Apolipoprotein A-I-Containing Lipoproteins, with or without Apolipoprotein A-II, as Progenitors of Pre-β High-Density Lipoprotein Particles†

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ABSTRACT: Apoliprotein A-I-(apoA-I-) containing lipoproteins isolated by immunoaffinity chromatography can be divided into two general subfractions on the basis of the presence [Lp(AI + AII)] or absence [Lp(AI – AII)] of apoA-II. The Lp(AI – AII) subfraction can be further subfractionated into two subgroups with pre-β mobility as well as those of α mobility. We have characterized the Lp(AI – AII) and Lp(AI + AII) subfractions after the removal of pre-β high-density lipoproteins (pre-β-HDL) to compare only the two subfractions with α mobility. The Lp(AI – AII) and Lp(AI + AII) of α mobility, while both heterogeneous subfractions, share many gross features in common. Both subfractions were predominantly spherical in shape, had similar conformation of apoA-I as investigated by circular dichroism and specific endoproteases, and had similar contents of phospholipids, phospholipid species, triglycerides, and cholesterol ester. However, there was significantly less protein (–10%) and more free cholesterol (+46%) in the Lp(AI – AII) subfraction than in the Lp(AI + AII) subfraction. We investigated the generation of pre-β-HDL from both the Lp(AI – AII) and Lp(AI + AII) subfractions during incubation with low-density lipoproteins and cholesteryl ester transfer protein. We found that both Lp(AI – AII) and Lp(AI + AII) subfractions were capable of generating pre-β-HDL-like particles. Our results suggest that the formation of pre-β-HDL involves dissociation of apoA-I from both Lp(AI – AII) and Lp(AI + AII) subfractions. These results refine a model describing the cycling of apoA-I between pre-β-HDL and α-HDL linked to the movement of cholesteryl esters through HDL.

High-density lipoproteins (HDL) are a collection of discrete species that vary in composition and structure. Since apolipoprotein A-I (apoA-I) is present in virtually all human HDL particles, isolation of HDL can be performed using selected affinity immunosorption which utilizes antibodies specific for apoA-I (Kunitake et al., 1982; McVicar et al., 1984). This technique allows the isolation of all the apoA-I-containing lipoproteins with minimal pertubation of their native structures.

In our laboratory we have separated apoA-I-containing lipoproteins into two subfractions, one with pre-β electrophoretic mobility (pre-β-HDL) and one with α mobility (α-HDL) (Kunitake et al., 1985).[1] Using this method, the majority of pre-β-migrating apoA-I, isolated from the plasma of fasting normal lipidemic subjects, appears to reside in particles of approximately 60 kDa (Kunitake, unpublished results). Compositional analysis of the pre-β-HDL subfraction showed that they are distinct from the α-HDL subfraction in physical structure and chemical composition (Kunitake et al., 1985, 1987, 1990a). Most important to this study is that pre-β-HDL do not contain apoA-II.

Since only a portion of apoA-I-containing lipoproteins contain apoA-II, a number of researchers have employed secondary immunoaffinity chromatography to separate the apoA-I-containing lipoproteins into two general subfractions on the basis of the absence [Lp(AI – AII)] or presence [Lp(AI + AII)] of apoA-II (Cheung & Albers, 1984; James et al., 1988; Kilsdonk et al., 1990; Luc et al., 1991; Ohta et al., 1988; Puchois et al., 1987). This separation was not intended to divide HDL into two homogeneous subfractions but into subgroups with possibly different functional properties. The chemical compositions of these two subfractions have been analyzed previously by two groups (Cheung & Albers, 1984; Kilsdonk et al., 1990). There are some apparent differences in the observations of these two reports. One group observed significant differences in the chemical composition of Lp(AI – AII) and Lp(AI + AII) (Kilsdonk et al., 1990) but the other did not (Cheung & Albers, 1984). Within the Lp(AI – AII) subfraction are pre-β-HDL, a subfraction with distinct characteristics (Kunitake et al., 1985). The removal of the pre-β-HDL subfraction would allow the direct comparison of Lp(AI – AII) and Lp(AI + AII) subfractions with α mobility.

The existence of these two types of α-HDL subfractions leads to another interesting point of inquiry. We have assembled a model in which apoA-I cycles between pre-β-HDL and α-HDL in conjunction with the generation of cholesteryl esters and their transfer from HDL (Kunitake et al., 1992). The pre-β-HDL particles are generated from α-HDL during the transfer of cholesteryl esters to acceptor lipoproteins. It is unknown whether pre-β-HDL arise from Lp(AI – AII) or Lp(AI + AII) exclusively or whether pre-β-HDL can be generated from both types of α-HDL.

In this study we analyzed the chemical composition and physical structure of two α-migrating Lp(AI – AII) and Lp(AI + AII) subfractions in order to investigate the generation of pre-β-HDL-like particles from these subfractions with α mobility.

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[1] The precise definition of HDL is those lipoproteins ultracentrifugally isolated in the density interval 1.063–1.21 g/mL. Lipoproteins isolated by selected affinity immunosorption all contain apoA-I. As apoA-I is the dominant protein in HDL, we used HDL as shorthand for the immunoisolated particles. We then named the α-HDL and pre-β-HDL subfractions on the basis of their general electrophoretic mobilities. These terms are not intended to imply any structural feature or lipid content to the two subfractions.

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EXPERIMENTAL PROCEDURES

Lipoprotein Isolation

Blood Drawing. Plasma was obtained from the venous blood of fasting healthy volunteers (both male and female). Immediately after the blood was drawn, the following preservatives were added to the indicated final concentrations: ethylenediaminetetraacetic acid (EDTA; 0.08%), gentamicin (10.0 μg/mL), phenylmethanesulfonyl fluoride (PMSF; 10 μg/mL), benzamidine (250 μg/mL), ϵ-aminocaproic acid (2 mg/mL), and sodium azide (0.1%). Plasma was isolated by low-speed centrifugation at 1000*g* for 45 min at 4 °C. The above listed preservatives and α-2 macroglobulin (10 μg/mL) were added to all subsequently isolated lipoprotein fractions.

