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Decrease of elastic moduli of DOPC bilayers induced by a macrolide antibiotic, azithromycin

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Abstract

The elastic properties of membrane bilayers are key parameters that control its deformation and can be affected by pharmacological agents. Our previous atomic force microscopy studies revealed that the macrolide antibiotic, azithromycin, leads to erosion of DPPC domains in a fluid DOPC matrix [A. Berquand, M. P. Mingeot-Leclercq, Y. F. Dufrene, Real-time imaging of drug-membrane interactions by atomic force microscopy, Biochim. Biophys. Acta 1664 (2004) 198-205.]. Since this observation could be due to an effect on DOPC cohesion, we investigated the effect of azithromycin on elastic properties of DOPC giant unilamellar vesicles (GUVs). Microcinematographic and morphometric analyses revealed that azithromycin addition enhanced lipid membranes fluctuations, leading to eventual disruption of the largest GUVs. These effects were related to change of elastic moduli of DOPC, quantified by the micropipette aspiration technique. Azithromycin decreased both the bending modulus (k_c, from 23.1±3.5 to 10.6±4.5 k_BT) and the apparent area compressibility modulus (K_{app}, from 176±35 to 113±25 mN/m). These data suggested that insertion of azithromycin into the DOPC bilayer reduced the requirement level of both the energy for thermal fluctuations and the stress to stretch the bilayer. Computer modeling of azithromycin interaction with DOPC bilayer, based on minimal energy, independently predicted that azithromycin (i) inserts at the interface of phospholipid bilayers, (ii) decreases the energy of interaction between DOPC molecules, and (iii) increases the mean surface occupied by each phospholipid molecule. We conclude that azithromycin inserts into the DOPC lipid bilayer, so as to decrease its cohesion and to facilitate the merging of DPPC into the DOPC fluid matrix, as observed by atomic force microscopy. These investigations, based on three complementary approaches, provide the first biophysical evidence for the ability of an amphiphilic antibiotic to alter lipid elastic moduli. This may be an important determinant for drug: lipid interactions and cellular pharmacology.

Keywords: Micropipette; Giant vesicle; Bending modulus; Area compressibility modulus; Azithromycin; DOPC

1. Introduction

Interactions of drugs with biological membranes may account for their activity and/or toxicity, thus represent an important area of investigation. Artificial lipid bilayers are increasingly used as models of cell membranes for biophysical studies of lipid:lipid, lipid:protein, and lipid:drug interactions.

Using atomic force microscopy on supported artificial phospholipid bilayers, we showed that azithromycin, a macrolide dicationic antibiotic [2,3] (Fig. 1), not only leads to the erosion and eventual disappearance of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) gel phase domains surrounded by a fluid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) matrix, but also increases the fluidity at the hydrophilic/hydrophobic interface of DOPC:DPPC [1,4]. To test whether these alterations resulted from changes in membrane cohesion of the bulk lipid phase, we here addressed the effect of azithromycin on the elastic properties of DOPC bilayers.

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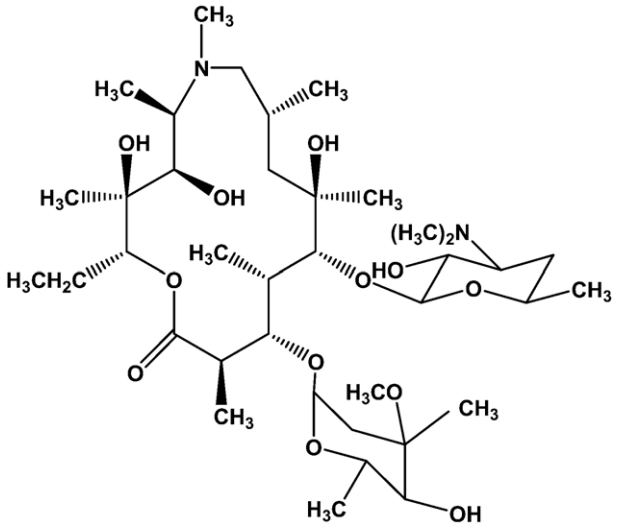

Fig. 1. Structural formula of azithromycin (9-deoxo-9a-methyl-9a-azahomoerythromycin A).

Following the pioneering work of Helfrich [5,6], numerous efforts have been devoted over the last three decades to determine the biophysical parameters of membrane cohesion and elasticity, in particular the bending properties which control bilayer fluctuations and vesicle shape in relation to intrinsic bilayer properties such as adhesion, in- or e-vagination and lipid: protein interactions [7–9]. The two major parameters reflecting the elastic properties of a bilayer are the bending modulus (k_c), reflecting the energy associated to spontaneous thermal fluctuations of the membrane, and the apparent area compressibility modulus (K_{app}), reflecting the energy required to stretch a bilayer.

In present work, we used giant unilamellar vesicles (GUVs) made of DOPC to study the effect of azithromycin on the overall shape of vesicles, and to measure the bending modulus and the apparent area compressibility modulus by the micropipette aspiration technique [10,11]. Results were related to the location of azithromycin at the hydrophilic/hydrophobic interface, the mean surface occupied by each DOPC molecule and the energy of interaction between DOPC molecules as determined by molecular modeling.

