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Voltage-driven counting of phospholipid vesicles with nanopipettes by resistive-pulse principle☆

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ABSTRACT

We herein systematically investigate the voltage-driven counting of phospholipid vesicles with single conical nanopipettes by resistive-pulse principle in liquid phase. With increasing the positive bias voltage, the translocation-event frequency increases and the dwell time decreases. We analyze the effect of applied voltage on nanoparticle dynamics based on the calculation of transfer frequency and translocation velocity of the vesicle at the nanopipette orifice and find that the applied voltage shows an obvious influence on analytical performance and can serve as a useful parameter to optimizing the analytical performance of vesicles. This study demonstrates that the nanopipette could be used as an easily operated platform for vesicle counting.

1. Introduction

Quantification and characterization of vesicles have attracted increasing attention over the last two decades due to the crucial role in biological processes and applications, such as cellular transport, neurotransmission or drug delivery [1,2]. In comparison with traditional techniques for vesicle analysis, for instance transmission electron microscopy (TEM), electrochemical methods are able to achieve individual nanoparticles analysis in solution with higher time resolution and sensitivity [3–7]. Along with the development in nanometer scale machining technology, nanopore and/or nanochannel sensors based on the resistive-pulse principle have emerged as powerful techniques for single entity analysis both in fundamental studies and practical applications, which bears the capacity for simultaneously determining the size distribution, surface charge, and concentration of particles according to ionic current signal, and can achieve individual particle analysis in a solution phase without electrochemical probes [8–11]. In this case, various biological or solid-state nanopores and/or nanochannels have been developed for single entity analysis based on the resistive-pulse technique [12]. Unlike protein channels and those prepared from silicon-based materials or polymers, the nanopipette is fabricated with more flexibility, repeatability, stability and size tunability from borosilicate or quartz capillaries by applying heat to soften the capillary to obtain conical shape tips [13]. Hence, there has been growing interest for using nanopipettes to study various analytes because of their potential applications for single entity analysis. For example, nanopipettes have been used for the analysis of hard particles, such as polystyrene [14] or single molecules labeled with nanoparticles [15]. Moreover, for the soft particles (e.g., liposomes or vesicles), White et al. have reported the use of glass nanopore to investigate the pressure-driven translocation process of multilamellar liposomes, providing insight into the liposome temperature-dependent deformation property [16]. Although the possibility to detect vesicles with nanopipettes has been conducted before [17,18], either the translocation process of the vesicle from the nanopipette orifice outside to inside under only voltage drive or the role of applied voltage to the vesicle analysis has not been discussed in detail.

In this communication, we systematically studied the influence of applied voltage on translocation-event frequency, translocation velocity and dwell time. Moreover, we found that the analytical sensitivity could

☆ The parameters used are as follows: $T = 298\,K$, $D_{K^+} = 1.957 \times 10^{-9}\,m^2\,s^{-1}$, $D_{Cl^-} = 2.032 \times 10^{-9}\,m^2\,s^{-1}$, $D_{Na^+} = 1.334 \times 10^{-9}\,m^2\,s^{-1}$, $D_{HPO4^{2-}} = 0.759 \times 10^{-9}\,m^2\,s^{-1}$, $D_{H2PO4^-} = 0.959 \times 10^{-9}\,m^2\,s^{-1}$, $D_{vesicle} = 3.06 \times 10^{-12}\,m^2\,s^{-1}$, $\varepsilon_r = 78$, $\eta = 1 \times 10^{-3}$ Pa·s, $c_{K^+} = 11.8$ mM, $c_{Cl^-} = 10$ mM, $c_{Na^+} = 16.4$ mM, $c_{HPO4^{2-}} = 8.2$ mM, $c_{H2PO4^-} = 1.8$ mM, $\rho = 1000\,kg{\cdot}m^{-3}$, $u_{K^+} = 7.62 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$ $u_{Na^+} = 5.19 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$ $u_{Cl^-} = 7.91 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$, $u_{HPO4^{2-}} = 5.91 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$, $u_{H2PO4^-} = 3.73 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$, $u_{vesicle} = 2.54 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$, $\sigma_s = -1\,mC{\cdot}m^{-2}$, $\lambda_D = 3.10$ nm.

