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Effect of bilayer charge on lipoprotein lipid exchange

Kathryn Louise Browning[a,b,*], Tania Kjellerup Lind[c], Selma Maric[c], Robert David Barker[d,1], Marité Cárdenas[c,**], Martin Malmsten[a,b]

[a] Department of Pharmacy, Uppsala University, Uppsala, Sweden
[b] Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[c] Department of Biomedical Sciences and Biofilms – Research Centre for Biointerfaces, Malmö University, Malmö, Sweden
[d] Institut Laue-Langevin, Grenoble, France

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ABSTRACT

Lipoproteins play a key role in the onset and development of atherosclerosis, the formation of lipid plaques at blood vessel walls. The plaque formation, as well as subsequent calcification, involves not only endothelial cells but also connective tissue, and is closely related to a wide range of cardiovascular syndromes, that together constitute the number one cause of death in the Western World. High (HDL) and low (LDL) density lipoproteins are of particular interest in relation to atherosclerosis, due to their protective and harmful effects, respectively. In an effort to elucidate the molecular mechanisms underlying this, and to identify factors determining lipid deposition and exchange at lipid membranes, we here employ neutron reflection (NR) and quartz crystal microbalance with dissipation (QCM-D) to study the effect of membrane charge on lipoprotein deposition and lipid exchange. Dimyristoylphosphatidylcholine (DMPC) bilayers containing varying amounts of negatively charged dimyristoylphosphatidylserine (DMPS) were used to vary membrane charge. It was found that the amount of hydrogenous material deposited from either HDL or LDL to the bilayer depends only weakly on membrane charge density. In contrast, increasing membrane charge resulted in an increase in the amount of lipids removed from the supported lipid bilayer, an effect particularly pronounced for LDL. The latter effects are in line with previously reported observations on atherosclerotic plaque prone regions of long-term hyperlipidaemia and type 2 diabetic patients, and may also provide some molecular clues into the relation between oxidative stress and atherosclerosis.

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1. Introduction

Arteriosclerosis and its clinical complications have been identified as the largest cause of mortality this century [1]. Development of atherosclerotic plaques and lesions is thought to be initiated by transfer of cholesterol from low density lipoprotein (LDL), oxidized LDL, lipoprotein(a), and other lipoproteins to the blood vessel wall. This induces foam cell formation and eventually calcification. The latter results in a dramatic stiffening of the blood vessels and an effective reduced diameter, increasing the risk of blockage and/or rupture of the vessel, leading to ischemic heart attack and stroke [2,3].

Lipids are transported to and from peripheral cells by lipoproteins; nanoscopic packages containing primarily cholesterol esters and triglycerides, coated by a monolayer of lipids and apolipoproteins [4]. Lipoproteins are categorised by density and size, and different classes are thought to play important roles in the development of, and protection from, atherosclerosis [4]. Among these, LDL and high density lipoprotein (HDL) are of particular interest to atherosclerosis. LDL particles are larger than HDL and contain higher levels of cholesterol esters. High levels of LDL in the blood are linked to *increased* risk of atherosclerosis [5], whereas high HDL levels are associated with *reduced* atherosclerotic risk [6]. Consequently, LDL (also known as the 'bad cholesterol') levels are currently used as an atherosclerotic risk indicator, although the ratios of LDL to either HDL ('good cholesterol') or total cholesterol content are becoming more prevalent due to HDL's role in reverse cholesterol transport to the liver and the atheroprotective effects of HDL [4].

* Corresponding author at: Department of Pharmacy, University of Copenhagen, Universitetsparken 2, Copenhagen, 2100, Denmark.
** Corresponding author at: Department of Biomedical Sciences and Biofilms, Malmö University, Malmö, 20506, Sweden.
E-mail addresses: kathryn.browning@sund.ku.dk (K.L. Browning), marite.cardenas@mah.se (M. Cárdenas).
[1] School of Physical Sciences, University of Kent, Canterbury, UK.