Isolation of Anti-ApoA-II Antibodies. HDL ($1.063 < d < 1.21$ g/mL) were isolated from plasma by sequential ultracentrifugation (Havel et al., 1955). ApoA-II was isolated from delipidated apoHDL in two steps; first by gel filtration on a Sephadex G-100 (Pharmacia) column and second by passage over an anti-apoA-I affinity column to ensure complete removal of apoA-I. The purity of apoA-II was evaluated by western blots (Towbin et al., 1979) using apoA-II antibodies (Boehringer-Mannheim, Indianapolis, IN) and apoA-I antibodies (Tago, Burlingame, CA). The purified apoA-II was then used for the generation of antisera in rabbits. Monospecificity of the resulting serum was verified by immunoelectrophoresis and western blots (Towbin et al., 1979) of HDL, very low (VLDL), and low-density lipoproteins (LDL). Selected-affinity immunosorption columns directed against apoA-II were made in a similar manner to our columns directed against apoA-I (McVicar et al., 1984). Briefly, antibodies to apoA-II were selected from antisera by using an apoHDL–Sepharose column. The column was washed with 150 mM NaCl and 5 mM Tris, pH 7.4 (TBS buffer) and a pauciclonal fraction of antibodies was eluted with 0.2 M acetic acid, pH 3.0. The antibodies were immediately neutralized with 2 M Tris, dialyzed against phosphate-buffered saline, pH 6.5, and conjugated to cyanogen bromide-(CNBr-) activated Sepharose 4B (Pharmacia).

Isolation of α-ApoA-I-Containing Lipoproteins with ApoA-II and without ApoA-II. All the apoA-I-containing particles [(Lp(AI)] were isolated from plasma by selected affinity immunosorption (Kunitake et al., 1982; McVicar et al., 1984). Lp(AI) were then separated on the basis of their electrophoretic mobilities (pre-β and α) by starch block electrophoresis as previously described (Kunitake et al., 1985).

The α-migrating Lp(AI) were further separated into LpAI without apoA-II [Lp(AI – AII)] and with apoA-II [Lp(AI + AII)] by use of the secondary selected-affinity immunosorption column directed against apoA-II. The α-migrating subfraction was applied to the immunoaffinity column and washed with TBS buffer; the unbound fraction was recovered and contain Lp(AI – AII). Lp(AI + AII) were recovered by elution of the column with 0.2 M acetic acid, pH 3.0, and then neutralized immediately with 2 M Tris, pH 10.0. The samples were concentrated and exchanged into TBS buffer with stirred concentrators fitted with YM10 membranes (Amicon, Beverly, MA) before compositional analysis. Recovery of the apoA-I-containing lipoproteins after passage over an anti-apoA-II immunoaffinity column chromatography was 95.2% ($n = 2$, data not shown). The absence of apoA-II in the Lp(AI – AII) subfraction was confirmed by western blot analysis (Towbin et al., 1979) (data not shown). In addition, we confirmed by immunoelectrophoresis that the two subfractions both retained α mobility (Figure 1).

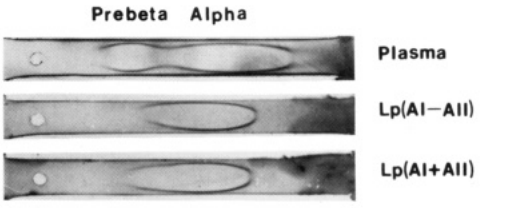

FIGURE 1: Immunoelectrophoresis of Lp(AI – AII) and Lp(AI + AII) subfractions. After isolation by secondary immunoaffinity chromatography, Lp(AI – AII) and Lp(AI + AII) were analyzed by immunoelectrophoresis using antisera against apoA-I. Whole plasma is shown for comparison.

Analysis of Isolated Lp(AI – AII) and Lp(AI + AII)

Chemical Analysis. Chemical composition of the Lp(AI – AII) and Lp(AI + AII) were determined in six normolipidemic subjects (four males and two females). Protein content was determined by the method of Lowry et al. (1951). Cholesterol and cholesteryl ester were measured by a fluorometric enzymatic method (Huang et al., 1975), triglyceride was measured by an enzymatic method (Nemeth et al., 1986), and phospholipid was determined from the measurement of lipid-associated phosphorus content (Stewart & Hendry, 1935). Phospholipid species were analyzed by thin-layer chromatography as described by Touchstone et al. (1980). ApoA-I and ApoE contents in the Lp(AI – AII) and Lp(AI + AII) subfractions were determined by radial immunodiffusion (Tago, Burlingame, CA) and by radioimmunoassay (Fainaru et al., 1977), respectively. Statistical analyses were performed using the nonparametric Wilcoxon test. Results were considered significantly different when $P < 0.05$.

Electrophoretic Analysis. The electrophoretic mobilities of the isolated subfractions were determined by agarose electrophoresis and by immunoelectrophoresis in agarose. Agarose electrophoresis was performed as previously described (Pagnan et al., 1977) except that the agarose strips were stained with a solution of 0.2% Amido Black in water–methanol–acetic acid (5:5:1). Immunoelectrophoresis was performed as described by Grabar and Williams (1955) using antisera directed against apoA-I (Tago, Burlingame, CA).

The apolipoproteins present in each subfraction were determined by electrophoresis in 5–25% sodium dodecyl sulfate–polyacrylamide gradient gels (SDS–PAGE) (Laemmli, 1970) in 0.1% sodium dodecyl sulfate, 0.025 M Tris-HCl, and 0.192 M glycine buffer, pH 8.9. For each subfraction, equal amounts of total protein were loaded onto the gels. The samples were prepared by the addition of an equal volume of solubilizing buffer containing 4% SDS, 0.125 M Tris, and 20% glycerol, pH 6.8, and were heated at 95 °C for 30 s. The identity of apoA-I and apoA-II in the two subfractions was determined by western blot analysis (Towbin et al., 1979).

Particle Size Determination. The apparent diameters and molecular weights of the Lp(AI – AII) and Lp(AI + AII) subfractions were estimated by gel-permeation chromatography and nondenaturing gradient polyacrylamide gel electrophoresis. Gel-permeation chromatography was performed with a Pharmacia Superose 12 column (Uppsala, Sweden) eluted with TBS buffer at a flow rate of 0.4 mL/min. The globular proteins ovalbumin (45 kDa), human albumin (65.6 kDa), transferrin (80 kDa), and ceruloplasmin (151 kDa) were used as standards. The elution volumes were plotted against the log M_r. Nondenaturing gradient polyacrylamide gel electrophoresis was performed using a 6–34% polyacrylamide gradient minigel (Bio-Rad) as previously described (Blanche et al., 1981). The calibrating proteins (Pharmacia)

thyroglobulin (17.0 nm) apoferritin (12.2 nm) catalase (10.4 nm) lactate dehydrogenase (8.2 nm), and bovine albumin (7.5 nm) were used as standards. Particle migration was plotted against the log of the diameter.