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be increased by increasing the applied voltage. This study demonstrates that the nanopipette could be used as an easily operated platform for vesicle counting, and could be potentially useful for implantable vesicle analysis.

2. Experimental details

2.1. Preparation and characterization of phospholipid vesicles

Phospholipid vesicles were prepared according to the methods reported previously with slight modification [19]. Typically, L-α-phosphatidylcholine (Type II-S, Sigma-Aldrich) was dissolved in chloroform to 10 mg/mL. A 1 mL of this solution was transferred to a round-bottomed flask and the solvent was then removed on a rotary evaporator at room temperature. A 10 mM KCl solution containing 10 mM phosphate buffer solution at pH 7.6 was carefully added to a lipid concentration of 1 mg/mL. The lipid film was allowed to swell overnight at 4 °C and the sample was sonication about 10 min in ice-water finally. Size, ζ-potential and electrophoretic mobility measurements were carried out by dynamic light scattering (DLS) characterization with Malvern Zetasizer Nano ZS (Malvern Instruments Ltd.). Herein, the concentrations of the phospholipid vesicles can be estimated by assuming complete transformation from phospholipid solution into bimolecular unilamellar spherical vesicles, and a phospholipid molecular radius is ~4 Å [20]. The cryo-transmission electron microscopy (cryo-TEM) images of the phospholipid vesicles were taken with JEM-2100F microscope (JEOL).

2.2. Fabrication and characterization of nanopipette

Nanopipettes were fabricated by pulling borosilicate glass capillaries (1.50 mm O.D. and 1.10 mm I.D., with filament, Sutter Instrument Co.) with a CO_2-laser-based pipette puller (P-2000, Sutter Instrument Co.). A two-cycle pulling program is shown as follows: Cycle 1 (heat = 323, filament = 5, velocity = 20, delay = 128, pull = 50) and Cycle 2 (heat = 350, filament = 4, velocity = 15, delay = 130, pull = 175), resulting in a nanopipette with ~250 nm tip diameter. The scanning electron microscopy (SEM) images of the nanopipette were taken with an SU8020 microscope (HITACHI).

2.3. Experimental setup and data acquisition

The nanopipette was filled with and immersed in 10 mM KCl solution (pH 7.6). Current traces were obtained by applying various voltages between two Ag/AgCl electrodes. One was embedded into the nanopipette, and the other was placed in the external bath. Axopatch 200B (Molecular Devices) in V-Clamp mode with a 10 kHz low-pass Bessel filter was used to record the current and the signal was digitized by Digidata 1440A Series (Molecular Devices) at 50 kHz sampling rate. In this experimental case, the typical level of noise is in the range of 3–5 pA. The variation in current that is greater than the level of noise is considered as signal.

3. Results and discussion

Schematic illustration of the experimental setup is shown in Fig. 1A. According to the DLS measurement, the average diameter (Fig. 1B) and zeta potential of the phospholipid vesicle were 139.3 ± 0.6 nm and −36.1 ± 3.8 mV. The quasi-spherical morphology of phospholipid vesicles was imaged by cryo-TEM (Fig. 1C). A single conical nanopipette with ~250 nm-diameter orifice (Fig. 1D) was used for the phospholipid vesicle translocation experiments. Ion current is generated when bias voltage is applied between two Ag/AgCl electrodes. When the vesicle approaches the nanopipette orifice and then enters the conical side, it occupies a limited section of the electrolyte solution at the sensing zone near the tip, leading to the decrease of ion current. The height, width and frequency of these pulses reflect the particle size, surface charge and concentration, respectively.

