Evidence that endothelial lipase remodels high density lipoproteins without mediating the dissociation of apolipoprotein A-I

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Abstract **Endothelial lipase (EL) is a triglyceride lipase gene family member that has high phospholipase and low triglyceride lipase activity. The aim of this study was to determine whether the phospholipase activity of EL is sufficient to remodel HDLs into small particles and mediate the dissociation of apolipoprotein A-I (apoA-I). Spherical, reconstituted HDLs (rHDLs) containing apoA-I only [(A-I)rHDLs], apoA-II only [(A-II)rHDLs], or both apoA-I and apoA-II [(A-I/A-II) rHDLs] were prepared. The rHDLs, which contained only cholesteryl esters in their core and POPC on the surface, were incubated with EL. As the rHDLs did not contain triacylglycerol, only the POPC was hydrolyzed. Hydrolysis was greater in the (A-I/A-II)rHDLs than in the (A-I)rHDLs. The (A-II)rHDL phospholipids were not hydrolyzed by EL. EL remodeled the (A-I)rHDLs and (A-I/A-II)rHDLs, but not the (A-II)rHDLs, into smaller particles. The reduction in particle size was related to the amount of phospholipid hydrolysis, with the diameter of the (A-I/A-II)rHDLs decreasing more than that of the (A-I)rHDLs. These changes did not affect the conformation of apoA-I, and neither apoA-I nor apoA-II dissociated from the rHDLs. Comparable results were obtained when human plasma HDLs were incubated with EL. These results establish that the phospholipase activity of EL remodels plasma HDLs and rHDLs into smaller particles without mediating the dissociation of apolipoproteins.**—Jahangiri, A., D. J. Rader, D. Marchadier, L. K. Curtiss, D. J. Bonnet, and K-A. Rye. **Evidence that endothelial lipase remodels high density lipoproteins without mediating the dissociation of apolipoprotein A-I.** ***J. Lipid Res.*** **2005.** 46: **896–903.**

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In 1999, a new member of the triglyceride lipase gene family, endothelial lipase (EL), was identified (1, 2). EL shares 45% homology with LPL, 40% homology with HL, and 27% homology with pancreatic lipase (1). Although these enzymes are all similar in structure, their substrate specificities vary widely. For example, pancreatic lipase hydrolyzes dietary triacylglycerols (TGs) (3), whereas LPL hydrolyzes phospholipids and TGs in chylomicrons and VLDL (4). LPL also hydrolyzes HDL TG (5). HL has both TG lipase and phospholipase activities. Its substrates include VLDL remnants, intermediate density lipoproteins, LDLs, and HDLs (6).

EL differs from the other triglyceride lipase gene family members in that it has high phospholipase and low TG lipase activity (1, 7). The principal substrates of EL are HDLs (7). EL also regulates HDL levels. For example, overexpression of EL in mice transgenic for human apolipoprotein A-I (apoA-I) reduces HDL-cholesterol and apoA-I levels, whereas infusion of anti-EL polyclonal antibodies into mice transgenic for human apoA-I increases plasma levels of total cholesterol, HDL-cholesterol, phospholipids, and apoA-I (1, 8). HDL-cholesterol levels are also increased in EL knockout mice. This is accompanied by a compensatory upregulation of HL and LPL activities (9).

Previous work from this laboratory has shown that HL remodels TG-enriched HDLs into small particles in a process that is accompanied by the dissociation of apoA-I (10–12). This is largely attributable to the TG lipase activity of HL depleting the HDLs of core lipids and generating an excess of surface constituents. This imbalance is cor-

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Abbreviations: apoA-I, apolipoprotein A-I; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; EL, endothelial lipase; RAM-Fc, rabbit anti-mouse Fc; rHDL, reconstituted high density lipoprotein; TG, triacylglycerol; UC, unesterified cholesterol.

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rected by the dissociation of lipid-poor apoA-I from the particles (10–12). Given that EL has very low TG lipase activity, the aim of the present study was to determine whether its high phospholipase activity is sufficient to remodel HDLs into small particles and mediate the dissociation of lipid-free or lipid-poor apoA-I.

As EL does not hydrolyze all HDL phospholipids equally well (13), it was important to use particles in which the phospholipid content was well defined. To ensure that interpretation of the results was unequivocal, it was also necessary to use HDLs that did not contain TG. This was achieved by using homogeneous preparations of spherical reconstituted HDLs (rHDLs) that were comparable in size and composition and contained only cholesteryl esters (CEs) in their core (14, 15). When these rHDLs were incubated with EL, phospholipids were the only constituents hydrolyzed.

