Intramembrane Electrostatic Interactions Destabilize Lipid Vesicles

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ABSTRACT Membrane stability is of central concern in many biology and biotechnology processes. It has been suggested that intramembrane electrostatic interactions play a key role in membrane stability. However, due primarily to a lack of supporting experimental evidence, they are not commonly considered in mechanical analyses of lipid membranes. In this paper, we use the micropipette aspiration technique to characterize the elastic moduli and critical tensions of lipid vesicles with varying surface charge. Charge was induced by doping neutral phosphatidylcholine vesicles with anionic lipids phosphatidylglycerol and phosphatidic acid. Measurements were taken in potassium chloride (moderate ion-lipid binding) and tetramethylammonium chloride (low ion-lipid binding) solutions. We show that inclusion of anionic lipid does not appreciably alter the areal dilation elasticity of lipid vesicles. However, the tension required for vesicle rupture decreases with increasing anionic lipid fraction and is a function of electrolyte composition. Using vesicles with 30% charged (i.e., unbound) anionic lipid, we measured critical tension reductions of 75%, demonstrating the important role of electrostatic interactions in membrane stability.

INTRODUCTION

As self-assembled structures, the mechanical properties of membranes are derived from noncovalent forces such as the hydrophobic effect, steric forces, and electrostatic interactions. The electrostatic force has drawn considerable attention, as most biological membranes are rich in anionic lipids and are therefore charged in aqueous solution. Plasma membranes of mammalian cells often consist of 10–20% anionic lipid (Yeagle, 1992), whereas bacterial membranes contain as much as 80% (Kates, 1964; for reviews on membrane electrostatics, see Cevc, 1990; Langner and Kubica, 1999).

Modulating the electrostatic interactions can tip the careful balance of forces in the bilayer and thus have implications on the mechanical properties of lipid membranes. For example, several authors have considered the effect of electrostatics on the various elastic moduli of lipid membranes both experimentally (Song and Waugh, 1990) and theoretically (Bivas and Hristova, 1991; Kozlov et al., 1992; Lekkerkerker, 1989; May, 1996). Of special interest is a series of papers regarding the rupture of red blood cell membranes placed in low ionic media (Betterton and Brenner, 1999; Cortez-Maghelly and Bisch, 1995; Gallez and Coakley, 1986). Betterton and Brenner (1999) described this using an electrostatic argument: as the salt concentration is lowered, the surface charges in the membrane are less screened. Eventually, the repulsive nature of the charge-charge interactions overpowers membrane cohesive forces, and the cell ruptures. Their conclusions are contrasted by the findings of Diederich et al. (1998). These authors, using an induced tension argument, expected a reduction in the stability of charged black lipid membranes (BLMs) to electroporation. However, their experimental findings were that BLM stability is not affected by surface charge or electrolyte concentration. Clearly, additional experimental work is needed, preferably using spherical lipid vesicles that are structurally more relevant to cellular membranes than BLMs.

In this paper, we use the micropipette aspiration technique to determine the mechanical properties of charged lipid vesicles. Our results demonstrate that the introduction of surface charge has little effect on bilayer elasticity but dramatically lowers the tension that can be applied to vesicles before rupture. This effect is dependent on the fraction of charged lipid present in the bilayer, with critical tension reductions up to 75%. Similar results are seen for the anionic lipids phosphatidic acid (PA) and phosphatidylglycerol (PG). Data show the effect of electrolyte identity as higher stabilities are measured in moderately binding potassium chloride (KCl) than in poorly binding trimethylammonium chloride (TMA-Cl). We hypothesize that the reductions in mechanical stability are due to electrostatic interactions and demonstrate that the destabilization scales with an electrostatically induced tension. Finally, we comment on key experimental issues, especially regarding glass surface coatings, that must be addressed for the micropipette technique to be confidently used in the mechanical characterization of charged lipid membranes.

