Colloids and Surfaces A: Physicochem. Eng. Aspects xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Bending rigidity of phosphatidylserine-containing lipid bilayers in acidic aqueous solutions

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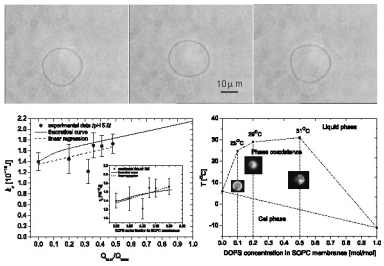
HIGHLIGHTS

- Surface charge effect on lipid membranes' bending rigidity was studied at low pH.
- Analysis of the thermal shape fluctuations of giant lipid vesicles was applied.
- The white-noise contribution to vesicles' fluctuations was taken into account.
- The surface potential was measured vs. pH and phosphatidylserine content in bilayers.
- Two-phase coexistence depending on temperature was found in studied membranes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 October 2013
Received in revised form
17 December 2013
Accepted 23 December 2013
Available online xxx

Keywords:
pH
Lipid membranes
Bending elasticity
Giant vesicles
Thermal fluctuations

ABSTRACT

The elastic properties of anion lipid-containing bilayers were studied in aqueous solutions with fixed ionic strength at pH ≤5. Giant vesicles from stearoyl-oleoyl-phosphatidylcholine (SOPC) with various contents of dioleoyl-phosphatidylserine (DOPS) were obtained via electroformation and observed in phase contrast and fluorescence. Two structural phases were found to coexist in vesicle membranes. Thermal fluctuation spectroscopy of quasispherical vesicles with homogeneous liquid membranes was applied for the quantification of their bending rigidity. The white-noise contribution, significantly influencing the calculated value of the bending modulus, was taken into account in the analysis of the thermal shape fluctuations of vesicles. Electrokinetic measurements with SOPC/DOPS liposomes provided information about their surface potential dependence on pH and also as a function of the DOPS molar content in bilayers. The experimentally obtained values for the bending rigidity of SOPC bilayers containing various amounts of DOPS are reported and discussed in the light of the existing theory for the electrostatic contribution to the bending modulus of charged lipid membranes.

1. Introduction

Numerous processes in biological cells are directly related to the membrane bending elasticity [1]. Furthermore, in view of the liposome usage for drug delivery in pharmacology, the membrane ability to bend is determinant for the successful transportation of the medicine throughout the blood capillary vessels, thus justifying the interest to the thorough investigation of the membrane mechanics altered by biologically relevant factors. One of these is pH whose impact on the elastic properties of lipid bilayers has recently attracted growing attention [2–5]. Even if the most of the 'physiological' liquids being neutral (pH∼7), cell membranes can

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http://dx.doi.org/10.1016/j.colsurfa.2013.12.059

be exposed to extreme pH values like for example, some mammalian stomach cells in contact with acid solutions attaining pH values lower than 1 [6]. Evidences were found for the close relation between the variations in the surrounding pH and the membrane morphology [7,8]. Moreover, pH-sensitive liposomes were explored as promising carriers for drug delivery purposes [9–11]. It has been established that the lipid packing, cluster formation [12–15] and the membrane rigidity [16–19] depend on the ionization state and the adsorption of ions at the lipid polar heads. The surface charge density of lipid membranes as well as their electrostatic interaction with inorganic ions and other ionized solutes is determined by the surrounding water solution's pH. As important phospholipid components of cellular membranes [20] the role of phosphatidylserines in the mechanics of lipid bilayers has been studied previously for various lipid compositions and aqueous solutions [3,4,21–23].

Here, we report new experimental results for the surface charge effect and the pH influence on the mechanical properties phospholipid membranes containing various molar parts of phosphatidylserine. Measurements of the membrane bending rigidity were carried out with giant unilamellar vesicles serving as a rough model of biomembranes by reproducing their basic physical properties [24]. The bending (or mean curvature) modulus of lipid membranes, k_c, is defined by the free energy of bending, f_c, which for a symmetrical lipid bilayer surrounded from both sides by identical solutions and with spontaneous curvature $c_0 = 0$ reads $f_c = k_c(c_1 + c_2)^2/2 + \overline{k_c}c_1c_2$ [25]. Here c_1 and c_2 are the two principal curvatures and $\overline{k_c}$ stands for the Gaussian curvature modulus. When using closed membrane structures such as lipid vesicles, we are able to measure only the mean curvature modulus k_c (for a review see [26]). Thermal fluctuation spectroscopy of giant unilamellar vesicles [27–29] was applied for the determination of the membrane bending modulus. The correlation between the surface charge and the pH value of the aqueous solution surrounding bilayers, was probed through electrokinetic measurements on liposomes. The results are discussed in the light of the theoretical predictions for the electrostatic contribution to the bending elasticity of lipid bilayers as well as in comparison with our previous results from micropipette experiments on lipid vesicles [23].

