Bulgarian Chemical Communications, Volume 52, Special Issue E (pp. 35 - 40) 2020

Bending rigidity of lipid bilayers in electrolyte solutions of sucrose

Victoria Vitkova*[1], Christophe Minetti[2], Angelina Stoyanova-Ivanova[1]

[1]*Georgi Nadjakov Institute of Solid State Physics, Bulgarian Academy of Sciences, 72 Tzarigradsko Chaussee Blvd., 1784 Sofia, Bulgaria*

[2]*Université libre de Bruxelles, Service de Chimie Physique EP CP 165/62, 50 Avenue Franklin Roosevelt, 1050 Bruxelles, Belgium*

Received November 20, 2019; Accepted December 02, 2019

The morphology and dynamics of lipid bilayers are related to their mechanical parameters such as the bending elasticity and the intermonolayer friction. The thermal shape fluctuation analysis of nearly spherical giant lipid vesicles has been established as a versatile tool for the non-invasive measurement of the membrane bending modulus. We applied this method for probing the membrane mechanics in electrolyte solutions of sucrose. The bending rigidity of lipid membranes in aqueous solutions of mono-, di- and polysaccharides was quantified by means of a holographic tool that allows performing quantitative phase measurements for reconstituting the vesicle shape. The analysis of the time autocorrelations of vesicles' diameters along the optical axis yields the membrane tension and bending modulus. The obtained results indicate that the presence of sodium chloride modifies the effect of sucrose on the bending rigidity of lipid bilayers. Our finding is discussed in the light of the calorimetric and molecular dynamics data in the literature about the ion-induced modification of the membrane interactions with this disaccharide. We present further evidences about the softening of lipid bilayers in sucrose-containing aqueous environment.

Keywords: Lipid bilayers, bending elasticity, holographic microscopy, carbohydrates, sodium chloride

INTRODUCTION

Lipid bilayers (or membranes) are biomimetic, two-dimensional liquid crystalline structures, readily obtainable in laboratory conditions. The strong interest towards them is due in a great extent to the potential of their application in biomolecular electronics [1,2], drug delivery, cryo- and biopreservation in food industry and medicine [3,4]. The experimental and theoretical investigation of the fundamental physicochemical properties of lipid bilayers with different chemical compositions and in various aqueous environments represents an important step to the realization of numerous applications. A significant part of the experimental and theoretical studies on model lipid membranes aims at clarifying the role of the lipid environment on the membrane proteins and other functional inclusions in membranes. At present, it is known that the physical state of membranes (composition, structure, elasticity, surface charge, etc.) determines the deformability of the lipid matrix, which is substantial in the cases, when the activity of the guest molecule is governed by its conformational changes. On the other hand, the ability of lipid bilayers to deform easily, when external forces are applied, for example in hydrodynamic flows, underlies the successful use of functionalized lipid structures in pharmacology for transporting medicinal substances into the bloodstream of human organisms [3]. Hence, it is of importance to investigate all factors impacting the membrane deformability. In aqueous medium, lipid bilayers spontaneously form closed structures, which in the case of unilamellar objects with characteristic sizes of the order of tens of micrometers are known as giant unilamellar vesicles (GUVs), successfully applied to probe the membrane properties [5] as well as cell hydrodynamics in flows [6]. Following elaborated experimental protocols, GUVs are readily prepared in laboratory conditions with good control of the chemical composition of membranes and bathing solutions. Advanced experimental methods have been developed so far for the measurement of the membrane bending rigidity [7-10]. The thermal shape fluctuation analysis (TSFA) or flicker spectroscopy applied on GUVs is one of the most elaborated ones [11,12]. After the approach based on the Fourier decomposition of the thermal fluctuations of the vesicle radius [12,13] the fluctuation spectroscopy method has been further refined with the introduction of Legendre analysis of the autocorrelation function of the vesicle contour and accounting for the white noise contribution to the calculation of the membrane bending modulus [11,13]. Here we study single-component, electrically neutral phosphatidylcholine membranes to determine the membrane bending modulus in aqueous solutions of carbohydrates as well as in electrolyte solutions of sucrose.

