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Research Article

Effect on the Liposome Morphology of DOPS in Ionic Solution

Arteaga-Jiménez A[1], Martínez-Balbuena L[2]*, Hernández-Zapata E[3], and López-Esparza R[4]

[1]*Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, México*

[2]*Unidad Multidisciplinaria de Docencia e Investigación - Facultad de Ciencias, México*

[3]*Departamento de Recursos de la Tierra, Universidad Autónoma Metropolitana-Lerma, México*

[4]*Departamento de Física, Universidad de Sonora, México*

***Corresponding author**

Martínez-Balbuena L, Unidad Multidisciplinaria de Docencia e Investigación, Universidad Nacional Autónoma de México - Facultad de Ciencias-Campus Juriquilla, Boulevard Juriquilla, 3001, C.P. 76230, Juriquilla, Querétaro, México, Tel: (442)1926224; Email: mtz.luciano@gmail.com

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- Debye length

Abstract

DOPS liposome formation experiments using the rehydration technique in ionic solutions are reported. We present observations on the ionic strength dependence of the liposome morphology and size. Shape changes from spheres to coiled cylindrical vesicles as the salt concentration increases. From optical microscopy observations and a Winterhalter-Helfrich theoretical model we infer that the counter ion presence leads to an increase in the liposome curvature elasticity, in such a way that small mechanical perturbations are able to radically transform the liposome morphology.

INTRODUCTION

Biological membranes are thin interfaces that enclose the cell and its organelles. Its basic structure is a lipid bilayer formed by complex lipids (phospholipids) and immersed proteins. Lipid bilayers form spontaneously to avoid contact between water and the hydrophobic regions of the phospholipid molecules. Its stability is maintained by a combination of hydrophobic interactions, covalent bonds and other kinds of attractive and repulsive forces such as van der Waals and electrostatic forces [1].

Biological membranes have multiple functions. They act as a boundary for both cells and the organelles of eukaryotic cells [2], constituting a semi permeable barrier for the flow of substances in both directions through the membrane, taking part in both passive and active transport [3,4]. They play an important role in catalytic processes since enzymes may be incorporated into the membranes or interact strongly with it, and typically the enzymatic reactions occur at the membrane surface [5]. Membranes are also the place where protein receptors interact with specific substances of biological importance triggering biochemical processes inside the cells.

Liposomes are a good model system for the study of physicochemical properties of biological membranes. They are vesicles with a central aqueous cavity covered by one or more phospholipid bilayers that may be separated from each other by aqueous spaces. The bilayer structure of a liposome is a good model to understand certain biological properties such as permeability, surface charge, elasticity, and morphology and membrane structure [6,7]. The simplest studies are carried out using isolated liposomes in aqueous solutions where it is possible to directly observe its deformations and structural changes.

The study of liposome physical properties is of interest for several topics of applied science, such as the analysis of biological processes such as endocytosis and exocytosis [8-10], the production of appropriate vehicles for the controlled transport, delivery and release of drugs in the human body [4,11], applications in the food and cosmetic industries [12], as well as in the administration of therapeutic genes [13]. On the other hand, characterization of the aggregate mechanical properties is of vital importance to reliably control their stability in physiological environments.

In the present paper we analyze the behavior of negatively charged liposomes immersed in aqueous solutions in the presence of monovalent salt at different concentrations. Specifically, we study the change in morphology and size of the aggregates as the salt concentration is modified. The observed morphologies are discussed in the context of a Winterhalter-Helfrich theoretical model [14], which describes the interplay between electrostatic and thermodynamic interactions and the bilayer geometric properties from and expression of the system free energy.

OVERVIEW

In this section we review a theoretical model, due to

Winterhalter and Helfrich [14], that describes a charged membrane behavior in the presence of ions in solution. An expression for the free energy of a charged interface immersed in an electrolyte solution can be obtained from the Helfrich theory [15]. Considering the Poisson-Boltzmann equation and the Debye-Hückel approximation [16], it can be derived an expression for the total membrane electrostatic energy (per unit area), which allows to obtain the electrostatic contributions to the Helfrich parameters (surface tension, bending rigidity, gaussian curvature modulus, and spontaneous curvature [15].

