Characterization of phospholipids in pre-α HDL: selective phospholipid efflux with apolipoprotein A-I

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Abstract **Previously, we have shown that lipid-free apoA-I, when incubated with fibroblasts, will produce lipoproteins of pre-α mobility (Asztalos, B. F., et al. 1997. *Arterioscler. Thromb. Vasc. Biol.* 17: 1630–1636). In order to understand the nature of these pre-α particles, we further characterized their lipid content. The pre-α particles are high density lipoproteins, having a median density of 1.08 g/ml. They have a surface charge of −18.45 mV. The phospholipid composition of these particles showed that they have 4% each of phosphatidyl ethanolamine and inositol; 69% phosphatidyl choline and 18% sphingomyelin. This phospholipid composition is different from those of plasma HDL (81% phosphatidyl choline, 13% sphingomyelin), plasma membrane on the fibroblasts, and whole fibroblast phospholipid. To demonstrate that the pre-α mobility resides in the lipids, lipids from pre-α lipoproteins were reconstituted with lipid-free apoA-I. The resultant particles retained their pre-α mobility. We conclude that apoA-I may react with specific regions of plasma membrane to acquire this unusual lipid composition and that pre-α mobility is caused in part by the unusual phospholipid composition.—**Zhang, W., B. Asztalos, P. S. Roheim, and L. Wong. **Characterization of phospholipids in pre-α HDL: selective phospholipid efflux with apolipoprotein A-I. *J. Lipid Res.* 1998.** 39: **1601–1607.**

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High density lipoproteins (HDL) are a major component of plasma lipoproteins. Unlike their low density lipoprotein counterpart, the function of HDL is not well understood. There is evidence that high levels of HDL are protective against coronary heart disease (CHD). In large scale epidemiological studies such as the Framingham study, subjects with high levels of HDL cholesterol have been shown to have lower incidence of CHD even when they have high levels of LDL (1).

Three decades of research into the structure and function of various lipoproteins have shown HDL to be heterogeneous. The particles can be separated by charge, density, and apolipoprotein composition into various fractions (2–4). There is also evidence that the different HDL particles have different metabolic functions (5).

Recently, we developed a quantitative two-dimensional agarose non-denaturing polyacrylamide gradient gel electrophoresis system (2DE) to analyze plasma high density lipoproteins (6). Using this procedure, we have observed free apoA-I-like particles in human plasma (7). We have also shown that free apoA-I is capable of causing accelerated cholesterol efflux from cells, and in the process several pre-α migrating lipoprotein particles are formed (8).

The purpose of the present study is to characterize the pre-α lipoproteins further. We wish to determine the density distribution of pre-α lipoproteins and the reason for their pre-α mobility. In this report, we will demonstrate that the particles appear to be HDL, having a density peak at 1.08 g/ml, and their surface charge is considerably greater than the plasma HDL particles. The phospholipid composition of the pre-α particles is different from plasma HDL particles. It is also different from the phospholipid composition of apoA-I-free media that has been incubated with cells, plasma membrane, and whole cells. Using the lipid from pre-α lipoproteins, we reconstituted the lipid with lipid-free apoA-I and were able to demonstrate that the reconstituted particles retained their pre-α mobility.

METHODS

Cell culture and lipid labeling

Normal human fibroblasts GM 3468A (Coriel Institute, NJ) from passage 10–20 were grown in T75 plastic flasks in Dulbecco's modified Eagle's media (DMEM) containing 10% fetal bovine serum (FBS) (Life Science Technologies, Gaithersburg, MD) at 37°C in a humidified incubator equilibrated with 5% CO_2 and 95% room air.

Subconfluent fibroblasts in each T-75 flask were incubated for 72 h with 100 μCi of [^{32}P]orthophosphate mixed with DMEM containing 10% FBS. Labeled cells were washed four times with

Abbreviations: HDL, high density lipoprotein; apoA-I, apolipoprotein A-I; 2DE, two-dimensional non-denaturing gradient polyacrylamide gel electrophoresis

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Dulbecco's phosphate-buffered saline (D-PBS) before incubation with apoA-I.

For the production of native pre-α lipoproteins, 10 μg/ml apoA-I was dissolved into serum-free media (BioRich, ICN; Costa Mesa, CA) and incubated with the fibroblasts for 24 h.