* To whom all correspondence should be sent.
E-mail: victoria@issp.bas.bg

EXPERIMENTAL

A Giant unilamellar vesicles (GUVs) were prepared from two phosphatidylcholine (PC) lipids, namely 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-choline (SOPC) or 1-palitoyl-2-oleoyl-sn-glycero-3-phospho-choline (POPC) (Avanti Polar Lipids Inc., AL, USA) by means of the well-established electroformation method [14, 15]. Methanol and chloroform (for analysis grade) used for dissolving the lipid were provided from Fluka Inc. (Germany). Sodium chloride was purchased from Sigma-Aldrich (Germany). An electroformation chamber with two indium tin oxide (ITO)- coated glass plates, separated by a silicone spacer, was used. The spacers of the experimental chamber for the preparation of GUVs were made from polydimethylsiloxane (PDMS) – from Dow Corning (Germany). The 0.5 mm-thick (CoverWell®) spacers that were utilized in the observation chamber were provided by Sigma-Aldrich Inc. (USA). The detailed procedure of GUVs' electroformation has been described in detail elsewhere [16]. Sucrose solutions with the desired concentration of sugar and 0.01 mol/L of NaCl were prepared with bidistilled water from a quartz distiller.

The shape of a bent tension-free membrane is characterized by its principal curvatures $c_1 = 1/R_1$ and $c_2 = 1/R_2$, where R_1 and R_2 are respectively, the largest and the smallest radii of curvature in two mutually orthogonal directions. To the second order, the density of the bending energy is given by the expression $g_s = (1/2)k_c(c_1 + c_2 - c_0)^2 + \bar{k}_c c_1 c_2$, where k_c and $\bar{k}_c$ are the moduli of curvature (bending) and saddle curvature with dimension of energy, and c_0 is the so-called spontaneous curvature. It vanishes, for symmetrical membranes as in the cases studied here [17].

For measurements by digital holographic microscopy vesicular suspensions were prepared so that the vesicle membranes enclose 0.2 mol/l of sucrose in water, while the suspending medium was the iso-osmolar aqueous solution of glucose containing 1 wt% of the biocompatible polymer of glucose, dextran. Sucrose and dextran were provided by Sigma-Aldrich Chemie (Germany), while glucose was purchased from Merck (Germany). Bidistilled water with pH~5.5 was used for the preparation of sucrose and glucose solutions. The measurements of the membrane bending rigidity were performed at room temperature (~22°C).

The observations of the vesicles' shape fluctuations were conducted by means of an inverted microscope Axiovert 100 (Zeiss, Germany). The visualization of the membrane fluctuations was carried out in phase contrast regime, using an oil-immersed objective (100x, NA 1.25). The image-recording was performed using a CCD camera (C3582, Hamamatsu Photonics, Japan). The video signal from the camera was fed to a frame grabber board (DT3155, Data Translation, USA, 768x576 8-bit pixels, pixel size: 0.106 μm/pix). For each chosen vesicle, at least several hundred images were captured once per second and processed for deducing the bending elasticity modulus and membrane tensions [11, 18]. The stroboscopic illumination of the observed vesicles, synchronized with the camera, permitted the fastest modes of fluctuations to be recorded and processed [19].

A particularly important step in the image treatment consisted in precisely locating the position of the vesicle contour (Figure 1), corresponding to the equatorial cross-section of the vesicle with the focal plane of the microscope in each image from the recorded sequence of snapshots.

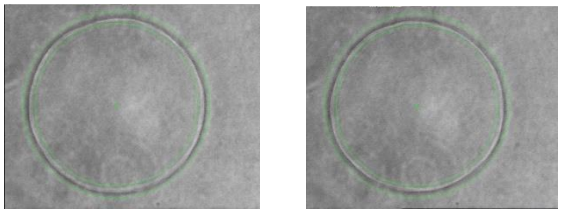


Fig. 1. Phase-contrast images of a POPC vesicle (Rv = 19.5 μm) for the analysis of the thermal shape fluctuations of the vesicle contour; 7 s time lapse.

