Short Note

Shapes of Mixed Phospholipid Vesicles

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Abstract. We studied the shape of phospholipid vesicles prepared by hydration of a mixture of phosphatidylcholine (SOPC) and phosphatidylserine (SOPS) in different proportions. The aim of the work is to obtain some insight into the influence of the chemical composition of a biomembrane on its shape. The optical microscopy results show that the shape of the vesicles depend on the SOPC:SOPS composition. For low SOPS contents, coiled cylindrical vesicles are observed. The results suggest that specific compositions of the SOPC:SOPS vesicles produce some spontaneous curvature on the membrane and then a coiling instability.

Key words: biological membranes, vesicles, phospholipids, vesicle coiling

Introduction

Biological membranes are highly heterogeneous systems. They are involved in endocytosis, exocytosis, cell fusion, drug delivery, etc. [1]. Furthermore, some cellular organelles, such as the Golgi apparatus and the endoplasmic reticulum, display characteristic morphologies, which are related to their specific functions. The basic structure of a biomembrane is a bilayer composed of several different phospholipids that serves as support for transmembrane proteins, polysaccharides and cholesterol. The influence of the chemical composition of lipid bilayers on the shape and function of biological membranes has not been fully understood.

The aim of this work is to obtain some insight into the influence of chemical composition on the shape of vesicles formed with mixtures of two phospholipids. For this, we prepared vesicles with a neutral lipid, phosphatidylcholine (SOPC), and a negatively charged lipid, phosphatidylserine (SOPS), in different proportions and we have studied by optical microscopy their morphology as a function of lipid composition. Our results show that the shape of the vesicles depends on the SOPC:SOPS composition. Interestingly, for low SOPS contents, coiled cylindrical vesicles are observed.

Experimental Results

Vesicles were made of 1-Stearoyl-2-Oleoyl-SN-Glycero-3-Phosphocholine (SOPC) and 1-Stearoyl-2-Oleoyl-SN-Glycero-3-Phospho-L-Serine (SOPS). Both phospholipids were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Appropriate amounts of SOPC and SOPS in chloroform solutions were mixed. The samples were prepared by vacuum drying at room temperature (at least 3 h); the total phospholipid mass in every sample was 200 μg . The samples were hydrated with 1 ml of a 60 mM sucrose solution. This method produces multilamellar vesicles. Samples prepared with higher lipid concentrations gave qualitatively similar results.

The aggregates formed were observed with the phase contrast optical microscopy technique in a LEICA DMIL inverted optical microscope with objective lenses 20 $\times$ and 40 $\times$. From the microscopy pictures we characterized the vesicle morphologies and measured the corresponding dimensions using a graduated reticule of 2 microns/division. Note that above the concentration range 2–8 mM there is non-ideal mixing of PC and PS [2]. Thus, our samples are prepared below this range, i.e. 0.2 mM. Additionally, differential scanning calorimetry experiments performed in our system showed a transition peak, which seems to indicate that in our concentrations there is ideal mixing. All the experiments were conducted at room temperature, well above the chain melting transition of both lipids.

We prepared samples with SOPC:SOPS proportions ranging from 100:00 (pure SOPC) to 0:100 (pure SOPS). A graphical survey of our results for all the studied proportions is shown in Figure 1. Because of the characteristics of the vesicle shapes, we have divided the results in three SOPC:SOPS proportion ranges: (a) 100:0–95:5, (b) 90:10–70:30, and (c) 60:40–0:100. In the following, we describe the main characteristics of vesicles in each range.

(a) 100:0–95:5. The vesicles obtained with pure SOPC are polydisperse in shape and size. That is, the vesicles are spherical, cylindrical, and compound aggregates (spheres inside spheres, spheres or cylinders aggregated on spheres, etc.), ranging roughly between 2 μm and 50 μm are observed. The average size of the spherical vesicles is 17 μm . This size changes only slightly with different SOPS concentrations. A characteristic value of the cylindrical diameter is 5 μm ; the cylinders can be as long as 100 μm and may undergo thermal fluctuations. The same kind of aggregates are observed for a SOPC:SOPS proportion of 95:5.

