Apolipoprotein A-I-containing lipoproteins with pre-beta electrophoretic mobility

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Abstract Among the apoA-I-containing lipoproteins isolated by selected-affinity immunosorption from human serum and plasma, we have identified a subpopulation which, unlike the bulk of high density lipoproteins, has pre-beta electrophoretic mobility. This pre-beta subpopulation can be observed directly in fresh plasma by immunoelectrophoresis. It contains phospholipid and free and esterified cholesterol, but protein constitutes 90% of its mass. Apolipoprotein A-I is the predominant apolipoprotein in this subpopulation; apolipoprotein A-II and the B lipoproteins are not detected. The protein moiety of this subpopulation exhibits markedly lower helicity than that of high density lipoproteins isolated by ultracentrifugation. —**Kunitake, S. T., K. J. La Sala, and J. P. Kane.** Apolipoprotein A-I-containing lipoproteins with pre-beta electrophoretic mobility. *J. Lipid Res.* 1985. **26:** 549–555.

Supplementary key words HDL subspecies

The high density lipoproteins (HDL) are a set of particles which are both structurally and functionally heterogeneous, differing with respect to particle size, composition, and biological activity. This heterogeneity has been demonstrated by a number of different strategies of subfractionation (1–5).

Sequential ultracentrifugal flotation of HDL, often used as the primary method or as an early step in the separation of subfractions of HDL, allows gross observation of HDL heterogeneity but cannot yield individual subspecies. This technique appears to perturb lipoproteins, at least in that apolipoproteins are lost from the lipoprotein complexes (6–10). Also, ultracentrifugation does not isolate apolipoprotein (apo) A-I-containing lipoproteins that fall outside the density range of $1.063 < d < 1.21$ g/ml.

For the first step in isolation of HDL subspecies, we have developed a new technical strategy, selected-affinity immunosorption (11, 12), which permits the quantitative isolation of apoA-I-containing lipoproteins with minimal perturbation. A recent study by Cheung and Albers (13) has confirmed the suitability of this technique for isolating apoA-I-containing lipoproteins.

From the immunoisolated apoA-I-containing lipoproteins, we have isolated a subpopulation that has pre-beta mobility in contrast to the alpha mobility of most HDL. These pre-beta-migrating lipoproteins contain approximately 90% protein. The predominant apolipoprotein is apoA-I; neither apoB nor apoA-II were detected.

METHODS

Preparation of human serum or plasma

Blood donors had fasted for at least 14 hr. Plasma was prepared by drawing venous blood into vacuum tubes containing EDTA. The blood was centrifuged immediately at 4°C, 1000 *g*, for 15 min. The plasma was recovered and EDTA, azide, and gentamycin were added as preservatives in final concentrations of 0.15%, 2.0 μg/ml, and 5.0 μg/ml, respectively. The plasma was analyzed within 30 min.

Serum was isolated after venous blood was allowed to clot 30 min at 4°C. The serum was recovered in the same manner as the plasma and the same preservatives were added. The serum was stored at 4°C before use. The plasma of three normolipidemic subjects (one female, two male) was analyzed completely. In addition, the plasma of three normolipidemic subjects and three patients with low HDL cholesterol was analyzed for its content of the pre-beta subpopulation.

Isolation of apoA-I-containing lipoproteins

The apoA-I-containing lipoproteins were isolated from plasma or serum by selected-affinity immunosorption, described in detail previously (12). Briefly, antibodies against apoA-I were isolated by binding them to an apoA-I-Sepharose column and eluting a subpopulation of antibodies that dissociated with an elution buffer we selected.

Abbreviations: HDL, high density lipoproteins; CD, circular dichroism.

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The antibodies were then themselves crosslinked to Sepharose to form the anti-apoA-I-Sepharose column we used to isolate the lipoproteins. Plasma or serum was applied to the anti-apoA-I-Sepharose column, and a 0.015 M Tris, pH 7.4, 0.15 M NaCl buffer was used to wash out the unretained protein. The buffer was then changed to 0.2 M acetic acid, pH 3.0 (the same buffer used to isolate the antibodies), and the apoA-I-containing lipoproteins were recovered. The eluate was immediately neutralized with 2.0 M Tris, concentrated to 2 mg of protein/ml by ultrafiltration with an Amicon YM-10 membrane under a pressure of 20 psi, and then dialyzed against saline which contained the same preservatives as were added to plasma.

