Acylglycerols of Palmitic and Oleic acid as New Candidates for Effective Stigmasterol Delivery - the Impact on Physicochemical Properties of Liposomes and Human Serum Albumin

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MATERIALS

The subjects of this study were eight newly synthesized hybrid molecules in which stigmasterol was linked *via* carbonate or succinyl linker with 1,3- and 1,2- acylglycerols of palmitic and oleic acid. The obtained conjugates were used to form lipid nanoparticles (liposomes) in combination with a common membrane lipid, dipalmitoylphosphatidylcholine (DPPC, Fig. 1).

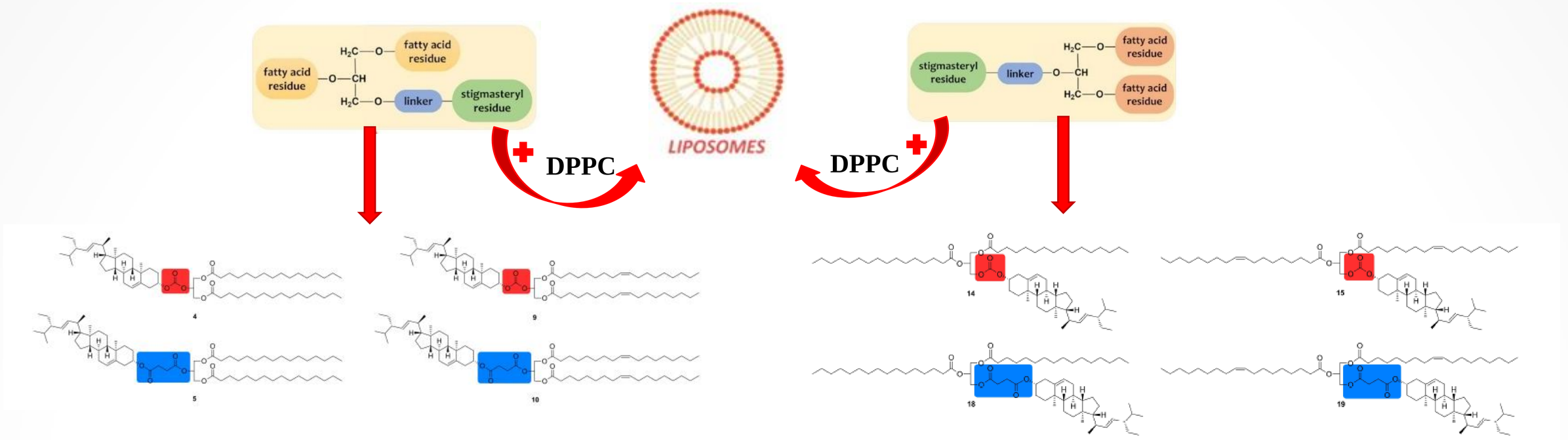

Fig. 1. The structures of the studied acylglycerol-stigmasterol conjugates (compounds 4, 5, 9, 10, 14, 15, 18 and 19) and scheme of liposome formation.

AIM OF RESEARCH

In order to design stigmasterol-enriched liposomal carriers with higher thermo-oxidative stability for their potential application in the food industry, the first step in this process was to **determine the effect of the new conjugates on the physicochemical properties of the liposomes, such as the order, fluidity and temperature of the main DPPC phase transition (Tm).** In addition, it was checked whether these mixed liposomes would bind to the albumin, the main transporting protein of human blood plasma. For the practical use of liposomes as carriers of active substances their stability and the longest possible circulation time in the bloodstream is very important. A stiffer bilayer and higher Tm results in a slower release of the active compounds from the liposomes and a longer time for their circulation in the bloodstream.

METHODS AND RESULTS

Using two fluorescence probes: Laurdan and DPH located in different areas of the membrane (Fig. 2), the effect of compounds on the order, fluidity and temperature of the DPPC phase transition was determined. Values of GP indicate changes in the packing order in the hydrophilic-hydrophobic interface of the bilayer (Fig. 3) and the values of anisotropy indicates changes in the fluidity in the hydrophobic region of the lipid bilayer (Fig. 4).

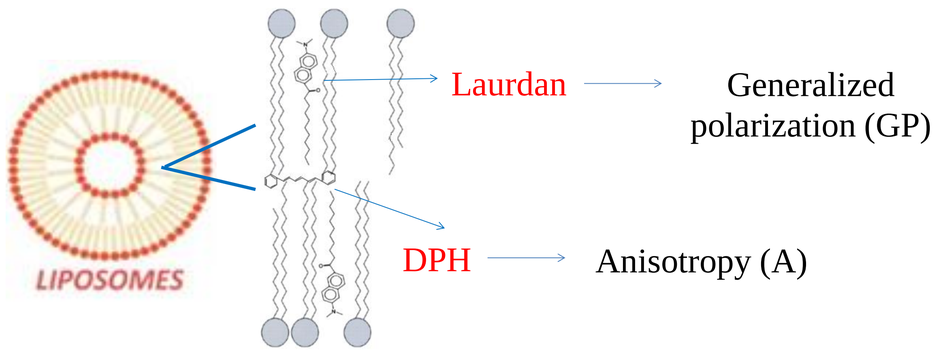

Fig 2. Schematic presentation of the location of two fluorescence probes in the lipid bilayer.