The HDLs in human plasma have been classified on the basis of apolipoprotein composition into two main populations of particles: those containing apoA-I but not apoA-II [(A-I)HDLs] and those containing apoA-I as well as apoA-II [(A-I/A-II)HDLs] (16). A minor population of HDLs that contain apoA-II but not apoA-I [(A-II)HDLs] has also been identified (17). Recent studies carried out in this laboratory have shown that the rate of EL-mediated phospholipid hydrolysis in (A-I/A-II)rHDLs is enhanced relative to that in (A-I)rHDLs and that EL-mediated hydrolysis of (A-II)rHDL phospholipids is minimal (18). These results raised the possibility that apolipoproteins may regulate the EL-mediated remodeling of HDLs. To determine whether this was the case, the remodeling of (A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs was investigated.

The results confirmed that EL hydrolyzed (A-I/A-II)rHDL phospholipids to a greater extent than the phospholipids in (A-I)rHDLs and that there was minimal hydrolysis of the phospholipids in (A-II)rHDLs. EL also remodeled the (A-I/A-II)rHDLs and (A-I)rHDLs, but not the (A-II)rHDLs, into smaller particles. This remodeling was not accompanied by the dissociation of either apoA-I or apoA-II from the particles. Comparable results were obtained when HDLs from human plasma were incubated with EL.

EXPERIMENTAL PROCEDURES

Purification of apoA-I and apoA-II

HDLs were isolated from samples of pooled, expired, autologously donated human plasma (Gribbles Pathology, Adelaide, Australia) by sequential ultracentrifugation in the $1.07 < d < 1.21$ g/ml density range. ApoA-I and apoA-II were obtained by delipidation of the HDLs (19). The resulting apoHDL was chromatographed on a Q-Sepharose Fast Flow column attached to a fast protein liquid chromatography system (Amersham Biosciences, Uppsala, Sweden) (20). The purified apoA-I and apoA-II appeared as single bands after electrophoresis on a homogeneous 20% SDS-polyacrylamide Phast Gel (Amersham Pharmacia Biotech) and Coomassie Blue staining. The apoA-I and apoA-II were reconstituted in 10 mM Tris and 3 M guanidine hydrochloride (Gdn HCl), pH 8.2, followed by extensive dialysis against 0.01 M Tris-buffered saline (pH 7.4) containing 0.15 M NaCl, 0.005% (w/v) EDTA-Na_2, and 0.006% (w/v) NaN_3.

Isolation of LCAT

LCAT was prepared from samples of pooled, expired, autologously donated human plasma (21). LCAT activity was assessed using discoidal rHDLs containing POPC (Sigma), unesterified cholesterol (UC; Sigma), apoA-I, and a trace amount of radiolabeled UC ([1α,2α-^{3}H]UC; Sigma) as the substrate (22). The preparation of LCAT used in this study generated 286 nmol CE/ml LCAT/h.

Preparation of spherical (A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs

Discoidal rHDLs containing POPC, UC, and either apoA-I or apoA-II (initial POPC/UC/protein molar ratio of 100:5:1) were prepared by the cholate dialysis method (23). Spherical (A-I)rHDLs containing CEs as the only core lipid were prepared by incubating the discoidal rHDLs with LDL and LCAT as described previously (14). Spherical (A-II)rHDLs were prepared by displacing all of the apoA-I from the spherical (A-I)rHDLs with lipid-free apoA-II (14). As judged by nondenaturing gradient gel electrophoresis, the spherical (A-I)rHDL and (A-II)rHDL preparations did not contain lipid-free apolipoproteins.

Spherical (A-I/A-II)rHDLs, with both apoA-I and apoA-II on the same particle, were prepared as described (15). Briefly, discoidal (A-I)rHDLs (45 mg of apoA-I) were incubated at 37°C for 24 h with discoidal (A-II)rHDLs (15 mg of apoA-II), LDL (180 mg of apoB), fatty acid-free BSA (final concentration, 40 mg/ml), β-mercaptoethanol (final concentration, 4 mM), and LCAT (90 ml) in a final volume of 164 ml. These conditions generated spherical (A-I/A-II)rHDLs and a small amount of spherical (A-I)rHDLs. The rHDLs were isolated by ultracentrifugation in the $1.07 < d < 1.21$ g/ml density range, dialyzed against TBS (3×1 liter), and subjected to immunoaffinity chromatography on a column containing an anti-human apoA-II polyclonal antibody coupled to CNBr-activated Sepharose 4B (Amersham Biosciences). The (A-I)rHDLs, which did not bind to the column, were eluted with TBS. The (A-I/A-II)rHDLs that bound to the column were eluted with 0.1 M acetic acid (pH 2.7) and adjusted immediately to pH 7.4 with 1 M Tris, pH 11.0 (final concentration, 0.1 M). The (A-I/A-II)rHDLs were concentrated 30-fold by ultrafiltration at 4°C (Millipore, Bedford, MA).

