Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1717 (2005) 104 – 108
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Effect of salt concentration on membrane lysis pressure

Catherine C. Logisz, Jennifer S. Hovis *

Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

Received 7 June 2005; received in revised form 4 October 2005; accepted 6 October 2005
Available online 25 October 2005

Abstract

Cell membranes are capable of withstanding significant osmotic stress, the exact amount of which varies with the lipid composition. In this paper, we examine the effect that salt concentration has on the lysis pressure of membranes containing anionic lipids. Vesicles containing varying amounts of phosphatidylcholine and phosphatidylglycerol were osmotically stressed using NaCl as the osmolyte. The lysis pressure was observed to vary linearly with the Debye screening length and the extent of the variation was linear with anionic lipid content. The implications these results have for cells that frequently encounter low solute environments are discussed.

Keywords: Anionic lipid; Lysis pressure; Osmotic pressure; Membrane stability

1. Introduction

Cell membranes are capable of withstanding many atmospheres of pressure [1]. The amount of pressure the membrane can withstand depends on the composition of the lipid bilayer; inclusion of micelle forming lipids or negatively charged lipids lowers the pressure while inclusion of inverted micelle forming lipids raises the pressure [2–6]. Additionally, it has been shown that calcium can modulate the mechanical properties of anionic membranes [6]. In recent years, a great deal of attention has been focused on the role that lipids play in cellular function with a key question being—how is lipid activity regulated? The mechanical properties of membranes can be altered by changing the lipid composition (e.g. through the action of phospholipases) or if negatively charged lipids are present by changing the electrolyte concentration. The use of electrolyte concentration to regulate membrane mechanical properties is the focus of the present study, particularly the effect of monovalent salts.

The standard technique for studying the mechanical properties of membranes is micropipette aspiration. However, the method for forming the giant unilameller vesicles used in the micropipette studies requires very low electrolyte concentrations making it impossible to work with physiologically relevant monovalent salt solutions [5]. Information about mechanical properties can also be gained using large unilameller vesicles and osmotic pressure; large amounts of monovalent salt can be tolerated and thus the effect of monovalent salts on the stability of anionic membranes can be examined [2,7–9]. When lipid bilayer vesicles are exposed to a low solute environment a net flux of water into the membrane will occur if the osmotic pressure is greater than the Laplace pressure. A vesicle is capable of only a small amount of swelling before a pore opens to relieve the tension. The interior solutes escape through the pore lowering the pressure difference; the pore closes when the osmotic pressure is reduced to the point that it balanced by the Laplace pressure and a stationary state with no net flux of water across the membrane is established. The pore closure pressure can be determined by examining the amount of leakage, if the radius of the vesicles is known then the critical surface tension and the line tension can also be determined [10,11].

In this study, the effect of NaCl concentration on the stability of vesicles containing phosphatidylglycerol (PG) and phosphatidylcholine (PC) is examined. In brief large unilameller vesicles containing a quenched amount of a fluorophore (100 mM carboxyfluoroscein) and a high salt concentration (700 mM NaCl with 50 mM HEPES, pH 7.4) are exposed to lower osmolarity environments (varying amounts of NaCl with 50 mM HEPES, pH 7.4) and the amount of leakage from the vesicles is quantified. It will be shown that as with divalent

* Corresponding author. Fax: +1 765 494 0239.
E-mail address: jhovis@purdue.edu (J.S. Hovis).

0005-2736/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2005.10.002

salts monovalent salts can quite significantly alter the mechanical properties of anionic lipid bilayers.

2. Experimental methods

2.1. Material

Chloroform stock solutions of L-α-phosphatidylcholine from egg yolk (eggPC) and L-α-phospatidylglycerol made by transphosphatidylation of egg lectin in the presence of glycerol (eggPG) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL), and used without further purification. (N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) (HEPES), Sephadex G-50, and Triton X-100 detergent were purchased from Sigma Chemical Co. (St. Louis, MO). 5-(and-6)-carboxyfluorescein was purchased from Molecular Probes (Eugene, OR). The carboxyfluorescein (CF) buffer was composed of 100 mM carboxyfluorescein, 700 mM NaCl, 50 mM HEPES; adjusted to pH 7.4 with 1550 mM NaOH. The osmolarity of this solution is estimated to be ~1440 mosM, it is assumed that each HEPES and CF molecule contribute one particle and that the NaCl contributes 1.84 particles (CRC handbook [12]). An approximately iso-osmotic buffer was composed of 750 mM NaCl, 50 mM HEPES, adjusted to pH 7.4 with 1550 mM NaOH.