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MATERIALS AND METHODS

Vesicle preparation

Giant unilamellar vesicles (GUVs) were created using a modification of the electroformation method (Angelova and Dimitrov, 1987; Longo et al., 1997). Neutral palmitoyloleoylphosphatidylcholine (POPC) was combined with anionic lipids palmitoyloleoylphosphatidylglycerol (POPG), or palmitoyloleoylphosphatidic acid (POPA) (Avanti Polar Lipids, Alabaster, AL) to make 0.5 mg/ml solutions in chloroform with the desired anionic lipid

fraction. 50 μl of lipid solution was spread on platinum electrodes that were held 5 mm apart in a Teflon/glass cell. Films were dried under vacuum overnight to remove trace solvent. Vesicle interior solution was added to the cell, and vesicles were formed by the application of a 1.0-V sine wave across the electrodes. Interior solutions consisted of 150 mM sucrose, 1 mM electrolyte (KCl or TMA-Cl), and 10 μM EDTA and were titrated to pH 7.4 with base (KOH or TMA-OH).

Similar to previous studies (Akashi et al., 1996; Needham and Hochmuth, 1989), a small amount of anionic lipid (at least 4%) was required to form well-behaved GUVs in electrolyte solutions. Neutral POPC vesicles did form in electrolyte solutions; however, they frequently had nonlinear stress/strain curves and were therefore deemed unsuitable for mechanical testing. Vesicles at low to moderate anionic lipid fractions had extremely high yields, with vesicles numbering in the tens or hundreds of thousands. At larger anionic fractions, yields decreased dramatically, limiting the experimentally accessible range. Additionally, yields of GUVs dropped rapidly with increasing electrolyte concentration, limiting experiments to ~1 mM salt. Before micromanipulation, vesicles were mixed with an equal volume of vesicle exterior solution (170 mM glucose, 1 mM matching electrolyte, 10 μM EDTA, pH 7.4). The osmotic imbalance causes vesicles to slightly deflate, aiding aspiration. Using glucose improves optical contrast and forces vesicles to sink, resulting in an accumulation of vesicles on the bottom of the sample chamber.

Determination of vesicle mechanical properties

The micropipette technique was used to determine the elasticity and critical tension of charged vesicles. Briefly, suction pressures were applied with a glass micropipette to individual GUVs, creating an isotropic membrane tension. Vesicle deformations from increased suction pressures allow calculation of vesicle elasticity. The applied areal strain at rupture is defined as the critical strain (for a general review of the technique, see Needham and Zhelev, 1996).

Using the concepts of Helfrich (Helfrich and Servuss, 1984), the relationship between stress, τ, and strain, α, for vesicles under aspiration is (Rawicz et al., 2000):

$$\alpha = \left(\frac{kT}{8\pi K_b}\right)\ln\left(1 + \frac{c\tau A}{K_b}\right) + \tau/K, \qquad (1)$$

where A is the total membrane area, K_b is the elastic bending modulus, K is the elastic dilation modulus, k is Boltzmann's constant, T is absolute temperature, and c is a constant, ~0.1. At low tensions, the logarithmic term dominates, and the change in membrane area is due to the smoothing of thermal undulations. At larger tensions, the linear term dominates and the vesicle approaches the expected elastic behavior described by $\tau = K\alpha$. However, even at the largest tensions, there is still a small contribution from the logarithmic term (Rawicz et al., 2000). As a result, linear fits to the high-tension regime commonly reported in micropipette aspiration studies overestimate K by 10–20%.

In this paper, we follow common convention and report the slope of stress versus strain in the high-tension regime ($\tau > 0.5$ mN/m) as the elastic dilation modulus. One must use caution here, as changes in the bending modulus (which may occur with changing surface charge) may manifest themselves in changes in the apparent dilation modulus. As it was difficult to experimentally determine K_b for highly charged vesicles, we performed calculations to assess this effect. Using experimental results (Song and Waugh, 1990) or theoretical predictions (May, 1996), electrostatically induced changes in bending moduli do not alter fits to high-tension stress/strain data (i.e., K for both charged and neutral membranes will be similarly overestimated). We therefore neglect this effect.

