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Effect of phospholipid composition on discoidal HDL formation

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ABSTRACT

Discoidal high-density lipoprotein (HDL) particles are known to fractionalize into several discrete populations. Factors regulating their size are, however, less understood. To reveal the effect of lipid composition on their formation and characteristics, we prepared several reconstituted HDLs (rHDLs) with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), and sphingomyelin at phospholipid to apolipoprotein A-I ratios of 100 and 25. When reconstitution was conducted at 37 °C, the efficiency of rHDL formation from POPC was decreased as compared with that conducted at 4 °C. Moreover, large rHDLs with a Stokes diameter of 9.6 nm became dominant over small rHDL with a diameter of 7.9 nm, which was distinctly observed at 4 °C. The aminophospholipids POPS and POPE promoted the formation of small rHDLs at 37 °C, but fluorescence experiments revealed that they did so in a different fashion: Fluorescence lifetime data suggested that the head group of POPS reduces hydrophobic hydration, especially in small rHDLs, suggesting that this lipid stabilizes the saddle-shaped bilayer structure in small rHDLs. Fluorescence lifetime and anisotropy data showed that incorporation of POPE increases acyl chain order and water penetration into the head group region in large rHDLs, suggesting that POPE destabilizes the planar bilayer structure. These results imply that these aminophospholipids contribute to the formation of small rHDLs under biological conditions.

1. Introduction

In high-density lipoprotein (HDL) biogenesis, apolipoprotein A-I (apoA-I) interacts with transmembrane ATP-binding cassette transporter ABCA1 [1–4]. This reaction brings about the formation of discoidal nanoparticles, in which apoA-I molecules wrap around the edges of the lipid bilayer. HDLs show discrete size distribution in plasma [5]. Discoidal HDLs are transformed into a spherical form through cholesterol esterification by lecithin:cholesterol acyltransferase (LCAT) [6,7]. Mature spherical HDLs accept more cholesterol from peripheral tissues and transport it into the liver for recycling and secretion into the bile [8]. Reverse cholesterol transport is, thus, believed to play a crucial role in cholesterol homeostasis and protection against arteriosclerosis.

Similar discoidal nanoparticles can be reconstituted with phospholipids (PLs), cholesterol, and apoA-I in vitro [9–11]. Physicochemical and biological studies with reconstituted HDLs (rHDLs) have revealed that efficiencies of cellular cholesterol efflux [12] and activation of LCAT [13,14] increase with the increase in size of rHDLs. Favari et al. have revealed that rHDLs with mean Stokes diameters (d_S) of 7.8 nm promote both ABCA1-mediated cellular cholesterol efflux and ABCG1-mediated cellular cholesterol efflux, while larger 9.6-nm rHDLs can be acceptors for only ABCG1-mediated cholesterol efflux but not ABCA1-mediated cholesterol efflux [15]. Toledo et al. have recently shown that 7.8-nm rHDLs are active to remove PLs from CHO cells compared to 9.6-nm rHDLs [16]. These results imply that the structural difference of rHDLs modifies interaction with cells and thereby determines their metabolic fate. Therefore, it is important to elucidate the structural differences among rHDLs with various dimensions and factors that regulate their size.

In our previous study, the structure of rHDLs consisting of apoA-I and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was investigated using 2 fluorescence techniques, i.e., fluorescence lifetime of dansyl phosphatidylethanolamine (dansyl PE) and excimer formation of 1,2-bis(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine (C_{10}dipyPC) [17]. The former is sensitive to the change of head group packing and the latter correlates to the lateral pressure in the acyl chain region. These approaches have demonstrated that small rHDLs with d_S of 7.9 and 9.0 nm have a curved lipid bilayer that forms a saddle surface, while large (9.6 nm) rHDLs have a planar bilayer.

Abbreviations: apoA-I, apolipoprotein A-I; C_{10}dipyPC, 1,2-bis(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine; dansyl PE, dansyl phosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; LPR, lipid-to-protein ratio; PL, phospholipid; rHDL, reconstituted HDL; Rho-DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(Lissamine Rhodamine B Sulfonyl)

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Phosphatidylserine (PS), phosphatidylethanolamine (PE), and sphingomyelin (SM) are major components of the plasma membrane as well as phosphatidylcholine (PC), and they are components of HDLs [18]. To reveal the effect of these lipids on the formation of discoidal HDLs, we prepared rHDLs with PC, PS, PE, and SM, and evaluated their size distribution and lipid-packing state by gel filtration chromatography and fluorescence methods, respectively. The results of these studies provide information on the physicochemical roles of the individual lipids. In particular, the finding that small rHDLs are stabilized in the presence of PE with negative spontaneous curvature reconfirms the saddle-shaped bilayer structure of these particles.

2. Experimental procedures

2.1. Materials

POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), egg yolk SM, and 1,6-diphenyl-1,3,5-hexatriene (DPH) were from Sigma-Aldrich (St. Louis, MO, USA). 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(Lissamine Rhodamine B Sulfonyl) (Rho-DOPE) and dansyl PE were from Avanti Polar Lipids (Alabaster, AL, USA). C_{10}dipyPC was from Invitrogen (Eugene, OR, USA). ApoA-I was isolated from pig plasma using procedures described previously [17]. The purity of apoA-I, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, was greater than 95%. All other chemicals were of the highest reagent grade.

