Lipids (2009) 44:283–289
DOI 10.1007/s11745-009-3280-z

ORIGINAL ARTICLE

Shapes and Coiling of Mixed Phospholipid Vesicles

Gerardo Paredes-Quijada · Helim Aranda-Espinoza · Amir Maldonado

Received: 11 September 2008 / Accepted: 15 December 2008 / Published online: 27 January 2009

Abstract We have studied some physical properties of mixed phosphatidylcholine (SOPC)–phosphatidylserine (SOPS) vesicles. In a previous work (Paredes et al. in J Biol Phys 32:177–181, 2006) it was shown that the shape of the vesicles depends on the SOPC:SOPS composition, and that coiled cylindrical vesicles exist in samples with low SOPS contents. In this work, we further studied the same system of mixed vesicles. Differential scanning calorimetry (DSC) experiments displayed peaks characteristic of lipid mixing in the liquid state, ruling out a possible phase transition as an explanation of vesicle coiling. In addition, small-angle X-ray scattering (SAXS) experiments allowed us to estimate the periodicity distance inside the vesicles. This distance is $d \approx 60$ Å, as revealed by the Bragg peaks observed in the experiments. Finally, the coiling transition of a cylindrical vesicle was observed under solvent flow. This observation indicates that the vesicle coiling reported previously for this system (Paredes et al. in J Biol Phys 32:177–181, 2006) does not depend on the SOPC:SOPS composition alone, but also on mechanical perturbations during the preparation steps.

G. Paredes-Quijada (✉)
Posgrado en Polímeros y Materiales, Universidad de Sonora, Apdo. Postal 130, 83000 Hermosillo, Sonora, México
e-mail: gtparedes@correo.fisica.uson.mx

H. Aranda-Espinoza
Fischell Bioengineering Department, University of Maryland, 3135 Kim Engineering Building, College Park, MD 20742, USA

A. Maldonado
Departamento de Física, Universidad de Sonora, Apdo. Postal 1626, 83000 Hermosillo, Sonora, México

Keywords Biological membranes · Vesicles · Phospholipids · SOPC · SOPS · Vesicle coiling

Introduction

Giant vesicles have been used as models for studying membrane properties that can be relevant in biological phenomena [1, 2]. For this reason, the development of new methods for the preparation of vesicles of controlled properties (size, shape, mono- or multilamellarity) is of current interest, as well as the understanding of the mechanisms underlying the established methods. From all these, the simplest one is the hydration method, where a dried phospholipid film is hydrated with an appropriate amount of a solvent, with the vesicles forming after shaking or stirring [3]. Regardless of its simplicity, the mechanisms that favor vesicle formation at each stage of the procedure (drying, hydration, shaking) are not fully understood so far [3].

In a previous work, we reported experimental results regarding the shapes of mixed phospholipid vesicles [4]. The aggregates were prepared by the hydration method from mixtures of phosphatidylcholine (SOPC) and phosphatidylserine (SOPS) in different proportions. Using optical microscopy, we showed that the shape of the vesicles depends on the SOPC:SOPS composition. The main results of reference [4] are summarized in Table 1. It is interesting to note that among the observed vesicle shapes, there are coiled cylindrical vesicles for SOPC:SOPS molar proportions between 90:10 and 70:30 [4]. The variety of structures was interpreted in terms of the packing parameter model [5], in which the geometry of the molecules determines the aggregate structure.

Table 1 Summary of the SOPC:SOPS vesicle shapes and sizes observed in [4]

SOPC:SOPS composition	Shapes of the observed vesicles	Characteristic dimensions of the observed vesicles (μm)
0:00–95:05	Spherical	$D_{spheres} = 17$
	Cylindrical	$D_{cylinders} = 5$
90:10–70:30	Spherical	$D_{spheres} = 16$
	Cylindrical	$D_{cylinders} = 5$
	Coils	See Table 2
60:40–0:100	Spherical	$D_{spheres} = 16$

In this paper we report experiments on the same system

In this work, we report further experiments in the SOPC:SOPS system. Our aim was a better characterization of the vesicles described in reference [4], as well as the coiling transition described therein. We performed differential scanning calorimetry (DSC) experiments in order to determine the phase transition temperatures of the phospholipid mixtures. Small-angle X-ray scattering (SAXS) experiments were performed in order to have an idea of the periodicity distance inside the multilayered vesicles. In addition, the same vesicles were hydrated with solutions of monovalent and divalent salts in order to screen electrostatic interactions in the membrane and to assess if electrostatic effects are relevant in the coiling of cylindrical vesicles. Finally, the coiling of a cylindrical vesicle under solvent flow was followed using optical microscopy.

