Controlling the mechanoelasticity of model biomembranes with room-temperature ionic liquids

Chiara Rotella^{1,2} · Pallavi Kumari^{1,2} · Brian J. Rodriguez^{1,2} · Suzanne P. Jarvis^{1,2} · Antonio Benedetto^{1,2,3,4}

Received: 13 April 2018 / Accepted: 16 April 2018 / Published online: 12 May 2018

© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Room-temperature ionic liquids (RTILs) are a vast class of organic non-aqueous electrolytes whose interaction with biomolecules is receiving great attention for potential applications in bio-nano-technology. Recently, it has been shown that RTILs dispersed at low concentrations at the water-biomembrane interface diffuse into the lipid region of the biomembrane, without disrupting the integrity of the bilayer structure. In this letter, we present the first exploratory study on the effect of absorbed RTILs on the mechanoelasticity of a model biomembrane. Using atomic force microscopy, we found that both the rupture force and the elastic modulus increase upon the insertion of RTILs into the biomembrane. This preliminary result points to the potential use of RTILs to control the mechanoelasticity of cell membranes, opening new avenues for applications in bio-medicine and, more generally, bio-nano-technology. The variety of RTILs offers a vast playground for future studies and potential applications.

In the last two decades, a new family of ionic compounds, liquid around room temperature and with other peculiar characteristics, has emerged and attracted significant attention for its high tunability and “green” character. These ionic systems comprise an organic cation and either an organic or inorganic anion, and they are known as room-temperature ionic liquids (RTILs) (Welton 1999; Earle and Seddon 2000). With an initial aim to assess their toxicity, several studies have highlighted a strong affinity between RTILs and biosystems ranging from simple biomolecules to living cells and micro-organisms (Bernot et al. 2005; Pretti et al. 2006; Kulacki and Lamberti 2007). The results to date highlight on one hand the potential danger of releasing RTILs into the environment, and on the other, a scope for potential applications in bio-nano-technology (Benedetto and Ballone 2016a, b).

In this context, one avenue of research that has received special attention concerns the interaction between RTILs and biomembranes, mostly because the cytotoxicity of RTILs has been shown to be positively correlated with their lipophilicity (Ranke et al. 2006, 2007). An important part of these investigations has been focused on the chemophysical interactions of model biomembranes and RTILs, with the aim to understand the microscopic mechanism behind their toxicity. A review of this subject has been recently published (Benedetto 2017). It has been shown that the ability of RTILs to create pores and fully disrupt model biomembranes depends on aspects such as the length of the alkali chain of the cation, the hydrophobicity of the anion, and, of course, their concentration. More specifically, it has been highlighted that model biomembranes are disrupted by RTILs dissolved in water at concentrations above their critical micellar concentration (CMC). Below the CMC, however, RTILs do not affect the overall stability of the biomembranes, even after several hours of incubation. Our interest lies in this “low concentration regime.” In this respect, the first experimental determination of the interaction between model biomembranes and water solutions of RTILs has been performed by Benedetto and co-workers by means of neutron reflectivity (Benedetto et al. 2014). In the case of a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) phospholipid bilayer interacting with a water solution of [bmim][Cl] at 0.5 M, it has been found that cations (i) penetrate in the lipid region aligning their tails to the lipid tails, (ii) account for about 6% of the total bilayer volume

This article is part of a Special Issue on ‘Ionic Liquids and Biomolecules’ edited by Antonio Benedetto and Hans-Joachim Galla.

✉ Antonio Benedetto
antonio.benedetto@ucd.ie

¹ School of Physics, University College Dublin, Dublin 4, Ireland

² Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 4, Ireland

³ School of Chemistry, University College Dublin, Dublin 4, Ireland

⁴ Laboratory for Neutron Scattering, Paul Scherrer Institut, Villigen, Switzerland

(corresponding to one cation every 5 lipids), and (iii) remain in the bilayer even after rinsing with water.

Even though the presence of cations in the lipid region does not affect the overall stability of the phospholipid bilayer, as determined by neutron reflectometry, the presence of cations could affect the mechanoelastic properties of the bilayer. Molecular dynamics simulations (Benedetto et al. 2015), for instance, have suggested that the relaxation dynamics of both lipids and interfacial water, as well as the mechanoelasticity of the membrane itself, can be altered by the presence of absorbed RTILs. Recently, by means of quasi-elastic neutron scattering, a specific variation in the dynamics of lipids and hydration water due to the presence of RTILs in the membrane region has been observed (Sharma et al. 2017). In this letter, we investigate the influence of RTIL incubation on membrane mechanoelasticity. This study is also motivated by the fact that the mechanoelasticity of the cell membrane plays a major role in different biochemical processes, from cell replication to cell migration. Being able to tune these properties, in turn, can have important impacts in several fields of applications, e.g., biomedicine, diagnostic, therapeutics, and, more generally, bio-nano-technology, as recently reported by Benedetto and Ballone (2018).

