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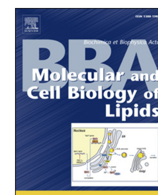

Acylation of lysine residues in human plasma high density lipoprotein increases stability and plasma clearance in vivo

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ABSTRACT

Although human plasma high density lipoproteins (HDL) concentrations negatively correlate with atherosclerotic cardiovascular disease, underlying mechanisms are unknown. Thus, there is continued interest in HDL structure and functionality. Numerous plasma factors disrupt HDL structure while inducing the release of lipid free apolipoprotein (apo) AI. Given that HDL is an unstable particle residing in a kinetic trap, we tested whether HDL could be stabilized by acylation with acetyl and hexanoyl anhydrides, giving AcHDL and HexHDL respectively. Lysine analysis with fluorescamine showed that AcHDL and HexHDL respectively contained 11 acetyl and 19 hexanoyl groups. Tests with biological and physicochemical perturbants showed that HexHDL was more stable than HDL to perturbant-induced lipid free apo AI formation. Like the reaction of streptococcal serum opacity factor against HDL, the interaction of HDL with its receptor, scavenger receptor class B member 1 (SR-B1), removes CE from HDL. Thus, we tested and validated the hypothesis that selective uptake of HexHDL-[^{3}H]CE by Chinese Hamster Ovary cells expressing SR-B1 is less than that of HDL-[^{3}H]CE; thus, selective SR-B1 uptake of HDL-CE depends on HDL instability. However, in mice, plasma clearance, hepatic uptake and sterol secretion into bile were faster from HexHDL-[^{3}H]CE than from HDL-[^{3}H]CE. Collectively, our data show that acylation increases HDL stability and that the reaction of plasma factors with HDL and SR-B1-mediated uptake are reduced by increased HDL stability. In vivo data suggest that HexHDL promotes charge-dependent reverse cholesterol transport, by a mechanism that increases hepatic sterol uptake via non SR-B1 receptors, thereby increasing bile acid output.

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1. Introduction

1.1. Atherosclerosis

Atherosclerosis, which is characterized by the accumulation of lipids, mostly free and esterified cholesterol, in the subendothelial space of the arterial wall, can be ischemic, ultimately restricting blood flow, which leads to heart attacks and strokes. Reverse cholesterol transport (RCT), which reduces the burden of cholesterol in the arterial wall, comprises cholesterol efflux from arterial wall macrophages to apolipoprotein (apo) AI or high density lipoproteins (HDL), esterification in plasma, and selective lipid uptake by hepatic scavenger receptor class B member 1 (SR-B1) [1]. Following hepatic uptake some cholesterol is converted to bile acids and ultimately excreted in the feces. HDL is central to RCT and although plasma HDL-cholesterol (HDL-C) concentration is a negative risk factor for atherosclerotic cardiovascular disease, most attempts to raise plasma HDL-C in a cardioprotective way have failed [2–5]. Several studies have shown that not all HDL are functionally equivalent and that one function of HDL, accepting cellular cholesterol, correlates better with reduced cardiovascular diseases (CVD) than HDL-C levels [6–8].

Abbreviations: AcLDL, acetyl LDL; apo, apolipoprotein; BSA, bovine serum albumin; CVD, cardiovascular disease; CE, cholesteryl ester; CERM, cholesteryl ester-rich microemulsion; CETP, cholesteryl ester transfer protein; FC, free cholesterol; GdmCl, guanidinium chloride; HDL, high density lipoproteins; HDL-C, HDL-cholesterol; HPTLC, high-performance thin layer chromatography; LCAT, lecithin:cholesterol acyltransferase; LF apo AI, lipid-free apolipoprotein AI; LDL, low density lipoprotein; CHO ldlA7, LDL-receptor null Chinese Hamster Ovary cells; CHO SR-B1, LDL-receptor null Chinese Hamster Ovary cells overexpressing SR-B1; PBS, Phosphate-buffered saline; PLTP, phospholipid transfer protein; PAGE, polyacrylamide gradient gel electrophoresis; RCT, reverse cholesterol transport; SR-B1, scavenger receptor class B member 1; SEC, size exclusion chromatography; SOF, streptococcal serum opacity factor; TBS, Tris-buffered saline.

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There is also a debate about the raising-HDL-C-is-better hypothesis and the regulatory mechanisms by which HDL-C is delivered to the liver (Reviewed) [9]. In mice, overexpression of SR-B1 in the liver reduces plasma HDL-C levels and is atheroprotective [10–13], whereas genetic deletion of SR-B1 in mice increases plasma HDL-C levels, but not in an atheroprotective [14–16]. As a consequence, there is continuing interest in HDL functionality that correlates with its cardioprotective properties, which may include faster clearance from plasma.

