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Signatures of chemical reactions in the morphology and fluctuations of giant vesicles

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Abstract

The behaviour of an amphiphilic membrane is determined by the physical and chemical properties of the molecules which form the bilayer and their interactions with the surrounding medium. Bulk or interfacial chemical reactions modify interaction parameters and/or affect directly the chemical composition of the membrane. We monitor the morphological response and the thermal fluctuations of giant lipid vesicles to chemical reactions in the external vesicle medium using phase-contrast microscopy. Observation of vesicle conformations as a function of time allows us to characterize the statics and dynamics of membrane response as well as the underlying chemical kinetics. As two examples, we present (a) a photochemical reaction of hexacyanoferrate which induces an increase in pH and (b) the enzymatic cleavage of phosphatidyl choline by the phospholipase C from *Bacillus cereus*.

1. Introduction

A chemical reaction occurring inside or outside a confined space will in general alter the free energy of the confining interface. For soft interfaces, this leads to an increase or decrease in interfacial spontaneous curvature. Specifically, we are interested in giant lipid vesicles in a reactive external environment. A reaction either changes bulk solution properties, such as salt concentration and pH, or chemically modifies the molecules of the external monolayer of the membrane. In either case, there is an increasing asymmetry across the bilayer as the chemical reaction progresses, leading to membrane curvature; more generally, the material parameters of the membrane change. As a result, giant vesicles will alter their shape and adopt a new equilibrium morphology which minimizes the elastic energy of the vesicle membrane [1, 2]. In addition to changes in vesicle mean shape, the (thermal) shape fluctuations around this equilibrium morphology change as well. Thus, giant vesicles may be used as convenient tools

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to probe the interactions of amphiphilic membranes employing both vesicle shapes and shape fluctuations. We will discuss the underlying physics in some more detail in section 2 and give two experimental examples in section 3. We close with a summary and an outlook.

There is a long history of giant vesicle research [3, 4] focusing on various aspects of biomembrane physics. However, it has become apparent recently [5] that biologically derived vesicles are only a small subset of what Nature has in store. Vesicles made of diblock copolymers were studied intensively [6–9]. The material and synthetic versatility of polymers promises to trigger a wide array of basic research and application development. Well-controlled polymer chemistry will offer almost endless possibilities for altering membrane properties according to the desired behaviour. Molecules can be modified during basic synthesis and/or after membrane self-assembly in solution. Thus, a general discussion and understanding of the effects of chemical reactions on vesicles is urgently needed.

2. Theory

2.1. Vesicle shapes and shape fluctuations

Giant vesicles are single, closed membrane bags formed by amphiphilic molecules with a typical size of 10 μm. For insoluble amphiphiles, vesicle area, $A = 4\pi R_A^2$, is fixed, but varies as a function of temperature. Enclosed volume, V, is determined by osmotic equilibrium across the vesicle membrane. At fixed volume and area, the shape of a vesicle stabilized by gravity on a non-adhesive (hard-wall) substrate minimizes the sum of the membrane bending energy and the gravitational energy of the vesicle [10, 11],

$$H = \kappa(H_{ADE}(v, \bar{c}_0) + g H_{grav}), \tag{1}$$

where κ is the bending modulus setting the energy scale. The reduced volume is defined by $v = V/(\frac{4\pi}{3}R_A^3)$, and $\bar{c}_0$ denotes the effective spontaneous curvature which includes contributions from area-difference elasticity as described in the ADE model [10]. The relative importance of gravity is measured via the dimensionless ratio $g = g_0\,\Delta\rho\,R_A^4/\kappa$, where g_0 is the acceleration due to gravity and $\Delta\rho$ denotes the density difference between the internal and external vesicle solutions.

At sufficiently large v and medium values of $\bar{c}_0$, the stable vesicle shape is an axisymmetric prolate form with its axis parallel to the substrate. At finite gravity g, this symmetry is broken and the vesicle is progressively flattened as g increases. Eventually, the vesicle shape becomes oblate, with its rotational symmetry axis perpendicular to the substrate. Decreasing the effective spontaneous curvature $\bar{c}_0$ at fixed reduced volume v and gravitational parameter g leads to a prolate–oblate transition as well. Viewed from the substrate, the equatorial contour of the oblate vesicle appears as a circle. Increasing $\bar{c}_0$ drives the prolate vesicle towards the budding transition where a small satellite is expelled from the parent vesicle along its long axis. As the vesicle moves towards the budding instability, the ellipsoidal shape becomes more and more elongated, since increasing spontaneous curvature counteracts gravitational flattening, i.e., the equatorial vesicle contour—as seen from the substrate—appears more ellipsoidal.