Physical Measurements. Circular dichroic measurements of the pre-β-HDL, α-HDL, Lp(AI – AII), and Lp(AI + AII) subfractions were performed between 240 and 200 nm on a Jasco J-500A circular dichrometer. The lipoproteins were diluted to a protein concentration of 100 μg/mL in 20 mM sodium phosphate buffer, pH 7.4. After dilution and circular dichroic analysis, the protein concentration of each sample was quantitated. Helicity was estimated for each lipoprotein subfraction on the basis of $[\theta]_{222}$.

Electron micrographs of the lipoproteins were also obtained to visualize the particles. The lipoproteins were negatively stained with 2% potassium phosphotungstate and photographed with a Siemens 101 electron microscope (Chen et al., 1984).

Limited Proteolysis of Lp(AI – AII) and Lp(AI + AII) Subspecies. The Lp(AI – AII) and Lp(AI + AII) subfractions were tested for susceptibility to limited protease digestion by various enzymes: 14 milliunits of plasmin (Boehringer-Mannheim, Mannheim, Germany), 2 μg of elastase (Boehringer-Mannheim, Mannheim, Germany), 1.2 units of thrombin (Sigma, St. Louis, MO), 10 μg of α-chymotrypsin (Worthington), 1.0 unit of arginine C-endoprotease (Sigma), 0.08 unit of *Staphylococcus aureus* V8 protease/50 mg of protein (Pierce), and 0.02 unit of thermolysin/50 mg of protein (Boehringer-Mannheim, Mannheim, Germany). Aliquots of 50 μg of total protein were incubated in 10 mM Tris-HCl, pH 8.0, in the absence or presence of one of the enzymes for 1 h at room temperature with the exception of plasmin, which was incubated at 4 °C. The enzyme reactions were terminated by boiling with sodium decyl sulfate (final concentration of 0.1%) for 1 min. The apoproteins were analyzed by SDS–PAGE. Each lane contained an amount of protein equivalent to 5 μg based on the protein content measured before the incubation with proteases.

Incubation of Lp(AI – AII) and Lp(AI + AII) Subfractions

To test whether pre-β-HDL-like particles can be generated from Lp(AI – AII) and Lp(AI + AII), each subfraction was incubated with cholesteryl ester transfer protein (CETP, recombinant, provided by Dr. P. Hass, Genentech, South San Francisco, CA) and LDL in the presence of protease inhibitors. LDL ($1.019 < d < 1.063$ g/mL) was isolated from plasma by ultracentrifugation (Havel et al., 1955). Aliquots (50 μg) of the Lp(AI – AII) or Lp(AI + AII) particles were incubated with 1 μg of CETP and 500 μg of the LDL fraction for 5 h at 37 °C. Lp(AI – AII) and Lp(AI + AII) particles without the additions of CETP and LDL fraction were also incubated at 37 °C. After incubation, each incubation mixture (5 μg of apoA-I) was analyzed by immunoelectrophoresis with either a goat anti-apoA-I or a rabbit anti-apoA-II antibody. The gels were washed to remove nonprecipitating protein and stained with 0.2% Amido Black. The incubation mixture was also analyzed by gel-permeation chromatography using a Superose 12 column (Pharmacia).

RESULTS

The two major Lp(AI – AII) and Lp(AI + AII) subfractions have many gross features in common. Both subfractions appeared to contain predominantly spherical particles when viewed by electron microscopy (Figure 2). The calculated

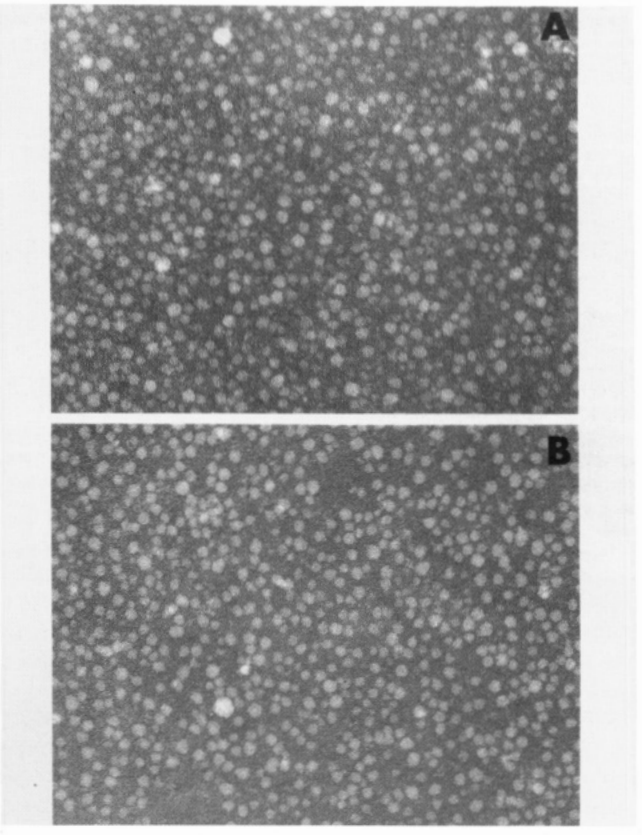

FIGURE 2: Electron micrographs of Lp(AI – AII) and Lp(AI + AII) subfractions. Lp(AI – AII) (A) and Lp(AI + AII) (B) were negatively stained with phosphotungstate and electron micrographs were taken. The magnification factor is 156 000.