Isolation of human (A-I)HDLs, (A-I/A-II)HDLs, and HDL_2 from human plasma

HDLs were isolated from human plasma by sequential ultracentrifugation in the $1.07 < d < 1.21$ g/ml density range. (A-I/A-II)HDLs were separated from (A-I)HDLs by anti-apoA-II immunoaffinity chromatography as described above for rHDLs. When the (A-I)HDLs and (A-I/A-II)HDLs were subjected to nondenaturing gradient gel electrophoresis, the (A-I)HDLs, but not the (A-I/A-II)HDLs, were found to contain lipid-free apoA-I. As lipid-free apoA-I was not present in the ultracentrifugally isolated HDLs, we concluded that it had dissociated from the (A-I)HDLs during immunoaffinity chromatography. As one of the aims of this study was to determine whether EL mediated the dissociation of lipid-free apoA-I from (A-I)rHDLs, these particles were not used further. For this reason, ultracentrifugally isolated HDL_2 ($1.07 < d < 1.12$ g/ml), which contains mainly (A-I)HDLs and only a small amount of (A-I/A-II)HDLs, was used instead of (A-I)HDLs in the plasma HDL incubations. As judged by nondenaturing gradient gel electrophoresis, HDL_2 did not contain lipid-free apoA-I. The HDL_2 were dialyzed against 3×1 liter of TBS, pH 7.4, before use.

Expression of EL

COS cells were grown in DMEM with 10% fetal bovine serum and 1% antibiotic/antimycotic at 37°C and 5% CO_2. Before infection, cells were brought to 90% confluence on 150 mm plates. Growth medium was removed and the cells were washed with 10 ml of serum-free DMEM without phenol red, then incubated with recombinant adenovirus encoding EL in 5 ml of the same medium at a multiplicity of 3,000 particles/cell. Two hours later, 9 ml of serum-free medium without phenol red, containing 10 U/ml heparin, was added to each of the plates. At 47.5 h after infection, an additional 10 U/ml heparin (280 μl of 500 U/ml) was added, and the cells were incubated for another 30 min. Medium was collected and clarified by centrifugation at 2,000 rpm for 10 min in 50 ml conical tubes, then frozen in 1 ml aliquots at −80°C. EL activity was assessed using as a substrate spherical (A-I) rHDLs containing only CE in the core and POPC as the only phospholipid. The rHDLs (final concentration, 1 mM phospholipid) were incubated with 20 μl of EL for 1.5 h at 37°C. NEFA mass was assayed using a commercially available kit (Wako Pure Chemical Industries, Osaka, Japan). The EL used in this study generated 135 nmol NEFA/ml EL/h.

Incubations

All incubations were carried out in 1.5 ml Eppendorf tubes in a shaking water bath maintained at 37°C. Nonincubated control samples were stored at 4°C. When the incubations were complete, the rHDLs were isolated by ultracentrifugation in the $1.07 < d < 1.21$ g/ml density range with a single 18 h spin at both the lower and upper densities. The spins were carried out at 100,000 rpm in a TLA-100.2 rotor using a Beckman TL-100 tabletop ultracentrifuge maintained at 4°C. The rHDLs were dialyzed against TBS, pH 7.4, before use.

Gradient gel electrophoresis

Plasma HDL and rHDL diameters were determined by electrophoresis on nondenaturing 3% and 40% polyacrylamide gradient gels prepared according to the method of Rainwater et al. (24). Stokes' diameters were calculated by reference to high molecular weight standards of known size (Amersham Biosciences).

Immunoblotting

HDLs and rHDLs (1 μg of apolipoprotein) were electrophoresed on 3% and 40% nondenaturing gradient gels, transferred electrophoretically to nitrocellulose membranes, and immunoblotted with either sheep anti-human apoA-I or goat anti-human apoA-II polyclonal antibodies (Calbiochem, San Diego, CA). Bound antibodies were detected by enhanced chemiluminescence (Amersham Biosciences).

Surface plasmon resonance analysis

A Biacore 2000 biosensor was used to measure the association rate constant (K_a) of seven unique apoA-I monoclonal antibodies to (A-I)rHDLs and (A-I/A-II)rHDLs. Saturating amounts of rabbit anti-mouse Fc (RAM-Fc) were immobilized on all four flow cells of a CM5 chip using amide coupling (25). The monoclonal antibodies, or an isotype control, were injected and captured by the RAM-Fc at dilutions predetermined to give 400 response units. This injection was followed by injection of the rHDLs. Data were collected at a high collection rate and evaluated by synchronizing the injection time and zeroing the sensogram baselines. Control antibody sensograms were subtracted from each specific antibody sensogram. Each antibody and rHDL pair was evaluated with a 1:1 (Langmuir) model. Microsoft Excel 2000 was used for statistical analysis. The *t*-test for two-tailed distribution and two-sample unequal variance was used to identify significant differences.