Phospholipid separation

Whole cell phospholipids were extracted by incubation with hexane–isopropranol 3:2 (v/v) overnight (9). For phospholipid extractions of other fractions, the method of Bligh and Dyer was used (10). Thin-layer chromatography (TLC) separation of phospholipids was by a modification of the method of Touchstone, Chen, and Beaver (11). Briefly, LK5D silica gel plates (Whatman; Clifton, NJ; 15 nm) were pre-run with chloroform–methanol 1:1 (v/v). The plates were then dried in an oven and the lipid samples were applied to the plates. The plates were then developed with chloroform–methanol–water–triethylamine–boric acid 30:35:4.5:35:1g (v/v/v/v/wt). The boric acid was dissolved in methanol–water before the addition of chloroform and triethylamine. After development, the plates were dried and the radioactivity was quantified on the PhosphorImager (Molecular Dynamics; Sunnyvale, CA).

For nonradioactive samples, the plates were charred by the method of Bitman and Wood (12) using a copper reagent.

ApoA-I purification

Apolipoprotein A-I was purified from normolipidemic human plasma (Southeast Blood Center, New Orleans, LA) HDL ($1.080 < d < 1.21$ g/ml) by modification of a procedure described by Nichols et al. (13) and Forte et al. (14). Solid guanidine hydrochloride (ICN, Irvine, CA) was added to 3 mg/ml HDL to give a final concentration of 2 m. This solution was incubated at 37°C for 3 h. After incubation, the mixture was dialyzed four times against a solution containing 150 mm NaCl, 10 mm Tris-HCl, and 0.01% EDTA at pH 8.0 to remove the guanidine-HCl. All chemicals were from Sigma (St. Louis, MO) unless otherwise noted. After dialysis, density of the solution was adjusted to 1.21 g/ml using solid KBr. The HDL solution was then transferred into an ultracentrifuge tube (Beckman, Fullerton, CA) and overlaid with an equal volume of KBr solution of the same density. The sample was then subjected to ultracentrifugation at 50,000 rpm for 24 h in a 50.3 Ti rotor (Beckman, Fullerton, CA) at 10°C. The bottom 1.5 ml was collected by tube slicing and dialyzed extensively against 0.01 m NH_4HCO_3. The apoA-I was stored sterile in solution for experimental use. Purity of the isolated apoA-I was determined by SDS polyacrylamide gradient gel electrophoresis followed by Coomassie Blue and silver staining. A single band corresponding to apoA-I was detected in all cases. In addition, apoA-I did not crossreact to antibodies to any known apolipoproteins except to anti-apoA-I by double immunodiffusion.

Reconstitution experiment

Media (120 ml) from incubation of lipid-free apoA-I with fibroblasts was adjusted to density d 1.25 g/ml with solid potassium bromide and ultracentrifuged to isolate total lipoproteins. After dialysis, protein was determined by the method of Lowry et al. (15) and 2 ml of the lipoprotein (157 μg/ml protein) was used for lipid extraction. The lipid extract was then dried under nitrogen and approximately 1.4 ml of Tris buffer was added (10 mm Tris HCl, 0.01% EDTA, 1 mm NaN_3, pH 7.4). The mixture was vortexed and 597 μg of sodium cholate in the same Tris buffer was added to the mixture. The mixture was left under nitrogen at 4°C for 1 h. At the end of the incubation, 314 μg of apoA-I in Tris buffer was added to the cloudy mixture. The final volume was 2 ml. The mixture was stirred at 4°C for 12 h. The clear solution was then dialyzed exhaustively to remove the cholate (3 × 1000-fold dialysis minimum). Samples were then taken for analysis.

Other methods

Separation of lipoprotein subpopulations by the technique of two-dimensional gel electrophoresis (2DE), agarose electrophoresis (1D) in the first dimension, and non-denaturing PAGE in the second dimension (2D) has been previously described (6). It should be noted that the agarose did not contain albumin, permitting the separation of α and pre-α migrating particles (6, 7). After transfer of lipoproteins to nitrocellulose membranes, apoA-I-containing lipoproteins were immunolocalized with monospecific goat anti-human apoA-I. Goat antibody was then immunodetected with ^{125}I-labeled anti-goat γ globulin. The radioactivity was quantified on the phosphorimaging device (Molecular Dynamics; Sunnyvale, CA). For both the 1D and 2D analyses, 15 μl of unconcentrated media was used. We defined the apoA-I-containing lipoproteins by their electrophoretic mobility on agarose gels as pre-β, α, and pre-α (6).

