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Probing the nanomechanical properties of lipid membranes: The effect of trodusquemine

B. LEONARDINI

Department of Physics, University of Genoa - Via Dodecaneso 33, 16146 Genoa, Italy

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Summary. — Atomic force spectroscopy was used to study the effects of trodusquemine, a promising drug candidate against neurodegenerative disorders, on the mechanical properties of supported lipid bilayers (SLBs) mimicking neuronal membranes. Both the force required to rupture the bilayer and the Young's modulus of the bilayer were found to increase in the presence of this molecule. The increase in mechanical strength could contribute to a stronger resistance of the membranes to the toxic action of misfolded protein oligomers.

1. – Introduction

Some neurodegenerative disorders, including Alzheimer's and Parkinson's diseases, are associated with protein misfolding processes leading to the formation of fibrillar aggregates called amyloid fibrils. The early oligomeric stages of fibrillogenesis are particularly harmful, as they exhibit cytotoxic activity [1]. Protein aggregation can be modulated by a variety of environmental factors, such as pH, temperature, ionic strength and by the interactions with a range of cellular components, including lipid membranes. It has been shown that trodusquemine, a natural aminosterol isolated from *Squalus acanthias*, has an inhibitory action on the formation of amyloid aggregates and on the interaction between misfolded oligomers and biological membranes. Trodusquemine is a cationic amphipathic aminosterol (MW 685, net charge + 3 at physiological *p*H) formed by a sulfate group, a sterol core group and a spermine polyamine tail. It was found that in the presence of this molecule the interaction between membranes of neuronal cells in culture and oligomers of α-synuclein and amyloid-β proteins, related to Parkinson's and Alzheimer's diseases respectively, is inhibited [2, 3]. This work was aimed to study the effect of this aminosterol on the membrane physico-chemical properties, to get insight into the mechanisms involved in the protective action of this molecule against toxic protein oligomers. In particular, the effect on the membrane mechanical properties were analyzed. The lipid matrix of the membrane plays a crucial role in the propagation of mechanical stimuli resulting in the activation of membrane channels and mechanisms regulating biological cell functions [4]. Using model membranes that mimic the lipid composition of neuronal cell membranes, we investigated the effects of trodusquemine on the nanomechanical properties of lipid bilayers on solid support. By employing atomic force spectroscopy, we measured the force necessary to rupture the bilayer (breakthrough force) and the bilayer Young's modulus. Our results show that trodusquemine increases the mechanical stability of the lipid bilayer.

2. – Materials and methods

2˙1. *Supported lipid bilayers (SLBs).* – Lipid vesicles with and without trodusquemine were prepared by the group of Prof. Fabrizio Chiti (Department of Experimental and Clinical Biomedical Sciences, University of Florence) according to the procedure described in [5]. To mimic the external leaflet of neuronal cells, a 2:1 (mol/mol) mixture of 1,2-dioleoyl-*sn*-glycero-3-phosphocoline and sphingomyelin, 1% (mol) cholesterol, 1% (mol) ganglioside GM1 was used, obtaining biomimetic membranes which exhibit phase separation as a consequence of the different main transition temperatures of the components. For the analysis with the atomic force microscope (AFM), we obtained SLBs via vesicle fusion on a mica substrate as reported in [5]. Briefly, suspended vesicles were deposited on a mica substrate to a final lipid concentration of 0.1 mg/mL, in the presence of 2 mM $CaCl_2$ to induce vesicle fusion. The samples were stored at room temperature for 10 min and then incubated at 60 °C for 15 min at 100% relative humidity. The samples were left at room temperature for 2 h, then rinsed with ultrapure water to remove undeposited vesicles. The AFM analysis started 1.5 h after rising. Membranes were studied in the absence and in the presence of 5 μM trodusquemine.