Experimental Methods

Materials

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). The molecular weights of these lipids are 788.14 g/mol and 812.05 g/mol respectively. Both phospholipids were purchased from Polar Avanti Lipids, Inc. (Alabaster, AL) and received as 10 mg/mL chloroform solutions. Purity is greater than 99%. They were stored at −20 °C. Sucrose was obtained from Sigma (St Louis, MO). Ultrapure water with a resistivity greater than 18 MΩ cm was used to prepare all solutions. For the samples hydrated with brine, we used two monovalent (NaCl, KCl) and two divalent ($CaCl_2$, $MgCl_2$) salts, all obtained from Sigma (St Louis, MO).

Sample Preparation

The samples were prepared by vacuum drying appropriate amounts of SOPC:SOPS mixtures at room temperature (3 h); the total phospholipid mass in every sample was 200 μg. In all our experiments, the samples were hydrated with 1 ml of a 60 mM sucrose solution in glass vials. The solvent of the sucrose solution was pure water, but brine solutions were used when we studied the effect of mono and divalent salts. Hydration with higher volumes of solvent gave qualitatively the same kind of aggregates. Note that above the phospholipid concentration range 2–8 mM there is non-ideal mixing of SOPC and SOPS [6]. Thus, our samples were prepared below this range, i.e. the phospholipid concentration was 0.2 mM, in order to ensure ideal SOPC–SOPS mixing. The SOPC:SOPS proportions of our samples were the same as those of reference [4] (see also Table 1). We worked in slightly acidic conditions (pH = 6). This pH is above the pK of the serine group in SOPS, which is thus not protonated.

Vesicle Shape and Size

In order to observe and to take images of the vesicles, 20 μl of the vesicle suspension were transferred to an observation chamber made with microscope cover slides and sealed with vacuum grease. The aggregates were observed using a LEICA DMIL inverted optical microscope with objective lenses 20× and 40×. From the microscopy pictures we characterized the vesicle populations and measured the aggregate dimensions by using a microscopic scale of 2 μl/division (Edmund Industrial Optics, Barrington, NJ). The vesicle dynamics was followed using a video camera (30 frames/s). All the experiments were conducted at room temperature, well above the chain melting transition of both lipids.

DSC and SAXS Experiments

Calorimetry experiments were performed in order to know the temperature transitions of the mixtures. A volume of 700 μl of the samples was injected in a calorimeter (Provo, UT) which measured the heat flow in the interval between 5 and 30 °C, at a rate of 1 °C/min. Small-angle X-ray scattering (SAXS) experiments were also performed in order to have some information about the interbilayer distance in the samples. We use a Rigaku rotating anode source that produces the CuKα lines (1.54 Å). The linear detector, with 512 channels, was placed 81 cm from the sample position. For the SAXS experiments, the samples were more concentrated in order to have a strong enough signal.

Experimental Results

In Fig. 1, we present DSC results for several SOPC:SOPS proportions. The DSC thermograms reveal a well-defined transition peak in the samples, indicating mixing of the phospholipids. The main transition for the mixtures lies between 8 and 18 °C, well below room temperature. All the transition temperatures lie between those of SOPC (≈6 °C) and SOPS (≈26 °C) [7]. This is an indication that effects related to the solid-liquid melting transition in the membranes can be ruled out. We can conclude that in our samples, the lipids are mixed in the liquid state.

Several vesicle shapes (spheres, cylinders, complex aggregates) appeared in the optical microscopy experiments, as reported in reference [4] and summarized in Table 1.

Since the vesicles were prepared by a hydration method, they should be multilayered aggregates. These vesicles can be pictured as onion-like structures, composed of layers of concentric spherical or cylindrical membranes. A representation of the vesicles is shown in Fig. 2. Due to the concentric layers, the aggregates have a local lamellar nature that should display a Bragg interference peak in scattering experiments. In order to have an idea of the