Mean curvature of liposomes in ionic solutions (Winterhalter-Helfrich model)

The membrane is modelled as a two-dimensional surface with a fixed surface density of elementary charges, σ. Its thermodynamic behavior can be described using a modified grand potential (per unit area) that satisfy the following equation [16]:

$$\frac{\Omega}{2K_BT} = \sigma\ \Phi\left(\vec{r}\right), \tag{1}$$

Where K_B the Boltzmann is constant, T is the absolute temperature, and Φ is the value of the dimensionless electrostatic potential at the surface. The effect of the salt ions on the membrane is obtained by calculating the electrostatic potential and substituting it into equation (1). When the potential is small, $\Phi(\vec{r}) \ll 1$, (high salt concentrations) the Poisson-Boltzmann (PB) can be linearized and directly solved, which in Gaussian units, for diluted solutions and small electrostatic potential is given as follows [16]:

$$\nabla^2\Phi\left(\vec{r}\right) = K_D^2\Phi\left(\vec{r}\right), \tag{2}$$

Where K_D^{-1} is the Debye length. It should be noted that this quantity depends explicitly on the salt concentration through $K_D^2 = \frac{8\pi n_s e^2}{\left[\epsilon k_B T\right]}$ [17]. Here n_s is the monovalent ion concentration, e is the elementary charge, and ε is the solution dielectric constant.

Equation (2) is solved for planar, spherical, and cylindrical geometries, obtaining in each case the corresponding electrostatic energy (per unit area). The electrostatic contribution to the Helfrich parameters (surface tension, γ, bending rigidity, κ_c^{elect}, gaussian curvature modulus, $\tilde{\kappa}_c^{elect}$, and spontaneous curvature C_0^{elect}) are obtained thus comparing this results to the corresponding Helfrich elastic energies [15]:

$$\kappa_c^{elect} = \frac{4\pi e^2\sigma^2}{\epsilon K_D}\left(\frac{3}{K_D^2}\right), \tag{3}$$

$$\tilde{\kappa}_c^{elect} = \frac{2\pi e^2\sigma^2}{\epsilon K_D}\left(\frac{1}{K_D^2}\right), \tag{4}$$

$$C_0^{elect} = \frac{2\delta K_D}{3}, \tag{5}$$

$$\gamma = \frac{4\pi e^2\sigma^2}{\epsilon K_D} \tag{6}$$

As can be observed in equations (3 - 6) an increase on the monovalent ion concentration leads to a decrease in the surface tension(γ) and in the modulus associated with the mean curvature elasticity (κ_c^{elect}) and the gaussian curvature elasticity ($\tilde{\kappa}_c^{elect}$). Thus, the salt ions inhibit the repulsive electrostatic interactions between the different parts of the membrane leading to a decrease in the membrane rigidity. In the results section these theoretical predictions are confirmed. We will observe there that negatively charged DOPS liposomes in an ionic environment modify their shape and they turn more flexible as the salt concentration increases.

MATERIALS AND METHODS

For the liposome formation it was used the phospholipid 1, 2-Dioleoyl-sn-glycero-3-phospho-l-serine (DOPS) dissolved in chloroform at a concentration of 10mg/ml. The phospholipid was provided by Avanti Polar Lipids. The DOPS molecule has a hydrocarbon double chain, with 18 carbon atoms and a double bond in each chain. The polar group is serine that has a negative charge. It has a molecular mass of 810.025u and a gel/fluid phase transition temperature of -11ºC [18].

In order to obtain the proper optical contrast it was used sucrose from Fluka with a purity of 95%. The salts employed in the present study were sodium chloride, NaCl, and potassium chloride, KCl. Both were provided by Sigma Aldrich with a purity of 99.5%. Water used in the solutions was deionized using the water purification system MilliQ with a conductivity of 18.2 $(MΩcm)^{-1}$.

Liposomes were prepared using the rehydration technique. The first step in this process consists on the dehydration of a 5 ml phospholipid solution in order to eliminate the organic solvent (ether and chloroform). To avoid sample contamination, evaporation of the organic solvent was carried out in a high vacuum chamber. Temperature was kept at 25ºC during the three-hour process. The next step was the hydration of the film with different aqueous solutions in a chamber formed by cover slips and finally the samples are analyzed using optical microscopy.

The samples used to form liposomes in pure (salt-free) water solution, and that were considered as the reference, were rehydrated with a 0.2 M sucrose solution. On the other hand, different phospholipid solutions were prepared by rehydrating the film with a mixture of100 µl of a sucrose solution and100 µl of electrolyte solution, for different salt concentrations varying in the range between 0.02M and2M.

