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Deformation of giant unilamellar vesicles under osmotic stress

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ABSTRACT

Biological membrane plays an important role in maintaining an osmotic equilibrium between the cytoplasm and the extracellular solution of cells. Here, the giant unilamellar vesicles (GUVs) as cell models were used to investigate the effect of osmotic stress on phospholipid membranes. The deformation of GUVs, including inward budding and outward budding, was systematically investigated by the osmotic press from glucose, sucrose, LiCl, and KCl solutions. The permeability (*P*) of DMPC, DMPC/10 mol% Chol GUVs, DMPC/25 mol% Chol GUVs, and DMPC/40 mol% Chol GUVs in glucose, sucrose, LiCl, and KCl solutions were all obtained. The *P* value decreases with the addition of more cholesterol in the bilayer. The monovalent cations caused higher permeability of lipid bilayer membranes due to their combination with phospholipids. The molar flux of water (*J*) value was found to be the key factor for determining the deformation state from mainly inward budding to mainly outward budding. The findings in this paper may help us to understand cell transformation triggered with osmotic stress.

1. Introduction

Biological membranes consist mainly of lipid bilayers and proteins. Giant unilamellar vesicles (GUVs) with different lipid compositions offer a simple and convenient experimental model for the study of important phenomena occurring in actual biological cells such as the permeability of the cytoplasmic membrane [1–3]. GUVs are self-assembled from amphiphilic phospholipid through simple rehydration or electroformation method [4–10]. The morphology, topology, and features of GUVs can change in response to various stimuli (pH [11], temperature [12], or osmotic stress [13,14]). It is important to understand the physicochemical mechanisms for the morphological changes in biological membrane structure in response to various internal and external stimuli because of the possible implications in membrane trafficking and endosomal systems [15].

Osmotic stress is one of the most important environmental factors involved in biological membrane homeostasis, which has been extensively used to induce the shape deformation of GUVs. They can shrink or swell due to the water penetration across the lipid bilayer membrane caused by the osmotic gradient. The water permeability through GUV membranes was investigated by the measurement of the size variation using microscopy [16]. Egg phosphatidylcholine (eggPC) vesicles were used to study the vesicle deformation and calculate the permeability *P* triggered by sucrose solution [16]. Triggered by sucrose and KCl solution vesicle showed several different types of deformation behavior, such as shrinking, opening hole, and keeping spherical shape [17]. The deformation of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) vesicles triggered by glucose solution exhibits shape transformations including prolate dumbbell shape, starfish-like shape, and tube shape [18]. In the most GUV deformation reports, they often focus on the phenomena. There is still no comprehensive study of the effect of the osmotic stress from different solution (electrolyte and nonelectrolyte solution) on GUV deformation in order to obtain the underlining decisive factors.

Herein, we investigated the GUV deformation triggered by osmotic stress from glucose, sucrose, LiCl and KCl solution respectively. The permeability *P* was calculated in different solution condition. Li^+ and K^+ caused higher permeability of lipid bilayer membranes due to their combination with phospholipids. The molar flux of water *J* was the key factor to determine the deformation of GUVs. The outcomes of this paper may help us to understand biophysics of cell membrane.

2. Materials and methods

2.1. Materials

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and cholesterol (Chol) were purchased from Avanti Polar Lipids (USA). Fluorescence-labelled 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) was obtained from

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Molecular Probes (Eugene, Oregon, US). Glucose, sucrose, LiCl, KCl, $MgCl_2$ and $CaCl_2$ were purchased from XiLong Chemicals (China). Glass slides coated with indium tin oxide (ITO, sheet resistance ≈8 to 12 Ω, thickness ≈160 nm) were purchased from Hangzhou Yuhong technology Co. Ltd (China). Chloroform and absolute ethanol were purchased from Sigma (China). Millipore Milli-Q water with a resistivity of 18.0 MΩ cm was used for solution preparation.

2.2. Preparation of GUVs

The GUVs were fabricated using electroformation method [4,19,20]. ITO-coated glass coverslips (25 mm × 45 mm) were cleaned in ethanol and water each for 15 min by sonication before being dried by N_2. Lipid solution (5 mg mL^{-1}) was composed of DMPC (or DMPC/Chol) and NBD-PE at a 99:1 M ratio. The lipid solution (7.5 μL) was deposited on ITO electrode surface using a needle to spread carefully back and forth 6 times, followed by drying under vacuum for 2 h. The coverslips were separated by a rectangular polytetrafluoroethylene (PTFE) spacer with a length, width and height of 35 mm, 25 mm and 2 mm, respectively. Subsequently, the AC-electric field (5 V, 10 Hz) was applied for 4 h at 40°C to generate GUVs.

2.3. Deformation of GUVs under osmotic stress

DMPC and DMPC/Chol (10%, 25%, and 40% Chol (mol%)) GUVs in pure water were produced as mentioned above for hypertonic condition study. The GUV solution and different osmotic stress (from −0.37 to −2.45 atm) salt solution (glucose, sucrose, LiCl, and KCl, 1/1, v/v) were mixed together at room temperature (25°C) for 5 min. The salt solution was prepared at room temperature and filtered on a calibrated 200 nm membrane before use. The morphology changes of GUVs were observed under a fluorescence microscope (Nikon 80i, Japan).

