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Two antibacterial nalidixate calixarene derivatives in cholesterol monolayers: Molecular dynamics and physicochemical effects

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ABSTRACT

The interaction of two antibacterial calixarene derivatives with cholesterol, a eukaryotic cell membrane lipid, was investigated with the aim to get more insight in the potential advers effects on our cells. The derivatives used had one or two nalidixic acid arms grafted on the lower rim of the calixarene aromatic crown. Monomolecular films spread at the air-water interface were used as model lipid membranes. Pure cholesterol and pure calixarene derivatives, as well as binary cholesterol – calixarene derivative mixtures were studied using surface pressure measurements, polarization-modulation infrared reflection absorption spectroscopy and molecular dynamics simulations. The properties of the mixed monolayers were described quantitatively using thermodynamic models.

The analysis of surface pressure-area isotherms of mixed monolayers shows that cholesterol may form homogenous but metastable domains with both nalidixate derivatives. This phenomenon is more clearly observed with mono-substituted calixarene. A detailed modeling analysis indicates that cholesterol favors dehydration of the calixarene polar headgroups and transfer of the derivatives from the aqueous to the gas phase. This effect, more pronounced in the case of the monosubstituted calixarene, can be linked to the hydrophobic interaction with cholesterol. This observation may be useful for developing new calixarene derivatives allowing us to control disease-causing bacteria without harming our own cells.

1. Introduction

Due to their intrinsic physico-chemical and chemical properties, calixarenes are often employed as carriers and spatial organizers of different substituents recognizing organic molecules or metallic cations [1–3]. For this reason, calixarenes are of interest in developing molecular drug organizers or dispensers containing drugs or probes for medical applications. In the former case, a drug carrier or a prodrug behavior could be engineered, with a biochemically controlled release of the bioactive agent. In the past, different polymeric structures with such possible applications were developed [4–8].

As reviewed by Schatz and co-workers [9], Mokhtari et al. [10], Ukhatskaya et al. [11] and recently Yousaf et al. [12], using calixarene derivatives for medical applications arouses an increasing attention. The latter is true in the case of anti-infectious and anti-cancerous treatments. Biological studies related to plasmid DNA binding and cell transfection were reported by Ungaro and co-workers [13,14]. In these applications calixarene derivatives are used as bioactive agents rather than as drugs carriers.

In the field of antibacterial applications, a pegylated calix[8]arene derivative, macrocyclon, was investigated in the 1950s as an anti-infective agent in treating leprosy and tuberculosis [15]. More recently, macrocyclon, pure *p-tert*-butylcalix[8]- and [4]arenes analogues were investigated *in vitro* and *in vivo* in view of developing therapeutic alternatives against multi-drug-resistant tuberculosis (MDR-TB); some new derivatives displaying a controlled polyethylene glycol (PEG) chain length were described in the literature [16,17]. Hailes and co-workers

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reported the synthesis and *in vivo* anti-mycobacterial activities of new derivatives of Macrocyclon [18]. Calixarene-based mimics of vancomycin were described as well [19].

In our group, new calixarene derivatives are synthesized with the aim to develope new molecular drug organizers or dispensers (prodrugs). These derivatives bear penicillin or quinolone moieties attached *via* an amide or ester bond at the lower rim of the aromatic crown. In view of an oral administration, a lipophilic calixarene moiety was chosen, to allow the release and absorption of the active moiety in the intestines and elimination of the calixarene, if not absorbed [20–24]. Because a synergistic antibacterial effect could occur through the incorporation of different antibiotics on the same spatial organiser, calixarene derivatives bearing various penicillin or quinolone subunits were also prepared [25]. Other derivatives incorporating the antiviral drug aciclovir were developed as well [26].

In vitro evaluation of the biological activity of these molecules is delicate due to their insolubility in water or buffers, even in the presence of dimethyl sulfoxide (DMSO). For this reason, these molecules were studied at the air-water interface. A controlled hydrolysis in the monolayers was recently described [26] and the role of cations in the supramolecular organization of calixarene derivatives within the film at the interface was elucidated [24]. Moreover, new insights were obtained concerning the interaction of some derivatives with model bacterial and eukaryotic lipid membranes [21,22,27].

The present study aims to clarify the behavior of *p-tert*-butylcalix[4]arene – nalidixic adducts upon interaction with cholesterol, a model eukaryotic cell lipid. Indeed, it is important to evaluate the effect of potential antibacterials on eukaryotic cells with the aim to control disease-causing bacteria without harming our own cells.

Nalidixic acid was chosen as a model of the quinolone family, due to its simple structure. It is the first commercially available derivative of this family of molecules, developed in the 1960s. It belongs to the first generation of quinolones, together with pipemidic acid and flumequine. The structure – activity relationship established with the first generation drugs allowed developing a second generation of quinolones, significantly more active in terms of concentration and antibacterial spectrum. The latter, highly potent antibacterials contain a fluorine atom in the nalidixic acid or piperazine moiety. They are active as inhibitors of the bacterial DNA gyrase, an enzyme essential for DNA replication and transcription, and display activity against Gram-negative bacteria (first generation), or against both Gram-positive and Gram-negative bacteria (second generation).