Experimental observations were carried out using an inverted optical microscope Oympus Ix2-ILL100 with 60X objective lens and a Hitachi camera coupled in order to obtain videos or photos of the samples.

RESULTS AND DISCUSSION

In order to understand the behavior of the liposomes in ionic environments, observations by optical microscopy in real time were carried out. As described in the experimental section, in order to register the evolution of the aggregated population, photographs were taken using a digital camera coupled to the microscope. The studied region was determined after analyzing the whole sample and it was chosen considering the observed diversity and dynamics of the liposome population. It was even possible to observe the transformation of a chosen individual liposome.

Liposomes used as reference were formed by using the rehydration technique in a (salt-free) sucrose solution to increase the optical contrast in the microscope. As shown in Figure (1), the population consists of spherical aggregates of different sizes (polidispersity). From the photographs, it was estimated that the liposome diameters were in the range between 5 and 40 μm. The aggregates obtained using the rehydration technique may be unilamellar or multilamellar, that is, they may be formed by many concentric spherical bilayers stacked on top of each other [19,20].

As explained in the experimental section, in order to obtain liposomes immersed in ionic environments the dehydrated DOPS film is rehydrated with a sucrose solution at different salt concentrations. This way we can ensure that the internal and external pressure are equal, eliminating a possible difference in osmotic pressure [21]. This let us rule out the possibility that the observed morphological changes are due to pressure differences and therefore the observed transformations were attributed only to the presence of salt. For salt concentrations larger than 2M, liposomes were not observed for both salts used.

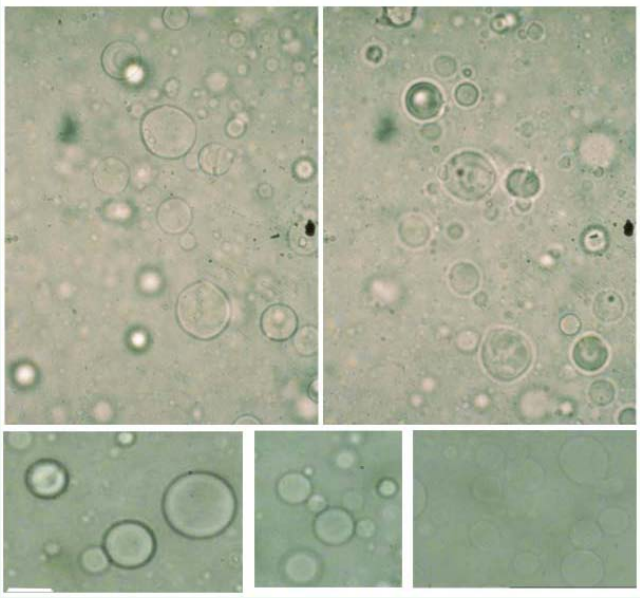

Figure 1 DOPS liposomes formed using the rehydration technique, as observed with optical microscopy. The aggregates are multilamellar and they typically have spherical shapes. The white bar at the lower left side of the figure represents 20 micrometers.

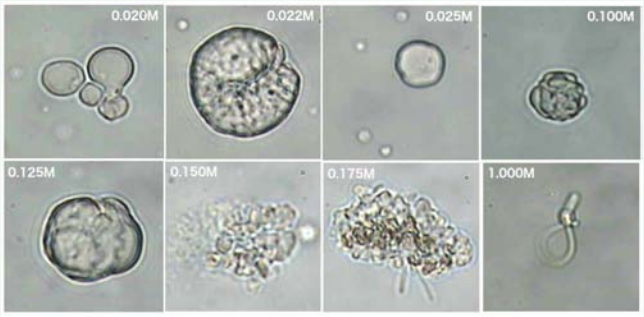

Figure 2 Changes in the DOPS liposome morphology as a function of the the NaCl concentration. The evolution of the aggregate geometry is due to the increasing membrane curvature elasticity. The electrostatic screening effect at the liposome surface leads to a decrease in its effective anionic charge. As a consequence the liposomes acquire an stick-like cylindrical structure.