Plasma membrane was purified by the method of Charalampous and Gonatas (16). The purity of the samples was monitored by using 5′ nucleotidase as a marker enzyme for plasma membranes. The assay for 5′ nucleotidase has been previously described (17). Ultracentrifugation was carried out by the method of Havel, Eder, and Bragdon (18).

Equilibrium ultracentrifugation was performed using the method of Kelly and Kruski (19). The result was expressed as fraction radioactivity divided by fraction volume and plotted against the density of the fractions. Surface charge of the particles was estimated by the method of Sparks and Phillips (20) using human serum albumin as standard. Because HDL migration is at the center of human serum albumin migration in agarose gel, we could not obtain an experimental error for α migrating particles.

RESULTS

Previously, we demonstrated that the five particles of pre-α lipoproteins are lipoproteins, having a flotation density of $d < 1.21$ g/ml. The lipoproteins contain phospholipid, free cholesterol, and apoA-I only. Other lipids, if present, were under the limit of our detection (8). One of the five lipoprotein particles (the lowest molecular weight particle) contained phospholipid and apoA-I and no cholesterol (8). To determine the density distribution of these HDL particles, fibroblasts were labeled with [^{32}P]-phosphate for 3 days. The labeled cells were then incubated overnight with 10 μg/ml apoA-I and the media were analyzed. The result, shown in **Fig. 1a**, demonstrated that all five pre-α particles were labeled. The media were then subjected to ultracentrifugation to isolate $d < 1.21$ g/ml particles. This is shown in Fig. 1b. Consistent with our previous findings (8), all five particles were recovered. Some of the smallest molecular weight pre-α particles were in the $d > 1.21$ g/ml fraction. The $d < 1.21$ g/ml particles were then layered onto a continuous density gradient and subjected to equilibrium density centrifugation. The result, illustrated in **Fig. 2**, showed that the five particles have a major density peak at 1.08 g/ml.

To characterize the phospholipid composition of the pre-α particles, the lipid from the particles was extracted