2. Materials and methods

2.1. Reagents

DOPC was purchased from Avanti Polar Lipids (Alabaster, AL, USA) and used without further purification; Tris (Tris-hydroxymethyl-aminomethane) was from Merck (Darmstadt, Germany). Bovine serum albumin (BSA), glucose and sucrose were from Fluka (Buchs, Switzerland). Azithromycin (potency = 94.4%, MW = 785 g/mol) was supplied as dihydrate free base by Pfizer (Groton, CT, USA). Since azithromycin is sparingly soluble in water at pH 7.0, but very soluble at acidic pH, a stock solution was prepared by dissolving 22.5 mg of the free base in 1 ml 0.1 M HCl (28.6 mM) and further diluted in Tris buffer (10 mM, pH 7.4) or in Milli-Q water just before the experiments, as indicated.

2.2. GUVs preparation

DOPC-GUVs were prepared by electroformation [12]. Ten μl of a DOPC solution (1 mg/ml in $CHCl_3$) were spread onto the Indium Tin Oxide (ITO)-coated side of a glass slide, followed by vacuum drying overnight. A growing chamber for vesicle electroformation was mounted by placing a covering plate over the conductive glass slide containing the lipidic film and by sealing both plates to each other with Vitrex wax (Vitrex, Denmark) with a 1-mm spacer. For direct morphological studies, the lipid film was hydrated with a 100 mM sucrose solution. For micropipette aspiration studies, the lipid film was hydrated with 100 mM sucrose supplemented or not with azithromycin solution (50 μM, final concentration). For both studies, GUVs were grown by applying an alternative voltage (2 V, 10 Hz) across the growing chamber for about 1 h.

2.3. Transfer of GUVs

Giant vesicles were transferred either into an observation chamber or a manipulation chamber filled with 102 mM glucose. The slight density difference between the 100 mM sucrose and 102 mM glucose solutions drived the vesicles toward the bottom plate where they could be easily handled and observed. The glucose and sucrose concentrations osmotically matched the outer and inner solutions (Osmometer Knauer, Osmo 2320, Berlin, Germany), so as to avoid vesicle swelling or shrinkage. A thin glass slide covered the spacer to avoid evaporation. In such conditions, GUVs were stable during at least 1 day [13].

For the observation of GUVs, we used a chamber made of a glass slide with a spacer (CoverWell, Grace Bio-Labs, Bend, OR, USA). To test the effect of azithromycin, the cavity of the chamber was filled with a mixture of 100 μl of GUVs suspension in 102 mM glucose and 30 μl of a solution containing glucose and azithromycin, to reach a final 50 μM azithromycin concentration. Since not all lipid initially deposited on the ITO-coated slide becomes organized in giant vesicles, it was not possible to evaluate either the exact amount of phospholipid transferred in the observation or manipulation chambers, nor the DOPC:azithromycin ratio.

For GUVs handling, we used a manipulation chamber made of a thin glass slide with a spacer (CoverWell). The inner volume of the spacer was around 0.6 ml. Hundred μl of a vesicle suspension were transferred from the growing chamber to the manipulation chamber containing 0.5 ml of a 102 mM glucose solution. To prevent leakage of azithromycin entrapped in GUVs during their preparation, azithromycin was added to the manipulation chamber to a final 25 μM concentration. For micromanipulation of GUVs in presence of azithromycin, the stock antibiotic solution was diluted in water instead of Tris buffer, in order to minimize charges in the manipulation chamber.

2.4. Morphological studies

Several hundreds of GUVs, preincubated or not with 50 μM azithromycin for 30 min, were recorded with an inverted microscope (Axiovert S100, Zeiss, Germany) to determine the vesicle size distribution (Axiovision 4.4 program, Zeiss, Göttingen, Germany). Briefly, after recording grey level images, individual objects were resolved by segmentation (binary images: white GUVs on a black background) and residual open structures were filled. Surfaces of individual GUVs were measured together with a shape factor (circle = 1; line = 0). Only GUVs with a shape factor >0.9 and a surface >8 pixels^2 (5.7 μm^2) were retained for the analysis. Each processed image was inspected one by one to ensure the complete and exclusive selection of GUVs (97% of recorded GUVs satisfied these criteria). The morphometric analysis was performed by an independent investigator, unaware of GUVs treatment.

2.5. Micropipette aspiration of GUVs

The bending modulus and the apparent area compressibility modulus were determined by controlled aspiration of GUVs into micropipettes made by pulling borosilicated capillary glass tubing (Phymep, Paris, France) with a microforge (F-1200, de Fonbrune, Alcatel, France). To avoid charge accumulation on the glass, the micropipette and the glass slide at the bottom of the manipulation chamber were first pretreated with a 0.05% (w/w) BSA solution in water during 10 min, then rinsed with distilled water to remove unbound BSA. The micropipette, with a final diameter of ~10 μm, was fixed on the stage of an inverted microscope and was driven by a *xyz* shift with an AIS2 micromanipulator device (CellBiology Trading, Hamburg, Germany) to contact selected vesicles. Then, a pressure difference between the interior and the exterior of the