2.2. Vesicle preparation

Large unilamellar vesicles (LUVs) were prepared by extrusion: the eggPC and eggPG lipids were combined at appropriate molar ratios in chloroform; the chloroform was removed under a nitrogen stream and the lipids were held under vacuum for 1 h; the dried lipids were re-suspended in the 100 mM CF, 700 mM NaCl, 50 mM HEPES buffer; the lipid suspension was then extruded through polycarbonate membranes with 50 nm pores a minimum of 15 times. The LUV solution was centrifuged for 5 min at 14,000 rpm (Eppendorf Minispin Plus) following extrusion and the solution was run down a Sephadex G-50 size exclusion column, equilibrated with iso-osmotic buffer, to remove any CF not encapsulated in the vesicles.

2.3. Vesicle stressing assay

This assay is same as one we have used previously [2] and is similar to others in the literature [7–9]. To osmotically stress the vesicles 30 μL of the vesicle solution was placed into a cuvette and 1970 μL of a lower osmolarity buffer (varying amounts of NaCl, 50 mM HEPES, pH 7.4) was added. In the solutions containing vesicles that leaked contents, a color change was observed as soon as the buffer solution was added, to ensure that the contents mixed the cuvettes were gently shaken; the solution in the cuvettes sat for 5 min before measurements were made. When the CF escapes from the vesicles it dequenches and the rise in fluorescence can be used to quantify the amount of leakage; previous papers have shown that the solutes are expelled indiscriminately [7–9]. The fluorescence was measured on a Jobin Yvon Fluorolog-3 (excitation λ=491 nm, emission λ=519 nm) equipped with a single excitation monochromator and dual emission monochromators. The amount of vesicle leakage was calculated with the following equation:

$$\text{Leakage}\,(\%) = 100 \times \left(\frac{F_{\text{signal}} - F_{\text{baseline}}}{F_{100\%} - F_{\text{baseline}}}\right)$$

where F_{baseline} is the fluorescence of the vesicles placed into an iso-osmotic environment, F_{signal} is the fluorescence of the stressed vesicles, and $F_{100\%}$ is the fluorescence after vesicle destruction by the addition of Triton X-100. The experiments were done at room temperature and repeated with at least three different sets of vesicles. For all compositions studied the $F_{100\%}$ and F_{baseline} values were very similar indicating that neither the encapsulation efficiency nor the leakage before stressing changed with composition.

2.4. Dynamic light scattering

A Coulter N4 dynamic light scattering system was used to determine the radii of the extruded vesicles. Data were fit using a cumulant analysis and the results were consistent with a narrow size distribution. The measured radii seem on the large size given that the pores are 50 nm in diameter; the discrepancy arises primarily from the fact that in the cumulant analysis the particle sizes are intensity weighted but the filter pore size is a number average quantity, thus two different statistical averages are being compared. Also, there are possibly two minor contributing factors to the size discrepancy: One, it has been shown that extrusion produces vesicles which are larger than the pore size and that the smaller the pore the larger the difference [13,14]. Two, dynamic light scattering is not very sensitive to small objects and is highly sensitive to large objects; therefore despite our best efforts to make sure no large vesicles are present a tiny fraction could skew the results.