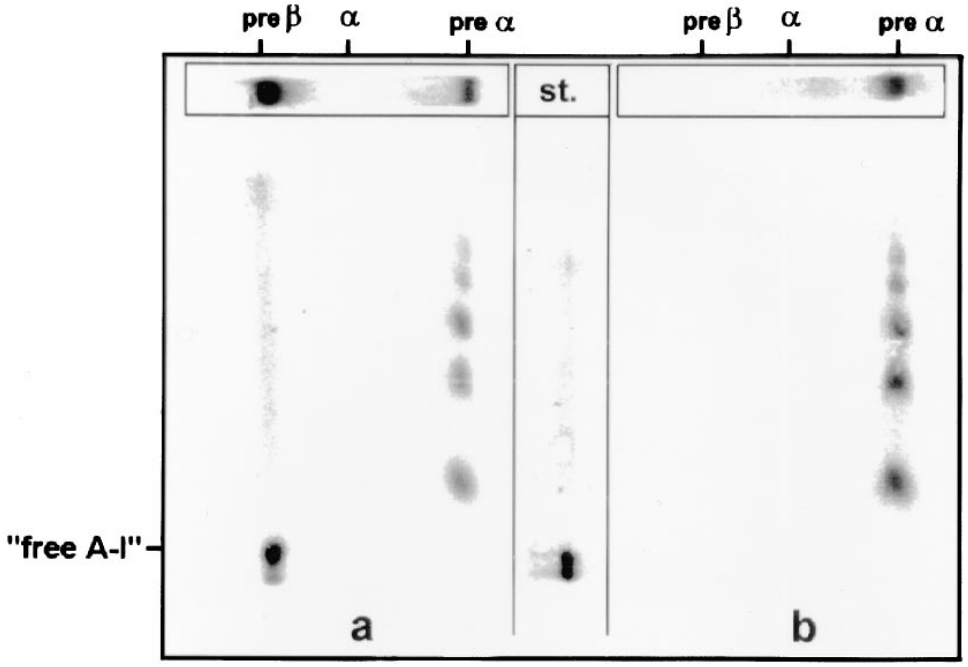

Fig. 1. Two dimensional electrophoresis of lipoproteins. a) Media containing apoA-I was incubated with ^{32}P-labeled fibroblasts. After the incubation, 15 μl of media was subjected to 2-DE. After electrophoretic transfer, the membrane was dried and subjected to phosphorimaging as described. "Free A-I" refers to lipid free apoA-I. b) Density < 1.21 g/ml lipoproteins isolated from media were subjected to 2-DE and phosphorimaging as described above. Note that the distribution of pre-α particles is similar to that of particles from the media, except free apoA-I was not present; st is lipid-free apoA-I run in the second dimension only.

and the phospholipid was separated on TLC. Because the phospholipid is labeled, the separated phospholipids could be quantified using a phosphorimager. The result, presented in **Fig. 3**, showed a typical separation of phospholipids of the different samples on TLC that was used for quantification. The phospholipid composition of media incubated with fibroblasts without apoA-I and media incubated with fibroblasts containing apoA-I is presented in **Table 1**. In the presence of apoA-I, TCA-precipitable counts were twice those recovered in media without apoA-I (3.8×10^5 vs. 7.3×10^5 cpm/ml). This suggested that phospholipid efflux from fibroblasts is doubled in the presence of apoA-I. The media phospholipid composition of effluxed phospholipid, in the presence of apoA-I, was very different from media incubated in the absence of apoA-I. In media without apoA-I, the effluxed phospholipid contained less phosphatidylcholine (45% vs. 66%), more unidentified lipid (20% vs. 7%), and more phosphatidylethanolamine (13% vs. 5%) than in media containing apoA-I.

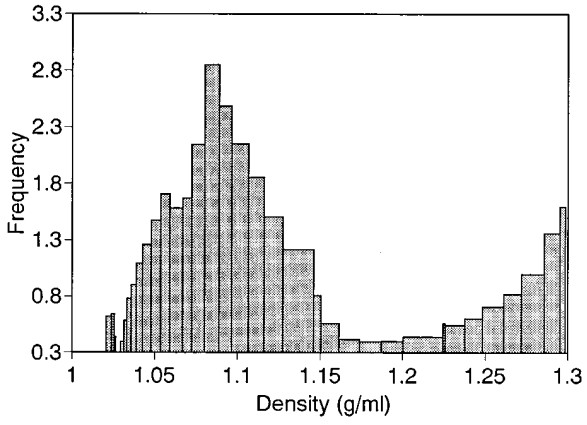

Fig. 2. Density distribution of ^{32}P-labeled lipoproteins. The lipoproteins were isolated from media containing 10 μg/ml of apoA-I and incubated with ^{32}P-labeled fibroblasts for 24 h. After the incubation, d < 1.21 g/ml lipoproteins were isolated. These lipoproteins were then subjected to density gradient ultracentrifugation as described in Methods. The density of the solutions, and the radioactivity were then determined. The result was plotted as frequency (defined as fraction of radioactivity divided by fraction of total volume) versus density.

Lipoprotein phospholipids from media, with and without apoA-I, were isolated into d < 1.21 g/ml and the d > 1.21 g/ml fractions by ultracentrifugation. With media containing no apoA-I, only 9% of the trichloroacetic acid (TCA)-precipitable radioactivity was in the d < 1.21 g/ml fraction. In contrast, 60% of the TCA-precipitable radioactivity was recovered in the d < 1.21 g/ml fraction with media containing 10 μg/ml apoA-I. The phospholipid composition of the d < 1.21 g/ml fraction isolated from media, with or without apoA-I, is presented in **Table 2**. Also included is the phospholipid composition of plasma HDL. There was a progressive increase of phosphatidylcholine from media without apoA-I to media with apoA-I to plasma HDL (54% < 69% < 81%). There was a progressive de-