The two instabilities of a prolate vesicle are connected with characteristic thermal fluctuations of the vesicle shape. The prolate–oblate transition, which is weakly first order, leads to pronounced fluctuations in the ellipticity of the vesicle in the vicinity of the transition [12]. At the budding transition, the up–down symmetry is broken which triggers strong pear-like fluctuations close to the instability of the prolate shape [13].

2.2. Non-equilibrium concepts

A giant vesicle reacting with its environment is not in chemical equilibrium. Let us denote by τ_c the typical equilibration time for completion of the chemical reaction. Concerning the interpretation of the experimental data, it is further important whether the vesicle is in mechanical equilibrium. In this respect, the characteristic timescale for mechanical relaxation is given by $\tau_m = \eta R_A^3/\kappa$, where η is the viscosity of the embedding medium [1]. In the limit $\tau_c \ll \tau_m$, the observed vesicle dynamics after initiation of the reaction reflects relaxation into the new mechanical equilibrium determined by the new elastic constants. Measurements of mean values should be taken on timescales $\tau_{exp} \gg \tau_m$. In the limit $\tau_c \gg \tau_m$, there is an ongoing chemical reaction as the vesicle is observed. There are basically two cases depending on the character of the chemical reaction: either the vesicle membrane is in a mechanical quasi-equilibrium with an adiabatic change of material parameters; or one has a genuine non-equilibrium situation. In the first case, characterization of an adiabatic mean shape and measurements of thermal shape fluctuations are still possible when $\tau_c \gg \tau_{exp} \gg \tau_m$. The second case is realized for active membranes. For example, the activity of the light-driven proton pump Bacteriorhodopsin leads to non-thermal momentum transfer to the embedding membrane [14]. Nevertheless, this is a special situation, in the sense that there are well-defined and *constant* elastic parameters. Thus, the notion of an equilibrium mean shape still exists. However, the vesicle shape fluctuations are non-thermal and will depend, e.g., on the viscosity of the medium.

3. Experiment

3.1. Morphological characterization

We stabilize fluctuating prolate vesicles by gravity—employing a small density difference between the solvent inside and outside of the vesicles—on the bottom of a temperature-controlled microchamber which allows exchange of the external vesicle solution *during* observation [15]. The focal plane of a phase-contrast microscope is adjusted to include the long axis of the vesicle, and shape contours are obtained by real-time video image analysis; for details see [16]. Choosing a coordinate system in which the x-coordinate lies along the long axis of the vesicle, the contours are then represented in polar coordinates (r, φ) as

$$r(\varphi) = r_0\left(1 + \sum_n a_n \cos(n\varphi) + \sum_n b_n \sin(n\varphi)\right), \tag{2}$$

where the angle φ is measured from the positive x-axis. The time-dependent amplitudes $\{a_n, b_n\}$ encode the full experimental information. Here, we will focus on the a_2-mode which encodes vesicle ellipticity.

3.2. A photochemical reaction

Under illumination in aqueous solution, the iron complex $Fe(CN)_6^{4-}$ undergoes photoaquation:

$$Fe(CN)_6^{4-} + H_2O \overset{h\nu}{\rightleftharpoons} Fe(CN)_5H_2O^{3-} + CN^-. \tag{3}$$

This reaction is accompanied by an increase in solution pH depending on light intensity. We have calibrated the pH change using the fluorescent probe 4-methylumbelliferone. Illuminating a sample with potassium hexacyanoferrate in the external vesicle solution leads to a pronounced signature in the vesicle ellipticity [17] as shown in figure 1. We find that increasing pH is correlated with more elongated vesicle contours and smaller fluctuations in vesicle

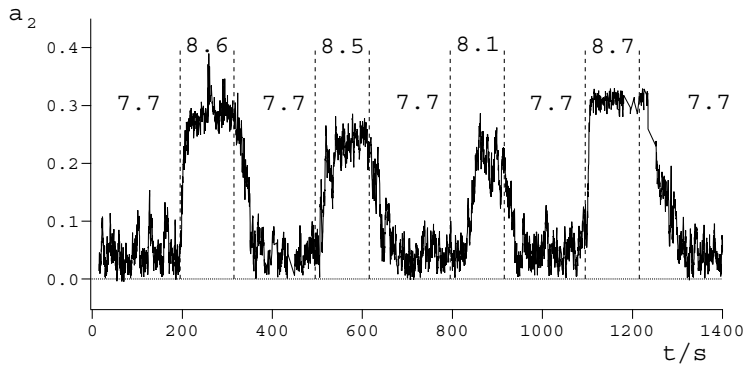

Figure 1. The time-dependent response of the vesicle ellipticity a_2 to a sequence of varying illumination intensities, which are characterized by the pH induced in the external vesicle solution. Times of variation in intensity are indicated by vertical dashed lines. The vesicle is seen to jump between an oblate ($a_2 \sim 0.05$) and a prolate ($a_2 \sim 0.2$–0.3) shape. SOPC vesicles were swollen in 88 mM raffinose solution and incubated in an iso-osmolar glucose solution with an admixture of 50 μM potassium hexacyanoferrate.