To characterize the mechanoelasticity of model biomembranes before and after incubation with a RTIL/water solution below the CMC, we utilized atomic force microscopy (AFM), a scanning probe microscopy technique that has been successfully used to study the mechanoelasticity of model biomembranes and living cells in different environments (Garcia-Manyes and Sanz 2010; Monocles et al. 2010; Roa et al. 2011; Ferenc et al. 2012; Kilpatrick et al. 2015; Stetter et al. 2016). AFM is essentially composed of a laser, a photodiode, and a cantilever ending with a sharp tip that acts as the probe and can be used to image the surface of the system and to perform force measurements (Fig. 1). In our experiments, the force that the AFM tip applied on the surface of the biomembrane is recorded as a function of the distance between the tip and the substrate on which the biomembrane is deposited. By approaching the biomembrane surface, the AFM tip “feels” the force due to the interaction between the tip and the surface of the sample. With increasing force, the tip elastically deforms the biomembrane until it ruptures. From the elastic portion of the force-distance curve, the elastic modulus of the biomembrane can be determined. The value of the force at the rupture event is defined as the rupture force and is a measure of the rigidity of the system. The distance between the substrate and the contact point between tip and system surface provides a measurement of the biomembrane thickness (Fig. 1).

POPC (Avanti Polar) and 1-butyl-3-methyl-imidazolium chloride ([bmim][Cl]) (Sigma-Aldrich) have been used for the lipid and RTIL components, respectively. Deionized milliQ water with a resistivity of 18 M Ω cm was used both during sample preparation and the AFM experiments. The

sample preparation protocol was quite similar to the one adopted in the neutron reflectivity experiment cited above (Benedetto et al. 2014): 100 nm vesicles were prepared by extrusion and incubated for 20 h on freshly cleaved mica supports, which were then rinsed, resulting in a single bilayer on their surfaces. A RTIL/water solution of [bmim][Cl] at a concentration of 0.5 M was used. Imaging and force measurements were performed using an MFP-3D AFM (Asylum Research) and V-shaped cantilevers (DNP A, Bruker) with a nominal spring constant and tip radius of 0.35 N/m and 20 nm, respectively. Topographic images were recorded using amplitude modulation AFM. Mechanical measurements were performed using force-volume spectroscopy.

After verifying the quality of the model biomembrane by AFM (Fig. 2a), a force map was collected on an extended area of its surface, as illustrated in Fig. 1c. Then, the water in contact with the biomembrane was replaced by the RTIL/water solution, and again the sample quality was checked by AFM (Fig. 2b). A second series of force-distance curves were then collected after about 1.5 h of incubation. Furthermore, to check the stability of the RTIL-doped biomembrane, a series of force-distance curves were collected up to 4 h after the incubation with the RTIL/water solution (e.g., Fig. 2c–f). The AFM images of the biomembrane surface before and during incubation with the RTIL/water solution remain basically unchanged following the introduction of RTIL (Fig. 2a–f). This is in agreement with the neutron reflectivity study of Benedetto and co-workers (Benedetto et al. 2014), and with the confocal laser scanning microscopy study of Maginn and co-workers (Yoo et al. 2016a, b), in which for concentrations below the CMC, no effect has been observed on the overall stability of the 2D membrane structure. By analyzing the force-distance curves, thickness, rupture force, and elastic modulus of the undoped and RTIL-doped uniform biomembranes were extracted. The distributions of the thicknesses, rupture forces, and elastic moduli for the undoped and the RTIL-doped biomembranes are shown in Fig. 2g–i. From these distributions, the means and standard deviations were determined. The error in the variation, from undoped to RTIL-doped bilayers, is reported as the mean square deviation of the standard deviation of each observable.