3. Results and discussion

The results from the osmotic stressing assay are shown in Fig. 1A (for clarity the 20 mol% PG data is omitted). It can be seen that when vesicles are placed in high osmolarity environments the Laplace pressure balances the osmotic pressure and no leakage occurs. For the eggPC (phosphatidylcholine from egg) vesicles content leakage begins ~1000 mosM and by ~750 mosM the amount of leakage is linear with respect to the external osmolarity. Fitting the linear section the pore closure pressure, C_{diff}, can be determined and knowing the radius of the vesicles the approximate critical surface tension and line tension can also be determined. The equation used to determine the pore closure pressure is,

$$\text{Leakage} = \left(1 - \frac{C_{\text{diff}} V}{C_{\text{in,o}} V_{\text{o}}}\right) + \left(-\frac{V}{C_{\text{in,o}} V_o}\right) C_{\text{out}} \qquad (1)$$

where $C_{\text{in,o}}$ is the initial internal osmolarity, V is the new volume and V_{o} is the initial volume [2]. Hallet and co-workers have shown that the fit can also be used to extract related

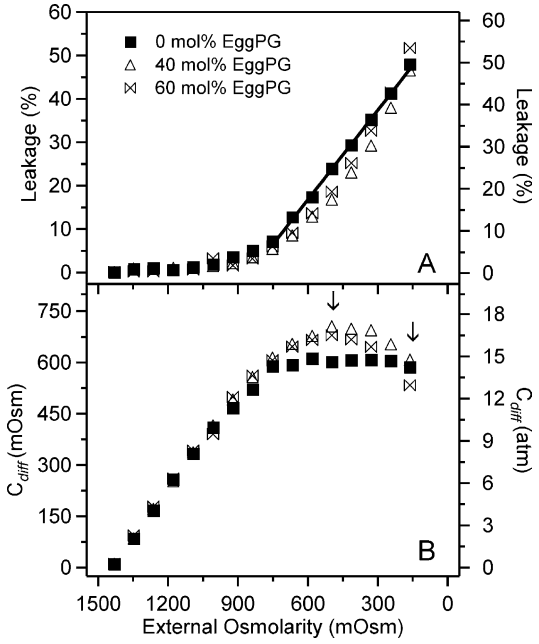

Fig. 1. Effect of lipid composition on: (A) Leakage from vesicles subjected to hypo-osmotic gradients. (B) Pore closure pressure. Results are shown for eggPC vesicles containing increasing amounts of eggPG. Error bars are not shown; they are the size of the markers and represent at least three independent measurements. Arrows in B indicate external salt concentrations of 240 mM NaCl (left) and 60 mM NaCl (right).

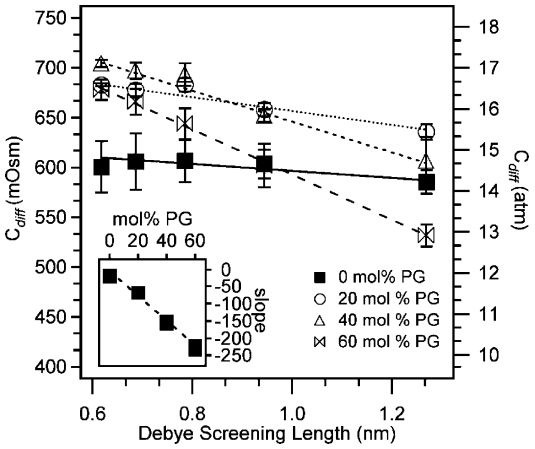

Fig. 2. Pore closure pressure for eggPC vesicles containing increasing amounts of eggPG plotted as a function of the external Debye screening length; only the data from vesicles in external salt concentrations from 240 to 60 mM NaCl is shown (see arrows in Fig. 1B). Linear fits to the data are shown; in the inset the slope of each of the fits is plotted as a function of mol% PG.

information, e.g., the area compressibility and Young's modulus [8]. The Laplace pressure is inversely proportional to the vesicle radius, we have a distribution of vesicle sizes and as a consequence the larger vesicles leak at lower pressure differences. As a result, the data between ~1000–750 mosM cannot be included in our determination of C_{diff}, which to be accurate is the average pore closure pressure. The vesicles used in this study have a narrow size distribution, as a result once the Laplace pressure has been exceed for all of the vesicles the polydispersity in vesicle size can be neglected.