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Surface sensitive techniques can be employed to investigate the interaction of lipoproteins with (model) cell membranes by supporting them on solid surfaces. We previously used neutron reflection and quartz crystal microbalance with dissipation (QCM-D) to study the interaction of LDL and HDL with synthetic supported lipid bilayers (SLBs) [7–9]. Using deuterated as well as non-deuterated SLBs formed of 90 mol% dimyristoylphosphatidylcholine (DMPC) and 10 mol% dimyristoylphosphatidylserine (DMPS), resulting in a negative charge close to that expected for endothelial cell membranes [10,11]. Both HDL and LDL were shown to remove lipids from the SLB and also to deposit hydrogenous material into the bilayer [9]. However, lipid deposition from lipoprotein to SLB was higher for LDL when compared to HDL, whereas HDL displayed considerably higher lipid removal from the SLB. These results correlate well to the clinically observed 'bad' and 'good' effects of LDL and HDL on atherosclerosis development, respectively.

In the present work, we aim to extend our studies into the molecular mechanisms underlying atherosclerosis by investigating the effect of bilayer charge on the adsorption of lipoproteins and lipid dynamics between the particles and the bilayer, as the literature contains seemingly conflicting data on this. For example, lipoproteins bind extensively to negatively charged surfaces, forming the basis for removal of lipoproteins from bloodstream circulation through apheresis using various polyanionic macromolecules (e.g., heparin, dextran sulfate, and sulfated poly(vinyl alcohol)) [12–17]. Therefore, one would expect lipoprotein binding to increase with increasing negative membrane charge. However, long-term hyperlipidaemia and type 2 diabetic patients display a reduction of anionic groups in endothelial cells in atherosclerotic lesion-prone regions, which could suggest greater interaction of LDL with lower charged membranes [18–20]. Consequently, there is a need to better understand the role of membrane charge on the extent of lipoprotein binding and their dynamics at model cellular membranes.

In addressing the effects of membrane charge density, DMPC/DMPS bilayers were prepared at different molar ratios, and their effect on LDL binding and lipid dynamics was monitored by neutron reflectometry and QCM-D. Morphological changes and phase separation of PC- and PS-lipids perpendicular to the bilayer plane have previously been reported for multilamellar vesicles above 30 mol% PS. Therefore, concentrations of PS up to 25 mol% were used to ensure the formation of homogenous flat bilayers at silicon surfaces [21]. Through this, increasing membrane charge was demonstrated to result in an increase in the amount of lipids removed from the SLB, an effect particularly pronounced for LDL, whilst the amount of hydrogenous material deposited from the lipoprotein to the SLB was found to be largely independent of membrane negative charge.

2. Experimental

2.1. Materials

Tail deuterated DMPC (1,2-dimyristoyl-d54-*sn*-glycero-3-phosphocholine) and DMPS (1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine), as well as their non-deuterated equivalents of >99% purity, were obtained from Avanti Polar Lipids (Alabaster, USA). Ultrapure water (18.2 MΩ cm^{-1}, Millipore) and D_2O (99.9% deuterated, Sigma Aldrich) were used throughout. Tris buffer was prepared by dissolving a buffer tablet (Sigma Aldrich) to obtain buffer solutions of 50 mmol L^{-1} Tris, 150 mmol L^{-1} NaCl, pH 7.6, pre-adjusted for pH in either H_2O or D_2O. Chloroform anhydrous ≥99% and calcium chloride dihydrate (≥99%) were obtained from Sigma Aldrich. All other chemicals used were of analytical grade.

