Spin label studies on effects of guest molecules on bilayer membrane

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1. Introduction

The study of liotropic liquid crystal features of biomembrane forming phospholipid bilayers has become an accentuated topic of soft condensed matters' physics in the last few years. Liposomes – supramolecular structures formed by the self assembly of amphiphilic lipid molecules (Fig.1) – are widely used for basic biomembrane research and biotechnological development. During the production of model membranes the rate and type of lipids are variable, and it is possible to study effects caused on the structure and stability of the membrane by certain guest molecules.

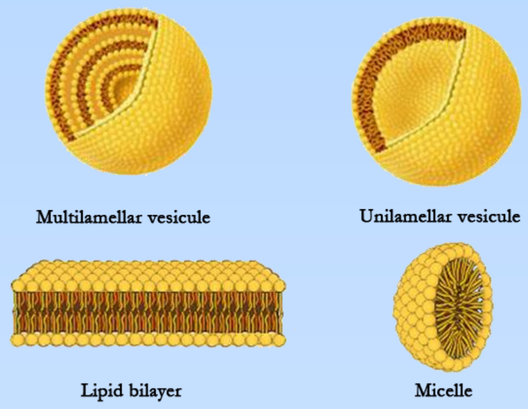


Fig. 1. Lipid bilayer structures

2. Materials

The studied samples were the following : DPPC liposomes were used as control samples; the second and the third samples were so-called ssls (sterically stabilized liposomes), which consisted of DPPC (dipalmitoylphosphatidyl-choline) and 10 mol% of DPPE-PEG:750, resp. DPPE-PEG:2000 (dipalmitoylphosphatidyl-ethanolamine bearing on the polar head polyethylene glycol), so these sterically stabilized liposomes consist of bilayer forming lipids and lipids having polymers covalently attached to the polar headgroup. Their stuctural and molecular dinamical characterization has become relevant after having been established (in some animal tests) that under particular conditions they act as very effective drug encapsulation and delivery systems and have a blood circulation time one or two orders of magnitude longer than conventional, unprotected liposomes. The fourth sample contained DPPC lipids and 10 mol% of an aromatic hydrophobic drug molecule (OL).

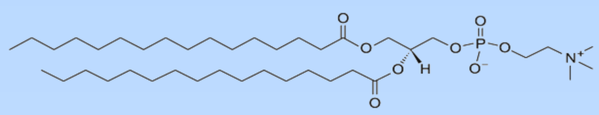


Fig. 2. Structure of DPPC molecule

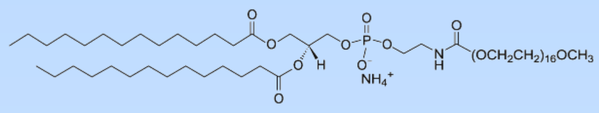


Fig. 3. Structure of DPPE-PEG:750 molecule

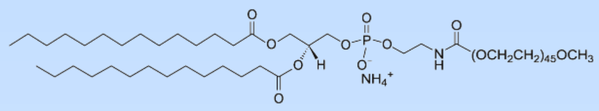


Fig. 4. Structure of DPPE-PEG:2000 molecule

3. Spin-labels in the membranes

As the lipid molecules do not provide EPR signal in themselves, so in order to study their molecular dinamical properties we use spin labels. The spin label we used was a nitroxyl free radical, which was atteched to the 16th carbon atom of a bilayer forming stearic acid through a doxil group. With this method we can monitor the inner hydrophobic zone of the membrane. The rate of the spin labelled lipids and the unlabelled ones was 1:100.

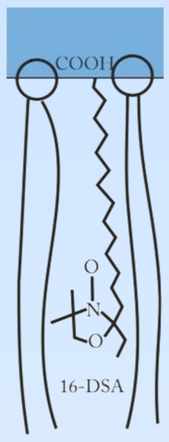


Fig. 5. 16-DSA molecules among the lipids

4. Experimental

A series of X-band EPR spectra were recorded in a BRUKER EleXsys E500 spectrometer in temperature range 280-320 K. This temperature range was choosen in order to contain all three transition temperatures of the pure DPPC membranes.

The spectra were evaluated by a program which fits EPR parameters g, coupling constatnts, correlation times etc. The spectra of all four samples were anisotropic on the beginning of the recording, while they are almost isotropic at high temperatures. The transition between the two spectra was more or less continual. The correlation time decreases with increasing temperature as the freedom of rotational motions increases. Furthermore in Fig. 6. it is noticable that the correlation time has a jump around 309 K, which is approximately the temperature of the pretransition, so it is quite likely that we can detect this transition with EPR method.