In contrast to the eggPC data the amount of leakage from vesicles containing eggPG (made by transphosphatidylation of eggPC in the presence of glycerol, consequently the tail distributions are the same) is not linear with respect to the external osmolarity, indicating that the pore closure pressure is sensitive to the external solution. Rather than fitting the data to extract a pore closure pressure we must then calculate the pressure for each individual point: $C_{\mathrm{diff}}=C_{\mathrm{in}}-C_{\mathrm{out}}$, C_{out} is known and C_{in} can be determined by using the leakage data to determine the fraction of contents left inside the vesicles and then multiplying that fraction by $C_{\mathrm{in,o}}$, which is calculated to be ~1440 mosM (see Materials and methods). In Fig. 1B, we show the calculated pore closure pressure for each of the data points in 1A. Examining the eggPC data we observe an initial linear rise in the data, in this regime, the vesicles are not leaking so it is inaccurate to label these values as pore closure pressures. The data then begins to deviate from linearity, this is the region where some but not all of the vesicles have lost contents. Finally, the C_{diff} values flatten out and are invariant with respect to the external osmolarity. The calculated weighted average of the individual pore closure pressures is 600±20 mosM while the fit to the data in Fig. 1A gives 570±20 mosM, in good agreement. The equation used to fit the data has the interesting feature that the initial internal osmolarity is not used in determining C_{diff} (the other values in the intercept are determined from the slope). To calculate the pore closure pressure for each individual point however the initial internal osmolarity is needed; that good agreement between the two methods is observed shows the initial osmolarity is as expected. In examining our previous work with eggPC vesicles containing eggPE (phosphatidylethanomaine, PE, from egg), lysoPC or lysoPE [2] we see that when plotting the data in the manner of Fig. 1B it also flattens out—at a higher pressure for the vesicles containing PE and at a lower pressure for those containing lysoPC or lysoPE (Hull, M.C., Sauer, D.B and Hovis, J.S., unpublished data).

Examining the data from vesicles containing eggPG in Fig. 1B, we observe that the values climb to a higher level than the eggPC data but then rather than flattening out they curve back down. Between the arrows, indicating external salt concentrations of 240 (left) and 60 mM (right) NaCl, the pore closure pressure drops ~22% for vesicles containing 60 mol% eggPG, ~14% for vesicles containing 40 mol% eggPG and ~7% for vesicles containing 20 mol% eggPG (shown in Fig. 2). We were unable to form vesicles containing 80 or 100 mol% eggPG. The Laplace pressure is inversely proportional to the radius of the vesicles and therefore the calculated pore closure pressures are only comparable in a meaningful way if the vesicle radii remain constant. Using dynamic light scattering (DLS), we have determined the average radii of 20, 40 and 60 mol% PG vesicles placed into external NaCl solutions of 240 and 60 mM NaCl (along with 50 mM HEPES, pH 7.4), the results are shown in Table 1; within the error of the measurement, ~4%, the average radii is not observed to change. Therefore, changes in vesicle size are not responsible for the bulk of the observed drops in pore closure pressure.

To examine why the pore closure pressure changes with the external salt concentration, we start by noting that it is proportional to the critical surface tension [10], a measure of the strength of the intermolecular forces.

$$C_{\mathrm{diff}} \approx \frac{2\sigma_c}{10^3 k_{\mathrm{B}} T N_{\mathrm{A}} R_{\mathrm{o}}} \tag{2}$$

where σ_{c} is the critical tension of the stretched membrane, k_{B} is the Boltzmann constant, N_{A} is the Avogadro number and R_{o} is the un-stretched vesicle radius. There is evidence in the literature that the headgroup area of PG can change substantially with salt concentration—specifically, it has been observed that the micelle-to-bilayer transition shifts for short chain PG lipids in the presence of salt [15] and that $di\mathrm{C}_{16}$PG increases in surface area 30% in going from the protonated form (pH 1.5) to the charged form (pH 8.0) at 1.5 M NaCl [16]. We hypothesize that the changing headgroup area modulates the intermolecular interactions by altering the extent of hydrogen-bonding and/or van der Waals tail–tail interactions. Phosphatidylglycerol has the potential to form intermolecular