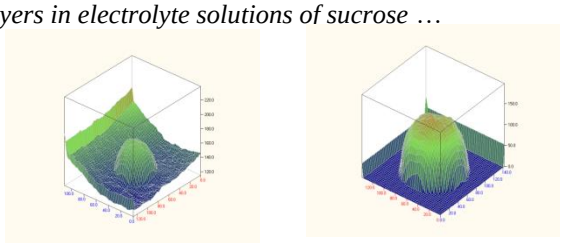
Optical techniques providing information about the fluctuations of vesicle membranes are developed to increase the precision of the bending rigidity measurements as in the case of digital holographic microscopy (DHM) [20]. The vesicle fluctuations were monitored and recorded using a microscopic holographic system described in details elsewhere [21]. The DHM approach utilizes the knowledge of the optical complex amplitude, namely amplitude and phase, to refocus numerically each slide in the experimental volume. In the experimental procedure, the vesicles were slightly defocused for a better visualization. As a result the optical phase map of the vesicles might be distorted because they do not correspond to the focus plane of the vesicle. Dry objectives (Olympus, Japan) with magnification ×20 and numerical aperture NA 0.3 were applied to achieve the appropriate optical resolution. The experimental parameters of our set-up are the following: laser wavelength of 633 nm; pixel size of 208 nm; frame

rate of 24 frames per second with exposure time of 200 μs. The measured phase $\phi(x,y)$ is related to the vesicle diameter, d and the refractive index difference, Δn, between the fluid, enclosed by the vesicle membrane and the surrounding aqueous solution.

$$\phi(x,y) = \frac{2\pi}{\lambda} d\Delta n \qquad (1)$$

The first step of the processing consists in the determination of the focus plane of each vesicle. The focus plane was determined by scanning along the optical axis a region of interest around the object under investigation and by computing for each plane the gradient of the amplitude modulus. Once the focus plane determined, we calculated the amplitude and the phase map in this plane. Due to the phase periodicity unwrapping procedure was applied in order to remove the discontinuities (phase jumps) with a period of 2π. The background phase, defined as the phase obtained when no object is present in the field of view, was approximated by a bi-dimensional quadratic phase map. For every studied vesicle the background phase map was fitted on the basis of the phase map in a neighborhood region where no objects were present. The numerical processing of the images consisted in removing the non-uniform background by successive segmentation and background fitting until the resulting background is completely flat and uniform as shown in Figure 2. In the present study only the fluctuations of the vesicle diameter laying on the optical axis of the microscope were extracted and analyzed. Hence, a segmentation process is performed on the vesicle to extract its contour and determine its center. The value of the phase in the center of the vesicle, proportional to the vesicle radius, is then deduced. Its time autocorrelation function is related to the bending modulus and the lateral tension of vesicle membrane. The experimentally acquired data for the fluctuations of the vesicle diameter with time were fitted with the theoretical expression for the autocorrelation function. The fit yielded the values of the two free parameters, which are the bending modulus and the lateral membrane tension for every analyzed vesicle [20].

The DHM method was applied for the evaluation of the bending elasticity modulus of SOPC membranes in aqueous solutions of 0.2 mol/L of sucrose.



(a) (b)

Fig. 2. (a) vesicle with non-uniform background; (b) flat and uniform background after numerical processing (see text); R_{ves}=8.11±0.05 μm

For the static thermal shape fluctuation analysis (TSFA) following Bivas et al. [13] we calculate the time average of the normalized angular autocorrelation function of the vesicle contour's radius (Figure 1) at N angular directions as described in detail elsewhere [11]:

$$\xi(\gamma,N) = \langle \frac{1}{2\pi R_v} \int_0^{2\pi} [R(\varphi,t) - R_v][R(\varphi+\gamma,t) - R_v] d\varphi \rangle \qquad (2)$$

where R_v is the volume of a sphere with volume equal to the vesicle volume. The function expressed by (2) is decomposed into a series with respect to Legendre polynomials with amplitudes depending on the bending elasticity modulus and the mechanical tension of the vesicle membrane. For the calculation of the bending constant we follow the fitting procedure described in detail in [11].

RESULTS AND DISCUSSION

Less than 10% of all recorded and processed vesicles satisfied the criteria of acceptance [6, 11] thus providing an ensemble of eleven vesicles with goodness of fit higher than 0.1, used for the evaluation of the membrane bending modulus as described in detail in [20]. This experimental result is presented in Figure 3 (open circle). It is comparable to the values of the bending constant of phosphatidylcholine membranes published in the literature [22, 23].