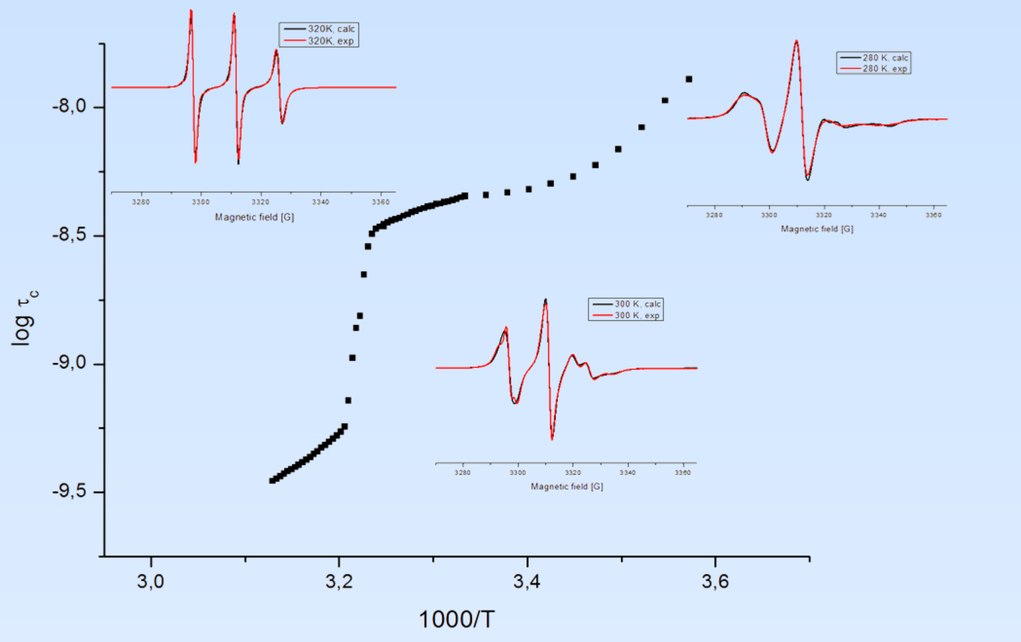
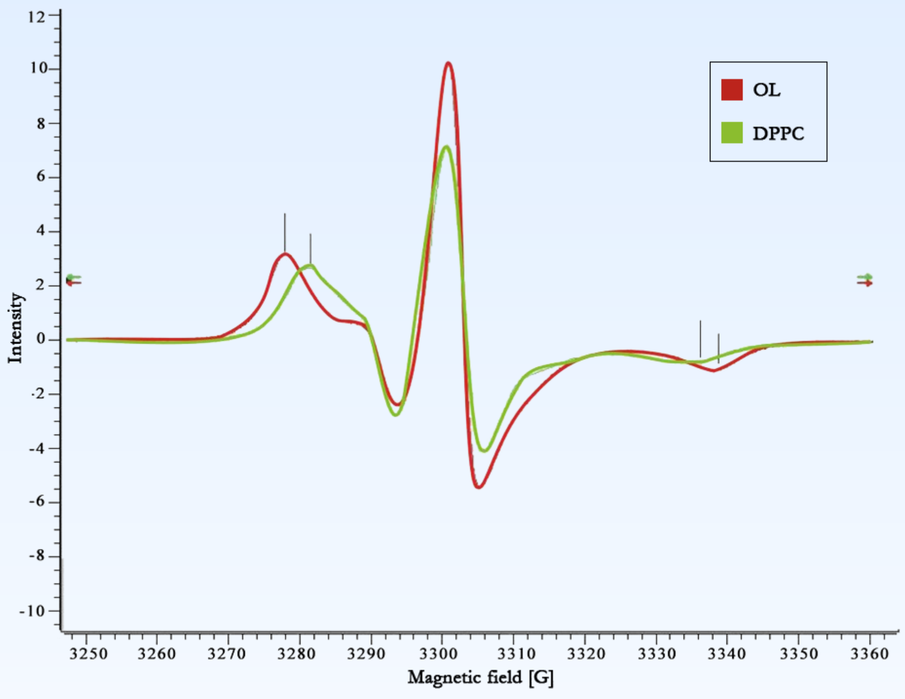


Fig. 6. Three representative recorded and simulated spectra of DPPC+DPPE-Peg:750, resp. the calculated correlation times on an Arrhenius-graph.