Table 1
Average vesicle radii in external salt solutions of 240 mM and 60 mM NaCl; change in pore closure pressure, C_{diff}, over the range from 240 to 60 mM NaCl

mol% PG	240 mM NaCl	60 mM NaCl	ΔC_{diff}
20	55±2 nm	55±2 nm	7%
40	53±2 nm	53±1 nm	14%
60	48±2 nm	46±2 nm	22%

hydrogen bonds between the hydroxyl groups and either the phosphate or carbonyl groups; from experiments [17,18] and MD simulations [19], there is evidence of limited formation of hydrogen bonds between PG molecules. It is logical to assume that the ability of PG to form intermolecular hydrogen bonds depends on the salt concentration—as the salt concentration decreases the electrostatic repulsion between the PG headgroups is increased and the ability to form hydrogen bonds should decrease. The van der Waals tail–tail interactions would vary in the same way, as the PG headgroups increase in size the tails will not be packed as tightly, decreasing the favorable van der Waals interactions. It might therefore be expected that the headgroup area and thus also the pore closure pressure would vary linearly with the Debye screening length. In Fig. 2, the pore closure pressures between the arrows in Fig. 1B are plotted as a function of the Debye screening length. It can clearly be seen that the pore closure pressures do indeed vary linearly with the screening length. Additionally the slopes of the fits are linear with respect to the mole fraction of anionic lipid, see inset in Fig. 2—the higher the concentration of anionic lipid, the more sensitive the lysis pressure is to changes in salt concentration. These results suggest that as the salt concentration decreases the amount of hydrogen bonding decreases and/or the tail–tail interactions decrease, either of which in turn decreases the critical surface tension and the pore closure pressure. Lastly, we note that our assertion that the PG headgroup area increases with decreasing salt concentration might seem contradictory to our previous assertion that within the ~4% error of the DLS measurements the vesicle radii do not change. A back of the envelope calculation shows that if 60 mol% of the lipids increase in area by 15% then the radius increases by 4.4%. A 30% increase in area was observed when PG went from the protonated to the charged form, if 60% of the lipids undergo that amount of a change then the vesicle radii would increase by 8%—larger than we measure but this large of an increase is not likely as the lipids are not being converted to the protonated form.

Assuming that the barrier to pore opening is on the order of k_BT the line tension can be calculated from the surface tension [10].

$$k_BT \approx \frac{\pi\gamma^2}{\sigma_c}, \tag{3}$$

where γ is the line tension. Thus, the pore closure pressure is related to the line tension squared and the effect that salt concentration has on the line tension can also be considered. Lipids that help to stabilize the pore will lower the line tension while lipids that are not well accommodated in the pore will raise the line tension. In general, it has been observed that micelle forming lipids lower the line tension while inverted micelle forming lipids raise the line tension [2–4]. As the salt concentration decreases, the distance over which the charges are felt increases, pushing the PG lipids father away from one another and increasing the average area of the PG headgroup—lipids with larger headgroup areas may be better accommodated in the pore, lowering the line tension and consequently pore closure pressure. As mentioned above, there is evidence in the literature that the headgroup area of PG can change substantially with salt concentration. As the ion concentration in the pore is changing with time, it is not straightforward to compare the line tension with the Debye screening length. Furthermore, recent theoretical work examining pores in the presence of electrolytes showed that for a small pore diameter (which is the case in these experiments), a screening cloud decreases the energetic cost of maintaining the pore [20]; so while the line tension favors shrinking the pore the electrostatic free energy favors growing the pore. The relationship between line tension and salt concentration is thus significantly more complicated than the relationship between surface tension and salt concentration. Work is ongoing to analyze this relationship further; for the moment changes in lipid shape cannot be ruled out or in as an explanation for why the pore closure pressure changes with salt concentration.

