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Effect of tetracycline antibiotic on the monolayers of phosphatidylcholines at the air–water interface

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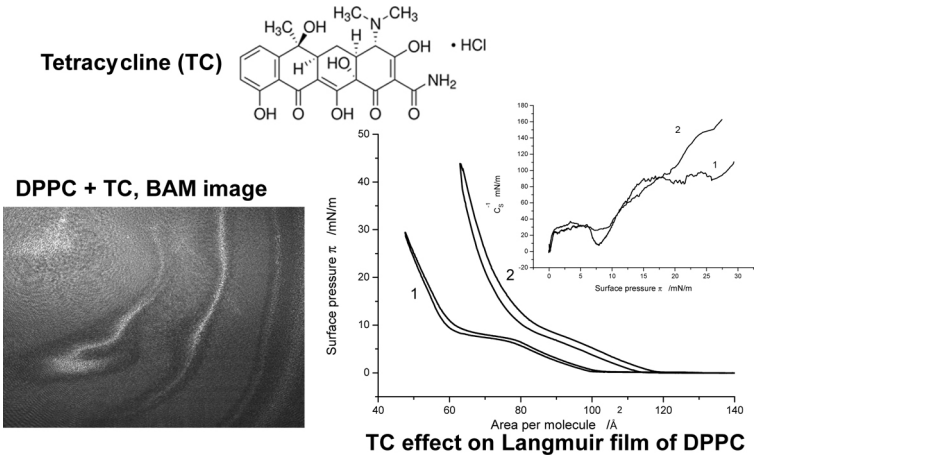
HIGHLIGHTS

- Tetracycline interacts with phosphatidylcholine monolayers through electrostatic interactions.
- Contact potential difference experiments point to the oriented adsorption at/in the head group region of phospholipid monolayer.
- Tetracycline induces disorder in the hydrophobic part, particularly visible in DMPC monolayer.
- Brewster angle experiments show polymorphic changes in the monolayers.

GRAPHICAL ABSTRACT



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ABSTRACT

Tetracycline is a broad-spectrum antibiotic belonging to the polyketide class. It is widely used against bacterial infections as well as food additive to a live-stock. Since tetracycline is resistant against degradation, it accumulates in the environment, leading also to antibiotic residues in animal and food products, which is now considered to be an important health risk because of increasing antibiotic resistance of pathogenic microorganisms. In this work we studied the interactions of tetracycline with phospholipid monolayers at the air/water interface in order to elucidate the mechanism of its action on cell membrane biomimetic system. We selected three phosphatidylcholines, having the same head-group structure, differing with respect to their hydrophobic chain length, the presence of unsaturated bonds between carbon atoms and, consequently, the phase transition temperature. Analysis of the results presented here suggests that tetracycline interacts with the three phosphatidylcholines mainly through the electrostatic interactions with hydrophilic groups of these lipids. On the other hand, the shape of presented pressure-area isotherms and their compressibility moduli show the synergistic effect of hydrophobic interactions that are larger for longer alkyl chains, promoting easier ordering of monolayer molecules with increased surface pressure when drug is present in the subphase, particularly for saturated DPPC. Based on our contact potential data we think that positively charged tertiary amino group of TC is facing negatively

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charged phosphate groups of phosphatidylcholines, penetrating only the hydrophilic head group region but not the hydrophobic moiety of these monolayers. However, the observed differences in CPD values for the case of DMPC point toward the drug disordering influence also on the organization of the hydrophobic region of this monolayer. This is because the drug penetration disturbs the van der Waals ordering interactions between its shorter hydrophobic chains to a greater extent comparing to DPPC and DOPC molecules.

1. Introduction

Since their discovery, the antibiotics have been used for treatment and prevention of microbial infections in humans and animals. The use of antibiotics is significant because they not only exhibit antibiotic activity against various bacteria and pathogenic microorganisms, but also significantly enhance growth when added to animal feed. However, the overuse of antibiotics has triggered the development of bacterial resistance, and leads to antibiotic residues in animal and food products, which is now considered to be an important health risk because of increasing antimicrobial resistance of pathogenic microorganisms [1,2].

Among most widely used antibiotics, tetracyclines play a significant role. Tetracycline (TC) is a broad-spectrum antibiotic, which has a wide antibacterial spectrum against both Gram (+) and Gram (−) microorganisms [3] and is frequently used as a feed additive to promote animal growth [4–6]. TC belongs to a subclass of polyketides having an octahydrotetracene-2-carboxamide skeleton, substituted with many hydroxy and other groups (see Fig. 1).

TC binds with high affinity to 30S subunit and with weak affinity to 50S subunit of bacterial ribosome, inhibiting protein synthesis which effects in the cell death.

