MEMBRANE CURVATURE INDUCED BY SUGAR AND POLYMER SOLUTIONS

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ABSTRACT

We monitor the effect of transversal membrane asymmetry on the morphology of giant uni-lamellar vesicles in sugar and polymer solutions. The shapes of fluid lipid vesicles are governed by the bending elasticity of their membrane which is characterized by the bending modulus and the spontaneous curvature of the bilayer. We present a recently developed technique for the measurement of the spontaneous curvature using quantitative phase contrast microscopy. Different mechanisms for elastic membrane asymmetry and the role of the bending energy concept for the morphology of cellular organelles are discussed.

INTRODUCTION

The membranes of cellular organelles and the plasma membrane define the boundary between the cytoplasm and the luminal or the extracellular space, respectively. Thus, each membrane has an inner and outer monolayer which usually have quite different lipid compositions. In addition to this membrane asymmetry [1], the adjacent aqueous environment of the two sides of the membrane varies with cellular function. The obvious morphological consequence is that membranes are usually not planar but show a prefered curvature towards one side reflecting transversal membrane asymmetry. Within the theory of bending elasticity [2, 3] such a behavior is described by the spontaneous curvature of the membrane. Despite its fundamental role, the latter quantity has received very little experimental attention so far [4, 5]. In this contribution, we study the morphological response of fluid lipid vesicles to transversal membrane asymmetry induced by asymmetric sugar and polymer solutions. After providing the theoretical framework, and reviewing the technique to extract spontaneous curvature from a quantitative measurement of vesicle shapes, we enumerate various mechanisms for elastic membrane asymmetry. Experimental results are discussed and put into perspective concerning the understanding of cellular morphology.

THEORY

ADE Model

There are two main contributions to the bending energy of a closed bilayer: i) The bending of the two monolayers at fixed bilayer area, and ii) the relative stretching and compression of the monolayers. This implies one considers the mean area $A = (A^{out} + A^{in})/2$ fixed, but allows for changes in the differential area $\Delta A = A^{out} - A^{in}$ during bending. These two contributions are combined in the Area-Difference-Elasticity model [6, 7]

$$H_{\mathrm{ADE}} = \kappa \left(\frac{1}{2} \int dA (C_1 + C_2 - C_0)^2 \; + \; \frac{\alpha}{2} \frac{\pi}{AD^2} (\Delta A - \Delta A_0)^2 \right) , \qquad (1)$$

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where the first term is the Helfrich energy describing monolayer bending with the modulus κ and the spontaneous curvature C_0. The second term gives the elastic contributions from the differential monolayer area ΔA, where the relative weight is determined by the ratio $\alpha = \bar{\kappa}/\kappa$ of the two bending moduli. The thickness of the membrane is denoted by D. The equilibrium area difference ΔA_0 describes the prefered curvature of the bilayer for $C_0 = 0$. In general, the proper dimensionless variable for prefered curvature [7] is

$$\bar{c}_0 = c_0 + 2\pi\alpha \, (\Delta a_0 - \Delta a) \, , \tag{2}$$

where $c_0 = C_0 R_A$, $\Delta a_0 = \Delta A_0 / 8\pi D R_A$, and Δa are reduced quantities scaled by $R_A = (A/4\pi)^{1/2}$. Vesicle shapes are obtained from minimizing Eq. 1 under the constraints of fixed area A and volume V [7]. Thus, a shape is characterized by the reduced volume $v = 3V/(4\pi {R_A}^3)$ and the effective spontaneous curvature $\bar{c}_0$. Experimental vesicle shapes are well described by this theory [8].

Spontaneous Curvature

The tendency of a closed membrane to bend in mechanical equilibrium has two principally different physical origins corresponding to the interactions of the two monolayers with the adjacent solutions and the bilayer architecture, respectively (see Eq. 2). Fig. 1 shows a cartoon of various sources for the effective spontaneous curvature of a vesicle. A relative

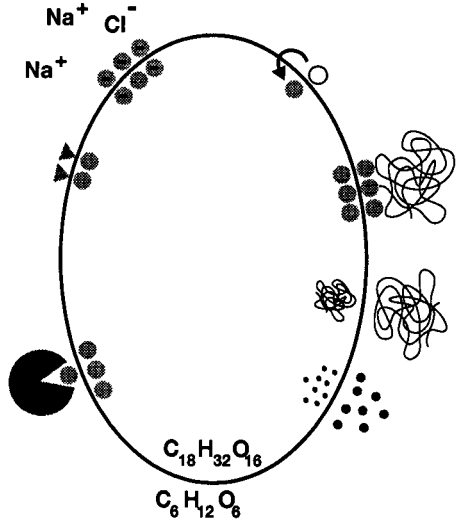

Figure 1: Cartoon of several mechanisms for membrane asymmetry discussed in the text.