2.2. Sample preparation

All rHDL particles were prepared by the sodium cholate dialysis method [11,19]. The required amounts of a chloroform–methanol solution of POPC, POPS, POPE, and SM were mixed in a round-bottomed glass flask. For samples labeled with a fluorescence probe, a chloroform–methanol solution of the probe was mixed with a lipid solution to yield 0.1 mol% (for Rho-DOPE or C_{10}dipyPC) or 0.2 mol% (for dansyl PE) of total lipids. After the organic solvent was removed by using an evaporator, the sample was dried overnight under vacuum. The dried mixture was dispersed in Tris-buffered saline (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.01 g/mL NaN_3, pH 7.4). Sodium deoxycholate was added at a molar ratio of lipid:deoxycholate of 1:2, and then the mixture was vortexed and incubated until clear. ApoA-I was added to the lipid mixture at a lipid-to-protein ratio (LPR) of 100 or 25 (mol/mol). After overnight incubation, the mixture was dialyzed for 2 days against Tris-buffered saline to remove deoxycholate.

2.3. Gel filtration chromatography

The rHDL samples prepared with 0.1 mol% Rho-DOPE were analyzed by gel filtration chromatography on a Superdex 200 column (GE Healthcare UK Ltd., Buckinghamshire, UK) eluted at room temperature with Tris-buffered saline at a flow rate of 0.4 mL/min. The elution profiles were monitored with a Hitachi F-2500 fluorescence spectrometer (Tokyo, Japan), which enabled the simultaneous detection of both fluorescence signals. Fluorescence from rhodamine was detected with excitation/emission wavelengths of 550 nm/590 nm, and tryptophan fluorescence was detected at 285 nm/350 nm. Elusion profiles were expressed as a function of K_{av}, which is given by the following equation:

$$K_{av} = (V_e - V_o)/(V_t - V_o) \tag{1}$$

where V_o is the void volume, V_t is the total column volume, and V_e is the elution volume. The Stokes diameter (d_S) of rHDLs was determined as described previously [17].

2.4. Fluorescence measurements

For structural evaluation of the lipid bilayer, rHDL samples containing 0.1 mol% C_{10}dipyPC were reconstituted at a temperature close to the phase transition temperature of membranes (i.e., 4 °C for POPC and POPC/POPS, 16 °C for POPC/POPE, or 28 °C for POPC/SM) and applied to the column for gel filtration chromatography. The elution profiles were monitored at room temperature with dual fluorescence from C_{10}dipyPC at 378 nm (monomer) and 478 nm (excimer) with excitation at 345 nm on the Hitachi F-2500 to determine the excimer-to-monomer fluorescence intensity ratio (I_e/I_m) of each subclass.

For structural evaluation of the lipid bilayer, rHDLs containing 0.2 mol% dansyl PE were reconstituted at an LPR of 100 and 25 and fractionated into each subclass by gel filtration chromatography. The fluorescence lifetime of dansyl PE in these samples was measured with a HORIBA NAES-550 Nanosecond Fluorometer (Kyoto, Japan) with a pulsed hydrogen lamp (full width at half-maximum: ~2 ns). The samples were excited through a HOYA U350 filter, and their fluorescence signal was detected through a HOYA Y48 filter at 25 °C. The mean fluorescence lifetime, $\langle\tau\rangle$, was determined as described previously [17].

Time-resolved fluorescence anisotropy of DPH was used to evaluate the order of lipid bilayers in rHDLs with different lipid compositions. To the rHDL preparation that was fractionated into each subclass by gel filtration chromatography, a small amount of a methanol solution of DPH was added and incubated at 25 °C for 1 h to incorporate 0.5 mol% DPH into the particles. The fluorescence was measured on the HORIBA NAES-550. Pulsed excitation light passing through a polarizing prism, HOYA U360 filter, and UV-34 filter excited the samples and their fluorescence was detected through a polarizing film, $CuSO_4$ solution (250 mg/mL), and HOYA L42 filter at 25 °C. The fluorescence polarization $r(t)$ was calculated as

$$r(t) = \frac{I_{VV}(t) - GI_{VH}(t)}{I_{VV}(t) + 2GI_{VH}(t)} \tag{2}$$

where I_{VV} and I_{VH} are the intensities of vertically and horizontally polarized fluorescent lights, respectively, when excitation light is vertically polarized. G represents the compensating factor for the anisotropy sensitivity of the instrument, which is expressed as follows:

$$G = \int_0^\infty I_{HV}(t)dt \Big/ \int_0^\infty I_{HH}(t)dt \tag{3}$$

where I_{HV} and I_{HH} represent the intensities of vertically and horizontally polarized lights, respectively, when excitation light is horizontally polarized. DPH wobbles within lipid bilayers and, thus, $r(t)$ can be expressed as the following equation [20]:

$$r(t) = (r_0 - r_\infty)\exp(-t/\phi) + r_\infty \tag{4}$$

where ϕ is the rotational correlation time, r_0 is the initial anisotropy at $t = 0$, and r_∞ is the residual anisotropy at infinite time. The value of r_0 was assumed as 0.395 according to Kawato et al. [21]. An apparent fluorescence anisotropy decay $r(t)$, which is obtained experimentally from Eq. (2), convolutes the intensity profile of pulsed excitation light; however, r_∞ can be directly identified by averaging the values of $r(t)$ for a longer time region, where the intensity profile of the light source no longer affects the anisotropy decay. The order parameter S is given by the equation.

$$S = (r_\infty/r_0)^{1/2} \tag{5}$$