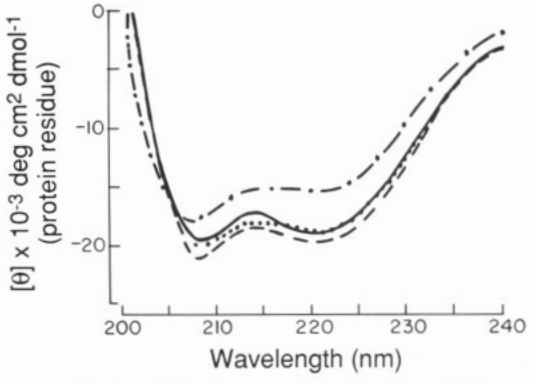

FIGURE 3: Circular dichroic measurements of apoA-I-containing lipoproteins. The ellipticities of (A) pre-β-HDL (- • -), (B) α-HDL (···), (C) Lp(AI – AII) (- - -), and (D) Lp(AI + AII) (—) were measured from 240 to 200 nm.

helicity (ellipticity at 222 nm) from circular dichroic measurements of the two subfractions was only different by 3% in contrast to the difference in helicity (–32%) when either subfraction was compared with the pre-β-HDL subfraction (Figure 3). The degree of sensitivity to endoprotease digestion of apoA-I in both Lp(AI – AII) and Lp(AI + AII) subfractions with a variety of enzymes was similar (judged by the intensity of Coomassie blue staining; Figure 4) as opposed to pre-β-HDL, which have increased sensitivity to digestion (Kunitake et al., 1987). The Lp(AI – AII) and Lp(Al + AII) subfractions were similar in phospholipids, phospholipid species (data not shown), cholesteryl ester, and triglyceride content (Table I).

However, with respect to apparent size the Lp(AI – AII) and Lp(AI + AII) subfractions were found to be slightly different as determined by gel-permeation chromatography and by nondenaturing gradient gel electrophoresis. The Lp-

Table I: Composition of Lp(AI – AII) and Lp(AI + AII) Subspecies[a]

	protein	phospholipids	free cholesterol	cholesteryl ester	triglycerides
Lp(AI – AII)	50.0 ± 7.8[b]	25.9 ± 2.6	3.5 ± 1.3[b]	16.1 ± 4.9	4.4 ± 1.8
Lp(AI + AII)	55.5 ± 10.8	22.7 ± 5.7	2.4 ± 0.8	15.4 ± 5.7	3.9 ± 1.5

[a] Values are expressed as % mass (mean ± SD, n = 6 subjects). [b] Significantly different from Lp(AI + AII), $P < 0.05$.

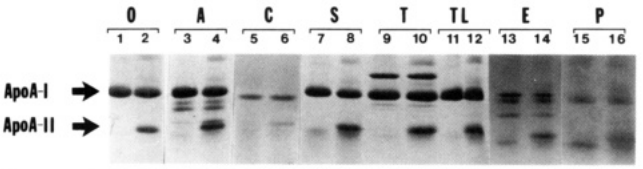

FIGURE 4: Endoprotease digestion of apoA-I in Lp(AI – AII) and Lp(AI + AII) subfractions. After Lp(AI – AII) and Lp(AI + AII) were subjected to limited digestion by a battery of endoproteases, samples were analyzed by 5–25% SDS–PAGE. Odd numbers represent the Lp(AI – AII) subfraction and even numbers represent the Lp(AI + AII) subfraction. O = control, i.e., no enzyme, A = arginine C, C = chymotrypsin, S = *Staphylococcus* protease, T = thrombin, TL = thermolysis, E = elastase, P = plasmin.

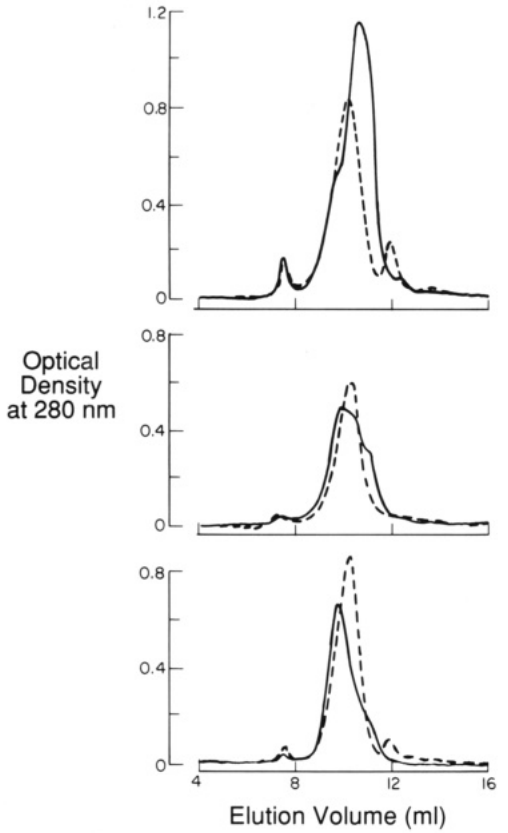

FIGURE 5: Gel-permeation chromatography of isolated Lp(AI – AII) and Lp(AI + AII) subfractions. The elution profiles (OD_{280nm}) of isolated Lp(AI – AII) (—) were compared with the elution profile of isolated Lp(AI + AII)(- - -) from three subjects. The profiles were generated by passage of the subfractions through a 1.0 × 30.0 cm Superose 12 column using TBS elution buffer. The peaks having retention volume of approximately 8 mL represent those particles eluting in the void volume; in the case of Lp(AI – AII) and Lp(AI + AII), it represents a few large apoA-I-containing lipoproteins.

(AI – AII) subfractions in all subjects were generally composed of two major size populations as evidenced by a bimodal elution profile obtained from gel-permeation chromatography (Figure 5, elution volumes of 10 and 11 mL) and two major bands observed on nondenaturing gels (Figure 6; lanes 1, 3, and 5). In contrast, the Lp(AI + AII) subfractions in the subjects were generally composed of one major size population of intermediate size as evidenced by the large, single peak obtained from gel-permeation chromatography (Figure 5;

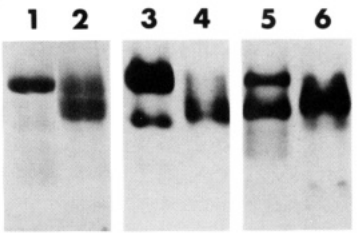

FIGURE 6: Particle sizes of Lp(AI – AII) and Lp(AI + AII) subfractions. Isolated Lp(AI – AII) and Lp(AI + AII) subfractions from three subjects were run on a 6–34% nondenaturing polyacrylamide gel. Lanes 1, 3, and 5 are Lp(AI – AII) and lanes 2, 4, and 6 are Lp(AI + AII).