3. Results and discussion

To systematically investigate the deformation behaviour of GUVs under hypertonic conditions, pure DMPC GUVs were studied as a function of osmotic pressure. The osmotic stress gradient across a membrane is related to the difference between the osmolarity of the inner and outer vesicle media: $\Delta\Pi = RT(c_{int} - c_{ext}) = RT\Delta c$. At $\triangle\Pi$ = −0.37 atm, the outward budding phenomena were often observed as shown in Fig. 1a. While the vesicle in vesicle (VIV) structure were the main outcomes when $\triangle\Pi$ = −0.74 atm, as shown in Fig. 1b. The splitting cases were found when $\triangle\Pi$ is higher than −1.47 atm. In nature, Chol is heterogeneously distributed in cellular membranes, with the highest content found in the plasma membrane [21]. Its predominant characteristic is its ability to modulate the physicochemical properties, such as, fluidity [22] and permeability [23] of the cell membrane. GUVs composed of DMPC and Chol were investigated under the same osmotic pressure. It is found that with $\triangle\Pi$ of −0.37 atm the VIV structures (Fig. 1e, i, m) were the predominated morphology, rather than outward budding for the pure DMPC GUVs. At medium osmotic pressure conditions ($\triangle\Pi$ = −0.74 atm, −1.47 atm), multicompartmental structures were mainly found for all Chol containing DMPC GUVs, as shown in Fig. 1f, g, j, k, n, o. With higher osmotic pressure ($\triangle\Pi$ = −2.45 atm), vesicle splitting were found for DMPC GUVs containing 10, 25% Chol (Fig. 1h, l), while multicompartmental structures were remained with 40% Chol containing DMPC GUVs (Fig. 1p).

From above mentioned results, under hypertonic conditions GUVs underwent two types of deformation, i.e., inward budding and ourward budding. The images in Fig. 1 are the representative images of those experimental conditions. In reality the inward budding and outward budding both existed in the solution. In order to analyze the data in a statistical way, Q value was defined as $N_{Inward\ budding}/N_{Outward\ budding}$, where $N_{Inward\ budding}$ is the number of inward budding GUVs, and $N_{Outward\ budding}$ is the number of outward budding. When Q > 1, the inward budding GUVs were the majority of deformed GUVs in solution; when 0 < Q < 1, the outward budding GUVs were the predominated structures. When Q = 0, all GUVs deformed in an outward budding manner. By analyzing as least 200 GUVs at each osmotic pressure, Fig. 2a was obtained. It is noted that Q > 1 for DMPC when -$\triangle\Pi$ is in the range between 0.49 and 0.98 atm, which means that most DMPC GUVs deformed as inward budding way in this osmotic pressure range. The largest Q value peaked at 0.74 atm. The peak Q value shifts towards higher osmotic pressure when increasing the Chol percentage in GUVs. Another point is that the Q value is small than 1 only at higher osmotic end with the addition of Chol. The higher Chol percentage is, the higher osmotic pressure needs to cause Q value smaller than 1.

The main factor for GUV deformation under hypertonic conditions is molar flux of water (J) through the lipid bilayer membrane. The actual driving force behind the deformation of GUVs is the osmotic stress between inside and outside of GUVs. The water freely penetrate through the phospholipid membrane to balance the osmotic stress. The osmotic flow of water through a membrane is generally described by the formula (1) as shown in below:

$$J = -P \triangle c \tag{1}$$

where $\triangle c$ is the difference in molar concentrations between the internal and external solutions, and P is an important physical quantity to describe the diffusion rate of water through lipid bilayer membrane, which is equivalent to the molar flux of water J when the difference in molar concentrations between the internal and external solutions is 1 mol L^{-1}. In order to calculate P value for each type of GUV, the ratio of R/R_0 for each type GUV was monitored against time with $\triangle\Pi$ = −0.74 atm, as shown in Fig. 2b. R and R_0 are the radius of the deformed GUV at each time point and initial GUV respectively. The P value can be determined by below equation [16].

$$P = \frac{R_0}{v_m \triangle_c} \frac{d(\frac{R}{R_0})}{dt} \tag{2}$$

where v_m is the water molar volume (18.04 ml mol^{-1}). The P values were calculated to be 95.4 ± 3.5 μm s^{-1}, 83.9 ± 3.0 μm s^{-1}, 75.2 ± 4.4 μm s^{-1}, and 62.1 ± 4.1 μm s^{-1} for pure DMPC GUVs, DMPC/10 mol % Chol GUVs, DMPC/25 mol % Chol GUVs, and DMPC/40 mol % Chol GUVs, respectively. The addition of Chol in lipid bilayer decreased the membrane permeability, which is consistent with reported findings [24,25]. The bending energy (K_c) of DMPC, DMPC/10 mol % Chol, DMPC/25 mol % Chol, and DMPC/40 mol % Chol bilayer membrane were listed in Table 1. In theory, the bilayer membrane bearing smaller K_c should have bigger permeability, which is consistent with our calculated P value sequence for those 4 type lipid bilayer membranes.

The critical molar flux of water J_{crit} is defined as the threshold value for GUVs deforming from mainly inward budding to mainly outward budding, i.e., from Q > 1 to Q < 1. From Fig. 2a and P values, J_{crit} values for pure DMPC GUVs, DMPC/10 mol% Chol GUVs, DMPC/25 mol% Chol GUVs, and DMPC/40 mol% Chol GUVs are 3.821 mol $m^{-2}s^{-1}$, 7.166 mol m^{-2} s^{-1}, 7.529 mol m^{-2} s^{-1} and 9.334 mol m^{-2} s^{-1}, respectively. For the same phospholipid composition of GUVs, P value is the same. Osmotic stress gradient determines the J value. According to Eq. (1), J value is proportional to –ΔΠ. From our observations, the larger or smaller J tends to make the GUVs outward budding. The possible explanation for these phenomena is that phospholipid bilayer membrane tends to form positive curvature due to the fast water loss rate at higher J. It is well known that the positive curvature of membrane leads to outward budding, and negative curvature of membrane causes inward budding [27,28]. The very slow water loss rate (lower J value) may also tends to induce the positive curvature of membrane, consequently leads to outward budding. In the suitable J value range, the phospholipid bilayer membrane shrinks to generate