Fig. 1E shows typical *i-t* traces obtained at the nanopipette under various applied voltages. When 0.25, 0.50, 0.75 and 1.0 V bias voltages were applied in ~5×10^{10}/mL vesicle solution, pulse signals could be detected successfully. The individual pulse signal (inset in Fig. 2A) was characterized by dwell time $\triangle t$, measured as the half-peak width, and the maximum decrease in current $\triangle I$ normalized to the baseline current I_0 ($\triangle I/I_0$). Fig. 2A depicts the scatter diagram of $\triangle I/I_0$ vs. $\triangle t$ analysed from the current-time trace at the bias potential of 0.50 V. Scatter diagrams at 0.25, 0.75 and 1.0 V were plotted in the same way (data not shown). The means of $\triangle I/I_0$ and $\triangle t$ at various voltages (Fig. 2D) were obtained for the same data set according to the log-normal function fitted results (Fig. 2B and Fig. 2C) from corresponding distribution histograms. Following applied positive bias voltage from 0.25 V to 1.0 V, event frequency (f) increased (Fig. 1F) and the dwell time $\triangle t$ decreased (Fig. 2D, red dots). The dwell time $\triangle t$ of the current signal was connected with the charge carried by the particle and it was sensitive to the applied voltages in our experiment. $\triangle I/I_0$ remained fairly static during various applied voltages (Fig. 2D, black squares), in accordance with the rule of particle translocation by the resistive-pulse principle [11]. The results suggest that positive voltage can drive and regulate the translocation of the phospholipid vesicle from the external solution to the interior through the nanopipette orifice within the sensing zone.

To further investigate the effect of the applied voltage, the driving force was estimated. Vesicles approached the nanopipette orifice depending on diffusion, migration and convection. The migration rate (f_{mig}) for vesicles in the electrolyte solution to arrive at the nanopipette orifice was estimated in Eq. (1) [21]:

$$f_{mig} = \frac{IC_{vesicle}}{eC_{ion}} \frac{u_{vesicle}}{u_{cation} + u_{anion}} \quad (1)$$

where I is the ionic current across the nanopipette (2.6 nA at 0.5 V), and e is the unit of charge. C and u refer to the concentration and electrophoretic mobility of ions or vesicle, respectively.

The diffusion rate (f_{dif}) for vesicles at the nanopipette orifice was calculated by Eq. (2) [22]:

$$f_{dif} = 3.35\pi D_{vesicle} C_{vesicle} r_0 \quad (2)$$

where $D_{vesicle}$ is the diffusion coefficient of the vesicles, and r_0 refers to the radius of the nanopipette orifice. The diffusion coefficient of vesicles is approximately calculated by the Stokes–Einstein Eq. (3):

$$D_{vesicle} = \frac{kT}{6\pi\eta r_{vesicle}} \quad (3)$$

where k is the Boltzmann constant, T is temperature, η is the viscosity of water, and $r_{vesicle}$ is the radius of a vesicle particle.

Here, because of no stirring and pressuring, the convection was a product of electroosmotic flow, which is the flow of the fluid caused by the counterions of the charged surface under an applied electric field and in opposition to the migration under this experimental setup. The convection rate (f_{con}) for vesicles to exclude from the nanopipette orifice was calculated by Eq. (4) [23,24]:

$$f_{con} = -\frac{\lambda_D \sigma_s E}{\eta} \pi r_0^2 C_{vesicle} \quad (4)$$

where λ_D is the Debye length, σ_s is the inner surface charge density of nanopipette, and E is the magnitude of electric field at the nanopipette tip (approximately $2.1 \times 10^5\ V\ m^{-1}$ at 0.5 V, half-cone angle ≈3° [25]).

In terms of the corresponding experiment conditions in Fig. 1E, f_{mig}, f_{dif} and f_{con} were estimated as 11.0, 0.2 and 1.6 s^{-1} at 0.5 V applied voltage, respectively. In this case, the motion that vesicles approached to the nanopipette orifice was dominated by migration. This result was consistent with the previous experimental fact that a translocation event could be observed at a certain positive voltage and event