(b) 90:10–70:30. The vesicle population for this intermediate SOPS proportions has very similar characteristics to those described in the preceding paragraph. The main difference with the lower SOPS proportion population is that some of the cylindrical vesicles display a coiled structure. In Figure 2 we present some of the observed coiled vesicles. The length of the helicoidal vesicles is in the range of 20–70 μm , while the average tube diameter and pitch are 5.4 μm and

Figure 1. Summary of the different vesicle shapes observed in all the SOPC:SOPS proportions.

Figure 2. Coiled vesicles observed in the 90:10–70:30 SOPC:SOPS proportion range. The bar represents 10 μm .

14.7 μm , respectively. These structures are double-coiled: two segments of the same cylinder wrap around each other.
(c)60:40–0:100. For these compositions, the cylindrical vesicles disappear and all the observed vesicles are spherical as a consequence of the high content of charged lipids in the bilayers.

DISCUSSION AND CONCLUSION

The appearance of large aggregates of small curvature in the SOPC-rich region can be understood in terms of the geometrical packing parameter $r = \frac{v}{l_c a_0}$ [3], where v is the hydrophobic volume of the phospholipid molecule, l_c is the length of the hydrophobic tail and a_0 the surface per polar head. It is well known that phospholipids where $r \sim 1$ (roughly cylindrical shape) pack together to form low-curvature membrane aggregates. This is observed in this study in which SOPC molecules pack together in large membrane aggregates: large spherical and cylindrical vesicles.

The same model partially explains why the cylindrical vesicles disappear when the SOPS concentration is large enough. As the hydrophobic tails of SOPC and SOPS are identical, any distinction arises from differences in the head group. In fact, the size of the SOPS head group is larger than that of the SOPC molecule, an effect that is increased because of the electrostatic repulsion between SOPS head groups. That is, SOPS is effectively a cone-shaped molecule that induces high curvature small aggregates. This effect explains the disappearance of large-curvature aggregates (cylindrical vesicles) when enough SOPS is added to the system. However, a more refined theory is needed since the packing model does not explain why large spherical vesicles are still present in the samples.

One striking structure observed in our mixtures is that of the helicoidal aggregates shown in Figure 2. These multilayer vesicles appear in the samples with proportions between 90:10 and 70:30. Similar structures, also called myelin figures, have been observed in other systems [4–10]. In some of these systems, an inclusion is needed to induce the coiling, for example Ca^{2+} ions [4] or an amphiphilic polymer [5, 6]. Similarly, in our case the SOPS molecule plays the role of an inclusion that induces coiling. Our coiled structures are similar to those predicted in references [5, 6] for intermediate polymer concentrations, where coils are maximally tight. They are Type I double helices in the geometric phase diagram of reference [6].

A variety of hypothesis exist on the formation of coiled vesicles [4–10]; here we only discuss the effect of spontaneous curvature. A theoretical model for vesicle coiling has been developed by Santangelo and Pincus [9]. They studied the bending and interaction energies for undulations of a cylindrical multilamellar aggregate and show that a twisting undulation, which may be related to coiling, can be induced by adding spontaneous curvature to the bilayers or by decreasing the interaction equilibrium distance between the layers. In the case of our SOPC:SOPS vesicles, the coiling depends on the composition of the sample because it is only observed for compositions between 90:10 and 70:30. However, the exact mechanism of coiling is unclear. It is possible that specific compositions of the SOPC:SOPS vesicles produce some spontaneous curvature on the membrane and then a coiling instability similar to that described in [9].

Note that another possible mechanism that explains vesicle coiling in our system could be the charge relaxation mechanism proposed to explain a pearling instability in DPPC-POPG and DMPC-POPG tubular structures [10]. In such systems, tubes

show instability towards pearling without the introduction of any external gradient that may affect the spontaneous curvature. Instead, the instability is driven by charge distribution. In order to study this issue in more detail, we are currently performing experiments varying the salt concentration of the solvent. The results will be reported in a future publication.

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