Fig. 3 recapitulates the experimental data acquired so far for the bending modulus of phosphatidylcholine bilayers in aqueous solutions of sucrose. Three experimental methods utilizing GUVs have been applied for quantifying the bending rigidity of lipid bilayers. Shape fluctuation spectroscopy data are presented in Figure 3 with full circles in pure water and at 50 mM of sucrose [16]. The full triangle stands for the result from the shape fluctuation analysis at 20 mM of sucrose, reported in [24]. As shown in [11] accounting for the white-noise contribution to fluctuations of the vesicle contour, $\Delta R^{noise}(\varphi,t)$, yields 30%-higher value of the bending modulus, which could be the

origin of the shift between the value reported in [24] and our results [16]. The open circle in Figure 3 corresponds to our DHM result for the bending modulus of PC membranes in carbohydrate-containing aqueous solutions. The obtained value is lower than the bending constant of PC bilayers in pure water, which is in accordance with the softening effect of sugars on lipid membranes reported earlier [6, 16, 25, 26].

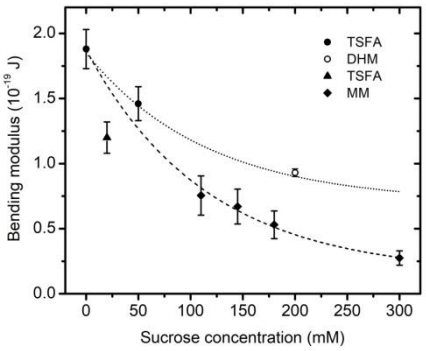


Fig.3. Bending modulus of phosphatidylcholine bilayers in aqueous solutions of sucrose acquired by different experimental methods: TSFA – thermal shape fluctuation analysis (full circles [16], triangle [24]); DHM – digital holographic microscopy (open circle, this study); MM – mechanical micromanipulation (full squares [25]).

The relative reduction in the bending modulus measured by fluctuation analysis is smaller than the k_c decrease obtained by GUVs micromanipulation (MM) [25]. The absolute values of the bending modulus in the micropipette study are lower than the values presented here and the sources of these differences being of various origins are discussed in [16]. Lower apparent bending modulus is expected from mechanical micromanipulation measurements without "pre-stressing" of vesicles [27] due to the contribution of the hidden area of the vesicle membrane [28]. Disregarding the white noise's contribution to the recorded fluctuations of the vesicle membrane, or a possible non-stationarity of vesicles due to uncontrolled deflation during measurements, or the non-uniformity of the mean vesicle's radius over all angular directions, could be responsible for the divergence of the bending rigidity values reported in previous fluctuation analysis studies in the literature. As pointed out in [16] measures have been taken to overcome the method-related side contributions to the reported values of the membrane bending rigidity deduced from GUVs' shape fluctuations analysis.

We further develop the approach proposed in [25] for fitting the micromanipulation data with an empirical exponential-decay equation (the dashed line in Figure 3) of the type:

$$k_c(c) = k_{c,min} + \Delta k_c \exp(-c/c_0) \quad (3),$$

where c is the sucrose concentration in the bulk phase, $k_{c,min}$ stands for the minimum value of the bending modulus, $\Delta k_c = k_c(0) - k_{c,min}$, and c_0 is the sucrose concentration at which the bending modulus decreases with $\sim 2\Delta k_c/3$ compared to its value in pure water $k_c(0)$. The fitting procedure with eqn. (3) yields $k_{c,min} = (0.151 \pm 0.004)10^{-19}$ J, $\Delta k_c = (1.73 \pm 0.06)10^{-19}$ J, $c_0 = (115 \pm 11)$ mM with goodness of fit (coefficient of determination) nearly 1.

TSFA and DHM data set of only three sucrose concentrations are not sufficient to perform the fitting procedure but nevertheless comparing the experimental data with an exponential-decay curve of the same type as Eqn. (3) and parameters $k_{c,min} = 0.73 \times 10^{-19}$ J, $\Delta k_c = 1.15 \times 10^{-19}$ J, $c_0 = 115$ mM, gives a qualitatively satisfying description of the observed sucrose concentration-dependent trend.