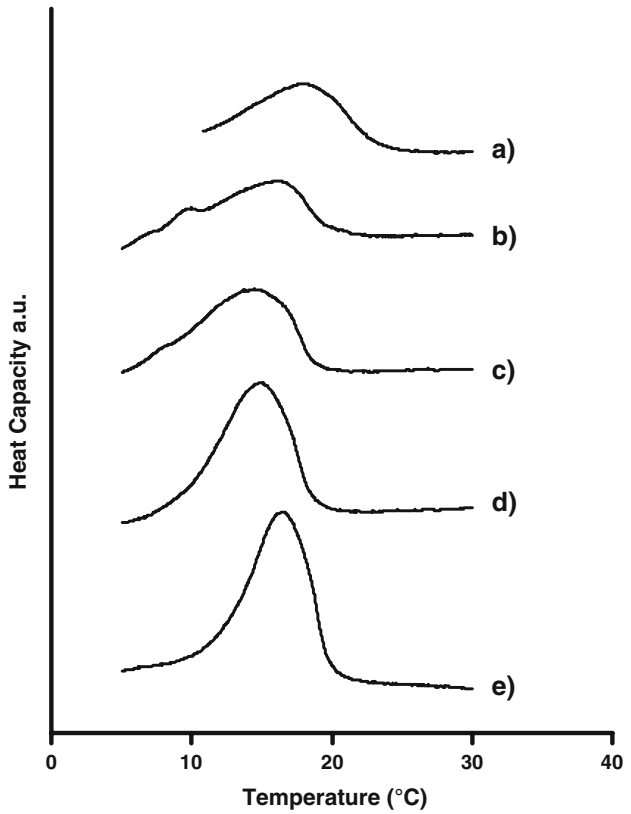

Fig. 1 DSC thermograms of SOPC:SOPS membranes with different proportions: *a* 90:10, *b* 70:30, *c* 50:50, *d* 30:70 and *e* 10:90. The transition temperatures are below room temperature

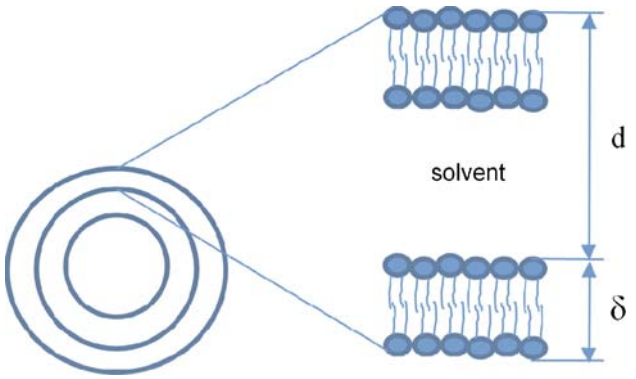

Fig. 2 Schematic representation of the vesicles prepared by the hydration method. They can be pictured as onion-like structures, composed of layers of concentric membranes. Note that the aggregates have a local lamellar nature, where d is the periodicity distance. δ is the membrane thickness. Note that the drawings are not at scale

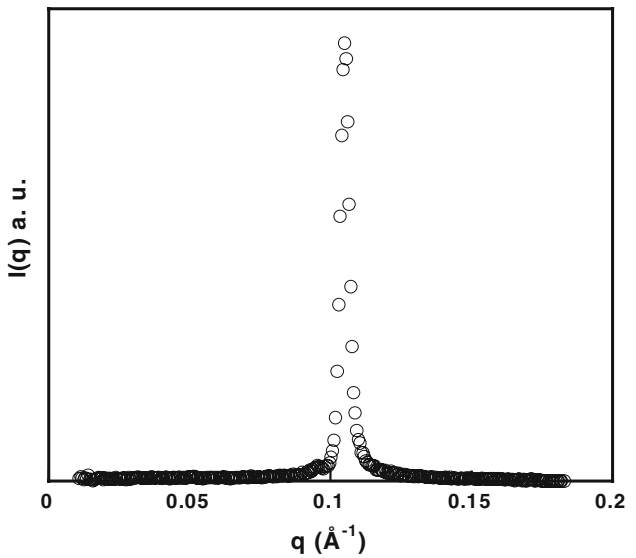

Fig. 3 SAXS spectra of a sample with 100:0 composition. The Bragg peak indicates a multilamellar structure. From the position of the peak, the periodicity distance is $d \approx 60$ Å

periodicity distance d of the lamellar structure inside the vesicles (Fig. 2), we have performed SAXS experiments. Since the scattering signal was too low with the concentrations used for the optical microscopy experiments, the samples for X-ray Scattering were more concentrated. The SAXS spectra displayed a well-defined Bragg peak (Fig. 3), indicating that the vesicles have a multilamellar structure. From the peak position we computed the periodicity distance d using the Bragg relationship, $d = 2\pi/q_{\max}$. In our vesicles, this interbilayer distance is $d \approx 60$ Å. Note that systematic SAXS experiments could be interesting in order to study properties such as the