TABLE 1. Physical properties of rHDLs and plasma HDLs

HDLs	Composition						Stokes' Diameter
	PL	UC	CE	TG	ApoA-I	ApoA-II	
	mass %						*nm*
(A-I)rHDLs	28.5	1.3	16.2	0	53.9	0	8.9
(A-I/A-II)rHDLs	36.6	0.8	17.6	0	30.7	14.4	9.4
(A-II)rHDLs	30.0	1.5	17.4	0	0	51.1	9.8
HDL_2	26.1	1.1	32.5	1.6	30.3	8.4	11.0
(A-I/A-II)HDLs	22.5	0.5	15.4	6.7	34.1	20.7	9.2

(A-I)rHDLs, reconstituted HDLs containing apolipoprotein A-I (apoA-I) only; CE, cholesteryl ester; PL, phospholipid; TG, triacylglycerol; UC, unesterified cholesterol. Plasma HDL_2, plasma (A-I/A-II)HDLs, (A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs were prepared as described in Experimental Procedures. The concentration of each constituent was determined as the mean of triplicate determinations that varied by less than 10%.

Chemical analyses

All assays were performed on a Roche Diagnostics/Hitachi 902 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany). An enzymatic kit was used to measure total cholesterol concentrations (Roche Diagnostics GmbH). ApoA-I, apoA-II, phospholipid, and UC concentrations were determined as described previously (26–28).

RESULTS

Characterization of rHDLs and plasma HDLs

The compositions of the (A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs are shown in **Table 1**. The larger size of the (A-I/A-II)rHDLs and (A-II)rHDLs, relative to (A-I) rHDLs, is consistent with earlier reports from this laboratory (14, 15). The compositions of the HDL_2 and the (A-I/A-II)HDLs are comparable to what has been reported

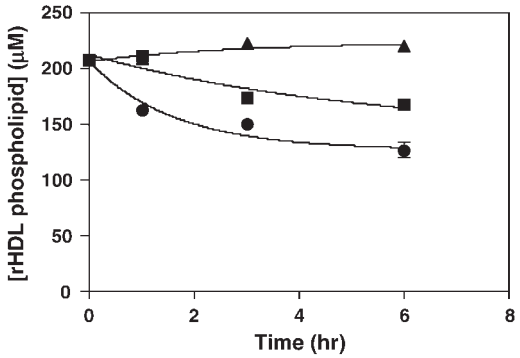

Fig. 1. Phospholipid hydrolysis in spherical reconstituted HDLs (rHDLs) containing apolipoprotein A-I (apoA-I) only [(A-I)rHDLs], apoA-I and apoA-II [(A-I/A-II)rHDLs], and apoA-II only [(A-II)rHDLs] during incubation with endothelial lipase (EL). (A-I)rHDLs (closed squares), (A-I/A-II)rHDLs (closed circles), and (A-II)rHDLs (closed triangles) (final concentration, 1 mM phospholipid) were incubated for 0–6 h at 37°C with fatty acid-free BSA (final concentration, 40 mg/ml) and a constant amount of EL (125 μl of a preparation that generated 135 nmol NEFA/ml EL/h). The final incubation volume was 312 μl. The rHDLs were isolated by ultracentrifugation, and phospholipid concentrations were measured enzymatically. Data are presented as means ± SEM (n = 3).

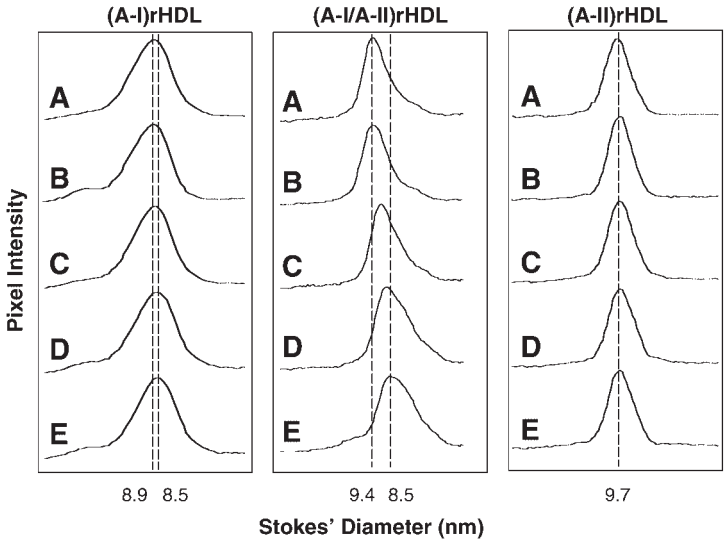

Fig. 2. Changes in (A-I)rHDL, (A-I/A-II)rHDL, and (A-II)rHDL size during incubation with EL. (A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs were maintained at 4°C (profile A), incubated at 37°C for 6 h in the absence of EL (profile B), or incubated at 37°C in the presence of EL for 1 h (profile C), 3 h (profile D), or 6 h (profile E). Details of the incubations are described in the legend to Fig. 1. The rHDLs were isolated by ultracentrifugation. Particle diameters were determined by nondenaturing gradient gel electrophoresis, with reference to high molecular weight standards of known size. Scans of the gels stained with Coomassie Blue are shown.

elsewhere (29, 30). ApoA-II constituted less than 10% of the total HDL_2 mass.