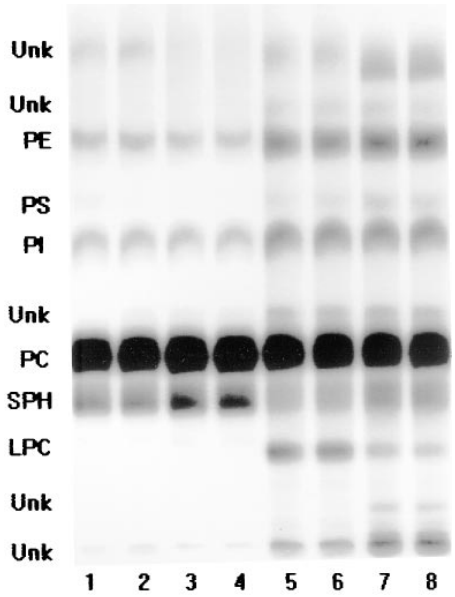

Fig. 3. Thin-layer chromatography of phospholipid. ^{32}P-labeled phospholipid was applied to thin-layer plates and the phospholipid was separated as described in Methods. The plates were then dried and subjected to phosphorimaging. PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PC: phosphatidylcholine, Sph: sphingomyelin, LPC: lysophosphatidylcholine; unk: unknown. Lanes 1, 2 are phospholipid content of media containing apoA-I after incubation with fibroblasts. Lanes 3, 4 are $d < 1.21$ g/ml lipoproteins isolated from media containing apoA-I after incubation with fibroblasts. Lanes 5, 6 are plasma membrane from fibroblasts and lanes 7, 8 are whole cell phospholipid.

crease in phosphatidylethanolamine (14% > 4% > 2%). Phosphatidylinositol was also decreased (9.9% > 3.9% > 2.8%). Sphingomyelin was the highest in HDL from media containing apoA-I (18%) and was relatively constant in the other two HDLs (13% and 14%). The phospholipid distribution in the $d > 1.21$ g/ml fraction generally reflects those of media shown in Table 1 (data not shown).

TABLE 1. Mole percent phospholipid composition of media with and without apoA-I after incubation with fibroblasts for 24 h

	Media with ApoA-I	Media without ApoA-I
	mol %	
Phosphatidylethanolamine	4.75 ± 0.69	13.3 ± 0.21
Phosphatidylserine	0.62 ± 0.26	0.47 ± 0.15
Phosphatidylinositol	4.44 ± 0.77	7.55 ± 0.36
Phosphatidylcholine	66.26 ± 1.19	45.04 ± 0.60
Sphingomyelin	16.36 ± 0.86	13.15 ± 0.19
Lysophosphatidylcholine	0.73 ± 0.05	0.46 ± 0.09
Unidentified	6.78 ± 0.5	20.03 ± 1.18
TCA-precipitable	7.3×10^5 cpm	3.8×10^5 cpm

The values are mean ± SD of three separate experiments performed in triplicate. The units are mole percent. Phospholipids, such as phosphatidylglycerol, phosphatidic acid, lysophosphatidic acid, and cardiolipin, were included in the unidentified section. Most of the unknown phospholipids are normally found in lysosomes, mitochondria, and in the cytosol. Also included is the trichloroacetic acid (TCA)-precipitable radioactivity (cpm/ml).

The phospholipid composition of whole fibroblasts, and a fraction enriched in plasma membrane (4.6-fold purification using 5′ nucleotidase as marker) was also determined. The results are summarized in **Table 3**. From the table, it can be seen that plasma membranes contained a higher concentration of phosphatidylethanolamine and phosphatidylinositol than effluxed lipids (compare Table 3 with columns 1 and 3 of Table 2). In whole cells, the concentration of phosphatidylethanolamine was further increased (to 16%) and there were more unidentified phospholipids. These would include phosphatidic acid, lysophosphatidic acid (in lysosomes), phosphatidylglycerol, and cardiolipins (the last two are found in inner mitochondrial membrane). The sphingomyelin content in both the plasma membranes and whole cells is considerably less than the 18% found in HDL of effluxed lipids when apoA-I is present.