elution volume of 10.6 mL) and one major band observed on nondenaturing gels (Figure 6, lanes 4 and 6). In only one of our subjects (Figure 6, lane 2) did the Lp(AI + AII) subfraction appear to have two major bands. In addition, we observed that the proportion of large and small particle populations in Lp(AI – AII) subfractions varied between individuals (Figure 5, lanes 1, 3, and 5). The apparent molecular masses determined by gel permeation were 227 ± 12.1 and 105 ± 17.2 kDa (SD, n = 3,) for the two major size populations in Lp(AI – AII) and 166 ± 11.5 kDa for the major size population in Lp(AI + AII). Similarly, the Stokes diameters of the major size populations determined from nondenaturing gels were 11.4 ± 0.1 and 9.0 ± 0.1 nm for the two Lp(AI – AII) subfractions and 9.7 ± 0.2 nm (SD, n = 3) for the major band of the Lp(AI + AII) subfraction. The size distributions of the Lp(AI – AII) and Lp(AI + AII) subfractions are not meant to indicate that these size populations are homogeneous HDL species since we recognize that protein heterogeneity still exits within these size populations.

Certain components of Lp(AI – AII) and Lp(AI + AII) subfractions also differed between the two subfractions. Lp-(AI – AII) contained 46% more free cholesterol and 10% less protein than Lp(AI + AII) (Table I). The apoA-I content relative to the particle protein content in Lp(AI – AII) was 11% higher (67.1 ± 14.9 vs 60.4 ± 14.7, SD, n = 6) than in Lp(AI + AII). In contrast the relative apoE content was 59% lower (0.03 ± 0.02 vs 0.08 ± 0.05, n = 5) in Lp(AI – AII) than in Lp(AI + AII).

To investigate the generation of pre-β-HDL-like particles, we incubated α-migrating Lp(AI – AII) and Lp(AI + AII) subfractions with LDL and CETP. Pre-β-HDL-like particles were detected in both incubation mixtures (Figure 7). No apoA-II was detected in the pre-β zone (not shown). In addition, no pre-β-HDL-like particles were detected when Lp(AI – AII) or Lp(AI + AII) was incubated alone (Figure 7). Further evidence corroborating the generation of pre-β-HDL-like particles was obtained from analysis of the incubated mixtures by gel-permeation chromatography. We observed the generation of particles with a similar size to pre-β-HDL isolated from plasma (Figure 8). Insufficient amounts of pre-β-HDL-like particles were generated to allow absolute identification.

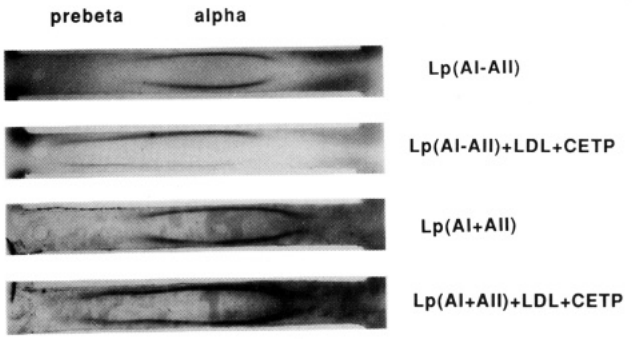

FIGURE 7: Generation of pre-β-HDL-like particles from Lp(AI – AII) and Lp(AI + AII) after incubation either alone or with $d <$ 1.063 g/mL lipoproteins and CETP for 5 h at 37 °C were analyzed by immunoelectrophoresis with antiserum directed against apoA-I. These findings are representative of the results obtained from three separate experiments.

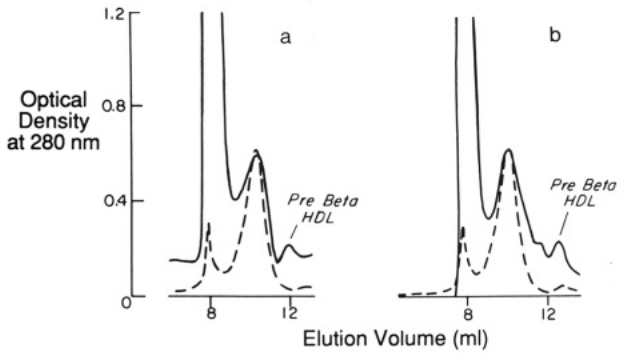

FIGURE 8: Gel-permeation chromatography of incubated Lp(AI – AII) and Lp(AI + AII). The Lp(AI – AII) and Lp(AI + AII) subfractions (100 μg) incubated with CETP (8 μg) and LDL (1 mg) (shown in Figure 7), were analyzed by gel-permeation chromatography using a Superose 12 column. The elution profile (OD_{280nm}) of the isolated (A) Lp(AI – AII) (- - -) is compared with the elution profile of the incubated Lp(AI – AII) + CETP + LDL (—) mixture. (B) Lp(AI + AII) (- - -) is compared with the elution profile of the incubated Lp(AI + AII) + CETP + LDL mixture (—). The elution volume of isolated pre-β-HDL is indicated by the arrow. The peaks having retention volume of approximately 8 mL represent those particles eluting in the void volume; in the case of Lp(AI – AII) and Lp(AI + AII), it represents a few large apoA-I-containing lipoproteins, and in the case of the mixture it represents mostly the LDL.

DISCUSSION

The separation of apoA-I-containing lipoproteins into two major heterogeneous subfractions, Lp(AI – AII) and Lp(AI + AII), has been performed by a number of groups (Cheung & Albers, 1984; James et al., 1988; Kilsdonk et al., 1990; Luc et al., 1991; Ohta et al., 1988; Oikawa et al., 1993; Puchois et al., 1987). There is significant evidence to indicate that these two subfractions differ with respect to a number of biological activities, such as choleseterol efflux, membrane binding, and metabolism (Barbaras et al., 1987; Barkia et al., 1988, 1991; De Crom et al., 1989; Kilsdonk et al., 1990; Rader et al., 1991; Schaefer et al., 1991). The results, however, are varied between groups. It is possible that this variation between groups may be an outcome of differences in the isolation methods and sources of the two subfractions.

ApoA-I-containing lipoproteins without apoA-II consist of both pre-β-HDL and α-HDL. Although pre-β-HDL constitute only 5% of the total apoA-I mass in the plasma of normolipidemic subjects (Ishida et al., 1987; Neary et al., 1991), they can account for approximately 12% of apoA-I mass in the Lp(AI – AII) fraction. Since pre-β-HDL are significantly different from α-HDL, we felt that removal of pre-β-HDL would allow a comparison of the two α populations of particles (those with and without apoA-II).