Time dependence of EL-mediated phospholipid hydrolysis in rHDLs and plasma HDLs

(A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs were incubated for 0–6 h at 37°C with a constant amount of EL. When the incubations were complete, the rHDLs were isolated by ultracentrifugation and the phospholipid concentration was determined at each time point (**Fig. 1**). After 6 h of incubation with EL, the phospholipid concentration in the (A-I)rHDLs (closed squares) had decreased from 207.3 ± 1.1 μM to 167.3 ± 3.4 μM. In the case of the (A-I/A-II)rHDLs (closed circles), the phospholipid concentration decreased from 207.3 ± 1.4 μM to 127.0 ± 5.4 μM. Phospholipid hydrolysis did not progress when the incubation time was extended beyond 6 h (results not shown). This is consistent with EL being thermally labile and not retaining its activity for more than 6 h at 37°C (D. Rader, personal communication). There was no measurable phospholipid hydrolysis in the (A-II)rHDLs (closed triangles).

The inability of EL to hydrolyze (A-I)rHDL and (A-I/A-II)rHDL phospholipids for more than 6 h is unlikely to be attributable to NEFA and lysophospholipids accumulating on the substrate surface. All of the incubation mixtures contained fatty acid-free BSA at a concentration that has been shown previously to bind all of the lysophosphatidylcholine and NEFA that is generated during hydrolysis of rHDL phospholipids (31).

When plasma HDL_2 and (A-I/A-II)HDLs were incubated with EL for 6 h, the HDL_2 phospholipid concentration decreased from 291.3 to 178.8 μM, whereas the concentration of the (A-I/A-II)HDL phospholipids decreased from 353.8 to 108.8 μM (results not shown).

Remodeling of rHDLs and plasma HDLs by EL

Nondenaturing gradient gel electrophoresis was used to determine whether the phospholipid hydrolysis mediated by EL is sufficient to remodel the (A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs into smaller particles (**Fig. 2**).

The rHDLs did not change in size when they were either maintained at 4°C (profile A) or incubated at 37°C for 6 h in the absence of EL (profile B). The diameter of the (A-I)rHDLs and (A-I/A-II)rHDLs decreased progressively when they were incubated with EL for 1 h (profile C), 3 h (profile D), or 6 h (profile E). By 6 h, the diameter of the (A-I)rHDLs had decreased from 8.9 to 8.5 nm. The diameter of the (A-I/A-II)rHDLs decreased from 9.4 to 8.5 nm. There was no further reduction in either (A-I)rHDL or (A-I/A-II)rHDL size when the incubation was extended to 24 h (results not shown). Incubation with EL had no effect on (A-II)rHDL size.

The composition of the rHDLs at each time point is presented in **Table 2**. Incubation in the absence of EL did not affect (A-I)rHDL, (A-I/A-II)rHDL, or (A-II)rHDL composition. When the (A-I)rHDLs and (A-I/A-II)rHDLs were

TABLE 2. Composition of (A-I)rHDLs, (A-I/A-II)rHDLs, and (A-II)rHDLs after incubation in the presence and absence of EL

Sample	Incubation Conditions	Additions	Composition				
			PL	UC	CE	ApoA-I	ApoA-II
			mass %				
(A-I)rHDLs	4°C, 6 h	TBS	24.7	1.2	19.9	54.2	0.0
	37°C, 6 h	TBS	24.4	1.0	20.2	54.4	0.0
	37°C, 1 h	EL	23.7	1.2	19.9	55.2	0.0
	37°C, 3 h	EL	21.8	1.3	20.7	56.2	0.0
	37°C, 6 h	EL	19.4	1.2	20.1	59.3	0.0
(A-I/A-II)rHDLs	4°C, 6 h	TBS	27.5	0.5	14.6	30.4	26.9
	37°C, 6 h	TBS	29.4	0.6	15.4	27.3	27.2
	37°C, 1 h	EL	23.8	0.3	15.8	32.9	27.1
	37°C, 3 h	EL	20.6	0.3	15.7	39.6	23.8
	37°C, 6 h	EL	16.3	0.4	15.4	36.7	31.1
(A-II)rHDLs	4°C, 6 h	TBS	22.1	1.3	18.4	0.0	58.2
	37°C, 6 h	TBS	21.7	1.3	18.1	0.0	59.0
	37°C, 1 h	EL	21.6	1.3	18.2	0.0	58.9
	37°C, 3 h	EL	21.8	1.4	20.6	0.0	56.2
	37°C, 6 h	EL	19.5	1.2	17.1	0.0	62.2

EL, endothelial lipase. Spherical rHDLs were incubated at 37°C for 0–6 h in the presence or absence of EL, then isolated by ultracentrifugation as described in the legend to Fig. 1. Each sample was assayed in triplicate to determine the concentrations of individual components.

incubated in the presence of EL, their phospholipid content decreased in a time-dependent manner, and there was a concomitant increase in the mass percentage of apolipoproteins. (A-II)rHDL composition was unaffected by incubation with EL.

