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Cholesteryl ester diffusion, location and self-association constraints determine CETP activity with discoidal HDL: Excimer probe study

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ABSTRACT

The transfer of cholesteryl ester by recombinant cholesteryl ester transfer protein (CETP) between reconstituted discoidal high-density lipoprotein (rHDL) was studied. Particles contained apolipoprotein A-I, unsaturated POPC or saturated DPPC and cholesteryl ester as cholesteryl 1-pyrenedecanoate (CPD) or cholesteryl laurate (CL) in donor and acceptor rHDL, respectively. Probe dynamics fulfilled the quenching sphere-of-action model. The cholesteryl ester exchange between donor and acceptor particles was characterized by a heterogeneous kinetics; the fast exchanging CPD pool was much higher in a case of POPC compared to DPPC complexes. Probe fraction accessible to CETP increased with temperature, suggesting a more homogeneous probe distribution. Noncompetitive inhibition of probe transfer by acceptor particles was observed. The values of V_{max} (0.063 μM min^{-1}) and catalytic rate constant k_{cat} (0.42 s^{-1}) together with a similarity of K_m (0.9 μM CPD) and K_i (2.8 μM CL) values for POPC-containing rHDL suggest the efficient cholesteryl ester transfer between nascent HDL with unsaturated phosphatidylcholine *in vivo*. The phospholipid matrix in discoidal HDL may underlie CETP activity through the self-association, diffusivity and location of cholesteryl ester in the bilayer, the accessibility of cholesteryl ester to cholesterol-binding site in apoA-I structure and the binding of cholesteryl ester, positionable by apoA-I, to CETP.

Introduction

The importance of cholesteryl ester transfer protein (CETP[1]) structure and function is determined by (1) the atheroprotective role of high density lipoproteins (HDL) in the development of coronary heart disease [1]; (2) the large effect of CETP gene polymorphism on the variance of HDL level [2]; and (3) the role of HDL in reverse cholesterol transport (RCT) from macrophages in the artery wall to the liver [3]. A decrease in CETP activity diminishes the transfer of cholesteryl ester from HDL to lower density lipoproteins – low density lipoproteins (LDL) and very low density lipoproteins (VLDL) – and elevates HDL levels [4]. The attractive idea of using CETP inhibitors to reduce cardiovascular risk is based on this elevation of HDL levels, the switch of the major cholesteryl ester reservoir in the RCT pathway from LDL and VLDL to HDL together with the overall constancy of RCT efficiency and remaining HDL functionality [5]. However, the last postulate has been questioned [6] and the downside effect of CETP inhibition has been attributed to decreased generation of small pre-beta HDL, which are the preferred acceptors of cell cholesterol and promote RCT [1].

The crystal structure of CETP [7] reveals that the protein contains two barrels at the N- and C-ends separated by central β-sheet and C-terminal helix proposed to be involved in lipoprotein binding. This structure is postulated to form a continuous tunnel for cholesteryl ester and triglyceride transfer with a total capacity of two neutral lipids within the tunnel and two phospholipid molecules plugging it at both ends. The concave surface of the CETP molecule can adopt a curvature of lipoprotein particle of 10 nm diameter; however, a recent electron microscopy study [8] largely assumes HDL binding directionality for CETP rather than a concave-convex surface alignment. Moreover, atomistic and coarse-grained simulations of CETP–HDL interaction [9] are inconsistent with deep penetration of the CETP N-terminal domain into the HDL particle core, as suggested by electron microscopy. Despite the availability of the CETP crystal structure, little is known about the details of CETP interaction with lipoproteins and mechanisms of neutral lipid transfer between lipoproteins [8]. Indeed, two basic non-coincident kinetic models include the formation of binary

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[1] *Abbreviations used*: CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; PC, phosphatidylcholine; PLPC, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; RCT, reverse cholesterol transport; rHDL, recombinant discoidal high density lipoproteins; VLDL, very low density lipoproteins.

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CETP–lipoprotein [10] (carrier hypothesis) or ternary donor–CETP–acceptor [11] (tunnel hypothesis) complexes. In the latter case, the concomitant twisting of two CETP domains with tunnel opening may be a prerequisite for lipid transfer between small HDL and large LDL and VLDL particles [12]. Moreover, the possible contribution of nascent discoidal HDL, with a limited number of cholesteryl ester molecules in phosphatidylcholine bilayer and originated from a very efficient lecithin:cholesterol acyltransferase reaction with this substrate [13,14], into cholesteryl ester exchange in plasma, yet undescribed, may influence a total RCT efficiency. Recently the heterogeneous distribution of cholesteryl ester molecules in recombinant discoidal high density lipoproteins (rHDL) has been uncovered by us [15]. The fluorescent probe cholesteryl 1-pyrenedecanoate (CPD) that forms in a diffusion- and concentration-dependent manner short-lived dimer of unexcited and excited molecules (excimer) was applied to measure CPD distribution in rHDL bilayer and the distance between pyrene moiety and apoA-I tryptophan residues. In the present study, we rationalized the dynamic probe properties to describe quantitatively cholesteryl ester exchange by recombinant CETP between donor HDL with probe molecules and acceptor HDL with cholesteryl laurate. The application of quenching sphere-of-action model to describe diffusion-related events permitted to obtain the kinetic parameters and construct a model of efficient CETP-catalyzed cholesteryl ester exchange between discoidal rHDL, the event not described yet but which seems to underlie significantly cholesteryl ester metabolic flow contributing in turn into reverse cholesterol transport.