Isolation of apoA-I-containing lipoproteins with pre-beta mobility

ApoA-I-containing lipoproteins with pre-beta mobility were isolated from immunoisolated lipoproteins by starch block electrophoresis (14). Potato starch (Giusto's, South San Francisco) was washed with distilled water and then suspended in 0.076 M barbital buffer, pH 8.6. The starch was poured into a 1 × 40 × 10 cm block. A concentrate of apoA-I-containing lipoproteins (approximately 15 mg of protein) was applied in a zone 5 cm from the anode end of the block and electrophoresed for 18 hr at a constant current of 80 ma. The block was divided into 1-cm segments along the direction of migration and the lipoproteins in each segment were recovered by two 3-ml washes of the starch with 0.15 M NaCl that contained preservatives. The apolipoprotein distributions in the fractions were determined by immunonephelometry. The apoA-I content of each fraction determined by immunonephelometry allowed the quantitative determination of the proportion of apoA-I found in the lipoproteins with pre-beta mobility. The appropriate fractions were pooled for further analysis.

Analyses of lipoproteins

Electrophoretic analyses of lipoproteins. The electrophoretic mobilities of the isolated lipoproteins were determined by agarose electrophoresis and verified by immunoelectrophoresis in agarose. Agarose electrophoresis was performed as was previously described (15), except that the agarose strips were stained with a solution of 0.5% Coomassie Blue R250 in water–methanol–acetic acid 5:5:1, as well as with the lipophilic stain.

Immunoelectrophoresis, modified from Grabar and Williams (16), was performed by placing 5 μl of sample (0.5–2.0 mg/ml of apoA-I) in a well cut in a 1% agarose (LE agarose, FMC) gel containing 0.05 M barbital buffer, pH 8.6. A potential of 165 volts was placed across the plate until the front had migrated 3.5 cm. After electrophoresis, troughs were cut between the sample wells, parallel to the direction of migration. One hundred fifty μl of goat anti-apoA-I antiserum was placed in each trough. After 18 hr of incubation at 25°C, immunoprecipitin lines formed. The gel was placed in saline for 48 hr and then dried and stained with Coomassie Blue R250. To determine the stability of the HDL subpopulations, the lipoproteins were radioiodinated by the McFarland method as modified by Bilheimer, Eisenberg, and Levy (17) and applied to the same agarose plates. They were then electrophoresed until the front migrated 10.0 cm. The agarose was then separated into 0.5-cm segments along the direction of migration and the radioactivity of each segment was measured. The isolated subpopulations were analyzed after storage at 4°C for 10 days and exposure to 37°C for 2 hr.

Chemical analyses. The protein, phospholipid, free cholesterol, esterified cholesterol, and triglyceride contents of the lipoproteins were determined as previously described (12).

Gel permeation chromatography. The sizes of the lipoproteins were estimated by exclusion chromatography. Standards of known molecular weight and lipoproteins were applied to an S-300 (1 × 90 cm) Sephacryl column (Pharmacia) and eluted with 5.0 mM Tris, pH 7.4, 0.15 M NaCl buffer pumped at 3 ml/hr. The samples were detected by absorption at 280 nm and their elution volumes were determined.

Analysis of apolipoproteins. Apolipoprotein components of the various lipoprotein subfractions were analyzed by electrophoresis in 8 M urea (18) and SDS gradient (2.7–27%, Isolab) polyacrylamide gels. The contents of apoA-I, A-II, and B in each subfraction were determined by immunonephelometric assay (19). The presence in lipoproteins of apolipoproteins C-I, C-II, and C-III was determined by double immunodiffusion (20).

Circular dichroism of each subfraction was measured 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 20.0 mM sodium phosphate, pH 7.4, and quantitated precisely after dilution. Helicity was estimated for each lipoprotein subfraction on the basis of $[\theta]_{222}$ (21).

RESULTS

In addition to classical HDL of alpha mobility among the immunosorbed lipoproteins, apoA-I-containing lipoproteins with pre-beta mobility were observed after electrophoresis when the agarose strips were stained with Coomassie blue for protein (**Fig. 1**). However, when a lipophilic stain was employed, only the alpha band was observed (not shown). Although the two bands were easily

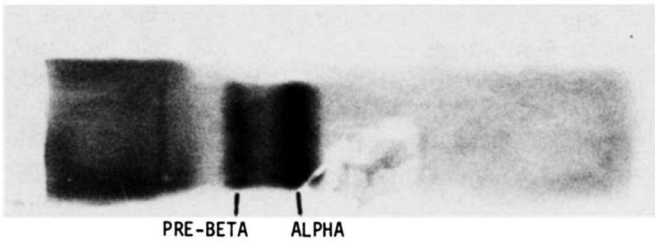

Fig. 1. Agarose electrophoresis of apoA-I-containing lipoproteins. ApoA-I-containing lipoproteins isolated by immunosorption were electrophoresed in agarose and then stained with Coomassie blue. Zones of alpha and pre-beta mobility are present.

visible, this staining method did not allow relative quantitation of the two zones.