Additional incubations were carried out to determine whether EL also remodeled the HDL_2 and (A-I/A-II)HDLs from human plasma into small particles. Most of the HDL_2 were 11.0 nm in diameter, compared with 9.2 nm for the (A-I/A-II)HDLs (**Fig. 3**, profile A). Neither the HDL_2 nor the (A-I/A-II)HDLs changed in size when they were incubated for 6 h in the absence of EL (profile B). After 6 h of incubation with EL, the diameters of the HDL_2 and (A-I/A-II)HDLs decreased to 10.4 and 8.3 nm, respectively (profile C).

Previous studies from this laboratory have shown that the reduction in size that occurs when HDLs are remodeled by plasma factors such as HL, cholesteryl ester transfer protein (CETP), and phospholipid transfer protein may be accompanied by the dissociation of apoA-I (11, 32, 33). To determine whether this is also the case for EL, the (A-I)rHDLs and (A-I/A-II)rHDLs were incubated at 37°C for 6 h with EL. The unprocessed incubation mixtures were subjected to nondenaturing gradient gel electrophoresis and immunoblotted for either apoA-I or apoA-II (**Fig. 4**). Aliquots of lipid-free apoA-I and lipid-free apoA-II were also applied to the gels. These results established that EL did not mediate the dissociation of apoA-I from the (A-I)rHDLs (Fig. 4A) or the (A-I/A-II)rHDLs (Fig. 4B). Similarly, apoA-II did not dissociate from either the (A-I/A-II)rHDLs (Fig. 4C) or the (A-II)rHDLs (Fig. 4D). Neither apoA-I nor apoA-II dissociated from the (A-I)rHDLs or the (A-I/A-II)rHDLs when additional EL was added after 6 h and the incubation was extended for an additional 18 h (24 h in total) (results not shown). Identical results were obtained when HDL_2 and (A-I/A-II)HDLs (Fig. 4E, F) were incubated for 6 h with EL.

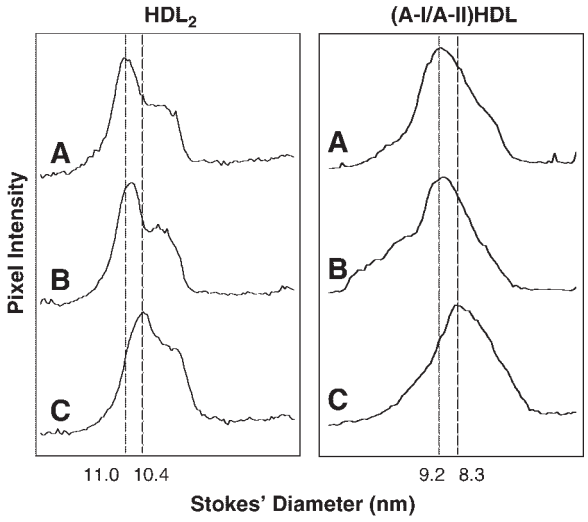

Fig. 3. Changes in plasma HDL_2 and plasma (A-I/A-II)HDL size during incubation with EL. HDL_2 and (A-I/A-II)HDLs were either maintained at 4°C in the absence of EL (profile A) or incubated for 6 h at 37°C in the absence (profile B) or presence (profile C) of EL. Details of the incubations are described in the legend to Fig. 1. The HDLs were isolated by ultracentrifugation, and particle diameters were determined by gradient gel electrophoresis. Scans of Coomassie Blue-stained gels are shown.

Influence of EL-mediated remodeling of (A-I)rHDLs and (A-I/A-II)rHDLs on the conformation of apoA-I

Surface plasmon resonance was used to determine whether the reduction in (A-I)rHDL and (A-I/A-II)rHDL particle size was associated with a change in the conformation of apoA-I. (A-I)rHDLs and (A-I/A-II)rHDLs were incubated for 6 h in either the absence or presence of EL. Binding of the rHDLs to seven well-characterized monoclonal antibodies that recognize unique epitopes spanning the entire length of the apoA-I molecule was examined. As determined by the affinity of binding of each epitope to its antibody, it was evident that the reduction in (A-I)rHDL and (A-I/A-II)rHDL size that occurred during incubation with EL could not be explained in terms of changes in the conformation of apoA-I (results not shown).

DISCUSSION

Previous work from this laboratory has shown that the TG lipase activity of HL remodels HDLs into small particles in a process that is accompanied by the dissociation of lipid-poor apoA-I (10–12). The aim of the current project was to determine whether these events are also mediated by the phospholipase activity of EL. This was achieved by using preparations of spherical rHDLs containing CE as the sole core lipid, such that phospholipids were the only constituents hydrolyzed by EL.