The experiments shown in this paper provide a clear demonstration that membrane stability can be controlled by monovalent salt concentration. Additionally, it was shown that the greater the anionic lipid content the more sensitive the membranes are to changes in electrolyte concentration. Bacterial cells are frequently osmotically shocked and it is well known that the accumulation of so-called compatible solutes helps protect against osmotic upshift [21,22] (high solute environment)—to our knowledge, an equivalent type protection against osmotic downshift has not been identified. The results in Fig. 2 show that bacterial cells (or any cells) can protect themselves from osmotic downshift, and the potentially fatal effect of pore opening, in one of two possible ways. One, the pore closing (opening) pressure drops with anionic lipid content, thus as bacterial and most other cells contain anionic lipids in the inner leaflet protection can be afforded by keeping the anionic lipid content low. Two, the higher the salt concentration the less of a difference it makes how much anionic lipid is present, therefore cells can protect themselves by keeping the internal salt concentration high enough to negate out the anionic lipids but low enough that it is unlikely a large enough gradient can be set-up across the membrane to cause pore opening.

As a last aside, we note that when bacterial cells are exposed to a low solute environment lysis of the lipid bilayer is prevented by the opening of a mechanosensitive channel, of these MscL, the bacterial channel of large conductance, is the best studied [21,22]. It has been shown in model systems that the presence of lipids with shorter tails lowers the activation barrier to channel opening and that the addition of lysoPC to one leaflet causes the channel to open [23]. As membranes swell and consequently thin when shocked it makes sense that a thinner bilayer would prime the channel for opening and a number of papers have examined the role that lipid tail length plays on the function of MscL [24,25]. That the addition of lysoPC opens the channel indicates that membrane curvature is crucial for the gating of this channel. How this curvature change is achieved when cells are downshocked is not, to our knowledge, understood. As it happens quickly, it seems unlikely that lipids could be added to or removed from the membrane fast enough to alter the curvature and open the channel. The inner leaflet of bacterial membranes is composed

of PE, PG and cardiolipin; [26] when these cells are downshocked the internal salt concentration decreases—based on the work in this paper, we suggest that when this happens the PG headgroup area increases, thus altering the membrane curvature and contributing to the opening of the channel.

4. Conclusions

The effect of monovalent salt (NaCl) concentration on the mechanical properties of anionic membranes (varying amounts of phosphatidylglycerol in phosphatidylcholine) has been examined. It was observed that over a range from 240 to 60 mM, the mechanical properties could be modulated quite significantly, a physiological range for the total monovalent salt concentration. Specifically, the lysis pressure was observed to drop ~22% for vesicles containing 60 mol% anionic lipid, ~14% for vesicles containing 40 mol% anionic lipid and ~7% for vesicles containing 20 mol% anionic lipid. For cells that are frequently downshocked, these results show that protection against lysis can be achieved by keeping the anionic lipid content low.

Acknowledgments

Jennifer S. Hovis is a recipient of a Career Award in the Biomedical Sciences from the Burroughs Wellcome Fund. We would like to thank Professor David Thompson for use of the dynamic light scattering system.

