Chemistry and Physics of Lipids 201 (2016) 21–27

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

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

Measurement of the bending elastic modulus in unilamellar vesicles membranes by fast field cycling NMR relaxometry

Gabriela A. Dominguez, Josefina Perlo[1], Carla C. Fraenza, Esteban Anoardo*

LaRTE—FaMAF, Universidad Nacional de Córdoba and IFEG—CONICET, Ciudad Universitaria, X2016LAE, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 22 September 2016
Accepted 31 October 2016
Available online 2 November 2016

PACS:
06.60.Mr
76.60.-k
87.64.kj

Keywords:
Liposomes
Lipid dynamics
Bending modulus
NMR relaxometry

ABSTRACT

The elastic properties of lipid membranes can be conveniently characterized through the bending elastic modulus κ. Elasticity directly affects the deformability of a membrane, morphological and shape transitions, fusion, lipid-protein interactions, etc. It is also a critical property for the formulation of ultradeformable liposomes, and of interest for the design of theranostic liposomes for efficient drug delivery systems and/or different imaging contrast agents. Measurements of κ in liposome membranes have been made using the fast field cycling nuclear magnetic relaxometry technique. We analyze the capability of the technique to provide a consistent value of the measured quantity under certain limiting conditions. Relaxation dispersions were measured acquiring a minimal quantity of points, within a reduced Larmor frequency range and, under inferior experimental conditions (in the presence of magnetic field in-homogeneity and lower power supply stability). A simplified model is discussed, showing practical advantages when fitting the data within the reduced frequency range. Experiments are contrasted with standard measurements performed in a state-of-the-art relaxometer. The methodology was tested in samples of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine with different percentiles of cholesterol. We observe a tendency to a decrease in κ with increasing temperature, and a tendency to increase with the cholesterol percentile.

1. Introduction

Lipid vesicles can be used as idealized model systems of real biomembranes. They have attracted much interest in biophysical research, particularly for the study of different processes related to the viscoelastic and mechanical properties of the membrane. The bending elastic modulus, a quantity reflecting the amount of energy needed to modify the intrinsic curvature of a bilayer, determines important biological functions of cells, like cell fusion, lipid–protein interactions and lipid-mediated protein activity (Katsaras and Guberlet, 2000; Groves, 2007; Park et al., 2010; Mouritsen, 2004). The effects of sterols (particularly cholesterol) on the membrane flexibility was frequently characterized through the bending elastic modulus (Méléard et al., 1997; Henriksen et al., 2004). Ultra-deformable liposomes used as transdermal carriers are formulated to have critical elastic properties through the addition of selected additives (Cevc and Gebauer, 2003). Therefore, reliable and non-invasive methods to characterize the elastic properties of membranes are attractive for both fundamental research and industrial applications.

In the last years, lipids and membranes came back into scene, with a tremendous need for the understanding of many lipid-mediated processes (Mouritsen, 2004; Rheinstädter and Mouritsen, 2013). The presence of proteins locally affects the elastic properties of the membrane thereby affecting the fluctuation spectrum of it. This has a direct impact on the lipid-protein dynamics and influence protein–protein processes like amyloid aggregation (Kotler et al., 2014) and other processes with direct impact on the human health (Tomaiuolo, 2014; Pretorius et al., 2016; Lasalvia et al., 2016). From a different perspective, non-specific interactions between proteins and the bilayer as a physical entity (chacterized by certain mesoscopic properties like e.g. elasticity or thickness) regulate protein activity (Lundbæk et al., 2010; Soubias et al., 2010; Brown, 2012; Epand et al., 2015). Today, it is clear that a close relationship exist between the elastic properties of the membrane, and a myriad of processes involving both lipids and embebed proteins.

* Corresponding author.
E-mail address: anoardo@famaf.unc.edu.ar (E. Anoardo).
[1] Current address: Magritek GmbH, Aachen, Germany.

http://dx.doi.org/10.1016/j.chemphyslip.2016.10.006
0009-3084/

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)–cholesterol mixtures have been studied by different authors in the past, not only using different experimental techniques, but also with computational resources. Still, uncler features persist concerning how the cholesterol modulates the viscoelastic properties of a DMPC membrane, even with no consensus on the corresponding phase diagram (de Meyer et al., 2010). How cholesterol modulates the elastic behaviour of the membrane strongly depends upon the saturation of the hidrocarbon chains of the lipids. When lipids have fully saturated chains, like DMPC, cholesterol increases κ. However, it does not have mayor effects for monoinsaturated chains (Pan et al., 2008a). Depending on the concentration, part of the lipids in the membrane will be in a cholesterol-induced ordered state (Fraenza et al., 2014). However, such ordered lipids are not necessarily isolated, they may tend to aglomerate into domains (or "rafts") in coexistence with a more "fluid" phase (Rheinstädter and Mouritsen, 2013). Plenty of questions remain on the lipid dynamics and order, and the conextion between these and the mesoscopic behaviour of the membrane.

