energetic barrier equal to the difference between energy values Φ in max. point and in point $r = 0$ in the absence of the pore. (6a) allows to easily determine the critical radius of pore r_* and the height of the energetic barrier Φ_* equal to

$$r_* = \gamma \Big/ \left(\sigma + \frac{C\varphi^2}{2}\right), \tag{7}$$

$$\Phi_* = \pi\gamma^2 \Big/ \left(\sigma + \frac{C\varphi^2}{2}\right). \tag{8}$$

The average time for the second stage of τ_2 poses the average time the radius first reaches the critical value pore. Let's consider a random walk of radius r in radius-dimensional space between its values 0 and r_*. The edge 0 is reflective, and edge r_* is absorptive. Let's assume the random walk of radius as walk of a particle between edges 0 and r_*. Introduce a probability function $F(t,r)$, of that a particle in point r at the moment t will reach the edge r_* for the first time. A differential equation in partial derivatives that describes a temporal change in function $F(t,r)$ is as follows [11]

$$\frac{\partial F}{\partial t} = D\frac{\partial^2 F}{\partial^2 r} - \frac{D}{k_B T}\cdot\frac{\partial \Phi}{\partial r}\cdot\frac{\partial F}{\partial r}, \tag{9}$$

where k_B is Boltzmann's constant, T is the absolute temperature, D is the coefficient of pore diffusion in the radius space. Initial and boundary conditions of equation (9) are as follows

$$F(0,r) = 0,\ 0 < r < r_*\ ,$$
$$F(t,r_*) = 1,\quad \left(\frac{\partial F(t,r)}{\partial r}\right)_{r=0} = 0. \tag{10}$$

From (9) equation can be derived for the average time of reaching a critical-size radius:

$$D\frac{\partial^2 \tau_2}{\partial^2 r} - \frac{D}{k_B T}\cdot\frac{\partial \Phi}{\partial r}\cdot\frac{\partial \tau_2}{\partial r} = -1. \tag{11}$$

Boundary conditions to equation (10) are

$$\tau_2(r_*) = 0,\ \left(\frac{\partial \tau(r)}{\partial r}\right)_{r=0} = 0. \tag{12}$$

Resolving equation (11) allows deriving the following expression for average time of the 2nd stage of τ_2:

$$\tau_2 = \frac{1}{D}\int_0^{r_*} \exp(\Phi(r)/(k_B T))\left(\int_0^r \exp(-\Phi(r')/(k_B T))dr'\right)dr. \tag{13}$$

Substituting free energy from (6a) into (13) and performing easy calculations allows derivation of the following approximate expression for average time of the second stage in form of

$$\tau_2 = \frac{(k_B T)^{3/2}}{4\pi D\sigma_p h\left(2Y_{\|}\cdot h\frac{\Delta S}{S_0} + \frac{C\varphi^2}{2}\right)^{1/2}}\cdot\exp\left(\frac{\pi(\sigma_p h)^2}{\left(2Y_{\|}\cdot h\frac{\Delta S}{S_0} + \frac{C\varphi^2}{2}\right)k_B T}\right). \tag{14}$$

Results and discussion. As seen from (5), the more the difference of OAS concentrations, the less τ_1. (5) also demonstrates that the presence of a trans-membrane potential difference on the vesicle leads to reduction of τ_1. The analysis (14) indicates that the average time of the second stage of τ_2 decreases in the presence of a transmembrane difference of potentials on the vesicle. The increase

in potential on the membrane induces a drastic reduction of time. As seen from (14), the average time of the second stage of τ_2 exponentially depends on the work of formation of a pore perimeter unit $\sigma_p h = \gamma$. With the increase in γ the average time of the second stage drastically increases. (14) indicates too that with the increase in the membrane elongation the average time of the second stage decreases.

Thus, this work proves that the presence of a transmembrane difference of potentials on the vesicle simplifies the pore formation process in the membrane. Sharp changes take place in critical elongation of the membrane, in pressure differential and critical difference of OAS at which the pore form. As indicated, the minimal pore radius is independent of a transmembrane potential difference.

Received 20.10.2008

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Proc. of the Yerevan State Univ. Phys. and Mathem. Sci., 2009, № 1, p. 49–53.

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Վեզիկուլների ուռչման կինետիկան պոտենցիալների անդրթաղանթային տարբերության առկայությամբ

Ցույց է տրված, որ վեզիկուլի վրա պոտենցիալների անդրթաղանթային տարբերության առկայությունը հանգեցնում է վեզիկուլի ուռչման ժամանակի փոքրաց-ման: Պոտենցիալի մեծացմանը զուգընթաց ուռչման ժամանակը էքսպոնենտային կերպով նվազում է: Ցույց է տրված նաև, որ վեզիկուլի ուռչման ժամանակը կտրուկ մեծանում է միավոր պարագծով ծակոտու առաջացման աշխատանքի աճին զուգընթաց:

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Кинетика набухания везикул при наличии трансмембранной разности потенциалов

Показано, что наличие трансмембранной разности потенциалов на везикуле приводит к уменьшению времени ее набухания. С увеличением потенциала время набухания везикулы экспоненциально уменьшается. Показано также, что время набухания везикулы резко увеличивается с ростом работы образования единицы периметра поры.