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Assembly of a model hydrophobic drug into cationic bilayer fragments

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Abstract

Our previous work has shown that dioctadecyldimethylammonium bromide (DODAB) bilayer fragments (BF) presented antimicrobial activity, solubilized fungicides, e.g., amphotericin B and miconazole (MCZ), stabilized hydrophobic drug particles and were effective in vivo. Here, the interaction between MCZ and DODAB BF is evaluated from determination of BF loading capacity and effects of drug-to-lipid molar proportion (MP) on particle size, zeta potential and gel-to-liquid-crystalline phase transition T_m. DODAB BF solubilized MCZ over a range of MP. BF loading capacity was 0.5 mM MCZ at 5 mM DODAB. Above this limit, the drug aggregated in the dispersion. At pH 6.3, BF zeta potentials decreased with MP, suggesting insertion of deprotonated drug into the bilayer. MCZ optical spectra in BF were similar to those in best organic solvent, confirming drug solubilization. At MP $\leqslant$ 1:10, BF T_m remained unchanged, suggesting drug capture at BF hydrophobic edges. At MP $\geqslant$ 1:10, T_m decreased, showing MCZ insertion into DODAB bilayer. However, drug was expelled from the bilayer core upon lowering temperature. Minimal fungicidal concentrations against *C. albicans* were synergically reduced by 10 times for drug/BF.

Keywords: Miconazole; Dioctadecyldimethylammonium bromide; Sodium dihexadecylphosphate; Bilayer fragments; Fungicidal activity; *Candida albicans*

1. Introduction

Over the past two decades membrane-derivatized colloidal systems have been introduced as potential applications to a broad set of problems involving chemical events on cell membrane surfaces [1–6]. Vesicles and other bilayer assemblies, such as bilayer fragments or disks, though ephemeral, produced useful devices by depositing onto polymeric [7,8], mineral [9,10], or drug particles [3,11] or cell surfaces [12–14] or by acting as templates for direction of inorganic matter deposition, redox processes, or polymerization reactions [15–17]. In biology and medicine, they offered a suitable matrix to solubilize and/or carry drugs [18–20], either as entire entities, as complexes with the drug to be carried, or as bilayer capsules surrounding the hydrophobic drug granule [21]. In medicinal chemistry, aggregation of hydrophobic drug molecules in aqueous solutions was shown to have an effect on drug bioavailability [22]. The uptake rate into systemic circulation would depend on having hydrophobic drug aggregates of appropriate size available for absorption [22].

On the other hand, there are two clinically important classes of antifungal hydrophobic drugs: the polyenes (e.g., amphotericin B) and the azoles (e.g., miconazole); see Ref. [23] for a review. The self-assembly of amphotericin B (AB) or miconazole (MCZ) at hydrophobic sites of synthetic bilayer fragments has been reported [24,25], as has the in vivo therapeutic activity of a novel AB formulation with cationic bilayer fragments [26] which exhibited low nephrotoxicity [27]. In fact, some double-chained synthetic lipids such as dioctadecyldimethylammonium bromide (DODAB) or sodium dihexadecylphosphate (DHP) self-assemble in aqueous solution yielding closed bilayers (vesicles) or disrupted vesicles (bilayer fragments, BF, or disks or lipid bilayer rafts), depending on the procedure used for dispersing the lipid [3]. Furthermore, sterically (PEGylated) stabilized

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BF have recently been employed for rapid evaluation of amphiphatic drug partition [28].

In this work, effects of the MCZ:DODAB molar proportion on physicochemical properties of the drug/BF assemblies were investigated. At room temperature, loading capacity of 5 mM DODAB bilayer fragments was 0.5 mM MCZ. Although the loading capacity increased as a function of temperature, returning to room temperature after a heating–cooling cycle caused drug expulsion from the bilayer and appearance of drug aggregates in dispersion at drug-to-lipid molar proportions M higher than 1:8. Below this limit, there was drug solubilization with excellent stability of the drug/bilayer fragment complex plus improvement of drug antifungal activity against *Candida albicans*.