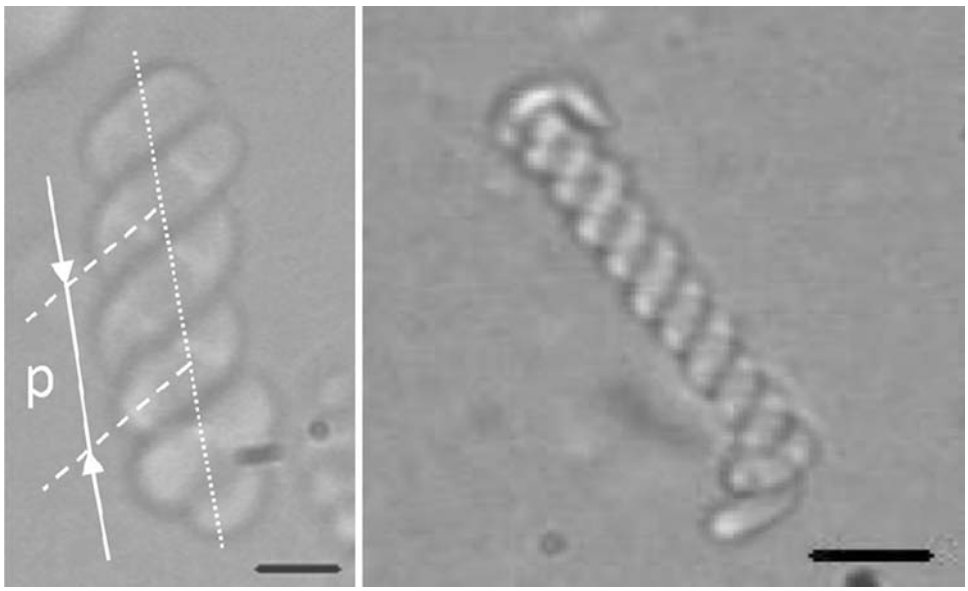

Fig. 4 Two of the coiled vesicles observed in the 90:10–70:30 SOPC:SOPS proportion range. *p* is the pitch of the helical structure. The *bar* represents 10 μm

interbilayer distance and the membrane elastic constants [8] as a function of concentration, temperature and pH.

In reference [4] it was reported that a striking structure observed for some SOPC:SOPS proportions is that of coiled vesicles. In Fig. 4 we present two of such coiled aggregates. We have measured the characteristic dimensions of the coiled vesicles. These dimensions are: the length of the coiled vesicle (l), the radius of the coiled cylinder (r) and the pitch (p) of the helical structure. Note that since the coiled vesicles are double helices [4, 9], the pitch p is defined as the repetition distance (for a single cylinder) along the helical axis (Fig. 4). The measured dimensions are shown in Table 2. As the ratio between the pitch of the helix p, and the radius of the cylinder r, is an indication of the tightness (or looseness) of the coiled structure, in Fig. 5 we have plotted p as a function of r. We see in this picture that the pitch is linearly related to the cylinder radius. The ratio of the radius to the pitch is obtained from the slope of this curve; its value is $r/p = 0.22 \pm 0.09$. This value is very near to that of the tightly wound helices observed in the phosphatidylcholine/cardiolipin mixture in the presence of calcium ions ($r/p = 0.214$) [10]. Looser helix formation has been observed in egg-PC vesicles, where the radius/pitch ratio is $r/p = 0.175 \pm 0.015$ [11].

In order to assess if electrostatic effects, such as the charge relaxation mechanism [12], do play a role in the coiling of our vesicles, we performed experiments with different salt concentrations in the solvent. For this, we

Table 2 Measured length, cylinder diameter and pitch of typical coiled vesicles observed in the proportion range 90:10–70:30

Vesicle	Length l	Cylinder radius r	Pitch p
1	18.09	2.92	8.33
2	22.50	3.33	4.17
3	23.75	5.02	18.33
4	25.83	4.17	10.43
5	31.43	4.17	9.32
6	35.42	3.33	4.17
7	48.32	3.25	9.58
8	52.29	6.67	17.98
9	57.21	7.96	29.36
10	62.7	8.92	25
11	66.65	9.51	25.00