change in the number of molecules in the two monolayers or an asymmetric change in mean molecular area will influence the equilibrium area difference Δa_0. This can happen because of lipid flip-flop between the monolayers [9] or the addition of molecules two one monolayer only (see below). Likewise, an enzyme modifying the lipid head group or the chains will

alter Δa_0. The 'molecular shape' in general tends to curve the monolayers [4]. This induces a spontaneous curvature c_0 of the bilayer whenever there is no exact balance in molecular species and/or number densities between the two sides of the membrane. The lipids may be neutral or carry a positive or negative charge. A difference of surface charge density or a different electrolyte concentration across the membrane then induces an electrostatic contribution to c_0 [11]. In general, electrostatic interactions will also change Δa_0. An example of special recent interest are interactions with polyelectrolytes, e.g., DNA. The entropic effects from depletion layers or restricted molecular configurations at the membrane [12] also lead to a spontaneous curvature whenever there is a difference in size or density of disolved species across the membrane [13, 14]. These may be small molecules, like sugars, polymeres, or in general colloids. They could be anchored or adsorbed or both [13, 14, 15]. Finally, specific interactions of proteins with membrane receptors do change the elastic parameters as well.

EXPERIMENT

Shape Measurement

Quantitative measurements of vesicular shapes are carried out using phase contrast microscopy [8, 16]. A careful comparison of experimental mean shapes with theoretical calculations allows to extract the effective spontaneous curvature of prolate vesicles [8]. In order to map experimental vesicle contours into the (v, c_0) shape space, one matches appropriate Fourier components of experimental and theoretical shapes. Recently, we succeeded to perform such a one-to-one mapping as a function of transbilayer asymmetry [16]. With single component phosphocholine vesicles in raffinose:glucose solutions, we found that the effective spontaneous curvature has indeed two parts corresponding to Eq. 2. One shows a linear correlation with the degree of sugar asymmetry across the membrane and could be explained in terms of varying depletion layers due to the different molecular sizes of the mono-saccharide glucose and the tri-saccharide raffinose [16]. Since glucose is the smaller molecule than raffinose, the entropy loss by the depletion layer is minimized when the membrane curves away from the smaller molecule, as observed [16, 17]. The other component is preparation dependent and may be related to the specific differential monolayer area of the particular vesicle under investigation.

Budding Induced by Glucose and Cholesteryl-Polyethylenglycol

Budding of single component vesicles swollen from stearoyl-oleoyl-phosphocholine (SOPC) or dimyristoyl-phosphocholine (DMPC) in sugar solution was induced at iso-osmolal conditions by either raising the glucose content of the vesicle exterior or adding cholesteryl-modified polyethylenglycol to the outer solution. Due to its amphiphilic character, the polymer will partition between the bulk solution and the outer monolayers. Incorporation into the membrane was verified by labeling the polymer (PEG-Flu-Chol) with Fluorescein [18]. In Figs. 2 and 3, typical examples of the budding transition for the two systems are shown. The expelled satellite vesicles stay close to their parents in the case of the sugar induced budding [8, 10]. In contrast, the incorporation of polymers leads to a separation of the buds. However, the two vesicles are still connected by an umbilical tube (see Fig. 3b). In both cases, the budded configuration is topologically a sphere. We may speculate, that

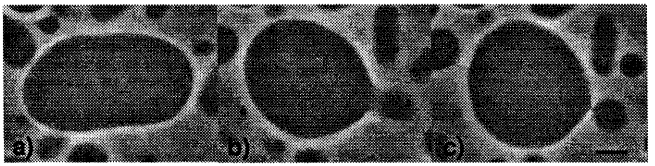

Figure 2: Glucose induced budding: a) Fluctuating prolate vesicle. b) Transient budding shape. c) Vesicle with attached bud. The bar denotes 5 μm.