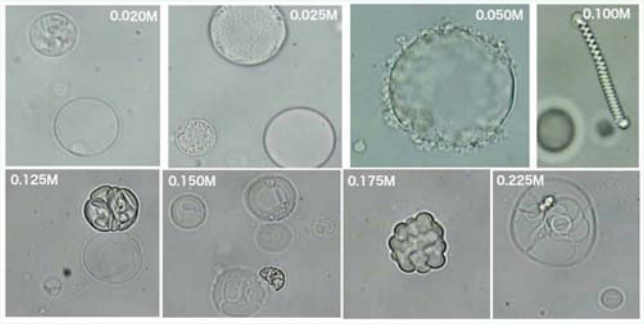

Figure 3 Changes in the DOPS liposome morphology as a function of the KCl concentration. The observed behavior is very similar to the one obtained with NaCl.

Figure (2) shows the morphological changes for a DOPS liposome population as the NaCl concentration increases. Figure (3) shows the corresponding results obtained when NaCl is substituted by KCl. As can be observed, the salt concentration diminishes the population and eliminates the regularity in the liposome shapes.

Figures (2) and (3) shows that the evolution in shape and size for liposomes immersed in NaCl and KCl solutions follow the same pattern. This result was to be expected since both are monovalent salts (however, the cylindrical structures present themselves for smaller KCl concentrations than in the case of NaCl). In the range of salt concentration between 0 and0.05M liposomes have a spherical shape but they present surface roughness and small deviations from the spherical shape that increase with the ionic strength. The addition of salt has also the effect of making more visible the multilamellarity of the aggregates. In the range between 0.05M and 0.2M the liposomes lose their spherical shape and transform into cylindrical and coiled cylindrical vesicles. For higher salt concentrations liposomes are not observed anymore and the scarce visible aggregates have irregular shapes with no apparent pattern.

The decrease in effective surface charge density for the aggregates (due to the presence of monovalent ions that screen the electrostatic interactions) is one of the main reasons for the

changes in morphology. The screening of the electrostatic forces increases the membrane curvature elasticity. This hypothesis is in agreement with the results obtained with the Winterhalter-Helfrich model (equation 3), according to which the bending rigidity decreases with the salt concentration.

The observed change in geometry has also been reported on mixtures of neutral and charged phospholipids. Paredes-Quijada et al. [19], analyzed the behavior of liposomes formed by SOPC and SOPS in the absence of salt. They observed that the neutral phospholipid (SOPC) form spherical and cylindrical liposomes while with the charged phospholipid (SOPS) only spherical liposomes are obtained. For SOPC: SOPS ratios of 90:10 and 70:30, they obtain a high density of cylindrical liposomes and coiled cylindrical vesicles (which are produced as a result of mechanical perturbations on the cylindrical liposomes). Thus, there are particular ratios for which the mixture of neutral and charged molecules increases the membrane curvature flexibility which allows for any fluctuation to modify the geometry of the aggregate. This behavior is similar to the one we observed on DOPS liposomes for monovalent salt concentrations between 0.05M and 0.2M.Therefore; we conclude that the aggregate bending flexibility depends on the effective surface charge of the membrane.

Finally, the results obtained in this work are in agreement with previous studies on EYPC liposomes in the presence of monovalent NaCl and KCl salt [22]. Sabin et al used dynamic light scattering and electrophoretic mobility to observe that, in the low salt regime, the liposome diameter decreases for higher salt concentrations. The decrease in size is explained by the membrane impermeability to the ions, which produces osmotic forces due to the difference in ion concentration in both sides of the membrane. As a consequence, water tends to migrate towards the liposome exterior leading to a decrease in vesicle size. This explains why in the DOPS experiments for high salt concentrations, aggregates are rarely observed and the visible structures do not have an irregular structure (See Figures 2 and 3). The structures obtained in the high salt regime may be thus the result of a squeezed liposome as a consequence of osmotic forces.

DISCUSSION

DOPS liposomes were produced using the rehydration technique in the presence of monovalent salt. It was shown that the charged phospholipid DOPS forms spherical liposomes in the absence of salt. High concentrations (above 2M) of monovalent salt were observed to inhibit the formation of aggregates. This is probably due to the osmotic pressure generated by the ion concentration in the exterior of the membrane leading to the extraction of water from inside the liposome and to the corresponding squeezing of the aggregate.

For intermediate salt concentrations (between 0.05M and 0.2M) the liposomes have a cylindrical shape due to a decrease in bending rigidity, as predicted by the Winterhalter-Helfrich model. The higher flexibility makes possible for small mechanical perturbations to transform the cylindrical structures into coiled cylindrical vesicles.

The comparison of our experimental results to theoretical predictions and other experimental observations reported in the literature makes possible to explain the pattern of morphology transformations of the aggregates in the presence of monovalent ions in solution.

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