A possible explanation of the experimental result that the bending modulus obtained from micropipette aspiration without pre-stressing of GUVs, was measured to decrease more steeply would be the hypothesis of that the hidden area of the vesicle membrane increases at higher sucrose concentrations. In a previous study we measured also the stretching elasticity of PC monolayers in sucrose solutions via mechanical micro-manipulation of emulsion droplets [25].Our results revealed the strong influence of sucrose dissolved in the water environment on the stretching elasticity modulus of phosphatidylcholine monolayers.

We performed a series of TSFA measurements to probe the membrane mechanics in sucrose salt solutions.

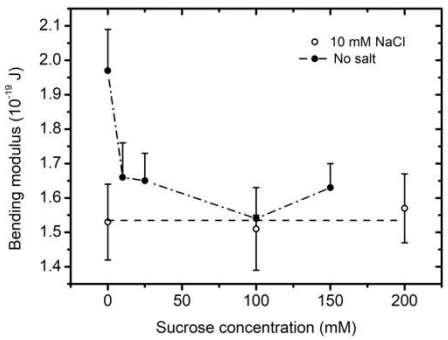


Fig. 4. Bending modulus of POPC bilayers in aqueous solutions of sucrose and NaCl at concentrations: 10 mM (open circles, dashed line) and 0 mM (full circles, dash-dotted line).

The membrane bending elasticity measured in sucrose solutions containing 10 mM of NaCl is independent of the disaccharide concentration in the aqueous surroundings as shown in Figure 4 (open circles, dashed line). This result can be considered in the light of the ion-induced modification of sucrose-phosphatidylcholine hydrogen bond (HB) network, reported in the literature [29, 30]. Molecular dynamics (MD) simulations and Fourier-transformed infrared (FTIR) spectroscopy studies revealed that the capability of disaccharides to replace water molecules [31] and to create a water-like HB network in the lipid surrounding contributes to retaining of the molecular properties of lipids [32]. The analysis of MD simulation results has provided evidences that the HB network of PC and sucrose is partially disrupted in the presence of NaCl ions [30].

CONCLUSIONS

The physicochemical properties of lipid structures are studied in relation to many biomedical applications including drug vectorization, liposome-based developments in beauty industry, cryopreservation and desiccation control in food industry and medicine. In the present work, unilamellar lipid vesicles with quasispherical shape and diameters of the order of tens of micrometers, GUVs, were used for the measurement of the bending elasticity modulus of single-component phosphatidylcholine bilayers at different sucrose concentrations in the surrounding aqueous environment. We reported on the determination of the bending rigidity of phosphatidylcholine membranes in electrolyte solutions containing carbohydrate molecules by means of experimental techniques utilizing GUVs. Using an empirical approach we analyzed the experimental data collected from micromanipulation measurements, thermal shape fluctuation spectroscopy and digital holographic microscopy on GUVs. For the assessment of the membrane bending modulus in aqueous solutions of mono-, di- and polysaccharides, we applied digital holographic microscopy based on quantitative phase measurements on fluctuating giant unilamellar vesicles and the subsequent analysis of the fluctuations of the vesicle diameter along the optical axis of the microscopic imaging system. The fully automated data treatment and the potential to reconstitute the tri-dimensional shape of lipid vesicles are only some of the advantages of the holographic approach for evaluating the bending constant of lipid bilayers.

Acknowledgements: *The authors thank Prof. Yu. A. Ermakov for the enlightening discussions. Financial support from the National Science Fund of Bulgaria (Grant DN08-7/2016), WBI, FRS-FNRS and the Bulgarian Academy of Sciences is acknowledged.*