The values are given in microns

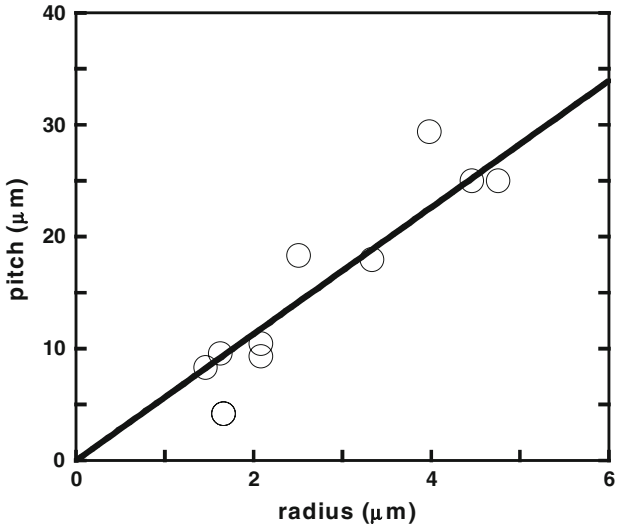

Fig. 5 Dependence of the measured pitch on the cylinder radius for coiled vesicles. The ratio of the radius to the pitch is 0.22 ± 0.09, as obtained from the slope of the linear fit

used monovalent (NaCl, KCl) and divalent ($CaCl_2$, $MgCl_2$) salts. The positive ions screen the repulsive electrostatic interactions between SOPS head groups. Our results indicate that the yield of vesicles depends on the type of ion. Monovalent salts inhibit the formation of vesicles. On the other hand, divalent ions promote the formation of mainly spherical vesicles. These results are somewhat similar to those obtained by Akashi and coworkers [13] with a slightly different hydration method. Interestingly, no coiling was observed in the few cylindrical vesicles obtained in our experiments. It is not clear if this effect is only due to the low yield of cylindrical vesicles or to electrostatic effects related with the screening of the Coulomb interactions between PS head groups.

Note that other possible source of electrostatic effects is the pH of the solutions. In our case, we work in a slightly acidic media (pH = 6), thus effects due to protonation of the phospholipids should be negligible. In fact, the phosphatidylcholine head group has a strong zwitterionic character [3], i.e, it bears a positive electrical charge (associated with the nitrogen atom) as well as a negative electrical charge (phosphorous atom). On the basis of titrimetric assays, PC is known to preserve its zwitterionic characteristics over the entire pH range, $1 < pH < 12$ [14]. Thus, it is expected that a modification of pH, at least in this range, does not change the SOPC properties. On the other hand, SOPS is an anionic phospholipid [3]. Its head group has three ionizable groups (the diester phosphoric acid, the α-amino group and the carboxyl group), and it bears a net negative charge above a pH = 4.5 [14]. Below this value of pH, the carboxyl group protonates and SOPS is in the zwitterionic form (two opposite-sign electrical charges) [14]. Thus, if pH decreases below 4.5, SOPS loses its net negative charge and its properties change.

This behavior of the PC and PS head groups has been confirmed by potentiometric titration and surface potential measurements in mixed PC:PS vesicles [15]. We expect that in the case of the shapes of our SOPC:SOPS vesicles, no modifications would be observed if the pH is varied in the range above 4.5. However, due to protonation of the PS head group, one would expect modifications in the shape and properties of the SOPC:SOPS vesicles below a pH = 4.5. It would be an interesting point to investigate if coiling is possible for these pH values. We leave this issue for a future work.

