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Differences in the interactions of a monoglyceride with cholesterol and with a bile salt

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ABSTRACT

The differences in the interaction of monoglycerides with bile acids or cholesterol have been investigated from a physico-chemical point of view. A Langmuir trough and fluorescence microscope was used to study mixed monolayers of DL-α-palmitin (a monoglyceride) and cholesterol or deoxycholic acid (a bile acid) at the air/aqueous interface. The surface pressure–area per molecule isotherms of the monolayers were analyzed to give the thermodynamic properties. The deoxycholic acid–DL-α-palmitin monolayer showed stronger repulsions between the film components than was observed with the cholesterol acid–DL-α-palmitin monolayer. Mixed monolayers containing DL-α-palmitin and cholesterol or deoxycholic acid phase separated at high surface pressures and high fractions of DL-α-palmitin, the conditions that resulted in the most repulsions between the two components of the monolayer. The mixed cholesterol and DL-α-palmitin monolayer phase separated in a random pattern. The deoxycholic acid and DL-α-palmitin mixed monolayer gave smaller domains that were distributed in a homogeneous fashion within the monolayer at high molecular packing densities. The difference in the cholesterol and deoxycholic acid interactions with DL-α-palmitin were explained by the fact that while both cholesterol and deoxycholic acid molecules do not pack efficiently with the DL-α-palmitin molecules, the attractive interactions between the alcohol groups on DL-α-palmitin and the carboxylic groups on deoxycholic acid cause attractive interactions between the deoxycholic acid and DL-α-palmitin domains, which causes the interaction abilities of deoxycholic acid with DL-α-palmitin to be higher than cholesterol.

1. Introduction

It is important to understand how fats interact with other substances in a body from a physico-chemical viewpoint, in order to better prevent or cure diseases associated with abnormal fat levels in the body, such as the metabolic syndrome. The fact that bile acids are made from cholesterol and that fats interact with bile acids in a body are reasonably well understood from a biological perspective [1]. However, studies have shown that the absence of bile acids causes an approx. 50% decrease in the absorption of dietary lipids in mice [2]. This result shows that cholesterol cannot efficiently transport fats within the body, in spite of the fact that cholesterol and bile acids have a similar structure. The causes for the difference in the ability of cholesterol or bile acids to interact with fats are not well understood.

The structural difference between cholesterol and bile acids may affect their interactions with fats due to the different possible physical and chemical interactions that occur as a result of the absence or presence of the carboxylic acid terminating side chain. Differences may also occur as a result of the differing orientation and structuring ability of the cholesterol or bile acid at a hydrophobic/hydrophilic interface, due to the increased hydrophilicity and bulkiness of the bile acid compared to the cholesterol as a result of the carboxylic acid side chain.

The cause for the difference in the interaction of cholesterol or bile acids with fats can be better understood by studying the surface properties of cholesterol and bile acid at an air (hydrophobic)/water (hydrophilic) interface, and the change in their structure and interactions in the presence of fats. The ring structure in cholesterol and bile acids give the molecules hydrophobicity. The hydroxyl groups in cholesterol and bile acids and the carboxylic groups in bile acids give the molecules hydrophilicity. The amphiphilicty of these molecules allow the formation of monolayers at an air/water interface. A systematic investigation of the change in the cholesterol or bile acid monolayer properties at an air/water interface upon the addition of fats to the monolayer can provide information about the molecular forces acting between fat, cholesterol, and bile acid molecules. A systematic comparison of the difference in

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these interactions allows us to determine the cause for the different interaction of fats with cholesterol and bile acids.

In this study, we determined how the presence of a carboxylic acid group on a steroid molecule affects its interaction with a fat. Deoxycholic acid was chosen as the bile acid, as the main difference in its structure with cholesterol is the presence of one carboxylic acid terminating side chain and one alcohol terminating side chain. The monoglyceride of DL-α-palmitin was chosen as the fat due to its amphiphilicity and as it has only one tail and one head group that can interact with the cholesterol or deoxycholic acid molecules. The interaction of cholesterol or deoxycholic acid molecules with DL-α-palmitin was studied by using the Langmuir trough to form mixed monolayers of DL-α-palmitin and cholesterol or DL-α-palmitin and deoxycholic acid at the air/aqueous interface. The surface pressure–area per molecule isotherms of the mixed monolayers were analyzed to give the thermodynamic properties of the mixed monolayers, in order to obtain information about the monolayer stability and the intermolecular forces within the monolayer [3–6]. The monolayers were imaged at the air/water interface by using a combined Langmuir trough-fluorescence microscope. This information furthers the understanding as to why fats interact differently with cholesterol and with bile acids.

2. Experimental

2.1. Materials

The materials used were cholesterol ("*Chol*", Wako Special Grade, Wako Pure Chemical Industries, Fig. 1A), deoxycholic acid ("*Deoxy*", ≥99% purity, Sigma, Fig. 1B), DL-α-palmitin ("*Palmitin*", ≥99% purity, Sigma, Fig. 1C), 25-[N-[(7-nitro-2-1,3-benzoxadiazol-4-yl)methyl]amino]-27-norcholesterol (25-NBD Cholesterol, >99% purity, Avanti Polar Lipids, Fig. 1D), chloroform ($CHCl_3$, 99.0%,Wako Pure Chemical Industries), ethanol (EtOH, >99.5% purity, 99.8%, Wako Pure Chemical Industries), and sodium chloride (NaCl, high purity, Wako Pure Chemical Industries). The water from a water purification system (Direct-Q3 UV, Millipore) was used in this experiment and had a resistivity of 18 MΩ cm and a total organic content of <5 ppm.