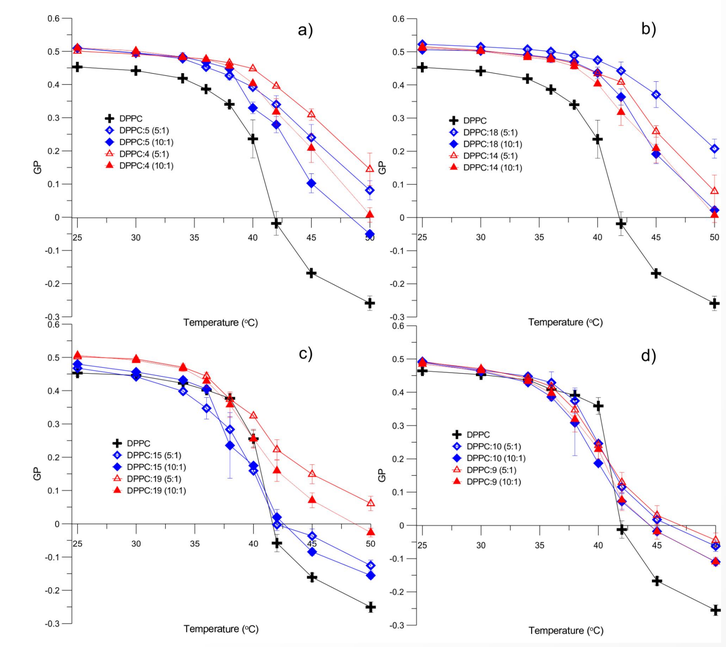

Fig. 3. Values of general polarization (GP) of Laurdan as a function of temperature for the liposomes formed with DPPC (control) and for the liposomes formed with DPPC:acylglycerol at 4, 5, 9, 10, 14, 15, 18 and 19 at 5:1 and 10:1 molar ratios. (The red color was used for compounds with carbonate linker and the blue one for compounds with succinyl linker).

The ability of compounds to bind to human serum albumin (HSA) makes it possible to know the bioavailability, distribution and consequently, the therapeutic efficacy of molecules. We observed that the addition of compounds caused a reduction/quenching of the natural fluorescence of albumin and these changes varied depending on the added compound. The decrease in HSA intensity depending on the added compound can be shown in the following order: HSA >DPPC >18>19>10>15>4>14>5>9.

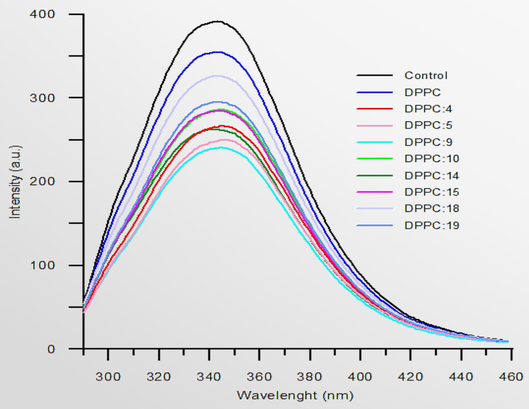

Fig. 5. The effect of the studied compounds at a compound/lipid molar ratio of 1:5 on the fluorescence intensity of HSA at 37 °C

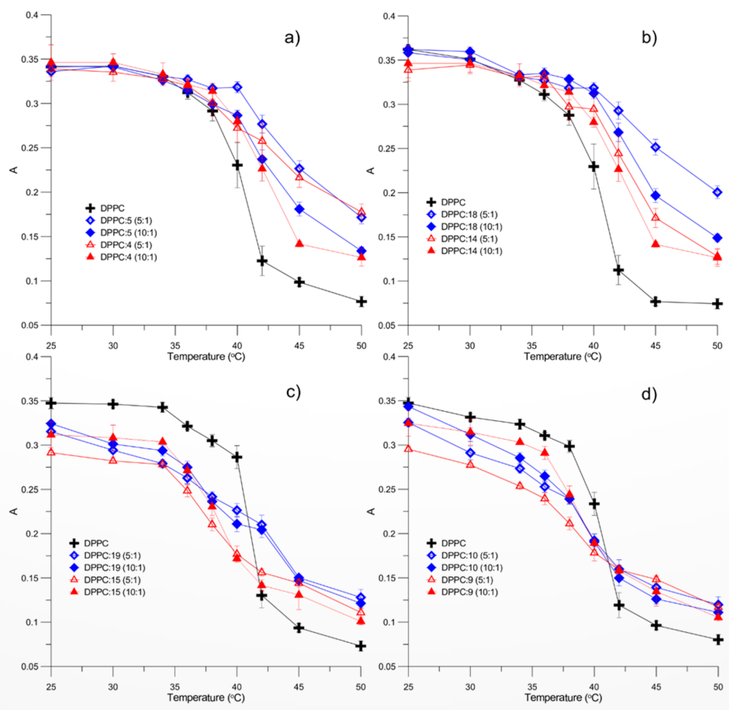

Fig. 4. Values of anisotropy of DPH as a function of temperature for the liposomes formed with DPPC (control) and for the liposomes formed with DPPC:acylglycerol at 4, 5, 9, 10, 14, 15, 18 and 19 at 5:1 and 10:1 molar ratios. (The red color was used for compounds with carbonate linker and the blue one for compounds with succinyl linker).

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CONCLUSIONS

The results indicate that acylglycerols with palmitic acid cause a significant increase in ordering and in stiffness. Moreover, these compounds also shift Tm towards higher values. In contrast, acylglycerols with oleic acid caused a decrease in ordering in the gel phase and an increase in the liquid phase of the lipid bilayer. In addition, an increase in membrane fluidity in the gel phase and a decrease in the liquid phase as well as a shift in Tm towards lower temperatures are observed.

The results allow us to conclude that conjugates with palmitic acid are a better candidate for use as potential stigmasterol nanocarriers for food applications compared to those with oleic acid. The obtained acylglycerol-stigmasterol conjugates included in liposomes can interact with HSA.