Proper pipette and cell preparation protocol was critical in obtaining reproducible results (see below). Borosilicate capillaries (0.9 mm o.d., 0.5 mm i.d.; Friedrich and Dimmock, Millville, NJ) were pulled to a fine point with a Kopf model 730 puller (Tujunga, CA) and forged to ~5–7 μm with a Narishige MF 830 microforge (Micron Optics, Cedar Knoll, NJ). Tips were then immersed in exterior solution doped with 1 wt % bovine serum albumin (BSA, 98% by electrophoresis; Sigma Chemical Co., St. Louis, MO) for 30 min. After incubation, the BSA solution in the tip was discharged and the tip was rinsed several times by aspirating and discharging water to insure removal of any nonadsorbed protein. The tip was then filled with water and flushed for at least 5 min by aspiration in the sample chamber. To reduce the possibility of artifacts, each vesicle batch was examined with at least two pipettes. The results of the two pipettes were in every case statistically identical.

Glass used for the sample chamber was coated with a self-assembled monolayer (SAM) of 2-[methoxy(polyethylenoxy)propyl] trimethoxysilane (Gelest, Tullytown, PA). For deposition, glass was immersed for 1 min in a 1 wt % SAM solution (95% ethanol, 5% water, to pH 5 with acetic acid), rinsed in ethanol, and then cured in a 110°C oven for 15 min. Air/SAM/water contact angles consistently measured 20°–25° with a Rame-Hart goniometer (Mountain Lakes, NJ). Chambers were manufactured by gluing two SAM-treated glass pieces to a 2.0-mm Teflon spacer with RTV sealant. Superior optical resolution was achieved by using a 1.0-mm-thick microscope slide as the top of the chamber and a 1½ coverslip for the bottom. Chambers had one side open to the atmosphere for micromanipulation and were held constant at 25.0°C by a circulating bath.

Vesicle aspiration tests were conducted using an inverted optical microscope fitted with differential interference contrast optics (Nikon TE200, Micron Optics). A Narishige MHW-3 micromanipulator (Micron Optics) was used for pipette manipulation. Digital images taken with a Kodak ES310 CCD camera were directly acquired on PC using a PIXCI-D imaging board (EPIX, Buffalo Grove, IL). (Capturing digital images directly provides greater image acquisition speed and resolution compared with an analog data source such as a VCR.) Both vesicle and pipette features were measured optically using the Subpixel Edger tool in the XCAP software package (EPIX). Suction pressure applied to vesicles was measured with Validyne pressure transducers (Advanced Controls, Warminster, PA) and recorded along with vesicle images. Pressure was stepwise increased to give membrane stress rates of 0.9 ± 0.1 mN m^{-1}min^{-1} until vesicle rupture. Mechanical properties reported are the averages of ~20 vesicles.

Chemicals and reagents

Unless otherwise stated, all chemicals were from Sigma, of the highest grade available, and used as received. Water used was produced by a Milli-Q UF unit (Millipore, Bedford, MA) and had a resistivity of 18.2 megohm-cm.

Micromanipulation of charged lipid vesicles

In this work, we used the micropipette aspiration method to assess the effect of electrostatic interactions on the mechanical properties of lipid membranes. Although this technique has become somewhat routine in the characterization of neutral membranes, we found alterations in standard micropipette protocols were essential to determine the mechanical properties of charged vesicles. Given the growing popularity of this versatile technique, we report on these new protocols here.

The most important factor in the success of charged membrane aspiration involves proper preparation of the pipette tip and sample chamber. Vesicles, both charged and uncharged, adhere to bare glass. This results in very irreproducible stress/strain curves and extremely low lysis tensions when vesicles are examined by micropipette aspiration. To alleviate this problem, most micropipette experimenters use BSA, a globular protein that adheres strongly to glass, either as a precoating on the chamber and tip or in the sample solution itself.