The α-migrating Lp(AI – AII) and Lp(AI + AII) subfractions had similar helical content and conformation of the apoA-I but had a different chemical composition. In our study group the protein content was 10% lower and the free cholesterol content was 46% higher in the Lp(AI – AII) than the Lp(AI + AII) subfraction. The higher content of free cholesterol found in the Lp(AI – AII) subfraction noted in this study and previous studies (Kilsdonk et al., 1990; Ohta et al., 1988) would support the observations that Lp(AI – AII) particles actively accept cellular free cholesterol (Barbaras et al., 1987; Barkia et al., 1988, 1991; Francone et al., 1989; Miida et al., 1990).

Previous studies have reported on the chemical composition of Lp(AI – AII) and Lp(AI + AII) subfractions; however, some observations in these reports are in apparent disagreement. Specifically, one group observed significant differences in the protein and free cholesterol content of Lp(AI – AII) and Lp(AI + AII) subfractions (Kilsdonk et al., 1990) but the other did not (Cheung & Albers, 1984). In this study we found a significant difference in the chemical composition of Lp(AI – AII) and Lp(AI + AII), agreeing with the results of Kilsdonk et al. (1990). The reason for the apparent discrepancy may be related to isolation procedure and selection of subjects for analysis. Kilsdonk et al. (1990) isolated HDL from plasma first by gel filtration and then by secondary anti-apoA-I and anti-apoA-II immunoaffinity chromatography, and this may have removed a portion or all of the pre-β-HDL in the plasma sample. The removal of pre-β-HDL could lower the protein content content in the Lp(AI – AII) subfraction, whereas the possible reason for the lack of any differences in chemical composition of the two subfractions reported could be due to the presence of pre-β-HDL in the Lp(AI – AII) since Lp(AI – AII) was isolated directly from plasma.

The compositional and functional differences reported between Lp(AI – AII) and Lp(AI + AII) subfractions could also be dependent on the subject. In our study we found that the proportion of large to small particles in the Lp(AI – AII) fraction varied between subjects. Similar particle size variation in the Lp(AI – AII) subfraction between subjects has also been reported by others (Cheung & Albers, 1984; James et al., 1988; Kilsdonk et al., 1990; Ohta et al., 1988). This variation in particle sizes in the Lp(AI – AII) subfraction adds an additional complication which could potentially mask compositional differences between the two subfractions or alter the function of the subfraction.

In addition to comparing the composition of Lp(AI – AII) and Lp(AI + AII) subfractions of α mobility, we wanted to investigate the ability of each fraction to generate pre-β-HDL. The importance of the α-migrating Lp(AI – AII) and Lp(AI + AII) in the interconversion of HDL subspecies is unclear. On the basis of the findings of a number of investigators (Castro & Fielding, 1988; Cheung & Wolf, 1989; Dieplinger et al., 1985; Eisenberg et al., 1978; Francone et al., 1989; Gambert et al., 1982; Holmquist & Carlson, 1985; Hopkins & Barter, 1984; Hopkins et al., 1985; Jonas et al., 1988, 1990; Lagrost et al., 1990; Nichols et al., 1981, 1984, 1985, 1987; Rajaram & Barter, 1986; Roheim, 1986; Rose & Ellerbe, 1982) as well as our own, we have assembled a model in which apoA-I cycles between pre-β-HDL and α-HDL in connection with the movement of cholesteryl esters through HDL (Figure 9) (Kunitake et al., 1992). As free cholesterol, derived from donors, enters the HDL and is esterified by lecithin:cholesterol acyltransferase (LCAT), pre-β-HDL are converted into α-HDL. Conversely, when cholesteryl esters that have

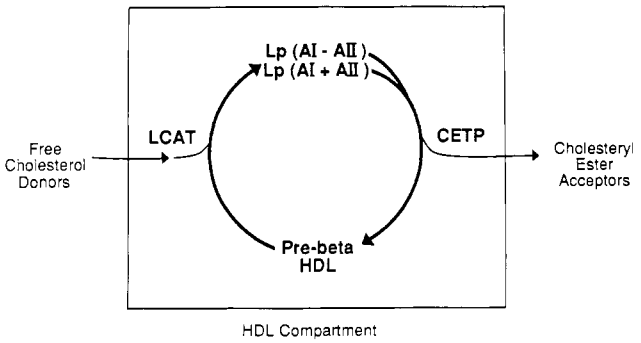

FIGURE 9: Refinement of previously proposed model (Kunitake, 1992) for the interconversion of pre-β-HDL and α-HDL. ApoA-I cycles between pre-β-HDL and Lp(AI – AII) and Lp(AI + AII) in response to the movement of cholesteryl esters through the HDL compartment. Adding cholesteryl esters to the HDL compartment (by LCAT-catalyzed esterification of free cholesterol) increases the proportion of α-HDL, whereas removing cholesteryl esters from the HDL compartment (by the CETP-mediated transfer to VLDL and LDL) increases the proportion of pre-β-HDL.

accumulated in HDL are transferred to acceptor lipoproteins (VLDL and LDL) by the action of CETP, pre-β-HDL are generated from α-HDL.

The generation of pre-β-HDL from α-HDL is not well understood. Pre-β-HDL could be formed by the removal of lipids from selected particles of Lp(AI – AII) or Lp(AI + AII) exclusively. On the other hand, it is possible that the pre-β-HDL is formed by dissociation of apoA-I from both α-HDL subfractions following removal of cholesteryl esters.

We hoped to further define this process by studying the Lp(AI – AII) and Lp(AI + AII) subfractions separately. We found that pre-β-HDL-like particles were generated from both Lp(AI – AII) and Lp(AI + AII) subfractions upon incubation with CETP and LDL. Evidence that these particles generated from Lp(AI – AII) and Lp(AI + AII) are pre-β-HDL-like and involve the transfer of lipid is supported by three findings. First, the particles had the same apparent size as purified pre-β-HDL. Second, no apoA-II was detected in the pre-β zone, implying that the particles contain only apoA-I and are not a degradation artifact. Third, when the Lp(AI – AI) and Lp(AI + AII) samples were incubated in the absence of CETP and LDL, no pre-β-HDL-like particles were generated. Since the generation of pre-β-HDL-like particles was dependent on CETP and LDL, we believed lipid transfer was involved. In analogous incubations involving LDL from patients with Tangier disease, we observed that the cholesteryl ester content of the LDL increased by 50%, indicating that cholesteryl esters were transferred from HDL to LDL (Kunitake et al., 1990b).