The presence of an apoA-I-containing lipoprotein with pre-beta mobility was verified by immunoelectrophoresis (**Fig. 2**). ApoA-I-containing lipoproteins of both alpha and pre-beta mobilities were found in plasma and among lipoproteins isolated by selected-affinity immunosorption, as seen by the presence of two immunoprecipitin arcs developed by antiserum directed against apoA-I. However, immunoelectrophoresis is only semiquantitative at best and when different amounts of protein are analyzed (as in Fig. 2), the relative size of the arcs cannot be directly compared.

The apoA-I distributed into two peaks upon starch block electrophoresis, a pre-beta (slow) and an alpha (fast) subpopulation (**Fig. 3**). ApoA-II was found only in the alpha component and apoB was detected in neither, for

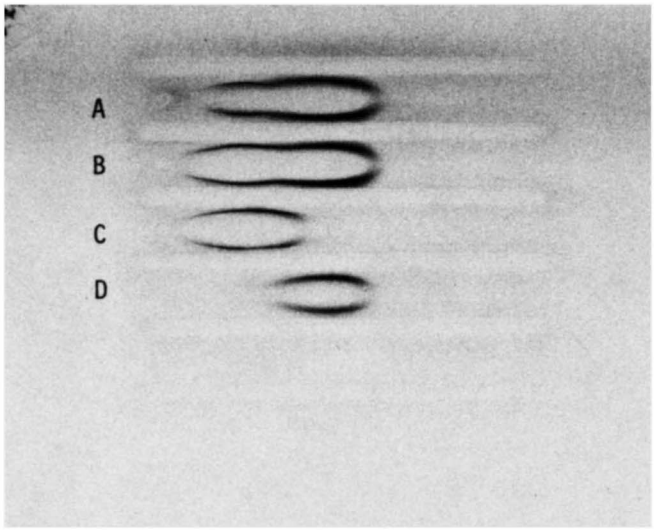

Fig. 2. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma (A); immunoisolated apoA-I-containing lipoproteins (B); apoA-I-containing lipoproteins with pre-beta mobility (C); and apoA-I-containing lipoproteins with alpha mobility (D) were electrophoresed in agarose and then reacted with a monospecific anti-apoA-I antiserum.

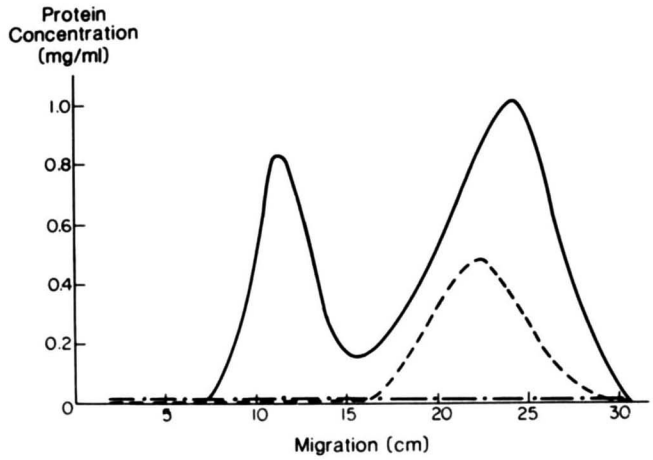

Fig. 3. Starch block electrophoresis of apoA-I-containing lipoproteins. ApoA-I-containing lipoproteins were subjected to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I (——), apoA-II (- - -), and apoB (— · —) content by immunonephelometry.

the three subjects so analyzed. In addition, we found the distributions of apoA-I in alpha and pre-beta subpopulations quantitated by immunonephelometry after starch block electrophoresis of plasma or immunoisolated apoA-I-containing lipoproteins to be the same (not shown). The pre-beta subpopulation displayed in Fig. 3 contained approximately 24% of the total apoA-I content, as determined by immunonephelometry of the starch block fractions. In six normolipidemic individuals we found a range of 5–25% (14.4 ± 5.4%, mean ± SD) of apoA-I in the pre-beta subpopulation. However, in three patients with abnormally low HDL cholesterol levels, the pre-beta component was virtually absent.