To demonstrate that the pre-α mobility was caused by the phospholipid composition of the pre-α lipoproteins, we produced pre-α lipoproteins by incubating lipid-free apoA-I with fibroblasts. At the end of the incubation, the pre-α lipoproteins were isolated by ultracentrifugation. Protein concentration was determined for the lipoproteins before lipid was extracted from the lipoproteins. We have previously determined that all the protein in pre-α lipoproteins is apoA-I, therefore, an equivalent amount of apoA-I was added to the extracted lipid and the particles were reconstituted by cholate dialysis. As control, a similar experiment was performed with plasma HDL_3. The result, shown in **Fig. 4** confirmed our hypothesis that the mobility of the particles resides in their lipid composition.

DISCUSSION

Previously, we have determined that the native pre-α particles contained only phospholipids, free cholesterol, and apoA-I (8). We therefore postulate that the high electronegativity of our pre-α lipoprotein particles may be caused by significant differences in their phospholipid composition as compared to plasma HDL that have α mobility. We undertook a comparison of the phospholipid composition of the pre-α particles, obtained after apoA-I-containing media was incubated with fibroblasts, with that of media incubated with fibroblasts without apoA-I, plasma membrane, and whole cell. As a reference, plasma HDL phospholipid composition was also measured. Indeed, the phospholipid composition of the pre-α particles was different from media incubated with fibroblasts without apoA-I, plasma membrane, whole cell, and plasma HDL. While it has long been recognized that microdomains exist on plasma membranes and that the phospholipid distribution is asymmetric across the membrane (21), we felt that we should compare the pre-α particle phospholipid content with that of plasma membrane. We assumed that if there is no specificity in apoA-I binding to plasma membrane, the phospholipid content of the pre-α particles may resemble that of the plasma membrane. Our results suggest that such is not the case.

TABLE 2. Mole percent phospholipid composition of d < 1.21 fraction isolated from media that has been incubated with fibroblasts for 24 h

	With ApoA-I	Without ApoA-I	Plasma HDL
		mol %	
Phosphatidylethanolamine	4.10 ± 0.41	13.93 ± 0.02	2.30 ± 2.19
Phosphatidylserine	0.59 ± 0.18	0.73 ± 0.07	0.00[a]
Phosphatidylinositol	3.85 ± 0.43	9.87 ± 0.05	2.81 ± 0.04
Phosphatidylcholine	68.62 ± 0.66	54.84 ± 0.55	81.21 ± 2.91
Sphingomyelin	18.36 ± 0.42	12.78 ± 0.01	13.55 ± 0.21
Lysophosphatidylcholine	0.89 ± 0.18	0.80 ± 0.11	0.14 ± 0.08
Unidentified	3.54 ± 0.25	7.05 ± 0.34	0.00[a]
TCA-precipitable	4.38×10^5 cpm	0.34×10^5 cpm	

The values are mean ± SD of three separate experiments performed in triplicate. The units are mole percent. Phospholipids, such as phosphatidylglycerol, phosphatidic acid, lysophosphatidic acid, and cardiolipin, were included in the unidentified section. Most of the unknown phospholipids are normally found in lysosomes, mitochondria, and in the cytosol. The phospholipid content of plasma HDL was determined using non-radioactive HDL and charring as described in Methods. Also included is the trichloroacetic acid (TCA)-precipitable radioactivity (cpm/ml). Note that 60% of the media TCA-precipitable radioactivity was recovered in d < 1.21 g/ml of media with apoA-I (7.3×10^5 cpm/ml) whereas only 9% of the radioactivity was recovered in the media without apoA-I (3.8×10^5 cpm/ml).

[a] Denotes that the phospholipid lipid content fell below our ability to detect.

It should be noted here that the phospholipid composition of the pre-α particles represents a composite of five particles. We believe that the five particles may be formed by a combination of lipid and apoA-I and may not be unique. Using a defined mixture of phosphatidylcholine, cholesterol, and apoA-I to make recombinant HDLs (rHDLs), Jonas, Steinmetz, and Churgay (22) found rHDLs with three different sizes, all with α electrophoretic mobility (data not shown).