Coiling of the SOPC:SOPS vesicles diluted with pure water depends on the composition of the sample because it is only observed for compositions between 90:10 and 70:30 [4]. However, it seems that the appearance of helicoidal vesicles is not only due to specific compositions but also to hydrodynamical stresses. In Fig. 6 we show the coiling of a SOPC:SOPS vesicle under a hydrodynamic perturbation. The composition of this vesicle is 80:20. The sequence was obtained following a long cylindrical vesicle after solvent dilution. Right after sample preparation, the cylindrical vesicle was observed for a period of time of 30 min and only thermal fluctuations were observed (Fig. 6a). Afterwards, the sample was diluted adding a small quantity (10 μl) of solvent with a pipette. The effect was to coil both ends of the vesicle (Fig. 6b). The vesicle remained stable in this state for several minutes. Only thermal fluctuations were observed. Again we added the same volume of solvent and both ends of the vesicle continued to coil (Fig. 6c). The resulting vesicle was again stable for 30 min. This procedure was repeated until a final highly packed coiled aggregate was obtained (Fig. 6i). Note that the scale in Fig. 6 was changed in the last two pictures because of the small size of the final vesicle. This kind of highly packed aggregate was also observed in other samples with these phospholipid compositions. It seems to be the final stage of a coiling dynamics triggered by a hydrodynamical perturbation. Thus, coiling does not only depend on the composition of the membrane, but also on mechanical perturbations. This is a possible explanation of why the shapes of the population of vesicles obtained by hydration methods depend on the mechanical treatment of the samples. Note that the added solvent volume in each step of Fig. 6 reduces the phospholipid concentration of the sample. By performing this series of dilutions, at the final stage (Fig. 6i) we changed the total phospholipid concentration to about 20% of its original value. This new concentration is well above the typical cmc of phospholipids [5]. It is possible that preparing the vesicles with this concentration could lead to modifications in the aggregate characteristics. However, in the experiments depicted in Fig. 6 we did not perform all the hydration steps (drying, hydration, shaking). Instead, we just added the solvent to the already hydrated vesicles, without shaking the sample. The effect was to modify the preformed vesicle in the form observed in Fig. 6. We did not measure the characteristics of the other remaining vesicles, but just followed this cylindrical aggregate.

Discussion

As reported in reference [4], coiled vesicles have been observed in other systems [9–13, 16–19]. In some cases, coiling is induced by adding an additional component to cylindrical phospholipid vesicles. This component is either an ion [10] or a polymer [9, 16].

From a theoretical point of view, several mechanisms have been proposed in order to understand the existence of helicoidal vesicles.

Some of these approaches explain the coiling of the vesicles as a result of the interplay between favorable and