The rupture force of the doped biomembrane increased by 0.5 ± 0.14 nN after about 1.5-h incubation with the RTIL/water solution from 1.0 ± 0.1 nN to 1.5 ± 0.1 nN (Fig. 2g). This increase could be explained by the penetration of the cations into the lipid region of the bilayer, consistent with neutron reflectivity results (Benedetto et al. 2014). Similarly, the elastic modulus of the doped biomembrane increased by 18 ± 9 MPa from 30 ± 7 MPa to 48 ± 5 MPa after about 1.5-h incubation with the RTIL/water solution (Fig. 2h). The elastic moduli for the undoped and the RTIL-doped biomembranes were obtained in the Hertz model approximation using a 20-nm spherical tip (Garcia-Manyes and Sanz 2010; Monocles et al. 2010; Roa

Fig. 1 **a** AFM force-separation curve collected while approaching (in red) and retracting (in blue) the AFM tip from our model biomembrane in milliQ water, as illustrated in **(b)**. **c** Force-mapping protocol of our

AFM measurement: a 16×16 array of force-distance curves have been collected to map an extended area of the biomembrane surface ($3 \times 3 \mu\text{m}^2$)

et al. 2011; Ferenc et al. 2012; Stetter et al. 2016). Even though the determination of the absolute modulus strongly depends on fitting parameters and assumptions inherent to the approximation (Kilpatrick et al. 2015), the relative change between the doped and undoped biomembranes are of interest here.

The thickness of the doped biomembrane increased by $0.5 \pm 0.4 \text{ nm}$ after about 1.5-h incubation with the RTIL/water solution from $4.2 \pm 0.3 \text{ nm}$ to $4.7 \pm 0.3 \text{ nm}$ (Fig. 2i). Previous experiments and computer simulations (Benedetto et al. 2014, 2015; Benedetto 2017), however, reported that the POPC bilayer thickness reduced by a few Angstroms after incubation with a RTIL/water solution of the same concentration. The increment reported here, determined with AFM, is almost within the error and is also affected by the determination of point (2) in Fig. 1a, which can introduce a non-negligible error. Further experiments may clarify this possible discrepancy.

To check whether the observed variations in rupture force and elastic modulus depend on the used protocol and technique, the same measurement protocol was applied on a second identical biomembrane incubated with just pure water rather than the RTIL/water solution. No measurable variations in rupture force or elastic modulus were observed. Furthermore, to check whether the observed variations are not due to a purely electrostatic effect, the same measurement protocol has been applied on a third biomembrane incubated with a water solution of NaCl at the same ionic strength of the RTIL/water solution used above. The results, not shown here, indicate that the rupture force remains almost unchanged and elastic modulus increases by 35%. As a result, the increment in rupture force and elastic modulus can be associated to the introduction of the RTIL. Given the similarity in the sample preparation for AFM results reported here and previously reported neutron scattering results, establishing that RTIL

Fig. 2 **a–f** AFM height images of a uniform POPC bilayer imaged in milliQ and RTIL/water solution up to 4 h of incubation. **g** Rupture forces, **h** elastic moduli, and **i** thicknesses for the undoped (in blue) and RTIL-

doped biomembrane after about 1.5 h of incubation (in red). The rupture force and the elastic modulus increase by about 50% upon the penetration of RTIL cations into the biomembrane

Fig. 3 Kinetics of a defective bilayer upon the interaction with the RTIL/water solution: the bilayer in (a) is at equilibrium, but the addition of the RTIL/water solution starts a kinetic process leading to a less defective bilayer over the course of about 6 h (b–f). **g** The normalized total area of

the defects versus time follows a power law behavior with a stretched exponent of about 1.2, suggesting a 1D process. The dashed orange straight line is the best fit to the experimental data

cations are inserted into the lipid region of the bilayer (Benedetto et al. 2014), it is reasonable to conclude that the cation insertion is responsible for the increased rupture force and elastic moduli observed here.

From these considerations, the hypothesis that the absorbed cations increase the stress of the biomembrane along its surface and, in turn, the line tension along any existing pore was formulated. As a result, the force needed to nucleate a pore on the biomembrane surface would be higher when doped with RTILs. This tentative explanation could be the reason behind the 50% increment in both rupture force and elastic modulus measured by AFM.

To check the validity of our hypothesis, a second set of AFM experiments have been performed on biomembranes with mesoscopic defects (Fig. 3a). These defects are stable while the biomembrane is in contact with water (data not shown). However, upon the interaction with the RTIL/water solution, an apparent kinetic process takes place, which leads to the disappearance of the defects, resulting in a nearly uniform biomembrane in about 6 h (Fig. 3b–f). By plotting the normalized total area of the defects as a function of time in a logarithmic scale, a linear behavior emerges (Fig. 3g), meaning that the observed kinetic process follows a power law. The stretched exponent is ~ 1.2 , highlighting that the dimension of the process is $\sim 1D$, in agreement with our hypothesis on the increment of the 1D line tension. Notably, the same trend in increasing rupture force and elastic modulus observed for the uniform system is observed here. For such a bilayer, when a uniform areas are selected, again, a $\sim 50\%$ increase in rupture force is observed, in agreement with the result reported in Fig. 2g for the uniform system.