2˙2. *Atomic force microscopy and spectroscopy.* – Tapping mode AFM images were acquired in liquid environment using a Dimension 3100 SPM (Bruker, Karlsruhe, Germany) and triangular silicon nitride cantilevers DNP-S10 (Bruker, nominal spring constant 0.06 N/m). Force maps containing 128×128 force-distance curves were acquired point-by-point on scan areas $5 \times 5\,\mu m^2$ with a Multimode SPM (Bruker) using the same experimental protocols and data analysis reported in [5]. In a second set of experiments, we acquired images with a Nanowizard IV microscope (Bruker) in *Quantitative Imaging* (QI) mode to simultaneously obtain topographic images and force-distance curve maps. DNP-10 cantilevers (Bruker, nominal spring constant 0.24 N/m) were used. Force-distance curves were recorded with a maximum applied force of 600 pN acquiring 1000 points per curve on scan areas of $2.5 \times 2.5\,\mu m^2$ (128×128 pixels). The length of the force curves was 50 nm and the approaching tip speed was 10 μm/s. The effective spring constant of each cantilever was determined in situ with the thermal noise method [6]. The cantilever deflection sensitivity was obtained by acquiring a force-distance curve on a mica disk, working in ultrapure water. Samples were always kept in liquid environment.

3. – Results and discussion

Nanomechanical maps of SLBs were obtained by acquiring force-displacement curves point-by-point while scanning the sample. A force-displacement curve is measured by holding the tip in a fixed xy position and changing the tip-sample relative distance along the vertical direction z. During this movement, the deflection of the cantilever, related to the tip-sample interaction force, is recorded as a function of the vertical displacement of the piezoelectric scanner (fig. 1). After tip-sample contact, the sample is initially in an elastic regime. When increasing the force exerted on the sample, the force value at which the tip penetrates the bilayer is called the breakthrough force. In our first set of experiments, we analyzed the morphological features of SLBs and evaluated the breakthrough force in the absence and in the presence of trodusquemine. Breakthrough maps and topographic images of SLBs are reported in fig. 2(a). In general, breakthrough maps features correspond to SLBs morphology. The latter exhibits a phase separation between a disordered, fluid phase L_d (corresponding to the dark image background) and

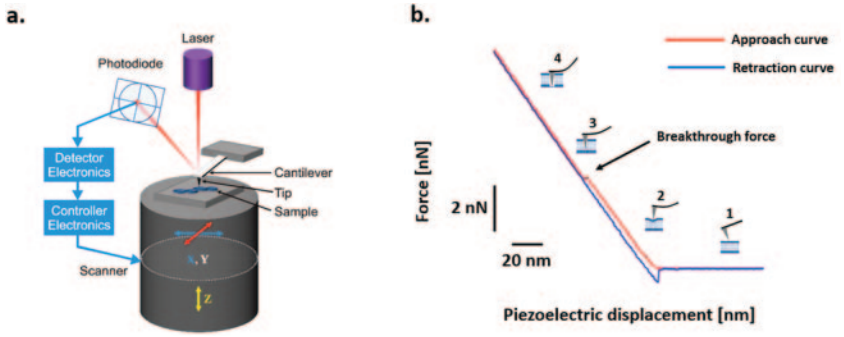

Fig. 1. – (a) AFM main components. (b) Mechanical behaviour of the sample due to the tip-sample interaction force as a function of piezoelectric scanner displacement (red, *approach curve*; blue, *retraction curve*). Initially, the tip-sample distance is large (1). At the contact point, the relative distance between the tip and the sample goes to zero. After that, the tip indents the sample deforming it (2). The breakthrough force is the force value at which the tip penetrates the bilayer (3). Immediately after breakthrough, the force drops, following bilayer rupture. The force increases further due to the interaction between the tip and the solid support (4).

an ordered, condensed and thicker phase L_β (corresponding to the domains in the lighter colour). In some samples, the ordered phase had a greater breakthrough force than the fluid phase, but in most cases samples did not show any breakthrough force in the L_β phase. Trodusquemine does not perturbate the intrinsic phase separation of SLBs. However, the presence of trodusquemine induces a clear increase in the breakthrough