2. Materials and methods

2.1. Drugs, lipids, and microorganisms

Dioctadecyldimethylammonium bromide (DODAB) and sodium dihexadecylphosphate (DHP) were purchased from Sigma at the highest purity available. Miconazole nitrate (MCZ) (batch BEA53, Jansen Research Foundation, Beerse, Belgium) was used as such from a 10 mg/ml (ca. 20 mM) stock solution in ethanol 43% v/v. This solution was incubated for 1 h at 50 °C to obtain complete dissolution of drug crystals. *Candida albicans* ATCC 90028 was obtained from the American Type Culture Collection. While methanol or ethanol were used as cosolvents percentages were always expressed as volume ratios.

2.2. Preparation of lipid dispersions and analytical determinations of lipid concentration

Lipids were dispersed in water, using procedures that yielded either large and closed vesicles (LV) or bilayer fragments (BF), depending on the dispersion method. Sonication with tip above the bilayer T_m [30] was the method employed for producing cationic DODAB or anionic DHP bilayer fragments [31]. At room temperature, BF were in the gel state. This procedure dispersed the amphiphile powder in water using a high-energy input, which not only produced bilayer vesicles but also disrupted these vesicles, thereby generating open bilayer fragments. Thus, lipids were dispersed in water using procedures that yielded either large and closed vesicles (LV) or bilayer fragments (BF). Sonication with tip at 60 °C was the method employed for producing cationic DODAB or anionic DHP bilayer fragments in the rigid gel state at room temperature [29–31]. DODAB was alternatively dispersed by vortexing at 60 °C to obtain large vesicles (LV). Analytical concentration was determined either by microtitration for DODAB [32] or by inorganic phosphorus analysis for DHP [33].

2.3. Determination of surface tension

Surface tension at the air–water interface was measured at 25 °C as a function of MCZ concentration at 5 mM DODAB BF in pure water with a Du Nouy ring. Controls were performed for methanol, which was the drug solvent, and for DODAB, which is soluble in this solvent.

2.4. Determination of miconazole optical absorption spectra in different media

UV–visible optical spectra for MCZ were determined using a Hitachi 2000 spectrophotometer in the double-beam mode against a blank of lipid dispersions and solvent (without drug). Light scattered by the lipid dispersions was subtracted from light absorption spectra for the drug. The MCZ–BF mixtures were obtained by adding 20–100 µl of MCZ stock solution in 43% ethanol (10 mg/ml MCZ) to 1 ml lipid dispersion (10 mg/ml, 15 mM DODAB). All optical spectra were obtained at room temperature (25 °C) just after mixing.

2.5. Determination of mean average diameter and zeta potential of dispersions

The size distributions for DODAB, MCZ, and DODAB/MCZ (mean zeta-average diameter, D_z) were determined by means of a ZetaPlus zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY) equipped with a 570-nm laser and dynamic light scattering at 90° for particle sizing [34]. The mean diameters referred to in this work from now on should be understood as the mean hydrodynamic diameters D_z. Zeta potentials (ζ) were determined from the electrophoretic mobility μ and Smoluchowski's equation, $\zeta = \mu\eta/\varepsilon$, where η and ε are medium viscosity and dielectric constant, respectively. For zeta potential and size distribution measurements, 0.3 ml MCZ (10 mg/ml) in 43% ethanol was added to 2.7 ml pure water or lipid dispersion. All size distributions were obtained at 25 and 50 °C just after MCZ and DODAB BF were mixed. As a control of formulation stability, mean sizes were determined also at 1, 24, 72, and 288 h after mixing.

2.6. Determination of temperature effects on turbidity of DODAB or DODAB/MCZ dispersions and assessment of bilayer phase transition

Incubation of DODAB LV and MCZ was done at 50 °C/18 h because the usual T_m for DODAB LV is ca. 45–47 °C [40] and drug insertion in the bilayer core of LV requires these relatively high temperatures and long incubation periods. For samples containing DODAB BF, such drastic conditions were not required, since the drug readily attached to available BF sites, as shown in Results. The gel-to-liquid-crystalline phase transition of DODAB BF or LV was investigated by means of turbidimetry at 310 and 440 nm as a function of temperature, respectively, in the presence or