Tetracycline is not completely metabolized by human and animal organisms, and most of it is excreted through the urine [7] in an unchanged form, therefore it is considered a harmful and persistent residue in the environment, favoring the contamination by pathogenic microorganisms that have developed antibiotic resistance [1,2]. Moreover, residues of TC have been frequently detected in the effluent of wastewater treatment plants, surface waters and sediments [8–10], posing an increasing potential risk to human health and ecosystem safety.

The antibiotic action of TC implies that its molecules are capable to penetrate through the cellular membranes, but a detailed mechanism of such process is still to be described. Whereas numerous works have been devoted to the studies of interactions of various antibiotics [11–13] with phospholipid monolayers spread at the air–water interface, studies on tetracycline influence are relatively scarce. In the excellent systematic studies of such interactions, the effect of lipid head groups and solution pH was elucidated [14,15].

Here, in an attempt to gain further insight in the mechanism of action of TC, we present our studies on the effect of tetracycline on Langmuir monolayers of lipids spread on the air/water interface. In this work, the interactions between tetracycline and phospholipid monolayers were studied in relation to the fatty acid chain structure, affecting the organization and state of these monolayers. Phospholipid monolayers were formed from the three

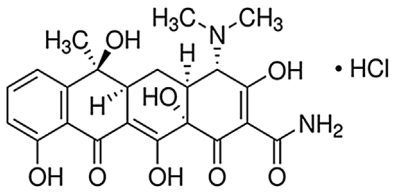

Fig. 1. Structure of tetracycline in a form of hydrochloride.

phosphatidylcholines, all having the same polar head group, but differing in their aliphatic chain length, phase transition temperature and the presence of unsaturated bond. Phosphatidylcholines belong to the group of phospholipids most frequently constituting the cell membranes and therefore monolayers formed from these molecules at the air/water interface are excellent models of biomembranes [16]. Here, we used three different phosphatidylcholines: (i) 1,2-Dimyristoyl-*sn*-gliceryl-3-phosphatidylocholine (DMPC), (ii) 1,2-Dipalmitoyl-*sn*-gliceryl-3-phosphatidylocholine (DPPC), and (iii) 1,2-Dioleoyl-*sn*-gliceryl-3-phosphatidylocholine (DOPC) (Fig. 2).

Among them, DOPC is of the longest fatty acid chain as well as the only one unsaturated, while DMPC is of the shortest fatty acid chain (see Fig. 2). The length and unsaturation of the fatty acid chains determine the value of transition temperatures of monolayers formed from these lipids, allowing for monitoring the interaction of TC with lipidic layers in the solid – DPPC (bulk $t_t = 41\,^{\circ}C$), liquid – DOPC (bulk $t_t = -20\,^{\circ}C$) and around the phase transition point – DMPC (bulk $t_t = 23\,^{\circ}C$). These interactions were monitored by acquiring the surface pressure-molecular area and surface potential-molecular area isotherms, as well as Brewster angle microscopy images at various surface pressures, depending upon the lipid molecules used. These studies allowed elucidating the mode of interaction of TC antibiotic with such monolayers.

2. Experimental

2.1. Materials

Tetracycline hydrochloride (>99%), 1,2-Dimyristoyl-*sn*-gliceryl-3-phosphatidylocholine (DMPC, $C_{36}H_{72}NO_8P$, >99%), 1,2-Dipalmitoyl-*sn*-gliceryl-3-phosphatidylocholine (DPPC, $C_{40}H_{80}NO_8P$, >99%), and 1,2-Dioleoyl-*sn*-gliceryl-3-phosphatidylocholine (DOPC, $C_{44}H_{84}NO_8P$, >99%) were supplied by Sigma–Aldrich. Chloroform (99.8%, from Chempur, Poland) was used as spreading solvent for all lipid solutions (2 mg/ml) via a Hamilton syringe. Milli-Q water was used for all experiments; resistivity 18.2 MΩ cm, pH 7, at 24 °C.

2.2. Methods

Langmuir monolayers were spread from 11.5 μl of appropriate chloroform solution applied carefully via a microsyringe on the aqueous subphase in a Langmuir trough (NIMA Technol., Model 611, Coventry, England) equipped with a Brewster Angle Microscope (KSV NIMA MicroBAM, Finland, fixed angle, 12 μm lateral resolution, and a field-of-view of 3600 μm × 4000 μm) and a vibrating plate surface potential sensor (Kelvin Probe SP1, NFT GmbH). After evaporation of the volatile solvent (ca. 10 min, as judged by the gradual return of the biased surface pressure to its zero value, pre-set for the pristine subphase), the monolayer was continuously compressed at a rate of 30 cm^2/min, to obtain pressure-area isotherms in an isotherm cycling mode set for 2 cycles of compression-decompression. This allowed us to obtain at least 2 isotherms, and to evaluate the quality of such monolayer and reproducibility of its surface behavior. In all figures that follow,