5. Results

According to Fig. 7. we believe that all three host molecules insrease the rigidity of the membrane in the region of the 16th carbon atom, and that the effect of the OL molecules is the most powerful. In Fig. 8. the larger 2Azz value of the OL spectra refers to a greater spectral anisotropy than measured at pure DPPC membranes. This experience is not surprising, as the strongly hydrophobic drug molecule (that consits of aromatic rings) is located presumably in the inner, hydrophobic zone of the membrane, so its effects on the motional properties of the spin label are more direct. It is also possible, that the aromatic rings of the guest molecule straddle parallel the ring of the doxil group, stemming the rotational motions around the axis perpendicular to the plane of the rings. In addition we presume, that the spectra of lipids with OL are superposition of two signals originating from two different surroundings.



	$T_{sub,min}$ [°C]	$T_{sub,max}$ [°C]	ΔT [°C]
DPPC/ water	34,0	36,0	2
DPPC + DPPE-PEG:750 / water	36,0	39,2	3,2
DPPC + DPPE-PEG:2000 / water	36,1	38,4	2,3
DPPC + DPPE-PEG:2000 / water	33,5	38,0	4,5

Table 1. Temperetures of the beginning an end of pretransition

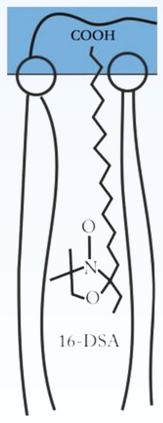
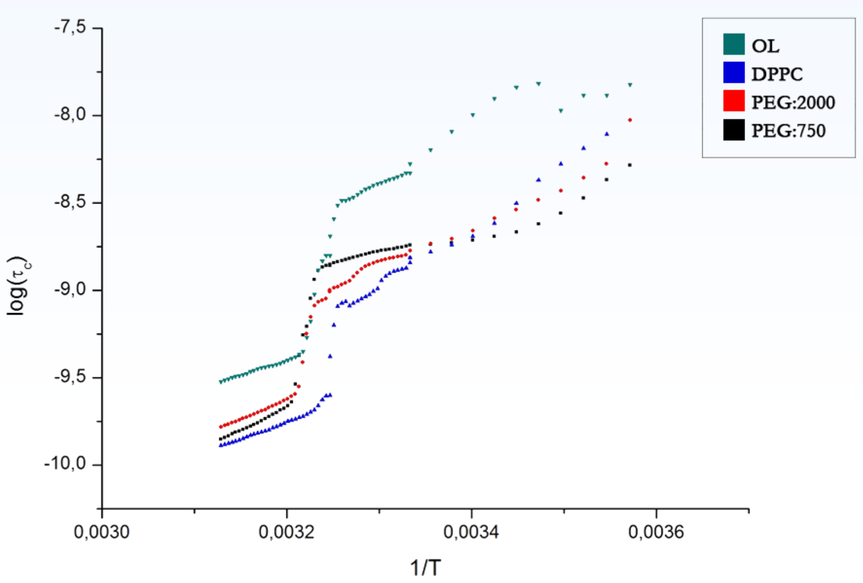


Fig. 7. A possible structure of membranes containing PEG lipids and 16-DSA spin labels

We can also see, that correlation times obtained from the spectra of samples containing DPPE-PEG are obviously larger on temperatures higher than 292 K, so these guest molecules have also an influence on rotational motions of the spin label in the central region despite we believe that the dominant part of the molecule is hydrated in the water. on the changes of correltion times. A possible explanation of the observation might be that the spin labelled lipids shift up in the bilayer so the COOH group gets closer to the water soluble polymer chain of PEG molecules. We can also see that there is no systematic connection between the molecular mass and their influence



5. Summary

A series of X-band EPR spectra were recorded in temperaure range 280-320 K of biomembran forming phospholipid bilayers containing different types of guest molecules. Our goal was to detect the effects caused on the stuctural and dinamical properties of the membrane. We simulated the spectra what allowed us to identify the EPR parameters such as the correlation times. The monitored region was the region of the 16th carbon atom of the carbon chains of DPPC, so the inner membrane region. We detected some effects on the rotational motions of the spin labels caused by the guest molecules. We presume in addition that the temperature of the pretransition was in each sample shifted by values in the table above.