Different experimental techniques are available for the study of membrane elasticity (Dimova, 2014; Monzel and Sengupta, 2016). However, easily available techniques implemented through benchtop instruments (generally based on optical microscopy) are only useful for studies in giant unilamellar vesicles (GUV, micrometer scale). Examples of this sort are video microscopy analysis of contour fluctuations (Méléard et al., 1997; Henriksen et al., 2004; Duwe and Sackmann, 1990; Minetti et al., 2016) and fluorescence confocal microscopy (Tian et al., 2009). On the other hand, successful techniques used for the study of membrane elasticity in large unilamellar vesicles (LUV, between 100 nm and 1 μm) tend to be based on large scale instrumentation like conventional nuclear magnetic resonance (NMR) (Althoff et al., 2002; Kinnun et al., 2015) or neutron spin-echo (NSE) systems (Yi et al., 2009; Armstrong et al., 2014). It is worth mentioning atomic force microscopy (AFM) as an exception, allowing the study of mechanical properties in LUV with small scale instrumentation, although more invasive than the previous methods (Delmore and Fery, 2006; Takechi-Haraya et al., 2016). In contrast to optical based techniques, NMR and NSE can be extended to small unilamellar vesicles (SUV, less than 100 nm).

NMR Relaxation is a powerful technique for the study of molecular dynamics. At high resolution, local positions of the acyl chains can be analyzed independently (Trouard et al., 1999; Brown et al., 2002; Martinez et al., 2002). A unified analysis of the frequency and temperature-dependence of ^{13}C and ^{2}H relaxation in DMPC revealed that individual segmental or molecular reorientations alone deficiently explain the low-frequency behavior of the observed results. In contrast, three-dimensional collective fluctuations can be argued consistently for the account of spin-relaxation in a broad MHz frequency range (corresponding to external magnetic field strengths between 0.382 and 14.6T) (Nevzorov and Brown, 1997).

Fast field-cycling (FFC) NMR relaxometry is an NMR technique already used in a series of compounds ranging from solid to liquids, and a large variety of soft materials (Kimmich and Anoardo, 2004; Fujara et al., 2014). The technique belongs to the "time-domain" NMR, since fast-switchable magnets having poor homogeneity (in terms of spectral resolution) are used. Proton relaxation rates obtained from this method are mainly driven by fluctuations of the ^{1}H–^{1}H dipolar couplings. It has been successfully used for the study of multilamellar vesicles (MLV) (Kimmich et al., 1983; Rommel et al., 1988; Struppe et al., 1997), and recently applied for the study of lipid molecular dynamics (strongly related with the viscoelastic properties) in LUV (Fraenza et al., 2014; Meledandri et al., 2009; Perlo et al., 2011). In these studies no attempt was made to use the FFC technique to measure a particular physical parameter.

Although the present work is heavily based on the previous studies described in Refs. (Fraenza et al., 2014; Meledandri et al., 2009; Perlo et al., 2011), now we concentrate on the limiting experimental conditions and model simplifications that would allow a systematic measurement of κ. It will turn out that κ can be measured within a restricted frequency range, using a simplified physical model, from data obtained using a FFC machine having a magnet with a lower homogeneity and a lower magnetic field stability (compared to the current state-of-the-art).

This work was planned with the idea of evaluating the feasibility for a small compact benchtop low-power & low-cost instrument, aimed for the measurement of the bending elastic modulus (κ). To do this, we measured the proton spin-lattice relaxation rate $R_1(\nu)$ within a restricted Larmor frequency (ν) range (typically from 100 kHz to 2.5 MHz), but using a standard instrument with degraded magnet homogeneity (~350 ppm/cm^3), and a lower magnet-current stability (~1:10^4). The idea supporting this study concerns the potential use of small-sized air-core field-cycling electromagnets (Kruber et al., 2013, 2014, 2015), resulting in important advantages in the electric parameters at the expense of a lower spatial homogeneity of the magnetic field. This fact in turn favors a lower technical demand on the power supply stability. Since the R_1 relaxation dispersion can still be measured at lower resolution, the main limitation of this approach concerns the signal to noise ratio of the NMR signal.

Since the total experimental time is also an important factor, we also reduced the quantity of measured R_1 experimental points and the number of signal acquisitions used for each R_1 measurement. We show here that even under extreme unfavorable conditions (just a few points having large errors), it is possible to measure κ within an uncertainty of ±20%. It is important to mention that at normal FFC conditions (20 ppm magnet and current stability better than 1:10^5) this error can be hardly decreased to less than ±10%.

In order to test the sensitivity of the measurements to a change in κ, experiments were done in DMPC liposomes prepared with different cholesterol content, a well known regulator of the membrane elasticity. However, it should be emphasized that the novel feature of this manuscript does not rely in this point. The behavior of the membrane in terms of cholesterol content has been studied in a previous work (Fraenza et al., 2014) and it is outside the scope of the present study.

2. Relaxometric analysis

We find it convenient to introduce the relaxometric properties using information already available in the literature. Fig. 1 shows the spin-lattice relaxation rate dispersion of DMPC liposomes having a radius $R_0 = 54$ nm (no cholesterol content). Measurements were performed at a temperature of 310 K. Data and the corresponding model are extracted from Ref. (Meledandri et al., 2009). The relaxation rate dispersion of lipid protons can be explained (black solid line) in terms of the following dynamical processes:

1. Local order fluctuations due to shape fluctuations of the liposome spheroid (OF).
2. Translational diffusion of the lipid molecules on a curved surface (D).
3. Rotations of the lipid molecules (R).
4. Fast internal motions within the lipid molecules (F).

The black solid line curve $R_1(\boldsymbol{\nu})$ corresponding to the model (see Fig. 1) was obtained after adding the contributions of each