Experimental

Materials

Phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were obtained from Avanti Polar Lipids (Alabaster, AL, USA), fluorescent probe cholesteryl 1-pyrenedecanoate (CPD) was purchased from Molecular Probes (Eugene, OR, USA), unlabeled cholesteryl laurate (CL) were obtained from Sigma (St. Louis, MO, USA). HDL were isolated from normolipidemic human plasma by preparative ultracentrifugation [16]. Apolipoprotein A-I was isolated from delipidated HDL by ion-exchange chromatography in denaturing conditions. ApoA-I purity was checked by SDS–PAGE in 8–25% precast gel (Pharmacia LKB Phast System, Sweden) or in a 5–20% gel prepared in the laboratory. Generally, the purity was greater than 95%. Recombinant CETP preparation was kindly donated by Dr. A. Tall (Columbia University, New York, USA) and stored unfrozen at −20 °C in potassium phosphate buffer (100 mM) at pH 7.4 with 50% glycerol.

Preparation and characterization of recombinant HDL

Complexes of apoA-I with phosphatidylcholine and fluorescent cholesteryl ester or unlabeled cholesteryl laurate were prepared in 0.01 M tris–HCl, pH 8.0, 0.15 M NaCl, 1 mM NaN_3, 0.01% EDTA, 25 μM phenylmethanesulfonyl fluoride (buffer *A*); complex isolation by gel-filtration was done in 50 mM tris–HCl, pH 8.0, 0.15 M NaCl, 0.02% NaN_3. The experimental procedures were described in details by us previously [15]. Briefly, phosphatidylcholine and cholesteryl ester were mixed in $CHCl_3$ and dried. The lipid film was solubilized in 7.5 mM sodium cholate. The initial probe or CL to PC ratio varied. After complete solubilization, apolipoprotein was added at PC:apoA-I weight ratio 2.5:1 (3:1 ratio was used for complexes as CETP substrate), the mixture was incubated at 42 °C (DPPC) or 23 °C (POPC) overnight and further diluted 4-fold with buffer *A* to reduce cholate concentration to 1.875 mM, lower than critical micelle concentration. Detergent was removed by double incubation with hydrophobic sorbent BioBeads SM-2 (Bio Rad, USA) at 30 mg of hydrated particles/ml for 3 h at the incubation temperatures as above with constant stirring followed by low-speed centrifugation to sediment Bio-Beads. The efficiency of cholate removal was greater than 99.5% as estimated from a control experiment by inclusion of a tracer quantity of [^{3}H] cholic acid into the initial mixture. Eighteen individual donor complexes with different CPD:phospholipid ratio (eight with DPPC and ten with POPC) and three acceptor complexes with different CL:phospholipid ratio (two with DPPC and one with POPC) were additionally separated by size-exclusion chromatography. The separations were performed at 23 °C on Superose 6PG column (1.0 × 48.5 cm) with simultaneous measurements of optical density at 280 nm and tryptophan emission at 330 nm for complexes with CL or probe monomer (398 nm) or excimer (475 nm) fluorescence for complexes with probe. To eliminate a nonspecific sorption of the material by a new column matrix, the column was saturated with liposomes until the coincidence of the elution profiles and recoveries of the complexes between different runs. Generally, PC and probe recoveries were 63–64% and up to 50%, respectively and no free PC or apolipoprotein were detected in elution profiles. The relative homogeneity of the complexes was estimated by comparison of PC and probe total content in fractions left and right to elution maximum. If required, fractions were combined and concentrated with Centricon 30 or 100 membranes (Millipore Corporation, Billerica, MA, USA) by low-speed centrifugation. Unfractionated complexes were also used when indicated. The chemical composition of the individual or combined fractions and unfractionated complexes was assayed as follows: DPPC and POPC and cholesteryl laurate as cholesterol were measured with kits from Biomerieux (Marcy-l'Etoile, France) and Boehringer (Mannheim, Germany), respectively, apoA-I content was determined as described [17] or by absorbance at 280 nm with weight extinction coefficient 1.15 ml/mg cm [18] and M_r value 28,082, probe content was determined with the molar extinction coefficient at 345 nm 38,000 M^{-1} cm^{-1} in chloroform.

Excimer formation in rHDL

The registration of CETP activity in donor–acceptor mixture of discoidal recombinant HDL is based on the dependence of excimerization efficiency of fluorescent probe cholesteryl ester 1-pyrenedecanoate (CPD) that forms in a diffusion- and concentration-dependent manner short-lived dimer of unexcited and excited molecules (excimer) on probe concentration in the lipid phase. Excimer formation is resulted in the quenching of monomer fluorescence and the ignition of excimer fluorescence at longer wavelengths. For isotropic media the ratio of fluorescence intensities of excimer and monomer fluorescence at 475 and 398 nm, respectively, linearly depends on probe concentration *C* [19] (Eq. (1)):

$$f = \frac{I_{475}}{I_{398}} = \frac{k_{fd}k_{dm}}{k_{fm}(k_d + k_{md})}C \quad (1)$$

where k_{fd} and k_{fm} are the emission rates of excimer and monomer, k_{dm} is the excimer formation constant, k_d is the sum of k_{fd} and radiationless deactivation rate constant and k_{md} is the excimer dissociation rate constant. The $(k_d + k_{md})^{-1}$ value for two-exponential fluorescence decay may be reasonably approximated by the short decay parameter [20], while the k_{fd}/k_{fm} ratio is a constant and equals to 7.7 [19]. Bimolecular rate constant k_{dm} at diffusion-controlled limit is directly related to the lateral diffusion coefficient of pyrene moiety (Eq. (2)):

$$k_{dm} = \frac{16\pi r N_A D_L}{1000} \quad (2)$$