The use of fluorescent probe ANS to determination of influence of tocopherol derivatives on the structure of the phospholipid membrane

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Introduction

Alpha-tocopherol (Toc) and its derivatives fulfils a number of biochemical and biological functions in the cell[1]. The wide spectrum of biological effects observed for them is related to their interactions with the components of the biological membrane. These interactions may in turn lead to modification of membrane properties (stiffness, fluidity, permeability). Research carried out concerned the assessment of the influence of selected Toc ester derivatives on the physical parameters of the lipid membrane in model systems - liposomes.

Materials and Methods

The tests were carried out for: alpha-tocopherol succinate (TS), alpha-tocopherol malonate (TM) and alpha-tocopherol oxalate (TO) incorporated into liposomes composed of dipalmitoylphosphatidyl choline (DPPC). The studies consisted in measurements of fluorescence emission of fluorescent probe 8-Anilino-1-naphthalenesulfonic acid (ANS) in temperature-dependent manner (in the range 25–60°C).

Results and Discussion

For pure DPPC membrane, ANS fluorescence intensity enhancement was observed around the temperature of 42°C with simultaneous shift of the fluorescence maximum towards shorter wavelength (Fig.1). It is known that ANS emission intensity increases and the wavelength of maximum emission undergoes a blue shift as ANS passes from a polar environment to a non-polar medium[2]. Observed sharp growth of ANS's fluorescence emission intensity expressed the increased hydrophobicity of the probe's environment. On the other hand, it is known, that membrane permeability increases at the temperature of the main membrane crystalline transition and is relatively low above and below this temperature. For pure DPPC membrane the main phase transition occurs at around 41-42°C. In this case, the observed perturbation of ANS fluorescence could be related to more effective penetration of the DPPC membrane by ANS molecules, caused by the increase in the membrane permeability as a results of DPPC membrane transition from gel to the crystalline phase.

For liposomes with incorporated Toc and its esters, ANS fluorescence intensity increase was observed around 39-41°C as consequence of the membrane permeability change. However only for Toc and TO a blue shift of ANS maximum emission was noted around temperature of transition. In the case of TS and TM, we observe the maximum shift towards longer wavelengths, what suggests another perturbation of the membrane structure caused by these esters.

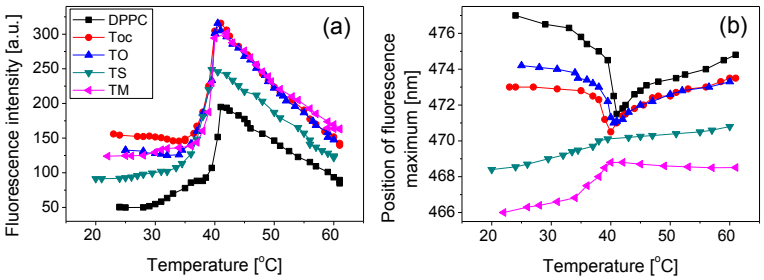

Figure 1: Changes of ANS fluorescence intensity (a) and position (b) of emission maximum in pure liposomal membranes and liposomes containing 2mol% of Toc, TO, TS and TM at different temperatures.

Conclusion

Presented results revealed that Toc ester derivatives embedded into DPPC membrane modify its structure much more compared to Toc. Also some differences is observed among studied esters which may be due to the different mechanism of interaction of esters molecules with phospholipid at bilayer interface, resulting from their location or number of hydroxyl groups.

Bibliography

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