References

[1] D. Boal, Mechanics of the Cell, Cambridge Univ. Press, Cambridge, 2002.
[2] M.C. Hull, D.B. Sauer, J.S. Hovis, Influence of lipid chemistry on the osmotic response of cell membranes: effect of non-bilayer forming lipids, J. Phys. Chem., B 108 (2004) 15890–15895.
[3] P.-H. Puech, N. Borghi, E. Karatekin, F. Brochard-Wyart, Line thermodynamics: adsorption at a membrane edge, Phys. Rev. Lett. 90 (2003) 128304.
[4] L.V. Chernomordik, M.M. Kozlov, G.B. Melikyan, I.G. Abidor, V.S. Markin, Y.A. Chizmadzhev, The shape of lipid molecules and monolayer membrane fusion, Biochem. Biophys. Acta 812 (1985) 643–655.
[5] S.D. Shoemaker, T.K. Vanderlick, Intramembrane elecrostatic interactions destabilize lipid vesicles, Biophys. J. 83 (2002) 2007–2014.
[6] S.D. Shoemaker, T.K. Vanderlick, Calcium modulates the mechanical properties of anionic phospholipid membranes, J. Colloid Interface Sci. 266 (2003) 314–321.
[7] S.D. Shoemaker, T.K. Vanderlick, Stress-induced leakage from phospholipid vesicles: effect of membrane composition, Ind. Eng. Chem. Res. 41 (2002) 324–329.
[8] F.R. Hallett, J. Marsh, B.G. Nickel, J.M. Wood, Mechanical properties of vesicles II. A model for osmotic swelling and lysis, Biophys. J. 64 (1993) 435–442.
[9] B.L. Mui, P.R. Cullis, E.A. Evans, T.D. Madden, Osmotic properties of large unilamellar vesicles prepared by extrusion, Biophys. J. 64 (1993) 443–453.
[10] Y. Levin, M.A. Idiart, Pore dynamics of osmotically stressed vesicles, Physica A 331 (2004) 571–578.
[11] M.A. Idiart, Y. Levin, Rupture of a liposomal vesicle, Phys. Rev., E 69 (2004) 061922.
[12] R.C. Weast, CRC Handbook of Chemistry and Physics, 56th ed., CRC Press, Cleveland, 1975.
[13] B.J. Frisken, C. Asman, P.J. Patty, Studies of vesicle extrusion, Langmuir 16 (2000) 928–933.
[14] J. Pencer, F.R. Hallett, Effects of vesicle size and shape on static and dynamic light scattering measurements, Langmuir 19 (2003) 7488–7497.
[15] J.H. Kleinschmidt, L.K. Tamm, Structural transitions in short-chain lipid assemblies studied by 31P-NMR spectroscopy, Biophys. J. 83 (2002) 994–1003.
[16] A. Watts, K. Harlos, D. Marsh, Charge-induced tilt in ordered-phase phosphatidylglycerol bilayers-evidence from X-ray diffraction, Biochem. Biophys. Acta 645 (1981) 91–96.
[17] A. Dicko, H. Bourque, M. Pezolet, Study by infrared spectroscopy of the conformation of dipalmitoylphosphatidylglycerol monolayers at the air–water interface and transferred on solid substrates, Chem. Phys. Lipids 96 (1998) 125–139.
[18] Y.P. Zhang, R.N.A.H. Lewis, R.N. McElhaney, Calorimetric and spectroscopic studies of the thermotropic phase behavior of the n-saturated 1,2-diacylphosphatidylglycerols, Biophys. J. 72 (1997) 779–793.
[19] Y.N. Kaznessis, S.T. Kim, R.G. Larson, Simulations of zwitterionic and anionic phospholipid monolayers, Biophys. J. 82 (2002) 1731–1742.
[20] M.D. Betterton, M.P. Brenner, Electrostatic edge instability of lipid membranes, Phys. Rev. Lett. 82 (1999) 1598–1601.
[21] B. Poolman, J.J. Spitzer, J.M. Wood, Bacterial osmosensing: roles of membrane structure and electrostatics in lipid–protein and protein–protein interactions, Biochem. Biophys. Acta 1666 (2004) 88–104.
[22] J.M. Wood, Osmosensing by bacteria: signals and membrane-based sensors, Microbiol. Mol. Biol. Rev. 63 (1999) 230–262.
[23] E. Perozo, A. Kloda, D.M. Cortes, B. Martinac, Physical principles underlying the transduction of bilyaer deformation forces during mechanosensitive channel gating, Nat. Struct. Biol. 9 (2002) 696–703.
[24] D.E. Elmore, D.A. Dougherty, Investigating lipid composition effects on the mechanosensitive channel of large conductance (MscL) using molecular dynamics simulations, Biophys. J. 85 (2003) 1512–1524.
[25] J. Gullingsrud, K. Schulten, Lipid bilayer pressure profiles and mechanosensitive channel gating, Biophys. J. 86 (2004) 3496–3509.
[26] W. Dowhan, Molecular basis for membrane phospholipid diversity: why are there so many lipids? Annu. Rev. Biochem. 66 (1997) 199–232.