In this study, Langmuir monomolecular film experiments combined with molecular dynamic simulations of monolayers were used to investigate the structure and properties of mixed monolayers formed with cholesterol (CHOL) and *p-tert*-butylcalix[4]arene-mono-propylnalidixate (calix I) or *p-tert*-butylcalix[4]arene-bis-propyl-nalidixate (calix II). The properties of mixed and pure films were compared.

2. Materials and methods

2.1. Materials and reagents

Synthesis of two nalidixate calixarene derivatives namely: *p-tert*-butylcalix[4]arene-mono-propylnalidixate and *p-tert*-butylcalix[4]arene-bis-propylnalidixate was described previously [23,24]. Synthetic cholesterol (CHOL) (~99% pure) and spectrophotometric grade chloroform (99.9% pure), used for preparing solutions, were purchased from Sigma-Aldrich. The chemical structures of calix I and calix II are shown in Fig. 1 (the insets). Calixarene derivatives and cholesterol were dissolved in chloroform to achieve a final concentration of 0.5 mg mL^{-1}. The stock solution of calix I, calix II and CHOL were used for preparing 0.1, 0.3, 0.5, 0.7 and 0.9 mol fraction mixtures. The solutions were stored at 4 °C. Ultrapure water (Milli-Q, Millipore) with resistivity of 18 MΩ cm and surface tension of 72.8 mN m^{-1} at 20 °C (pH 5.6) was used in all experiments.

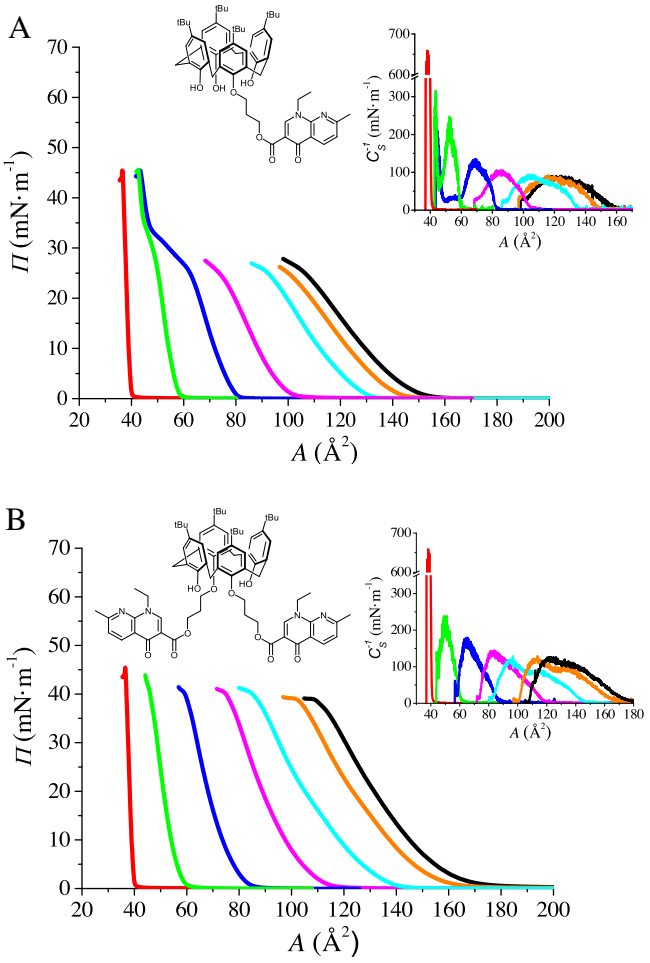

Fig. 1. *Π–A* isotherms of binary mixtures of calix I/CHOL (A) and calix II/CHOL (B) spread on water at 20 °C. x_{CHOL} = 0 (black), 0.1 (orange), 0.3 (cyan), 0.5 (magenta), 0.7 (blue), 0.9 (green), 1 (red). Insets: C_S^{-1}–*A* dependencies and chemical structures of calix I (A), and calix II (B). The errors in C_S^{-1} are 2–3 orders of magnitude smaller than the reported values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Surface pressure measurement

The surface pressure measurements were carried out using a KSV 2000 Langmuir balance (KSV Instruments Ltd, Helsinki). A Teflon trough with two hydrophilic Delrin barriers (symmetric compression) was used in compression isotherm experiments. The system was equipped with an electrobalance and a platinum Wilhelmy plate as a surface pressure sensor. The temperature was kept constant at 20 ± 0.1 °C. All impurities were removed from the subphase surface by sweeping and suction. After the equilibration time of 15 min, the film was compressed at a constant rate of 2.5 mm min^{-1} barrier^{-1}. A PC computer and KSV software were used to control the experiments. Each compression isotherm was performed at least three times. The standard deviation obtained from compression isotherms was ± 0.5 Å^2 on molecular area, and ± 0.2 mN m^{-1} on surface pressure.