Our current understanding of cellular cholesterol efflux defines such efflux into two distinct processes: specific and non-specific. A number of investigators have provided evidence that there is a specific cholesterol efflux. This specific efflux appears to be apoA-I specific (23, 24) although other apolipoproteins have also been shown to increase cholesterol efflux (25, 26). Such an efflux can be blocked by antibody to apoA-I (27) and by trypsinization of HDL particles (24). There is also evidence for non-specific efflux that can be promoted by phospholipid micelles (28) or cyclodextrin (29). By this definition, our pre-α HDL appeared to represent only the specific cholesterol efflux because no pre-α particles were formed in the absence of apoA-I (8). Also, agarose gel electrophoresis of d < 1.21 g/ml of media incubated with fibroblasts in the absence of apoA-I did not show any consistent banding that would suggest the formation of lipoproteins. In addition, when apoA-I was added to and incubated with media that had been previously incubated with fibroblasts, no pre-α particles were formed. Although cholesterol and phospholipid efflux can occur in the absence of lipid acceptors, it is to be noted that the phospholipid species that were effluxed in the absence of apoA-I are very different from those in the presence of apoA-I (see Table 1). The most likely explanation is that apoA-I is able to bind to specific sites on the plasma membrane, thus acquiring the unusual phospholipid composition. Apolipoprotein specificity for phospholipids has been reported by Forte et al. (14) who showed that the phospholipid released in the presence of apoA-I was different from that released in the presence of apoA-II.

TABLE 3. Mole percent phospholipid composition of plasma membrane and whole cell

	Plasma Membrane	Whole Cell
	mol %	
Phosphatidylethanolamine	10.58 ± 1.28	16.04 ± 2.05
Phosphatidylserine	1.22 ± 0.49	1.03 ± 0.25
Phosphatidylinositol	9.67 ± 0.38	8.84 ± 0.07
Phosphatidylcholine	53.86 ± 1.34	46.60 ± 4.47
Sphingomyelin	7.86 ± 0.95	8.93 ± 1.29
Lysophosphatidylcholine	5.81 ± 0.92	1.25 ± 0.56
Unidentified	11.00 ± 0.34	17.31 ± 3.81

The values are mean and standard deviation of three separate experiments performed in triplicate. The units are mole percent. Phospholipids, such as phosphatidylglycerol, phosphatidic acid, lysophosphatidic acid, and cardiolipin, were included into the unidentified section. Most of the unknown phospholipids are normally found in lysosomes, mitochondria, and in the cytosol. Plasma membrane was 4.6-fold purified over whole cell as determined by 5′ nucleotidase assay.

In the presence of apoA-I, most of the phospholipids that effluxed from the cell appeared to be different from those in media without apoA-I. This is evident by comparing data from Table 1 with that from Table 2. The pre-α HDL phospholipid subspecies (Table 2) is similar to that of conditioned media (Table 1). Consistent with our previous data from dog lymph, the nascent HDL has a higher concentration of sphingomyelin. It also has higher concentrations of phosphatidylethanolamine and phosphatidylinositol (see Table 2) than plasma HDL. We recovered 60% of the phospholipid in d < 1.21 g/ml range. The remaining 40% reflects the presence of the particle containing only phospholipid–apoA-I that we found previously, and that was in the d > 1.21 g/ml range, and phospholipids released from cells not associated with apoA-I.

The high sphingomyelin content of these nascent HDL suggests that they may be poor substrates for LCAT. Indeed, Bolin and Jonas (30) have provided evidence to sug-