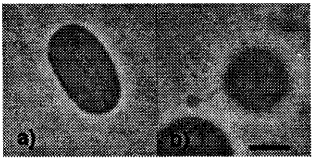

Figure 3: PEG induced budding: The bud separates but is still connected by an umbilical tube. The bar denotes 5 μm.

the polymer grafting density is different in the umbilical tube and the spheres connected by it. The transition from a stomatocyte vesicle to a discocyte in Fig. 4 is also driven by addition of polymers to the external solution. Note, that the last two frames (d, e) in Fig. 4 show two orthogonal cuts of the same shape. It is a flat biconcave disk with broken rotational symmetry. Reducing the volume of this shape, leads to the starfish-like vesicle, depicted in Fig. 5a, which belongs to the same symmetry class [19]. Again, budding of the vesicle arms (with a subsequent separation of the buds) could be induced by asymmetric incorporation of polymers into the membrane. Thus, in all examples the observed shape changes are in accord with an increase of the spontaneous curvature [3, 7, 8]. For the glucose induced budding, we already discussed a possible mechanism. In the polymer system, the anchoring in the membrane increases both components of the effective spontaneous curvature $\bar{c}_0$. The addition of the cholesteryl-anchor to the outer monolayer increases the outer monolayer area and thereby raises Δa_0. Further, the configurational restriction of the polymer at the membrane can be partially relieved when the membrane curves away from the polymer. The balance between entropy gain of the polymer and bending energy cost of the membrane results in a positive spontaneous curvature [13].

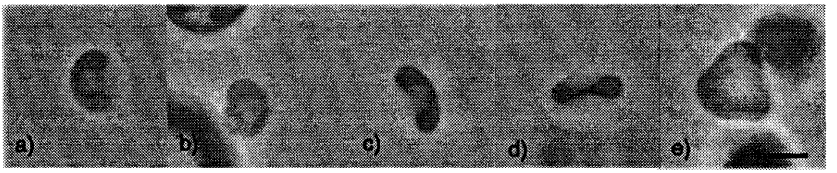

Figure 4: Shape change from a stomatocyte to a starfish-like vesicle by adding PEG-Flu-Chol solution (10^{-4} mol/l): a) Pure SOPC-vesicle without polymer. b) After polymer solution is added. Note that $\bar{c}_0$ is first decreasing and than increasing; c) 4 min from b); d) 6 min from b; e) 10 min from b). The bar denotes 5 μm.

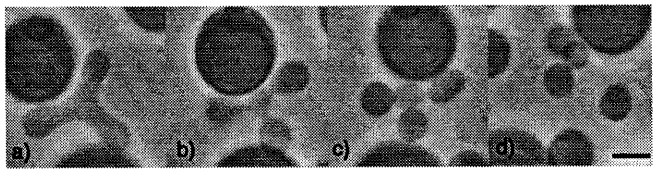

Figure 5: Budding of a starfish vesicle: The polymer solution added was PEG-Flu-Chol (10^{-4} mol/l). The changes from a) to c) were relatively fast (within 10 seconds). Separation of the spheres took about 1 minute. The spheres are still connected by small lipid tubes. The bar denotes 5 μm.

CELLULAR MORPHOLOGY

We have seen that morphological changes in giant vesicles can be induced by controlling the effective spontaneous curvature of the vesicle membrane. In particular, increasing this parameter either by changing the composition of the exterior solution or by addition of a polymer coat to the external monolayer leads to budding. In the cell, budding of vesicles from internal organelles or the plasma membrane is a ubiquitous process and key for cellular traffic. In most cases, budding seems to be related to an asymmetric protein coat [20] of the membrane which induces a local spontaneous curvature of the composite system. These protein patches could lead to domain induced budding [21, 22]. In any case, it seems that modeling the morphological aspects of the cellular budding processes within the framework of elasticity theory is reasonable. It has already been shown experimentally that the shape of large uni-lamellar vesicles with a typical size on cellular length scales can be understood by the ADE Model [9]. However, e.g., the clathrin coat of endocytic vesicles forms a tethered network and introduces a shear elastic energy contribution in addition to the bending energy of the membrane. Recently, a model of endocytosis has been proposed including such a term [23]. Nevertheless, the spontaneous curvature of the composite system remains a key parameter. It is thus worthwhile to study the effect of general membrane-solvent interactions on the spontaneous curvature of (bio-)membranes using our technique. We propose that a lot of the morphological changes in the cell, in particular the budding process, can be explained by the physical mechanism outlined above, whereas the elastic parameters of membranes are controlled and regulated biochemically.

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