2.2. Preparation of lipoproteins

Lipoproteins were prepared by sequential ultracentrifugation (densities of 1.065 and 1.019 g mL^{-1} for HDL and LDL, respectively) of plasma pooled from three healthy males. The samples were stored in 50% sucrose, 150 mmol L^{-1} NaCl, 24 mmol L^{-1} EDTA, pH 7.4, at −80 °C. Before use, buffer was exchanged to 50 mmol L^{-1} Tris, 150 mmol L^{-1} NaCl, pH 7.4, using a Sephadex G25 PD-10 desalting column (GE Healthcare, Uppsala, Sweden) and further purified by size exclusion chromatography (Superose 6 Increase 10/300 GL column, GE Healthcare, Uppsala, Sweden) at 25 °C. Each fraction was then stored away from light, at 4 °C, under an inert atmosphere. Prior to use the protein concentration was determined by Bradford analysis [22] and the solutions diluted to either 0.132 mg mL^{-1} (HDL) or 0.1 mg mL^{-1} (LDL), concentrations chosen to maintain a constant particle concentration of HDL and LDL, calculated from the protein content per lipoprotein particle [23]. Preliminary experiments showed pronounced changes to the bilayer, induced by the lipoproteins (either through exchange or destruction), so this concentration was chosen to allow studies of lipid removal and deposition effects without risking complete supported bilayer destabilisation.

2.3. Preparation of lipid bilayers

Phospholipid bilayers were deposited on the native oxide of polished silicon blocks (neutron reflection; NR) or silicon dioxide surfaces (QCM-D) by vesicle fusion. In short, lipid films were prepared by dissolving DMPC in chloroform and DMPS in a 2:1 chloroform:methanol solution. Non-deuterated lipids were used for QCM-D measurements and tail-deuterated lipids for NR. The lipids were then mixed in appropriate molar ratios and dried to the walls of clean glass vials by evaporation with nitrogen. The films were further dried under vacuum for 24 h at room temperature. Before use the lipid films, consisting of 100 mol% DMPC:0 mol% DMPS, 90 mol% DMPC:10 mol% DMPS, or 75 mol% DMPC:25 mol% DMPS, were hydrated in H_2O for 1 h at 40 °C in order to be above the phase transition temperature for both dDMPC (19 °C) and dDMPS (31 °C) [24]. Hydrated films were then sonicated before injection using a tip sonicator (Hielscher, Germany) intermittently for 5 min until clarity whilst ensuring the temperature did not rise above 50 °C. Silicon surfaces were first washed with 2 mmol L^{-1} $CaCl_2$ solution before a 1:1 mixture of vesicles and 4 mmol L^{-1} $CaCl_2$ was pumped into the cell to screen the charge and bridge between the negative silicon oxide surface and lipids. All bilayers were allowed to incubate for 20 min before rinsing with 5 mL of 2 mmol L^{-1} $CaCl_2$ solution, 5 mL H_2O and 15 mL Tris buffer before measurement.

2.4. Quartz crystal microbalance with dissipation

Experiments were performed on a Q-Sense E4 quartz crystal microbalance (Q-Sense, Göteborg, Sweden). All experiments were measured at 37 °C in duplicate. Tubing, cells, and o-rings were cleaned first in 2% Hellmanex solution (Sigma Aldrich), rinsed in ultra-pure water and ethanol (99.9%, Sigma Aldrich) before drying under nitrogen. Silicon oxide sensors were cleaned in the same way before UV-Ozone treatment for 10 min (BioForce Procleaner, Bioforce Nanosciences, Salt Lake City, USA), resulting in highly hydrophilic surfaces fully wettable by water (contact angles of less than 10°). Resonance frequencies were obtained in ultrapure water and bilayers formed as described above using a flow rate of 100 μL min^{-1} until stable signals, characteristic for complete bilayers ($\Delta F \approx -25\ s^{-1}$, $\Delta D \approx 0$ a.u.), were established [25]. The bilayers were then washed in Tris buffer at 100 μL min^{-1} for 20 min. Subsequently, 1 mL of either HDL (0.132 mg mL^{-1}, based on protein content) or LDL (0.1 mg mL^{-1} based on protein content)

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