

ESR quantification of liposome membrane microviscosity change induced by the addition of cholesterol and drugs.

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Electron spin resonance (ESR) is currently used to investigate the microenvironment of in membrane liposome. The relative anisotropy observed in an ESR spectrum of nitroxide spin probe is directly related to the rotational mobility of the probe, a term that can be correlated with the probe's microviscosity (η). Here, the microviscosity is defined as homogenous solution viscosity, which results in the same spectrum as that recorded in the microenvironment. Standard curves of microviscosities have been established by calibration of the ESR spectra of three n-doxyl stearic acids (n-DSA: n = 5, 12, 16) probes in glycerol-ethanol mixtures of known viscosities (*). These curves allow us to quantify the effective microviscosity at different depths inside liposomes by measuring the order parameter (S) and the correlation time (τ_c) on n-DSA ESR spectra. This method has been applied to measure the liposome membrane microviscosity change induced by molecules presenting a medical interest. The effect of cholesterol at various percentages on dimyristoyl-L- α phosphatidylcholine (DMPC) liposome has been studied quantitatively at room temperature. At the depth of 7.8 Å (5-DSA), the progressive addition of cholesterol induced an increase of η from 222.53 cP to a maximum value of 428.57 cP for a 20% mole fraction of cholesterol. For a depth of 16.95 Å (12-DSA), η increased from 64.09 cP to a maximum value of 171.39 cP for a 25% mole fraction of cholesterol. At a depth of 27.7 Å (16-DSA), η increased from 62.56 cP to 108.26 cP. In this case, a 40% mole fraction of cholesterol was necessary to reach the maximum value of viscosity. These results indicate clearly that the membrane saturation in cholesterol is made progressively from the head group to the centre of bilayer. It is also well known that cholesterol associates with fatty acid side chains and reduces their rotational motion. This work allowed the quantification of this effect.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. They can interact with appropriately sized molecules and form water-soluble inclusion complexes with many lipophilic water-insoluble drugs. Some cyclodextrins are known to extract cholesterol from biological membrane. In this study, we demonstrate that the addition of randomly methylated β -cyclodextrin (RAMEB) in cholesterol saturated DMPC liposomes solution leads to the expulsion of the spin label out of the membrane.

Miconazole, an antifungal agent, is used for skin infections such as athlete's foot and jock itch and for vaginal yeast infections. In this work, we show that the incorporation of miconazole inside Dipalmitoyl-L- α Phosphatidylcholine (DPPC) liposome at room temperature induces fluidizing effect: the microviscosity in the polar head decreased from 316.87 to 239.81 cP.

* Mohamed A. Bahri, Belinda J. Heyne, Pol. Hans, Alain E. Seret, Ange A. Mouithys-Mickalad, Maryse D. Hoebeke, *Biophysical Chemistry* 2005, in press.