The generation of pre-β-HDL-like particles from both Lp(AI – AII) and Lp(AI + AII) refines the previous cyclical model of apoA-I movement between pre-β-HDL and α-HDL. When cholesteryl esters that have accumulated in the HDL are transferred to acceptor lipoproteins (VLDL and LDL) by the action of CETP, pre-β-HDL can be generated from both Lp(AI – AII) and Lp(AI + AII) (Figure 9). This observation suggests the mechanism by which pre-β-HDL are generated is by the dissociation of apoA-I (and a small amount of lipid) from all α-migrating HDL as their cholesteryl esters are transferred.

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REFERENCES

Barbaras, R., Puchois, P., Fruchart, J., et al. (1987) Cholesterol efflux from cultured adipose cells is mediated by LpAI particles but not by LpAI:AII particles, *Biochem. Biophys. Res. Commun. 142*, 63–69.

Barkia, A., Barbaras, R., Ghalim, N., et al. (1988) Effect of different APO A-I containing lipoprotein particles on reverse cholesterol transport in fat cells, *Hormone Metab. Res. Suppl. 19*, 10–12.

Barkia, A., Puchois, P., Ghalim, N., et al. (1991) Differential role of apolipoprotein AI-containing particles in cholesterol efflux from adipose cells, *Atherosclerosis 87*, 135–146.

Blanche, P., Gong, E., Forte, T., et al. (1981) Characterization of human high density lipoproteins by gradient gel electrophoresis, *Biochim. Biophys. Acta 665*, 408–419.

Castro, G., & Fielding, C. (1988) Early incorporation of cell-derived cholesterol into pre-β-migrating high density lipoprotein, *Biochemistry 27*, 25–29.

Chen, G. C., Kane, J. P., & Hamilton, R. L. (1984) Thermal behavior of cores of human serum triglyceride-rich lipoproteins: A study of induced circular dichroism of β-carotene, *Biochemistry 23*, 1119–1124.

Cheung, M., & Albers, J. (1984) Characterization of lipoprotein particles isolated by immunoaffinity chromatography, *J. Biol. Chem. 259*, 12201–12209.

Cheung, M. C., & Wolf, A. C. (1989) In vitro transformation of apoA-I-containing lipoprotein subpopulations: role of lecithin:cholesterol acyltransferase and apoB-containing lipoproteins, *J. Lipid Res. 30*, 499–509.

De Crom, M., Van Haperen, M., Puchois, P., et al. (1989) Binding characteristics of high density lipoprotein subclasses to porcine liver, adrenal and skeletal muscle plasma membranes, *Int. J. Biochem. 21*, 649–656.

Dieplinger, H., Zechner, R., & Kostner, G. M. (1985) The in vitro formation of HDL_2 during the action of LCAT: the role of triglyceride-rich lipoproteins, *J. Lipid Res. 26*, 273–282.

Eisenberg, S., Chajek, T., & Deckelbaum, R. (1978) Molecular aspects of lipoproteins interconversion. *Pharm. Res. Commun. 10*, 729–738.

Fainaru, M., Havel, R., & Imaizumi, K. (1977) Radioimmunoassay of arginine-rich apolipoprotein of rat serum. *Biochim. Biophys. Acta 490*, 144–155.

Francone, O., Gurakar, A., & Fielding, C. (1989) Distribution and functions of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in plasma lipoproteins, *J. Biol. Chem. 264*, 7066–7072.

Gambert, P., Lallemant, C., Athias, A., et al. (1982) Alterations of HDL cholesterol distribution induced by incubation of human serum. *Biochim. Biophys. Acta 713*, 1–9.

Grabar, P., & Williams, C. (1955) Methode immunoelectrophoretique d'analyse de melanges de substances antigeniques, *Biochim. Biophys. Acta 17*, 67–74.

Havel, R. J., Eder, H. A., & Bragdon, J. P. (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins of human sera, *J. Clin. Invest. 34*, 1345–1353.

Holmquist, L., & Carlson, L. A. (1985) Subfractionation and characterization of native and enlarged human plasma high density lipoprotein particles by high performance gel filtration, *Lipids 20*, 378–388.

Hopkins, G. J., & Barter, P. J. (1984) Capacity of lipoproteins to act as substrates for lecithin:cholesterol acyltransferase, *Biochim. Biophys. Acta 794*, 31–40.

Hopkins, G. J., Chang, L. B. F., & Barter, P. J. (1985) Role of lipid transfers in the formation of a subpopulation of small high density lipoproteins, *J. Lipid Res. 26*, 218–229.

Huang, H., Kuan, J., & Guilbault, G. (1975) Fluorometric enzymatic determination of total cholesterol in serum, *Clin. Chem. 21*, 1605–1608.

Ishida, B. Y., Frolich, J., & Fielding, C. J. (1987) Pre-β-migrating high density lipoprotein: quantitation in normal and hyperlipidemic plasma by solid phase radioimmunoassay following electrophoretic transfer, *J. Lipid Res. 28*, 778–786.

James, R. W., Hochstrasser, D., Tissot, J.-D., et al. (1988) Protein heterogeneity of lipoprotein particles containing apolipoprotein A-I without apolipoprotein A-II and apolipoprotein A-I with apolipoprotein A-II isolated from human plasma, *J. Lipid Res. 29*, 1557–1571.

Jonas, A., Kezdy, K. E., Williams, M. I., et al. (1988) Lipid transfers between reconstituted high density lipoprotein complexes and low density lipoproteins: effects of plasma protein factors, *J. Lipid Res. 29*, 1349–1357.

Jonas, A., Hefele Wald, J. H., Harms Toothill, K. L., et al. (1990) Apolipoprotein A-I structure and lipid properties in homogeneous, reconstituted sperhical and discoidal high density lipoproteins, *J. Biol. Chem. 265*, 22123–22129.

Kilsdonk, E., Van Gent, T., & Van Tol, A. (1990) Characterization of human high-density lipoprotein subclasses LPA-I and LPA-I/A-II and binding to HepG2 cells, *Biochim. Biophys. Acta 1045*, 205–212.

