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Effect of antibiotic amphotericin B on structural and dynamic properties of lipid membranes formed with egg yolk phosphatidylcholine

Monika Hereć [a,b], Akhmed Islamov [c], Alexander Kuklin [c], Mariusz Gagoś [a,d], Wiesław I. Gruszecki [a,*]

[a] *Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, 20-031 Lublin, Poland*
[b] *Department of Theoretical Physics, The John Paul II Catholic University of Lublin, Lublin, Poland*
[c] *Frank Laboratory of Neutron Physics, Joined Institute of Nuclear Research, Dubna, Russia*
[d] *Department of Physics, Agricultural University, Lublin, Poland*

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Abstract

Amphotericin B (AmB) is a popular antibiotic applied in treatment of deep-seated mycotic infections. The mode of action of AmB is based upon interactions with biomembranes but exact binding properties of the antibiotic to the lipid membranes still remain obscure. Effect of incorporation of AmB into egg yolk phosphatidylcholine membranes in the concentration range from 0.01 to 5 mol% on structural and dynamic properties of lipid bilayers was studied with application of small-angle neutron scattering, X-ray diffractometry and Fourier-transform infrared spectroscopy (FTIR). The results of the experiments show that AmB is located predominantly in the headgroup region of the membranes at concentrations below 1 mol%. The process of AmB aggregation, at concentrations above 1 mol%, is associated with ordering effect within the acyl chain region and therefore indicates incorporation of AmB into the hydrophobic membrane core.
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1. Introduction

Antifungal polyene macrolide antibiotic amphotericin B (AmB; Fig. 1) is a representative of the group of chemotherapeutics which mechanism of action consists in change of membrane cell permeability (Aracava et al., 1981; Brajtburg et al., 1990; Gallis et al., 1990; Ellis, 2002; Baginski et al., 2006). Destroying natural selective membrane permeability is an effect of incorporation of amphiphilic molecules of amphotericin B into the lipid phase and their interaction with lipid molecules (Hartsel et al., 1991; Wolf and Hartsel, 1995). Rod-shaped structure of AmB molecule, with the polar head mycosamine, the hydroxyl groups on the one side of the macrolide ring and the polyene fragment on the opposite side, makes it possible to interact both with the polar part of the lipid membrane and acyl chains. The consequence of such a structure of AmB is also a formation of molecular aggregates (De Kruijff et al., 1974; De Kruijff and Demel, 1974; Bolard et al., 1991; Barwicz et al., 1993; Caillet et al., 1995; Fujii et al., 1997; Gruszecki et al., 2002)

* Corresponding author. Tel.: +48 81 537 62 52; fax: +48 81 537 61 91.
E-mail address: wieslaw.gruszecki@umcs.lublin.pl (W.I. Gruszecki).

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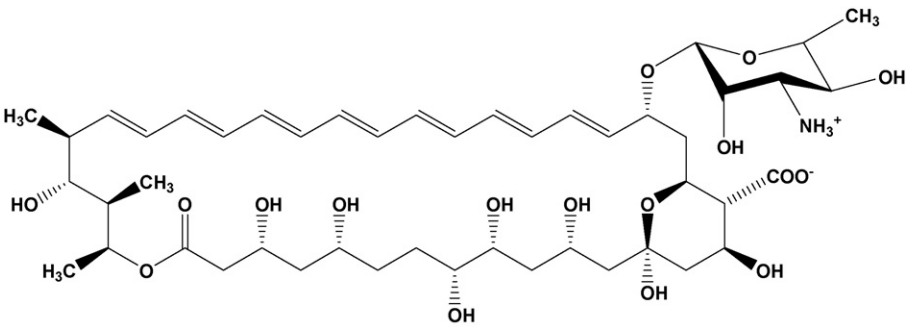

Fig. 1. Chemical structure of amphotericin B.

that play an important role in AmB mode of therapeutic action.

It has been postulated that not only formation of porous molecular structures by AmB, but also modification of the physical properties of the lipid bilayers modulates membrane permeability to ions (Barwicz and Tancrede, 1997; Fournier et al., 1998; Wojtowicz et al., 1998; Charbonneau et al., 2001; Gagos et al., 2001; Milhaud et al., 2002; Paquet et al., 2002; Zumbuehl et al., 2004; Gagos et al., 2005; Baginski et al., 2006; Gabrielska et al., 2006). Such mechanisms can be particularly effective at low concentrations of AmB, at which formation of aggregated molecular structures of the drug within the lipid phase cannot be expected (Gagos et al., 2001; Gruszecki et al., 2003a,b). On the other hand, the recent findings show that AmB increases the barrier for transmembrane ion transport, while incorporated to the lipid membranes at low concentrations instead of acting as an ionophore (Herec et al., 2005). In the present work, we address the problem of molecular interactions between AmB and lipids in the membranes containing very low and relatively high concentrations of the drug, in order to explore further, the molecular mechanisms responsible for action of this antibiotic with respect to biomembranes.

2. Materials and methods

Amphotericin B was purchased from Sigma Chem. Co. (St. Louis, USA). AmB was dissolved in and recrystallized from 2-propanol–water (4:6, v/v) and purified by means of HPLC directly before use. A Supelco PKB-100 column was applied (length 25 cm, internal diameter 4.6 mm) and the solvent mixture 2-propanol–water (4:6, v/v) was used as a mobile phase. The final concentration of AmB was calculated from the absorption spectra. The molar extinction coefficient in the absorption maximum at 408 nm was $1.3 \times 10^5\ M^{-1}\ cm^{-1}$. Owing to the low concentration of AmB eluted from the column (absorbance level below 0.2 at the optical path-length 1 cm) and owing to the fact that absorbance level decreased linearly upon dilution we considered the solution as composed mostly of monomeric AmB and the determination based on absorbance measurement accurate. Egg yolk phosphatidylcholine (EYPC) was obtained from Sigma Chem. Co. and used without further purification.

The AmB-containing EYPC membranes were investigated in the form of either unilamellar liposomes (SANS, electronic absorption) or multibilayers (X-ray, FTIR).

The multibilayers composed of 40 bilayers were formed on glass support or on ZnSe crystal with EYPC and AmB at molar concentration from 0.01 to 5 mol% according to the procedure described in detail previously (Gruszecki and Sielewiesiuk, 1990, 1991). Briefly, the lipid multibilayers were deposited to a solid support by means of evaporation from ethanol. After deposition the lipid films were transferred to a vacuum, for 30 min, in order to remove possible residuals of organic solvent, and then exposed for 30 min to relative humidity 80% in order to hydrate the lipid multibilayers.

The unilamellar liposomes were prepared in 100 mM Tricine buffer (pH 7.6) by extrusion technique, with application of liposome extruder (Avestin Inc., Canada) using filters with 50 nm pores, as described in detail previously (Herec et al., 2005).

The small-angle neutron scattering (SANS) with two-detector system measurements were performed at the small-angle time-of-flight axially symmetric neutron scattering spectrometer MURN at the IBR-2 fast pulsed reactor of the Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna. For complete description of technical details, see Kuklin et al. (2005). The samples were poured into quartz cells (Hellma, Müllheim, Germany) to provide the 2 mm sample thickness. The sample temperature was set and controlled electronically at $20.0 \pm 0.1\ ^\circ C$. The sample in quartz cell

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