In this letter, we have presented an exploratory study of the mechanoelasticity of a model biomembrane in low concentrations of aqueous RTIL solutions. By means of AFM,

we measured a 50% increment in rupture force and elastic modulus upon incubation with a RTIL/water solution. We have formulated a tentative explanation, i.e., the absorbed RTILs increase the stress along the bilayer surface and in turn the interfacial tension, which was supported by a second set of AFM experiments on a biomembrane with defects. Upon interaction with the RTIL/water solution, the defects disappeared over the course of 6 h following a power law behavior, with a value of the stretched exponent supporting our explicative hypothesis. The introduction of the RTIL therefore increases the energetic cost of biomembrane pore formation by an AFM tip. Preliminary measurements, carried out with a low-noise AFM (Fukuma et al. 2005, 2006, 2007, Ferber et al. 2011, Sheikh et al. 2011), also indicate that on the top of the RTIL-doped system, there is a layer of hydrated Cl^- ions instead of the simple water layer as in the case of the undoped system (Fig. 4). More specifically, for the RTIL-doped system, the

Fig. 4 AFM force-distance curves collected using a low-noise AFM while approaching a POPC bilayer with an AFM tip in milliQ water (blue) and after 1-h incubation in an RTIL/water solution (red)

measured layer thickness of the hydration layer closest to the bilayer surface of 0.34 nm is comparable to the one of hydrated Cl^- ions (Marcus 1988). Moreover, the force required to push them away from the doped bilayer is higher than the force needed to push the water layer away from the undoped bilayer. This observation supports the neutron reflectivity results on the insertion of the cation in the bilayer (Benedetto et al. 2014) and proposes a screening effect by the anions.

This study represents, to the best of our knowledge, the first investigation of the mechanoelasticity of model biomembranes hydrated in a RTIL/water solution at a RTIL concentration below the CMC. As such, the specific results need to be tested and expanded, for example, to take into account the effects of different lipids, and the RTIL concentration and chain length.

In conclusion, RTILs seem to offer a new and vast playground to change the mechanoelasticity of a biomembrane, and AFM appears to be a suitable technique to probe such changes. Because of the important implications of cell membrane mechanoelasticity, we hope this exploratory study inspires a series of new investigations and discoveries.

Acknowledgments The authors thank Prof. Pietro Ballone and Dr. Jason Kilpatrick for fruitful discussions. A.B. acknowledges the additional support provided by the School of Physics and the School of Chemistry, University College Dublin, Ireland, and the Laboratory for Neutron Scattering, Paul Scherrer Institute, Switzerland.

Compliance with ethical standards

Funding C.R. receives support from the ACRITAS (actuation and characterization at the single bond limit) program under Marie Curie Initial Training Networks grant agreement number 317348. B.J.R. receives support from the European Union's Horizon 2020 research and innovation program under Marie Skłodowska-Curie grant agreement number 644175. A.B. receives support from (i) Science Foundation Ireland (SFI) under the Starting Investigator Research Grant 15-SIRG-3538, and from (ii) the European Community under the Marie-Curie Fellowship Grants HYDRA (No. 301463) and PSI- FELLOW (No. 290605). The AFM used in this work was funded by Science Foundation Ireland (SFI SFI07/IN1/B931).