The solutions to be used in this study were prepared in the following way. Cholesterol was dissolved in chloroform to give a concentration of 1.05 mg/ml. Deoxycholic acid and DL-α-palmitin were dissolved in a mixed solvent of chloroform and ethanol (mixing ratio 9:1) to give a concentration of 0.99 mg/ml. The mixed solutions of cholesterol–DL-α-palmitin ("*Chol–Palmitin*") and deoxycholic acid–DL-α-palmitin ("*Deoxy–Palmitin*") were prepared by mixing cholesterol or deoxycholic acid with DL-α-palmitin to give mixing ratios of 25 vol%, 50 vol%, and 75 vol%. The mixed monolayers are designated X% *Chol–Palmitin* or *Deoxy–Palmitin*, where the X% refers to the percent of *Chol* or *Deoxy* in the mixed monolayer. The *Chol*, *Deoxy*, *Chol–Palmitin* and *Deoxy–Palmitin* solutions that were used in the fluorescence-Langmuir trough studies were prepared by adding the fluorescence probe (25-NBD Cholesterol) to give concentrations <1 mol%. Such low concentrations have been shown to have little or no effect on the observed phase behaviors of the monolayers [7]. The fluorescence probe of 25-NBD Cholesterol has a similar structure to *Chol* and *Deoxy*, and thus highlighted the *Chol* and *Deoxy* phases of the mixed *Chol–Palmitin* or *Deoxy–Palmitin* monolayers. The *Deoxy* saturated aqueous solutions were prepared by firstly adding *Deoxy* to water, dissolving *Deoxy* to saturation by hand-mixing the solution, sonificating for 1 h, and then by leaving the solution overnight. The solution was filtered the next day using filter paper (Whatman 101), in order to remove any undissolved *Deoxy*. The filtered solution was used as the subphase in the 100% *Deoxy* monolayer studies when stated in the manuscript.

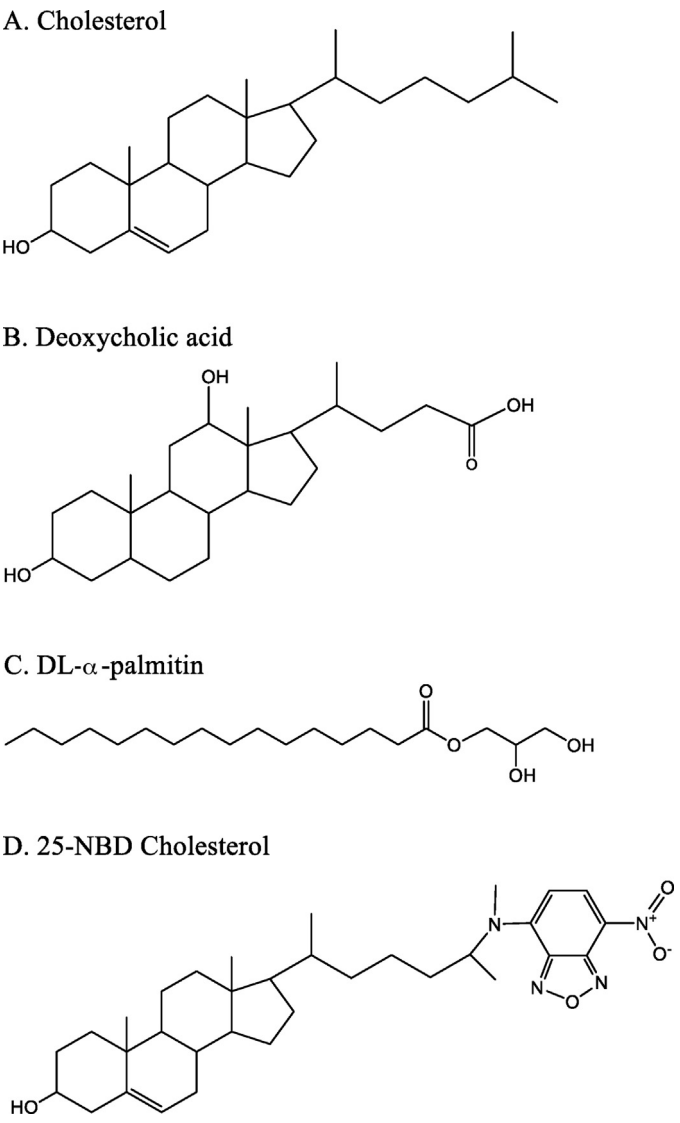

Fig. 1. Structures of cholesterol (A), deoxycholic acid (B), DL-α-palmitin (C), and 25-NBD cholesterol (D).

2.2. Methods

2.2.1. Langmuir trough

A Langmuir trough (Model 302M, Nima Technology Ltd) was used to prepare the monolayers at the air/water interface in this study. The trough was made from poly(tetrafluoroethylene) (PTFE) and was equipped with two PTFE barriers that compressed around the center of the trough (maximum surface area of trough = 290 cm^2). The surface pressure was monitored using a Wilhelmy plate of filter paper (No.2 240 mm, Toyo) that was wet with water [8] and suspended from a strain gauge (Nima PS4 surface pressure sensor, Nima Technology Ltd).

The surface pressure (Π)–area per molecule (*A*) isotherms were acquired by firstly cleaning the Langmuir trough with chloroform and then with ethanol. Water for cleaning was then added to the trough and subsequently removed. The aqueous solution to be used as the subphase (water, 0.1 M NaCl, or the *Deoxy* saturated aqueous solution) was then added to the trough, after which the barriers were compressed to maximum, the subphase surface between the barriers cleaned by suctioning, and the barriers decompressed. The monolayers were formed at the subphase surface by spreading 15 μL of a *Chol*, *Deoxy*, mixed *Chol–Palmitin*, or mixed