The results showed that EL remodels (A-I)rHDLs and (A-I/A-II)rHDLs into smaller particles in a process that is not accompanied by the dissociation of either apoA-I or apoA-II. These observations are consistent with earlier work from this laboratory showing that phospholipase A_2 remodels (A-I)rHDLs into small particles without mediating the dissociation of apoA-I (31). In that study, apoA-I dissociated from the rHDLs only when the phospholipid-depleted rHDLs were incubated with Intralipid® and CETP under conditions that depleted the particles of core lipids and generated an excess of constituents on the rHDL surface. When the current results are considered in light of the earlier study, it follows that the lack of dissociation of apoA-I from either the (A-I)rHDLs or the (A-I/A-II)rHDLs is probably attributable to the inability of EL to deplete the rHDLs of core lipids. The very low triglyceride lipase activity of EL makes it likely that this is also the case in vivo. This possibility is strengthened by the results shown in Fig. 4, which show that EL does not mediate the dissociation of either apoA-I or apoA-II from plasma (A-I/A-II)HDLs that contain substantial amounts of TG in their core (Table 1).

The immunoblots shown in Fig. 4B, C indicate that EL did not remodel all of the (A-I/A-II)rHDLs into small particles. This is at odds with the data shown in Fig. 2, which

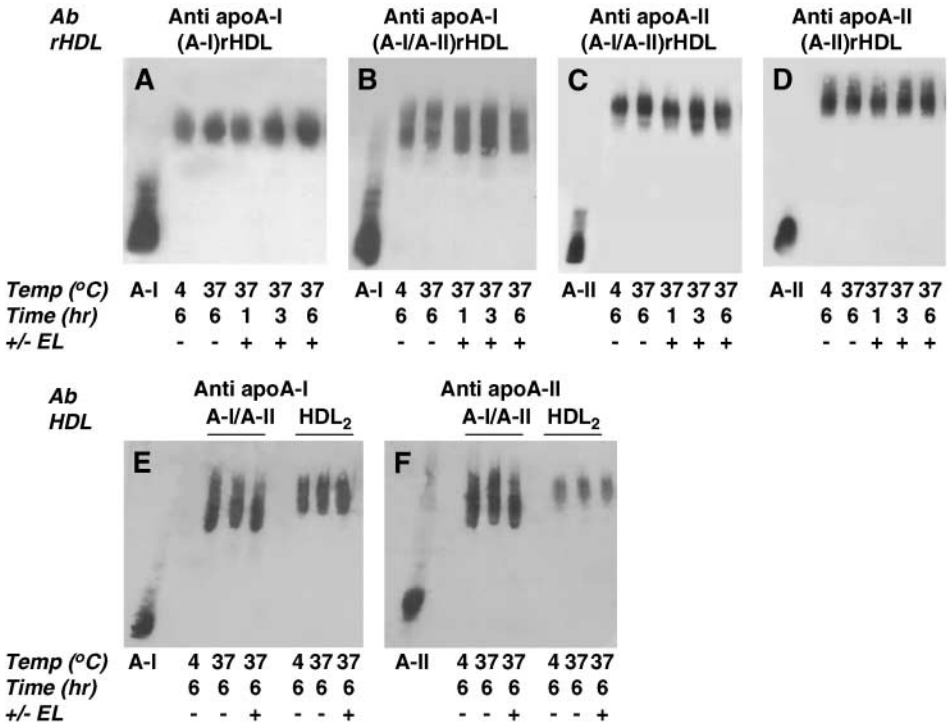

Fig. 4. Absence of dissociation of apoA-I and apoA-II during EL-mediated remodeling of plasma HDLs and rHDLs. (A-I)rHDLs, (A-I/A-II)rHDLs, (A-II)rHDLs, HDL_2, and (A-I/A-II)HDLs were incubated with EL as described in the legends to Figs. 1 and 3. The unprocessed incubation mixtures were electrophoresed on nondenaturing gradient gels and transferred electrophoretically to nitrocellulose membranes. The (A-I)rHDLs were immunoblotted for apoA-I (A). The (A-I/A-II)rHDLs were immunoblotted for apoA-I (B) and apoA-II (C). The (A-II)rHDLs were immunoblotted for apoA-II (D). HDL_2 (E) and (A-I/A-II)HDLs (F) were immunoblotted for apoA-I and apoA-II. Samples of lipid-free apoA-I or lipid-free apoA-II were applied to the gels as indicated. Ab, antibody.

show a quantitative conversion of rHDLs into smaller particles. Together, these results suggest that only a small amount of unmodified (A-I/A-II)rHDLs, which was not sufficient for detection with Coomassie Blue, remained after incubation with EL. These unmodified particles were enriched with apoA-II and apparently resistant to remodeling by EL. This is consistent with an earlier report from this laboratory showing attenuated remodeling of (A-I/A-II)rHDLs by CETP (34). It also suggests that EL-mediated remodeling of (A-I/A-II)rHDLs may generate small particles that are depleted of apoA-II.