Conflict of interest Chiara Rotella declares that she has no conflict of interest. Pallavi Kumari declares that she has no conflict of interest. Brian J. Rodriguez declares that he has no conflict of interest. Suzanne P. Jarvis declares that she has no conflict of interest. Antonio Benedetto declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Benedetto A (2017) Room-temperature ionic liquids meet bio-membranes: the state-of-the-art. *Biophys Rev* 9:309–320
- Benedetto A, Ballone P (2016a) Room temperature ionic liquids meet bio-molecules: a microscopic view of structure and dynamics. *ACS Sustainable Chem Eng* 4:392–412
- Benedetto A, Ballone P (2016b) Room temperature ionic liquids meet bio-molecules: a microscopic view of structure and dynamics. *Philos Mag* 96:870–894
- Benedetto A, Ballone P (2018) Room-temperature ionic liquids and bio-membranes: setting the stage for applications in pharmacology, biomedicine, and bio-nano-technology. *Langmuir*. <https://doi.org/10.1021/acs.langmuir.7b04361>
- Benedetto A, Heinrich F, Gonzalez MA, Fragneto G, Watkins E, Ballone P (2014) Structure and stability of phospholipid bilayers hydrated by a room-temperature ionic liquid/water solution: a neutron reflectometry study. *J Phys Chem B* 118:12192–12206
- Benedetto A, Bingham RJ, Ballone P (2015) Structure and dynamics of POPC bilayers in water solutions of room temperature ionic liquids. *J Chem Phys* 142:124706
- Bernot R, Brueske MA, Evans-White MA, Lamberti GA (2005) Acute and chronic toxicity of imidazolium-based ionic liquids on daphnia magna. *Environ Toxicol Chem* 24:87–92
- Earle MJ, Seddon KR (2000) Ionic liquids. Green solvents for the future. *Pure Appl Chem* 72:1391–1398
- Ferber UM, Kaggwa G, Jarvis SP (2011) Direct imaging of salt effects on lipid bilayer ordering at sub-molecular resolution. *Eur Biophys J* 40:329–338
- Ferenc R, Sanchez J, Blumich B, Herrmann W (2012) AFM nanoindentation to determine Young's modulus for different EPDM elastomers. *Polym Test* 31:425–432
- Fukuma T, Jarvis SP (2006) Development of liquid-environment frequency modulation atomic force microscope with low noise deflection sensor for cantilevers of various dimensions. *Rev Sci Instrum* 77:043701
- Fukuma T, Kimura M, Kobayashi K, Matsushige K, Yamada H (2005) Development of low noise cantilever deflection sensor for multienvironment frequency-modulation atomic force microscopy. *Rev Sci Instrum* 76:053704
- Fukuma T, Higgins MJ, Jarvis SP (2007) Direct imaging of individual intrinsic hydration layers on lipid bilayers at Angstrom resolution. *Biophys J* 92:3603–3609
- Garcia-Manyes S, Sanz F (2010) Nanomechanics of lipid bilayers by force spectroscopy with AFM: a perspective. *Biochim Biophys Acta* 1798:741–749
- Kilpatrick JJ, Revenko I, Rodriguez BJ (2015) Nanomechanics of cells and biomaterials studied by atomic force microscopy. *Adv Healthcare Mater* 4:2456–2474
- Kulacki KJ, Lamberti GA (2007) Toxicity of imidazolium ionic liquids to freshwater algae. *Green Chem* 10:104–110
- Marcus Y (1988) Ionic radii in aqueous solutions. *Chem Rev* 88:1475–1498
- Monocles MA, Young TJ, Di Maio D (2010) AFM indentation method used for elastic modulus characterization of interfaces and thin layers. *J Mater Sci* 45:3190–3197
- Pretti C, Chiappe C, Pieraccini D, Gregori M, Abramo F, Monni G, Intorre L (2006) Acute toxicity of ionic liquids to the zebrafish (*Danio rerio*). *Green Chem* 8:238–240
- Ranke J, Cox M, Müller A, Schmidt C, Beyersmann D (2006) Sorption, cellular distribution, and cytotoxicity of imidazolium ionic liquids in mammalian cells—influence of lipophilicity. *Toxicol Environ Chem* 88:273–285
- Ranke J, Müller A, Bottin-Weber U, Stock F, Stolte S, Arning J, Störmann R, Jastorff B (2007) Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. *Ecotoxicol Environ Saf* 67:430–438
- Roa JJ, Oncins G, Diaz J, Sanz F, Zegarra M (2011) Calculation of Young's modulus value by means of AFM. *Recent Pat Nanotechnol* 5:27–36

- Sharma VK, Ghosh SK, Mandal P, Yamada T, Shibata K, Mitra S, Mukhopadhyay R (2017) Effects of ionic liquids on the nanoscopic dynamics and phase behavior of a phosphatidylcholine membrane. *Soft Matter* 13:8969–8979
- Sheikh KH, Giordani C, Kilpatrick JJ, Jarvis SP (2011) Direct submolecular scale imaging of mesoscale molecular order in supported dipalmitoylphosphatidylcholine bilayers. *Langmuir* 27:3749–3753
- Stetter FWS, Hyun S-H, Brander S, Urban JM, Thompson DH, Hugel T (2016) Nanomechanical characterization of lipid bilayers with AFM-based methods. *Polymer* 102:326–332
- Welton FT (1999) Room-temperature ionic liquids. Solvents for synthesis and catalysis. *Chem Rev* 99:2071–2084
- Yoo B, Jing B, Jones SE, Lamberti GA, Zhu Y, Shah JK, Maginn EJ (2016a) Molecular mechanisms of ionic liquid cytotoxicity probed by an integrated experimental and computational approach. *Sci Rep* 6:19889
- Yoo B, Zhu Y, Maginn EJ (2016b) Molecular mechanism of ionic-liquid-induced membrane disruption: morphological changes to bilayers, multilayers, and vesicles. *Langmuir* 32:5403–5411