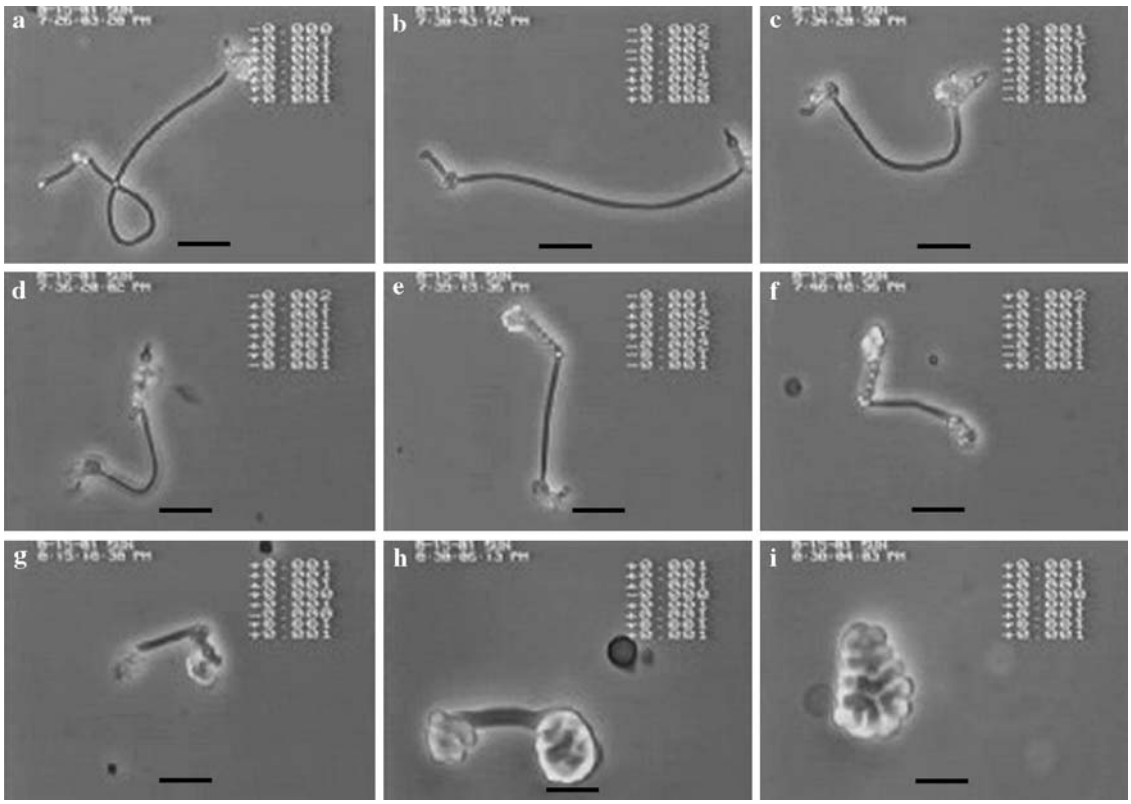

Fig. 6 Coiling dynamics of a SOPC:SOPS vesicle under a hydrodynamic perturbation (see text). The phospholipid composition of this vesicle is 80:20. **a** Unperturbed cylindrical vesicle undergoing thermal fluctuations. **b–i** Coiling of the vesicle observed after hydrodynamic perturbation (flow of solvent) in each step. **i** Final highly-packed coiled vesicle. The *bar* represents 10 μm (**a–g**) and 5 μm in (**h**) and (**i**)

unfavorable forces. For instance, Lin et al. [10] have explained the Ca^{2+}-mediated induction of helical liposomes in mixtures of DMPC and cardiolipin by membrane–membrane binding forces; they argue that weak membrane–membrane binding energies can overcome curvature elastic energies and stabilize coiled vesicles. On the other hand, Mishima et al. [11] have reported coiling of cylindrical vesicles of egg-yolk lecithin. In this work, the proposed mechanism of coiling is an interplay between intermembrane attraction and the forces opposing helix formation which arise from the bending energy of the membranes. In the case of the SOPC:SOPS vesicles studied in this paper, membrane adhesion should be weak because the SOPS proportions are relatively small and adhesion forces should be similar to the case of pure SOPC [9]. In addition, the electrostatic repulsion of SOPS is unfavorable to adhesion. We thus rule out a mechanism related to a balance between favorable and unfavorable forces.

Another possible mechanism is the coupling between membrane composition and local membrane curvature. This is the driving force in the case of coiling induced by anchoring a hydrophobically modified polymer into cylindrical phospholipid vesicles [9, 16]. It has been proposed that the added polymer diffuses and creates inhomogeneities that reduce the free energy of the system; this process locally induces a spontaneous curvature which drives the coiling of the cylinders [9, 16]. In our case, local inhomogeneities could appear after demixing of the two phospholipids composing the vesicle membranes. However, the DSC experiments show that our experiments were performed at a temperature well above the chain melting transition, regardless of the SOPC:SOPS proportion. Thus, phase transitions in the membrane can be ruled out as a driving force for coiling.

In our system, the exact mechanism of coiling is so far unclear. It is possible that the vesicles undergo a coiling transition due to some spontaneous curvature appearing at specific SOPC:SOPS compositions. This is a mechanism reported in the framework of a theoretical model by Santangelo and Pincus [19].

However, as shown in Fig. 6, coiling can also be induced by an appropriate flow of solvent. It is possible that the hydrodynamic perturbation produced by this flow creates asymmetric stresses or forces around the cylindrical vesicle so as to drag the cylinder to coil around itself. These forces could be of hydrodynamic origin, like those

explained by the Bernoulli equation of fluid dynamics [20]. One of the main effects predicted by this equation is a variation in pressure correlated with variations in the local velocity in an incompressible fluid. If the velocity of the fluid is higher in one point than in another, in the first point the fluid pressure is lower than in the second. This could give rise to two forces: a drag force, parallel to the free-stream velocity, and a lift force, perpendicular to the free-stream. The effect of the drag force is to push an object along the fluid flow. However, the lift force can lead to a bending of a flexible object. In the case of the vesicle of Fig. 6, it is possible that the solvent flow around the cylinder had an asymmetric velocity distribution. The side of the vesicle exposed to a low solvent velocity is subjected to a higher fluid pressure (as compared with the side exposed to a high solvent velocity). Thus, a lift force acts on the cylinder, pushing it from low-velocity to high-velocity regions. This effect, combined with appropriate shape or curvature fluctuations, could lead to the process depicted in Fig. 6. However, this is a point that needs more clarification, both theoretically as well as experimentally. Meanwhile, it is clear that in our case coiling is a process that depends on the way the system is prepared. This result has implications regarding the preparation of vesicles by hydration methods. Steps like stirring or shaking can induce flows that change the vesicle morphology as in the case of Fig. 6. In fact, these effects could explain some of the differences observed in the population of vesicles obtained by different hydration procedures.