1.2. HDL stability

HDL stability is measured according to its dose-dependent resistance to changes in structure in response to a perturbation, which may be physico-chemical or biological. Using these criteria, others showed that HDL resides in a kinetic trap from which it escapes in response to chaotropic and thermal perturbations [17]. HDL structure is also readily disrupted by several plasma factors—lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester and phospholipid transfer proteins (CETP, PLTP), endothelial and hepatic lipases, and Streptococcal serum opacity factor (SOF) [18–23]. Concomitant with their disruptive activities, many of these factors, especially SOF, release lipid-free (LF) apo AI into the surrounding aqueous phase. Thus, HDL instability and apo AI exchangeability are intrinsic to HDL functionality. Given that apo AI but not other HDL apos nor low density lipoprotein-apo B-100, is labile and readily released, we hypothesized that apo AI exchangeability is necessary or at least permitting for HDL functionality. We tested this hypothesis with an HDL particle containing apo AI modified in a way that increases its hydrophobicity thereby suppressing its exchangeability and release to the aqueous phase, and its negative charge, which may divert HDL to alternative receptors. Acylated HDL stability and function were tested by chemical and biological perturbation with guanidinium chloride (GdmCl), SOF and PLTP. Functionality was tested both in vitro as cellular cholesteryl ester (CE) uptake, and in vivo by plasma clearance, tissue uptake and CE metabolism. Earlier studies of acetylated low density lipoprotein (AcLDL) identified the LDL scavenger receptor on macrophages and other scavenger cells [24,25]. SR-B1 is a promiscuous receptor that binds ligands other than HDL—AcLDL, OxLDL, apoptotic cells, and unmodified LDL and VLDL, maleylated serum albumin, and anionic phospholipids (Reviewed) [26]. Our studies probed the interaction of Acylated HDL with this receptor but also imply the existence of one or more receptors comparable to the LDL scavenger receptor that promotes in vivo plasma clearance and liver uptake of acylated HDL, which is more negatively charged than native HDL.

2. Materials and methods

2.1. Acylation of HDL

HDL was isolated by flotation between d = 1.063 and 1.21 g/mL and further purified by size exclusion chromatography (SEC) over two 30 cm × 1 cm Superose HR6 columns in tandem with a flow rate of 0.5 mL/min [27]. This SEC system was also used for analysis of the HDLs after treatment with GdmCl, SOF and PLTP. Reproducibility of peak elution volumes for this system is SD = ±25 μL. HDL was acylated with acetic or hexanoic anhydrides with minor modifications as described [24]. Briefly, 1–2 μL aliquots were added at 30 min intervals for a total of acetic anhydride 0.744 mg (100 mg/mL in ethanol) and hexanoic anhydride 1.56 mg (100 mg/mL in ethanol) with mixing to HDL (2.0 mg/mL in 2.9 mL) at an anhydride/lysine molar ratio of 1.6:1. Incubations were continued overnight at room temperature (≥18 h). Addition of 20% more anhydride precipitated HDL. Commercial kits were used to determine the protein (DC protein assay, Bio-Rad, Hercules, CA) and lipid composition (total and free cholesterol, WAKO Life Sciences, Richmond, VA) of lipoproteins.

2.2. HDL particle analysis

The HDL, AcHDL and HexHDL were analyzed by electrophoresis in 0.78% and 1% agarose gels (90 mM Tris, 80 mM borate, pH 8.2). Lipoprotein samples (5–13 μg of protein in 30 μL) were loaded onto gels, and electrophoresed at 4 °C at 90 V for 3 h, as described [28]. The HDLs were also analyzed by nondenaturing polyacrylamide gradient gel electrophoresis (PAGE) (Life Technologies, Novex, 4–20% Tris-Glycine Gel). Lipoproteins (6–19 μg of protein in 20 μL) were loaded onto gels, and electrophoresed at 4 °C at 125 V for 2.5 h.

2.3. HDL acylation analysis

Fluorescamine was used to determine the extent of lysine acylation [29,30]. A series of doses of HDL, AcHDL, and HexHDL between 0 and 500 μg/mL in PBS were made; 150 μL aliquots of samples and 50 μL of 10.8 mM (3 mg/mL) fluorescamine (Sigma-Aldrich) dissolved in acetone were added to each well of 96-well black microplate with clear bottom (Costar). After the plate was shaken for 1 min, the fluorescence was determined using a UV–Vis fluorescence and absorbance plate reader (TECAN, SAFIRE II) with a 400 nm, 20 nm bandwidth, excitation filter and a 460 nm, 20 nm bandwidth emission filter [29,30]. The fluorescence vs. protein concentration curves were fitted to a first order polynomial in SigmaPlot and the number of free lysines in each HDL was calculated as the slope. The number acylated lysines was calculated as the difference between the number of lysines per particle (nonacylated HDL) and the number per particle labeled with fluorescamine.

2.4. Chaotropic perturbation

Stability of HDL, AcHDL and HexHDL was compared by chaotropic perturbation with GdmCl [17]. Various aliquots of Tris-buffered saline (TBS) and 9 M GdmCl were combined with HDL, AcHDL, and HexHDL (0.27 mg/mL in 3 mL). After 18 h, each reaction mixture was analyzed by SEC, which revealed shifts in the peaks for HDL, and the appearance of new peaks in the void volume and at an elution volume of ~34 mL, corresponding to fusion products and LF apo AI respectively [31]. The shift in the elution volume of the main HDL peak and the appearance of LF apo AI were correlated with the GdmCl concentration.

2.5. SOF kinetics

SOF was prepared as described [32]. The kinetics of the SOF reaction with HDL and acylated HDL were determined by right angle light scattering in real time and according to the distribution of reaction products by SEC as described [23,33]. Briefly, HDL (0.27 mg/mL in 3 mL) was placed in a fluorescence cuvette, which was transferred to the cell compartment of an Aviv spectrofluorometer. After equilibration to 37 °C, SOF (2.8 μg/2.5 μL) was added with constant stirring and the light scattering intensity (325 nm) monitored at 90° to the incident light. The light scattering intensity vs. time data were fitted to a two-parameter growing exponential function in SigmaPlot.

2.6. PLTP reactivity of HDL and acyl HDL

The disruptive effects of PLTP on HDL structure [34,35] were also used to assess HDL stability. A His-tagged PLTP, prepared according to Albers [36], was a gift from Xian-cheng Jiang, Ph.D. (SUNY Downstate Medical Center). HDL and acylated HDL (0.27 mg/mL) and rPLTP (4 μg/mL) were incubated at 37 °C overnight with stirring. Aliquots of the HDL and acyl HDL were then analyzed by SEC.

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