SPECTROPHOTOMETRIC ANALYSIS OF THE ORGANISATION OF POLYENE ANTIBIOTIC AMPHOTERICIN B IN LIPID MEMBRANES

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Amphotericin B (AmB) is a polyene antibiotic widely used in the treatment of deep-seated fungal infections. The mode of action of AmB is directly related to the effect of the drug on the lipid phase of biomembranes. In this research the effect of AmB on the properties of lipid bilayers formed with dipalmitoylphosphatidylcholine (DPPC) and the effect of the lipid phase on the molecular organisation of AmB were studied using UV-Vis light spectrophotometry. The absorption spectra of AmB in lipid membranes display a complex structure with hypsochromic- and bathochromic-shifted bands indicative of the formation of molecular aggregates of the drug. The formation of molecular aggregates was analysed at different concentrations of the drug in the lipid phase in the range (0.05 to 5 mol%) and at different temperatures in the range (5 to 55 ^{o}C). The aggregation level of AmB in the ordered phase of DPPC displayed a minimum corresponding to a concentration of 1 mol% with respect to the lipid. An increase in the aggregation level was observed in the temperature region corresponding to the main phase transition. The structure of molecular aggregates of AmB is analysed on the basis of spectroscopic effects in terms of the exciton splitting model. An analysis of the position of the absorption maximum of AmB in the lipid phase of DPPC in terms of the theory of solvatochromc effects allows the refractive index n=1.40 and n=1.49 to be ascribed to the hydrophobic core of the membrane in the L_{α} and the P_{β}' phases respectively. An analysis of the aggregation of AmB in the lipid phase relation to the physical state of the membrane reveals that the temperature range of the main phase transition of a lipid cluster in the immediate vicinity of AmB depends on its concentration. The termination of the phase transition temperature, as read from the AmB aggregation, varies between 42 ^{o}C at 1 mol% AmB in DPPC and 49 ^{o}C at 5 mol% AmB in DPPC. The exciton splitting theory applied to analyse the obtained spectroscopic data gives a AmB pore diameter of 3.2 Å in the gel phase and 4.2 Å in the fluid phase of the DPPC membrane, assuming that the pore is formed by 9 AmB molecules.