Conclusions

We studied some properties of phospholipid vesicles prepared by hydration of mixtures of phosphatidylcholine (SOPC) and phosphatidylserine (SOPS) in different proportions. We investigated this system because in a previous work it has been shown that the shape of the vesicles depends on the SOPC:SOPS composition [4]. Using SAXS experiments we have confirmed that the vesicles are multilayered and that the periodicity distance inside a vesicle is or the order of $d \approx 60$ Å. On the other hand, DSC experiments have shown that the membranes are formed by a homogeneous mixture of the two phospholipids, with melting transitions well below room temperature. This result rules out effects related to phase transitions as a driving force for the coiling of cylindrical vesicles observed in this system. Finally, coiling was induced in a cylindrical vesicle by a hydrodynamic perturbation. This result shows that this process not only depends on the SOPC:SOPS composition, but also on mechanical forces applied to the phospholipid vesicles. This observation has implications for understanding the mechanisms behind the differences observed in the shapes and properties of vesicles prepared by hydration methods.

Acknowledgments G.P.-Q. acknowledges a fellowship from Conacyt-Mexico. A.M. acknowledges financial support from Conacyt-Mexico (grants 32105E and 0074) and ANUIES-ECOS (MOOP03). H.A.-E. and G.P.-Q. thank Dennis Discher for allowing us to use his laboratory where initial experiments were carried out. We thank Raymond Ober and Ricardo López-Esparza for collaboration in the SAXS experiments.

References

1. Yeagle PL (2005) The structure of biological membranes, 2nd edn. CRC Press, Boca Raton
2. Svetina S, Žekš B (2002) Shape behavior of lipid vesicles as the basis of some cellular processes. Anat Rec 268:215–225
3. Cevc G (ed) (1993) The phospholipid handbook, Marcel Dekker, NY
4. Paredes G, Aranda H, Maldonado A (2006) Shapes of mixed phospholipid vesicles. J Biol Phys 32(2):177–181
5. Israelachvilli J (1992) Intermolecular and surface forces, 2nd edn. Academic Press, Orlando
6. Huang JR, Swanson JE, Dibble ARG, Hinderliter AK, Feigenson GW (1993) Nonideal mixing of phosphatidylserine and phosphatidylcholine in the fluid lamellar phase. Biophys J 64:413–425
7. Lygre H, Moe G, Skalevik R (2003) Interaction of Tricosan with eukaryotic membrane lipids. H Eur J Oral Sci 111:216–222
8. Helfrich W (1973) Elastic properties of lipid bilayers: theory and possible experiments. Z Naturforsch 28C:693–703
9. Tsafrir I, Guedeau-Boudeville MA, Kandel D, Stavans J (2001) Coiling instability of multilamellar membrane tubes with anchored polymers. Phys Rev E 63:031603
10. Lin KC, Weiss RW, McConnell HM (1982) Induction of helical liposomes by Ca^{2+} mediated intermembrane binding. Nature 296:164–165
11. Mishima K, Fukuda L, Suzuki K (1992) Double helix formation of phosphatidylcholine myelin figures. Biochim Biophys Acta 1108:115–118
12. Nguyen TT, Gopal A, Lee KYC, Witten TA (2005) Surface charge relaxation and the pearling instability of charged surfactant tubes. Phys Rev E 72:051930
13. Akashi KI, Miyata H, Itoh H, Kinosita JK (1998) Formation of giant liposomes by divalent cations: critical role of electrostatic repulsion. Biophys J 74:2973–2982
14. Hanahan DJ (1997) A guide to phospholipid chemistry. Oxford University Press, Oxford
15. Tsui FC, Ojcius DM, Hubbel WL (1986) The intrinsic pKa values for phosphatidylserine and phosphatidylethanolamine in phosphatidylcholine host bilayers. Biophys J 49:459–468
16. Frette V, Tsafrir I, Guedeau MA, Jullien L, Kandel D, Stavans J (1999) Coiling of cylindrical membrane stacks with anchored polymers. Phys Rev Lett 83:2465–2468
17. Buchanan M, Egelhaaf SU, Cates ME (2000) Dynamics of interface instabilities in nonionic lamellar phases. Langmuir 16:3718–3726
18. Huang JR, Zou LN, Witten TA (2005) Confined multilamellae prefer cylindrical morphology: a theory of myelin formation. Eur Phys J E Soft Matter 18:279–285
19. Santangelo CD, Pincus P (2002) Coiling instabilities of multilamellar tubes. Phys Rev E 66:061501
20. Shames IH (2003) Mechanics of fluids, 4th edn. Mc Graw-Hill, New York