After isolation, migration of the two components was similar to their migration before isolation (Fig. 2); the mobilities of the isolated subpopulations remained stable even when held for 10 days at 4°C or 2 hr at 37°C (**Fig. 4**). Analysis of plasma after storage for up to 10 days at 4°C did not appear to increase the prominence of the pre-beta subpopulation. These findings indicate that the two subpopulations are stable and suggest that the apoA-I-containing lipoprotein with pre-beta mobility is not an artifact generated from the storage of lipoproteins of alpha mobility.

The chemical compositions of the alpha subpopulations determined in three subjects were considerably different from the composition of their pre-beta subpopulations (**Table 1**). In each case the composition of the alpha subpopulation was similar to compositions of immunosorbed HDL (12). However, the pre-beta subpopulation contained approximately 90% protein. Both phospholipid and cholesterol (free and esterified) were present, but at low levels, and triglycerides were not detected. The apparent molecular weight of the complex was estimated to

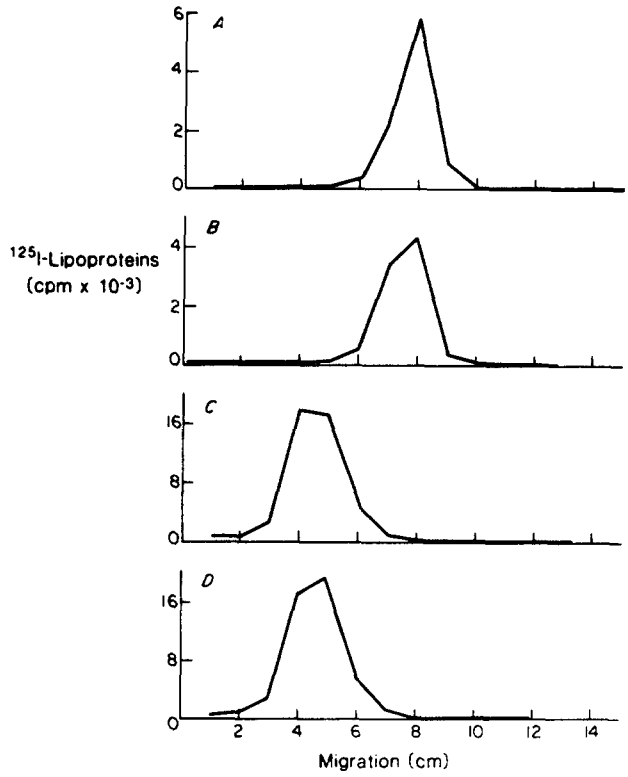

Fig. 4. Stability of apoA-I-containing lipoproteins. ApoA-I-containing lipoproteins with alpha mobility (A, B) and pre-beta mobility (C, D) maintained at 4°C (A, C) or 37°C (B, D) for 2 hr were electrophoresed in agarose gel.

be 80,000 by gel permeation chromatography, as compared to a series of globular proteins (not shown).

The predominant protein of the pre-beta subpopulation was apoA-I (**Fig. 5**). In addition to apoA-I, an apoprotein(s) of low molecular weight could be seen in 2.5–27% SDS gradient gels (**Fig. 6**). This apoprotein(s) did not appear to be any of the apoC species, as determined by its migration in alkaline urea gels and double immunodiffusion (not shown).

The helicity of the apoprotein of the pre-beta subpopulation was found to be significantly less than that for ultracentrifugally isolated HDL or the subpopulation with alpha mobility (**Fig. 7**). The helicity of the subpopulation with pre-beta mobility was 52% as determined by ellipticity at 222 nm, whereas the helicity of the ultracentrifugally isolated HDL and the subpopulation of alpha mobility were 66% and 64%, respectively. All the CD measurements were made within 4 hr of each other and the protein content of each sample was carefully determined on the solutions from which the CD measurements were made. The measurements were performed on three sets of samples; all three sets of samples showed the same difference in helicity.

DISCUSSION

We have identified a subpopulation of apoA-I-containing lipoproteins which, unlike the bulk of HDL, possesses pre-beta mobility on electrophoresis in agarose gels. These apoA-I-containing lipoproteins of pre-beta mobility are small, with a molecular weight of approximately 80,000, and they consist mostly of protein (predominantly apoA-I but no apoA-II). These lipoproteins contain no detectable apoB or triglyceride and therefore are not VLDL- or chylomicron-like particles. This pre-beta migrating subpopulation of apoA-I-containing lipoproteins can be observed by immunoelectrophoresis directly in fresh plasma and serum as well as in immunoisolated HDL. Furthermore, the isolated lipoproteins appear to be stable after isolation when stored for 10 days at 4°C or even during short term (2 hr) exposure to 37°C.