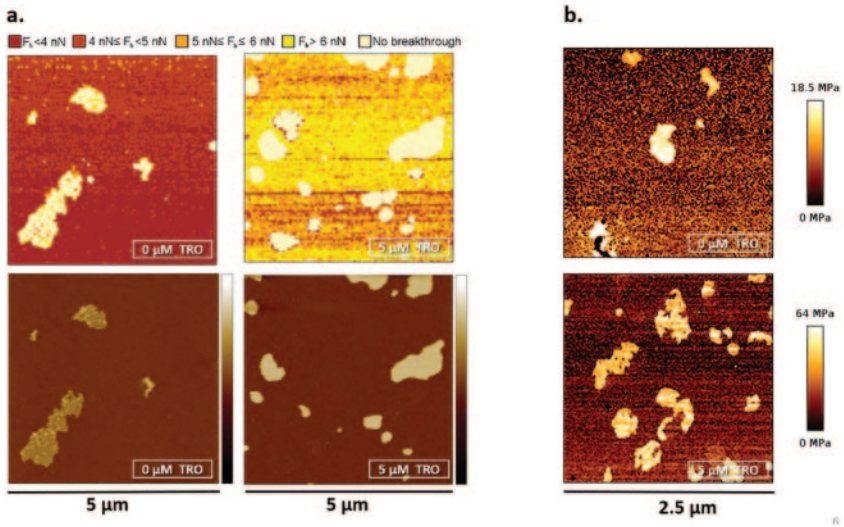

Fig. 2. – (a) Upper row, breakthrough force maps. The lighter areas correspond to the ordered phase where no breakthrough occurs. Lower row, corresponding topographic images that show a phase separation, where the ordered domains are thicker than the disordered phase. Z range 15 nm. (b) Young's modulus maps in the absence (top) and in the presence (bottom) of trodusquemine.

force of the fluid phase. From a statistical analysis of all measurements of different samples, we obtained a breakthrough force of 2.8 ± 0.1 nN in the absence of trodusquemine and 4.5 ± 0.3 nN in the presence of $5\,\mu$M trodusquemine. In a second set of experiments, we studied the effect of trodusquemine on the bilayer elasticity measuring the bilayer Young's modulus. In the region between the contact point and the breakthrough point, soft samples display a non-linear elastic behaviour, due to the indentation of the sample by the tip. The expressions relating force to indentation differ according to the geometry of the indenter [7]. The Sneddon formula relates the force between a parabolic tip and the sample,

$$F_{\text{Sneddon}}(\delta) = \frac{16}{9} E \sqrt{R} \delta^{3/2}, \tag{1}$$

where E is Young's modulus, R the tip radius, δ the indentation. This expression for indentations smaller than the tip radius well approximates the one obtained for a spherical tip geometry [8]. We fitted this model to the force-distance curves using the Nanowizard IV software, thus obtaining Young's modulus maps as those reported in fig. 2(b). These maps exhibit ordered domains that have a greater stiffness than the fluid phase. Again, the presence of trodusquemine has a stiffening effect on the bilayer, as the Young's modulus changes from 18.8 ± 0.1 MPa (L_d phase) and 30.0 ± 0.1 MPa (L_β phase) in the absence of trodusquemine to 25.7 ± 0.1 MPa (L_d phase) and 62.4 ± 0.3 MPa (L_β phase) at $5\,\mu$M trodusquemine. This increase in membrane stiffness could be related to the molecular arrangement of trodusquemine in the bilayer. Based on experiments and numerical simulations, it has been proposed that trodusquemine is positioned almost parallel to the membrane plane (and therefore perpendicular to the lipid molecules) [5]. Such molecular conformation can play a stabilizing role for the membrane with respect to mechanical stress perpendicular to the membrane plane. The increase in mechanical strength could contribute to a stronger resistance of the membranes to the toxic action of misfolded protein oligomers.

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