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Protocols

Disruption of gel phase lipid packing efficiency by sucralose studied with merocyanine 540

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ABSTRACT

Sucralose, an artificial sweetener, displays very different behavior towards membranes than its synthetic precursor sucrose. The impact of both sugars on model dipalmitoylphosphatidylcholine model membranes was investigated using absorbance and flourescence spectroscopy and the membrane probe merocyanine 540. This probe molecule is highly sensitive to changes in membrane packing, microviscosity and polarity. This work focuses on the impact of sugars on the outer leaflet of unilamellar dipalmitoyl phosphatidylcholine model membranes. The choice of lipid permits access to the gel phase at room temperature and incorporation of the dye after liposome formation allows us to examine the direct impact of the sugar on the outer leaflet while maximizing the response of the dye to changes in the bilayer. The results demonstrate that sucrose has no impact on the packing efficiency of lipids in unilamellar DPPC vesicles in the gel phase. Conversely sucralose decreases the packing efficiency of lipids in the gel phase and results in decreased microviscosity and increased membrane fluidity, which may be as a result of water disruption at the membrane water interface.

1. Introduction

The naturally occurring disaccharide trehalose has been shown to be effective at preserving lyophilized membranes [1–10] through the so called water replacement hypothesis [11]. This discovery led to a range of studies attempting to decipher specific interactions that may occur between sugars and membranes and their impact on biological and model membrane systems [12–17]. These studies discovered that trehalose also decreases solute permeability [18], likely due to a change in the packing of the lipids in the bilayer, as the sugar strongly interacts with the polar headgroup region of lipids. Additional studies on smaller monosaccharies demonstrated that they too were able to penetrate the bilayer to some degree although the interactions with lipids were not as strong [19].

As well as having an impact on the packing efficiency of lipids in bilayers sugars have also been shown to impact the phase behavior of membranes, although the effects vary by sugar and small structural changes between sugars can result in large differences in the way the impact the phase behavior. These studies demonstrate that the mode of interaction between sugars or analogues with membranes is highly complex and difficult to explain. In addition to the concentration of sugar present can greatly alter the types of interactions observed and at low concentrations it is believed that disaccharides strongly interact with bilayers and an event resembling binding may occur, however at higher concentrations these interactions are severely weakened and sugar may be expelled from the surface of the membrane [20].

Despite the interest in these types of interactions they typically focus on naturally occurring sugars and the fundamental interaction between artificial disaccharides and membranes has largely been ignored. The most common artificial sugar consumed is sucralose, which is synthesized by selective chlorination of sucrose and various studies have focused on how this disaccharide is metabolized and its potential impact on the environment, human and animal health [21–32]. Despite concerns few studies have sought to understand the fundamental interactions between this disaccharide and membranes although one study was able to use lipid coated membranes as a sensor for detecting this sugar [33]. This study demonstrated that sucralose may alter dipoles at the membrane water interface and directly impact lipid packing efficiency.

In order to effectively investigate the impact of sucralose on lipid packing in model membrane systems we employed the use of merocyanine 540 (MC 540) an amphipathic dye that is highly sensitive to changes in lipid packing as it is located slightly above the glycerol backbone of the lipid bilayer [34–38]. This dye can

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exist in monomeric and dimeric forms within the bilayer as well as monomers and dimers in the surrounding aqueous environment. It has been shown that the ratio between the monomer and dimer species within the membrane varies as the packing efficiency changes and the dimer is the predominant species in gel phase membranes, whereas the monomer dominates in the liquid crystalline phase [39,40]. Both of these species display unique absorption and emission bands which allow the equilibrium to be closely monitored when contributions from aqueous species of MC 540 are minimized or eliminated. This can be accomplished by using dye to lipid ratios of less than 1:600, where a two state system is observed, corresponding to lipid bound species only [41].

2. Materials and methods

2.1. Chemicals

Lipids were purchased from Avanti Polar Lipids (Alabaster, AL) as lyophilized powder (>99%) and stored at −20 °C. Sucralose (>98%) and merocyanine 540 (99%) were purchased through Fisher Scientific (Pittsburgh, PA). A stock merocyanine solution (0.222 mM) was prepared in chloroform/methanol (50/50) and stored in the dark at −20 °C. Stock sugar, sucrose or sucralose, solutions (44 mM) were prepared in phosphate buffer (pH 7.40).