2. Material and methods

In the present study we used the following synthetic phospholipids: 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) and 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS), provided from Avanti Polar Lipids Inc. (Alabama, USA). Fluorescence observations (see Appendix A) were performed on membranes with different SOPC/DOPS composition and containing a modified lipid, 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (Rh-DHPE, Molecular probes, USA) at concentration of 1.5 mol%. Sucrose, sodium chloride (NaCl), and hydrochloric acid (HCl) were purchased from Sigma Aldrich (Germany). Chloroform (high performance liquid chromatography, HPLC, grade) and methanol (HPLC grade) from Fluka Inc. (Germany) were used for the preparation of lipid solutions. Vesicles were prepared in 0.19 mol/L sucrose solutions with fixed ionic strength and pH. The acidity of the background electrolyte solutions was adjusted by the addition of HCl. A pH-meter with combined glass electrode (Lovibond, Germany) was applied for the determination of pH values of the aqueous solutions used for the preparation of vesicle suspensions. All measurements of the membrane bending rigidity and of the surface potential of liposomes were conducted at a fixed ionic strength (0.01 M NaCl or 0.01 M KCl).

2.1. Preparation of giant unilamellar vesicles

Flaccid quasispherical vesicles with diameters of several dozens of micrometers were obtained via electroformation [5,30]. A modified protocol adapted to high ionic strengths of the aqueous medium was used for preparing vesicles in electrolyte solutions [3,5]. As described in details in [4], deposits of 1 g/L lipid solution in chloroform/methanol (9/1 volume parts) solvent were uniformly spread on the conductive surface of indium-tin oxide (ITO) coated glass electrodes. Prior to vesicle preparation, the lipids were kept lyophilized under vacuum at −20 °C and only freshly prepared organic solutions of lipids were used. After the complete evaporation of the organic solvents (for at least 2 h under vacuum) the electroformation chamber (assembled of two ITO-coated glass plates and a 3 mm-thick polydimethylsiloxane from Dow Corning – Germany spacer) was filled with the appropriate aqueous solution with fixed pH and ionic strength. Thus, vesicles were obtained in symmetrical conditions with respect to their membranes (the same aqueous solution inside and outside the vesicles). The electroformation was performed at $T \approx 30$ °C in order to prevent phase separations in membranes for all lipid compositions studied here (for details, see Appendix A).

2.2. Bending elasticity measurements

Thermal shape fluctuations analysis (TSFA) of nearly spherical lipid vesicles was performed for determining the membrane bending rigidity [29,28]. This method is one of the most advanced ones for the measurement of this membrane mechanical constant. It is based on the tremendous softness of lipid membranes, thanks to which the thermal energy of the Brownian motion of water molecules surrounding a lipid bilayer is sufficient to induce observable shape fluctuations of the latter. In the present study the visualization of the membrane fluctuations was carried out in phase contrast regime of an inverted microscope Axiovert 100 (Zeiss, Germany) equipped with a thermo-controlled microscope stage and an oil-immersed objective (100×, numerical aperture 1.25). The image recording was performed using a CCD camera (C3582, Hamamatsu Photonics, Japan) and a frame grabber (DT3155, Data Translation, USA, 768 × 576 8-bit pixels, pixel size: 0.106 μm/pix). All measurements of the bending moduli were carried out at constant temperature of (30 ± 0.1) °C. For TSFA only well-fluctuating quasispherical vesicles with diameters higher than 15 μm were chosen (see Fig. 1). For each vesicle, at least several hundred images were acquired once per second and subjected to TSFA for determining their membrane bending elasticity moduli and tensions [28,29]. The stroboscopic illumination of the observed vesicles, synchronized with the camera, permitted the fast modes of fluctuations to be recorded and processed [31].

2.3. Electrokinetic measurements

Electrokinetic measurements were performed with suspensions of multilayer vesicles (MLV). Multilamellar liposomes were prepared by evaporation of chloroform from lipid solutions placed in a round-bottom flask mounted on a home-made rotary evaporator and kept in vacuum for about 1 h before the addition of the appropriate electrolyte followed by gentle shaking in the electrolyte. The final lipid content in liposome suspensions was 1 g/L. In order to adjust the pH value the proper amount of HCl was progressively added in the suspension. The same appropriate amount of HCl was added to a separate vessel containing only the aqueous medium (without liposomes) for indirect pH value control. Our control electrokinetic measurements on MLV, which were prepared by different methods, demonstrated that the electrokinetic properties of their membranes are independent of the pH

Please cite this article in press as: D. Mitkova, et al., Bending rigidity of phosphatidylserine-containing lipid bilayers in acidic aqueous solutions, Colloids Surf. A: Physicochem. Eng. Aspects (2014), http://dx.doi.org/10.1016/j.colsurfa.2013.12.059