REFERENCES

1. P. Facci, Biomolecular Electronics. 2014, Amsterdam: Elsevier Inc.
2. A. G. Petrov, The Lyotropic State of Matter: Molecular Physics and Living Matter Physics. 1999, Amsterdam: Gordon & Breach. 549.
3. T. M. Allen, P.R. Cullis, *Science*, **303**, 1818 (2004).
4. B. Stark, G. Pabst, R. Prassl, *Eur. J. Pharm. Sci.*, **41**, 546 (2010).
5. R. Dimova, in Advances in Planar Lipid Bilayers and Liposomes, A. Iglič (ed), vol. 16, Elsevier: Burlington. 2012.
6. V. Vitkova, C. Misbah, in Advances in Planar Lipid Bilayers and Liposomes, A. Iglic (ed), vol. 14, Academic Press: Burlington. 2011, p. 258-292.
7. M.B. Schneider, J. T. Jenkins, W. W. Webb, *Biophys. J.*, **45**, 891 (1984).
8. M. Kummrow, Helfrich, W., *Phys. Rev. A*, **44**, 8356 (1991).
9. E.A. Evans, *Biophys. J.*, **14**, 923 (1974).
10. R. Dimova, *Adv. Colloid Interface Sci.*, **208**, 225 (2014).
11. J. Genova, V. Vitkova, I. Bivas, *Phys. Rev. E*, **88**, 022707 (2013).
12. M.D. Mitov, J. F. Faucon, P. Méléard, P. Bothorel, in Advances in Supramolecular Chemistry, G. W. Gokel (ed), vol. 2, JAI Press Inc.: Greenwich, CT. 1992, p. 93-139.
13. I. Bivas, P. Hanusse, P. Bothorel, J. Lalanne, O. Aguerre-Chariol, *J. Physique*, **48**, 855 (1987).
14. M.Angelova, D.S. Dimitrov, *Progress in Colloid and Polymer Science*, **76**, 59 (1988).
15. V. Vitkova, K. Antonova, G. Popkirov, M.D. Mitov, Y.A. Ermakov, I. Bivas, *J. Phys. Conf. Ser.*, **253**, 012059 (2010).
16. D. Mitkova, V. Vitkova, *Russ. J. Electrochem.*, **52**, 1172 (2016).
17. W. Helfrich, Z. *Naturforsch*., **28**c, 693 (1973).
18. M.D. Mitov, J.F. Faucon, P. Meleard, P. Bothorel, G.W. Gokel (ed), vol. 2, JAI Press Inc.: Greenwich. 1992, p. 93-139.
19. J. Genova, V. Vitkova, L. Aladgem, M.D. Mitov, *J. Optoel. Adv. Mater.*, **7**, 257 (2005).
20. C. Minetti, V. Vitkova, F. Dubois, I. Bivas, *Opt. Lett*., **41**, 1833 (2016).
21. C. Minetti, T. Podgorski, G. Coupier, F. Dubois, Proc. SPIE 8429, 84291I (2012).
22. D. Marsh, *Chem. Phys. Lipids*, **144,** 146 (2006).
23. E. Evans, D. Needham, *J. Phys. Chem.*, **91**, 4219 (1987).
24. H.A. Faizi, S. L. Frey, J. Steinkuhler, R. Dimova, P.M. Vlahovska, *Soft Matter*, **15**, 6006 (2019).
25. V. Vitkova, J. Genova, M.D. Mitov, I. Bivas, *Mol. Cryst. Liq. Cryst.*, **449,** 95 (2006).

26. P. Shchelokovskyy, S. Tristram-Nagle, R. Dimova, *New J. Phys.*, **13**, 025004 (2011).
27. K. Olbrich, W. Rawicz, D. Needham, E. Evans, *Biophys. J.*, **7**9, 321 (2000).
28. V. Vitkova, Genova, J., Bivas, I., *Eur. Biophys. J.*, **33**, 706 (2004).
29. C.C. Valley, J.D. Perlmutter, A. R. Braun, J.N. Sachs, *J. Membr. Biol.*, **244**, 35 (2011).
30. D. Bakarić, D. Petrov, Y.K. Mouvencherya, S. Heiβler, C. Oostenbrink, G.E. Schaumann, *Chem. Phys. Lipids*, **210**, 38 (2018).
31. M.C. Luzardo, F. Amalfa, A.M. Nunez, S. Diaz, A.C. Biondi de Lopez, E. A. Disalvo, *Biophys. J.*, **78**, 2452 (2000).
32. S. Leekumjorn, A.K. Sum, *J. Phys. Chem. B*, **112**, 10732 (2008).