2.2. Addition of MC540 post liposome formation

Liposome suspensions were prepared by hydrating 1.25 mg of dry lipid powder in 2.5 mL phosphate buffer (pH 7.4) above the transition temperature for one hour with frequent mixing. The suspension was then subjected to three freeze thaw cycles and extrusion through a 100 nm polycarbonate membrane (11 passes). The vesicles were then stored at 4 °C overnight prior to the introduction of dye. In a glass vial the solvent from a 24 μL aliquot of MC 540 stock was removed under a slow stream of nitrogen and the film was dried under vacuum. The vesicle suspension was added to the dry powder for a dye to lipid ratio of ~1:2000 and the sample was mixed for 30 min at room temperature to ensure complete incorporation of dye into liposomes, prior to analysis.

2.3. Absorbance and fluorescence measurements

For both fluorescence and absorbance measurements 2.0 mL of the previously prepared liposome suspension was added to a 1 cm quartz cuvette. Spectra (absorbance or fluorescence) were recorded before addition of sugar. Either sucrose or sucralose was then introduced by adding 6 μL aliquots of stock sugar and mixing the solution for three minutes prior to acquisition of spectra and all spectra were corrected for dilution of dye. Absorbance measurements were made using a Shimadzu UV-2550 double beam spectrophotometer between 400 and 600 nm. Spectra were recorded against a phosphate buffer blank as spectra of the liposome suspension only revealed no obvious scattering or absorption. Fluorescence measurements were made in emission mode (λ_{ex} 495 nm) from 540 to 690 nm on a Photon Technology International QM-4CW spectrofluorimeter. No obvious scattering or emission was seen for the liposome suspension in the absence of dye. For all fluorescence experiments three emission spectra were averaged, and the spectra were identical during acquisition, indicating that osmotic stress is not a factor to be considered when evaluating the changing behavior of the dye.

3. Results

Prior to investigating the impact of sugars on liposome packing the control studies were carried out to investigate the possibility of direct interactions between the dye and sugars used was tested in the absence of liposomes. A solution of dye with the same

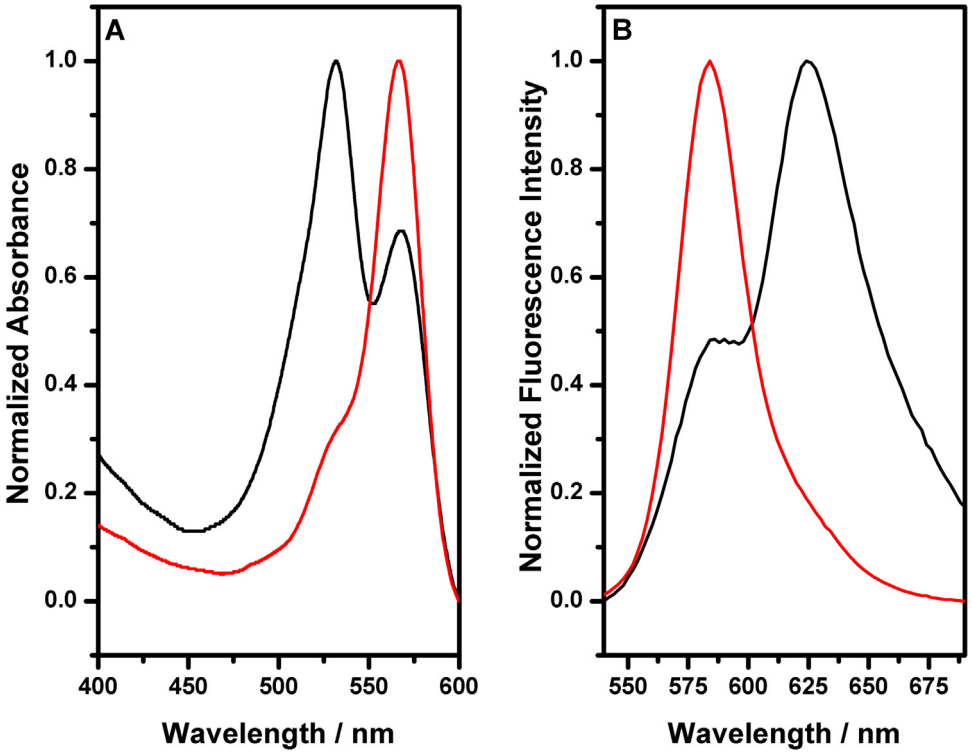

Fig. 1. Normalized absorbance spectra (Panel A) and fluorescence spectra (Panel B) of merocyanine 540 (MC 540) incorporated into liposome membranes are shown for liposomes in the gel phase (black line) and the liquid crystalline phase (red line). In each case the band associated with monomer species of MC 540 increases as membrane fluidity increases. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)