Several approaches were used to determine why the size of (A-I)rHDLs and (A-I/A-II)rHDLs decreased in the absence of core lipid depletion. The first possibility was that the reduction in particle size reflected a change in the conformation of apoA-I. ApoA-I is a flexible molecule with a hinged domain that may or may not associate with lipid (35, 36). If the size of an HDL particle decreases, the hinged region of apoA-I may be removed from contact with lipids. This would reduce the surface area requirements of apoA-I, thus enabling it to remain associated with the smaller particles. To determine whether this was the case, surface plasmon resonance was used to compare the conformation of apoA-I in (A-I)rHDLs and (A-I/A-II)rHDLs before and after incubation with EL. These results showed that incubation with EL did not change the conformation of apoA-I in either the (A-I)rHDLs or the (A-I/A-II)rHDLs. Given the high affinity of apoA-II for lipid (37), the possibility that the reduction in (A-I/A-II)rHDL size was caused by a change in the conformation of this apolipoprotein was considered unlikely.

The possibility that the remodeling of rHDLs into small particles was attributable entirely to phospholipid depletion was also investigated. This was achieved by comparing the reduction in rHDL surface area with the decrease in the area occupied by the phospholipids on the particle surface. Incubation with EL reduced the (A-I)rHDL surface area by 22 nm^2 (from 249 to 227 nm^2) (Fig. 2). To ascertain whether there was a comparable reduction in phospholipid area, it was necessary to determine how many phospholipid molecules were hydrolyzed by EL. The (A-I)rHDLs initially contained three molecules of apoA-I per particle (31). Given that apoA-I did not dissociate from these particles during incubation with EL, it follows that the small conversion products also contained three molecules of apoA-I per particle. As incubation with EL reduced the (A-I)rHDL phospholipid/apoA-I molar ratio from 17:1 to 12:1, it follows that the number of phospholipid molecules in the (A-I)rHDLs decreased from 51 to 36. Assuming that the reduction in rHDL size was associated with a reorganization of the unhydrolyzed phospholipids remaining on the rHDL surface from an expanded

(0.75 nm^2/molecule) to a condensed (0.45 nm^2/molecule) state (38), it follows that the area occupied by phospholipids decreased from 38 to 16 nm^2. This 22 nm^2 difference corresponds precisely to the decrease in particle surface area. It is also consistent with the reduction in (A-I)rHDL size being attributable to phospholipid depletion and a structural reorganization of the unhydrolyzed phospholipids that remained associated with the particles.

This was also the case for the (A-I/A-II)rHDLs, in which incubation with EL reduced the particle surface area from 278 to 227 nm^2 (Fig. 2). Earlier work from this laboratory established that (A-I/A-II)rHDLs contain two molecules of apoA-I and two molecules of apoA-II per particle (15). As two molecules of apoA-II occupy approximately the same area as one apoA-I molecule (39), it is reasonable to assume that the (A-I/A-II)rHDL surface contained the equivalent of three molecules of apoA-I. Incubation with EL decreased the (A-I/A-II)rHDL phospholipid/apoA-I molar ratio from 37:1 to 17:1. This equates to a reduction in the number of phospholipid molecules from ~111 to 51. Using the approach outlined above, this translates into a decrease in phospholipid surface area of 60 nm^2 (from 83 to 23 nm^2). As was the case for the (A-I)rHDLs, this is consistent with the remodeling of (A-I/A-II)rHDLs into smaller particles being attributable to phospholipid depletion.

The present results showed that (A-II)rHDLs are poor substrates for EL. Although this suggests that apoA-II inhibits the phospholipase activity of EL, it is not consistent with the results shown in Fig. 1, which show that EL-mediated phospholipid hydrolysis is increased in (A-I/A-II) rHDLs compared with (A-I)rHDLs. This is consistent with the presence of apoA-II on an rHDL particle that also contains apoA-I enhancing, rather than inhibiting, phospholipid hydrolysis and possibly reflects an increased affinity of EL for the rHDL surface or enhanced access of rHDL phospholipid acyl chains to the active site of the enzyme. This is in contrast with our previous results, which showed that the rate of HL-mediated phospholipid hydrolysis in (A-I/A-II)rHDLs is intermediate between that of (A-II) rHDLs and (A-I)rHDLs (40). These differences most likely reflect variations in EL and HL structure, particularly the lack of homology of their lid regions, which determines substrate specificity (1, 2).

The current results suggest that the role of EL in HDL metabolism is distinct from that of HL. In the case of HL, the phospholipase and triglyceride lipase activities act together to reduce HDL size and promote the dissociation of lipid-free or lipid-poor apoA-I (10–12). This apoA-I has the ability to accept unesterified cholesterol and phospholipids from peripheral cells in the first step of reverse cholesterol transport. It also forms new HDLs and thus maintains, or possibly increases, HDL levels. The present results suggest that EL is not able to enhance the initial step of reverse cholesterol transport and is therefore unlikely to contribute to the cardioprotective properties of HDLs.

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