Kunitake, S., McVicar, J., Hamilton, R., et al. (1982) Isolation of human high density lipoproteins by immunoaffinity chromatography, *Circulation (Suppl II) 66*, 240.

Kunitake, S., La Sala, K., & Kane, J. (1985) Apolipoprotein A-I-containing lipoproteins with pre-β electrophoretic mobility, *J. Lipid Res. 26*, 549–555.

Kunitake, S., La Sala, K., Mendel, C., et al. (1987) Some unique properties of apoA-I containing lipoproteins with pre-β electrophoretic mobility, in *Proceedings of the workshop on lipoprotein heterogeneity 87–2646* (Lippel, K., Ed.) pp 419–428, National Institutes of Health, Bethesda, MD.

Kunitake, S., Chen, G., Kung, S., et al. (1990a) Pre-β high density lipoprotein; unique disposition of apolipoprotein A-I increases susceptibility to proteolysis, *Arteriosclerosis 10*, 25–30.

Kunitake, S. T., Young, S. G., Chen, G. C., et al. (1990b) Conformation of apolipoprotein B-100 in the low density lipoproteins of Tangier disease, *J. Biol. Chem. 265*, 20739–20746.

Kunitake, S., Mendel, C., & Hennessy, L. (1992) Interconversion between apolipoprotein A-I-containing lipoproteins of pre-β and α electrophoretic mobilities, *J. Lipid Res. 33*, 1807–1816.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature 227*, 680–685.

Lagrost, L., Gambert, P., Dangremont, V., et al. (1990) Role of cholesteryl ester transfer protein (CETP) in the HDL conversion process as evidenced by using anti-CETP monoclonal antibodies, *J. Lipid Res. 31*, 1569–1575.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., et al. (1951) Protein measurement with the Folin phenol reagent, *J. Biol. Chem. 193*, 265–275.

Luc, G., Parra, H., Zylberberg, G., et al. (1991) Plasma concentrations of apolipoprotein A-I containing particles in normolipidaemic young men, *Eur. J. Clin. Invest. 21*, 118–122.

McVicar, J., Kunitake, S., Hamilton, R., et al. (1984) Characteristics of human lipoproteins isolated by selected-affinity immunosorption of apolipoprotein A-I, *Proc. Natl. Acad. Sci. U.S.A. 81*, 1356–1360.

Miida, T., Fielding, C., & Fielding, P. (1990) Mechanism of transfer of LDL-derived free cholesterol to HDL subfractions in human plasma, *Biochemistry 29*, 10469–10474.

Neary, R., Bhatnagar, D., Durrington, P., et al. (1991) An investigation of the role of lecithin:cholesterol acyltransferase and triglyceride-rich lipoproteins in the metabolism of pre-β high density lipoproteins, *Atherosclerosis 89*, 35–48.

Nemeth, P., Hitchens, O., Solanki, L., et al. (1986) Fluorometric procedures for measuring triglyceride concentrations in small amounts of tissue and plasma, *J. Lipid Res. 2*, 447–450.

Nichols, A., Gong, E. L., & Blanche, P. (1981) Interconversion of high density lipoproteins during incubation of human plasma, *Biochem. Biophys. Res. Commun. 100*, 391–399.

Nichols, A. V., Gong, E. L., Blanche, P. J., et al. (1984) Interaction of model discoidal complexes of phosphatidylcholine and apolipoprotein A-I with plasma components, *Biochim. Biophys. Acta 793*, 325–337.

Nichols, A. V., Blanche, P. J., Gong, E. L., et al. (1985) Molecular pathways in the transformation of model discoidal lipoprotein complexes induced by lecithin:cholesterol acyltransferase, *Biochim. Biophys. Acta 834*, 285–300.

Nichols, A. V., Gong, E. L., Blanche, P. J., et al. (1987) Pathways in the formation of human plasma high density lipoprotein subpopulations containing apolipoprotein A-I without apolipoprotein A-II, *J. Lipid Res. 28*, 719–732.

Ohta, T., Hattori, S., Nishiyama, S., et al. (1988) Studies on the lipid and apolipoprotein compositions of two species of apoA-I-containing lipoproteins in normolipidemic males and females, *J. Lipid Res. 29*, 721–728.

Oikawa, S., Mendez, A., Oram, J., et al. (1993) Effects of high-density lipoprotein particles containing apo-A-I, with or without apo-A-II, on intracellular cholesterol efflux, *Biochim. Biophys. Acta 1165*, 327–334.

Pagnan, A., Havel, R., Kane, J., et al. (1977) Characterization of very low density lipoproteins containing two electrophoretic populations, *J. Lipid Res. 18*, 613–622.

Puchois, P., Kandoussi, A., Fievet, P., et al. (1987) Apolipoprotein A-I containing lipoproteins in coronary artery disease, *Atherosclerosis 68*, 35–40.

Rader, D., Castro, G., Zech, L., et al. (1991) In vivo metabolism of apolipoprotein A-I on high density lipoprotein particles LpA-I and LpA-I, A-II, *J. Lipid Res. 32*, 1849–1859.

Rajaram, O. V., and Barter, P. J. (1986) Increases in the particle size of high-density lipoproteins induced by purified lecithin: cholesterol acyltransferase: effect of low-density lipoproteins, *Biochim. Biophys. Acta 877*, 406–414.

Roheim, P. (1986) Atherosclerosis and lipoprotein metabolism: Role of reverse cholesterol transport, *Am. J. Cardiol. 57*, 3C-10C.

Rose, H. G., & Ellerbe, P. (1982) Lipoprotein products of lecithin: cholesterol acyltransferase and cholesteryl ester transfer, *Biochim. Biophys. Acta 712*, 547–556.

Schaefer, E., Cheung, M., Hachey, D., et al. (1991) Apolipoprotein A-I and A-II metabolism in HDL subpopulations. *Arteriosclerosis Thromb. 11*, 1408a.

Stewart, C., & Hendry, E. (1935) The phospholipids of blood, *Biochem. J. 29*, 1683–1689.

Touchstone, J., Chen, J., & Beaver, K. (1980) Improved separation of phospholipids in thin layer chromatography, *Lipids 15*, 61–62.

Towbin, H., Staehelin, T., & Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications, *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.