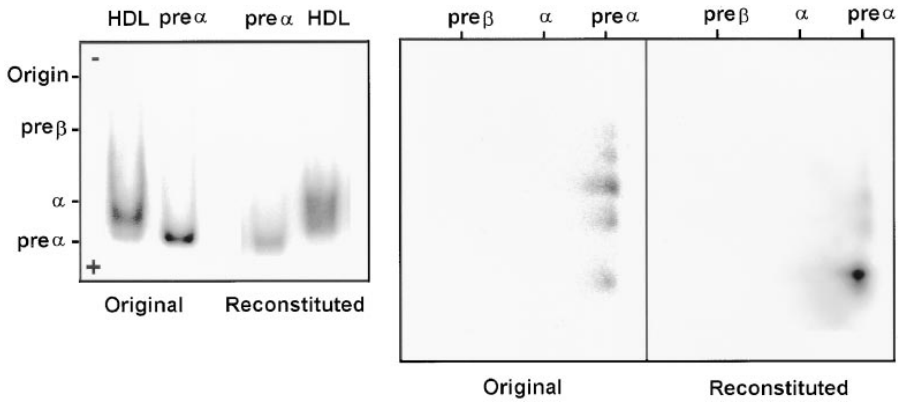

Fig. 4. One-dimensional (left) and two-dimensional (right) electrophoresis of reconstituted and original lipoproteins. Fibroblasts were labeled with [^{32}P]-phosphate and the reconstitution experiment was carried out as described in Materials and Methods. Orientation of the agarose gel is shown by − and + sign on the inside of the left panel. The left two lanes in the left panel showed 1-D of the original plasma HDL and pre-α lipoprotein (200 ng protein). The right two lanes in the left panel showed 1-D of the reconstituted pre-α lipoprotein and plasma HDL. The right panel showed the 2-D of the original and reconstituted pre-α lipoprotein. Because the polyacrylamide gels were not run to completion, no molecular weights could be determined. However, based on our previous work (8), where both 3–16% and 3–35% gradient gels were run on the same sample, we have determined the molecular weights of the particles to be 114,000, 302,000, 475,000, 592,000, and 684,000 respectively (from the lowest molecular weight particle to the highest). On 2-D, it could be seen that only four particles were recovered instead of the original five. Also, the lowest molecular weight particle is predominant in the reconstituted lipoprotein instead of the middle molecular weight particle in the original lipoprotein.

gest that high sphingomyelin content in HDL leads to a decrease in binding of LCAT to HDL resulting in lower LCAT activity. This would agree with our in vivo observation showing that interstitial fluid contains LCAT, but interstitial fluid HDL are poor substrates for LCAT (31). We should emphasize that the terms 'good' and 'poor' substrates of LCAT are kinetic terms and that the nascent HDLs are substrates for LCAT. Forte et al. (14) have shown that nascent HDLs very similar to the ones produced here could be converted to HDL_3-like particles when they were incubated with LCAT. Similarly, early work of Norum and Gjone (32) also showed that discoidal HDLs isolated from patients with LCAT deficiency could be acted on by LCAT to produce spherical particles.

Given our previous observation that four of the five pre-α lipoproteins contained apoA-I, phospholipid, and free cholesterol, and that the 5th particle (the lowest molecular weight one) contained only apoA-I and phospholipid (8), we hypothesized that the pre-α mobility of these particles may be caused by the phospholipid content of these particles. Considering the phospholipid composition of the pre-α particles (Table 2), we saw a 50% increase in phosphatidylethanolamine, inositol, and sphingomyelin, coupled with a 30% decrease in phosphatidylcholine. The surface charges of pre-β, α, and pre-α particles were determined to be −6.65 ± 0.19, −12.58 and −18.45 ± 0.54 mV, respectively. We questioned whether such a composition would be sufficient to confer the pre-α mobility on an apoA-I–phospholipid mixture. Through a series of studies, Sparks and Phillips (20), Sparks, Lund-Katz, and Phillips (33), and Davidson et al. (34) have shown that the surface charge of a lipoprotein molecule is dependent on the shape and phospholipid and cholesterol content of the lipoprotein. Four of the five particles had free cholesterol and one did not (8) and yet all five particles had exactly the same electrophoretic mobility on agarose. Therefore, we could eliminate cholesterol as having a significant impact on these particles. It is possible that the lipoproteins in the present study are discoidal, although lipid composition alone could not confirm the existence of a discoidal particle. A discoidal particle is more negatively charged (34). A lipoprotein with a high concentration of phosphatidylinositol could also have high negative charge (34). The particles we have described have 50% more inositol than normal HDL (although the phosphatidylinositol content is still less than 5% of the total phospholipid). We could not determine that this amount of phosphatidylinositol alone was sufficient to give these particles a higher electronegative charge.

We hypothesize that phospholipid alone is sufficient to give these particles pre-α mobility. We reasoned that if the lipid from these pre-α particles is reconstituted with lipid-free apoA-I to produce pre-α lipoproteins, then lipid alone is the cause of the mobility. Indeed, results in Fig. 4 confirmed our hypothesis.

In conclusion, we have provided data in this report to suggest that the high electronegativity of the pre-α particles is related to its phospholipid composition, which is most likely acquired by apoA-I binding to specific regions of the cell membrane.

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