ellipticity. As discussed in section 2.1, such a behaviour is indicative of an increase in membrane spontaneous curvature. This is mainly caused by partial charging of the external monolayer [18, 19]. Variation of pH and charging of the membrane are instantaneous on the timescale of the experiment. Thus, the observed vesicle dynamics reflects mechanical relaxation into the new stable shape. Note that for pH $= 8.1$, there is a delay before the oblate–prolate transition takes place. This indicates metastability of the oblate form at this pH value. Vesicle morphology is completely reversible.

3.3. Enzyme activity

The phospholipase C (PLC) from *Bacillus cereus* prefers phosphatidyl choline (PC) lipids as substrates, where it cleaves off the PC head group to yield a diacylglycerol [20]. In this study, we chose stearoyl-oleoyl-phosphatidylcholine (SOPC):

$$\text{SOPC} \longrightarrow \text{PC} + \text{SOG}. \tag{4}$$

Enzyme activity was checked by quantifying the number of PC head groups liberated into aqueous solution using a modified phosphate assay [21]. In figure 2, we show the sequence of events when immersing a SOPC vesicle into PLC solution. The increase in ellipticity and the diminished fluctuations signal an increase in spontaneous curvature. This is consistent with binding of the enzyme to the external monolayer, reducing interfacial tension. Enzyme activity results in a decrease in spontaneous curvature as signalled by a prolate–oblate transition. This is consistent with the production of SOG in the external monolayer, which has a much smaller head group than SOPC; see figure 3. Prolonged activity leads to an overall decrease in vesicle fluctuations. Eventually, domains rich in SOG phase separate as thick lenses, leaving the membrane intact [22]. This shows that most of the SOG produced in the external monolayer actually migrates across the membrane to the internal monolayer. Cleavage of PC head groups proceeds sufficiently slowly that vesicles are in mechanical equilibrium as regards their shape. However, whether membrane fluctuations during enzyme activity are thermally equilibrated is not clear yet.

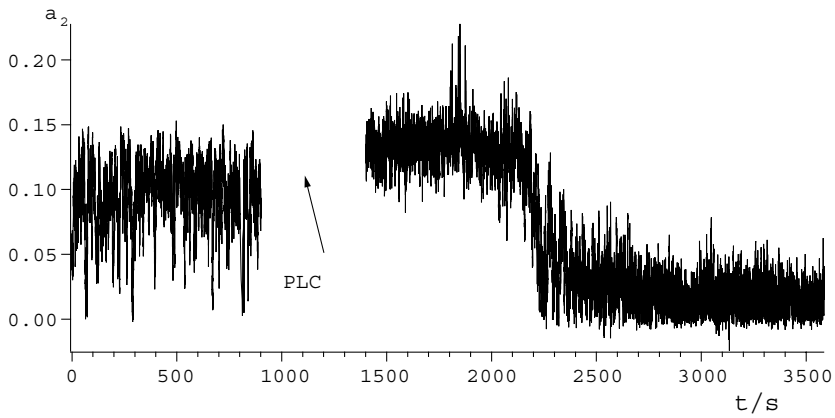

Figure 2. The time-dependent response of the vesicle ellipticity a_2 to the addition of 0.2 units/ml PLC at 25 °C, indicated by an arrow. SOPC vesicles were prepared in 100 mM sucrose/glucose and 10 mM HEPES pH 7.4.

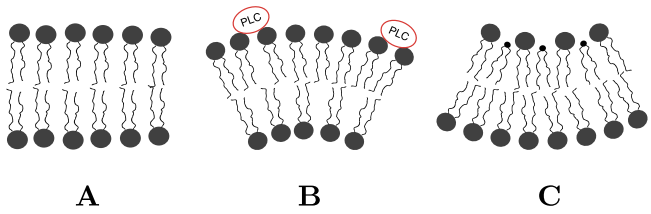

Figure 3. A mechanistic diagram of membrane curvature during PLC activity. (A) Without the enzyme. (B) With the bound enzyme. (C) After the reaction.

4. Summary and outlook

We have shown that giant vesicles can be used as probes to monitor chemical reactions involving amphiphilic membranes. We have identified typical response patterns both in vesicle shape and shape fluctuation. They can be further quantified employing advanced flicker spectroscopy of fluid membranes which allows us to measure both bending elastic constants, the modulus and the spontaneous curvature, simultaneously [23]. Promising future directions of giant vesicle research include studies of polymersomes and non-equilibrium phenomena of membranes. Clearly, there will be a lot more exciting experiments fostering our understanding of soft matter.

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