It does not appear that immunosorption generates this subpopulation artifactually because in three subjects the ratio of alpha to pre-beta-migrating apoA-I that was determined by immunonephelometry after starch block

TABLE 1. Composition of the apoA-I-containing lipoproteins[a]

	Protein	Phospholipids	Free Cholesterol	Cholesteryl Esters	Triglycerides
			%		
Subject 1					
Alpha subpopulation	69.1	18.9	1.2	11.4	3.7
Pre-beta subpopulation	93.0	6.0	0.3	0.7	ND[b]
Subject 2					
Alpha subpopulation	64.0	18.0	3.0	13.0	2.0
Pre-beta subpopulation	93.0	4.8	0.1	2.1	ND
Subject 3					
Alpha subpopulation	65.0	18.0	1.0	12.0	4.0
Pre-beta subpopulation	88.0	9.0	0.5	2.5	ND

[a]Compositions are given as % mass.
[b]Not detected at a level of 25 μg/ml.

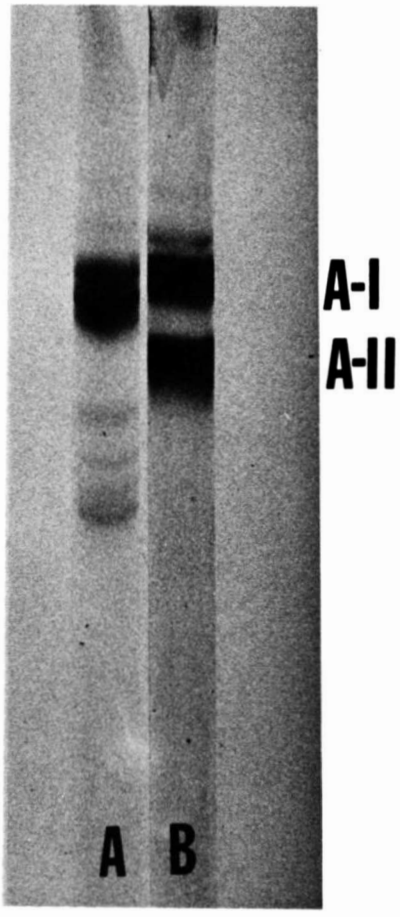

Fig. 5. Polyacrylamide gel electrophoresis in 8 M urea of the apoproteins of the pre-beta subpopulation (A). A gel containing apoA-I and apoA-II is shown for comparison (B).

electrophoresis of plasma was the same as that found after electrophoresis of immunoisolated HDL from the same subject. We also believe that this fraction is not an artifact of electrophoresis because we have found patients whose plasma contains almost none of the pre-beta subpopulation after starch block electrophoresis. If pre-beta material were generated by electrophoresis, we would expect to find some pre-beta migrating lipoproteins in all samples. Also, re-electrophoresis on starch block of alpha-migrating lipoproteins does not generate pre-beta lipoproteins. Further, Schonfeld, Balley, and Steelman (22) have demonstrated by an alternate method, molecular sieve chromatography, that a small HDL (mol wt 50,000) that contains only apoA-I exists in plasma. These investigators did not isolate or characterize this lipoprotein; hence, that lipoprotein may be different from the particle we described. However, they did provide corroborating evidence that a small molecular weight lipoprotein exists in plasma which contains apoA-I and no apoA-II.

Because the pre-beta subpopulation of apoA-I-containing lipoproteins contains mostly apoA-I, the method which best visualized this subpopulation is immunoelectrophoresis using antiserum against apoA-I. Indeed, nearly 20 years ago Levy and Fredrickson (23), and later Albers and Aladjem (24), detected immunoprecipitin arcs with pre-beta mobility in ultracentrifuged fractions using anti-HDL and anti-apoA-I serum, respectively. However, these investigators could not detect this subpopulation in plasma and postulated that it was an artifact of ultracentrifugation. We do not know the reason for this discrepancy; however, there are two possibilities. First, we have found that the apoA-I in the pre-beta subpopulation can vary from approximately 0 in some patients to 25% of total A-I in normolipidemic subjects. It is possible that the samples they analyzed had low levels of this subpopulation. Second, immunoelectrophoresis is only semiquantitative, in that only the proper ratio of antigen to antibody will form an immunoprecipitin line. It is possible that the antibody concentration used by these investigators was such that

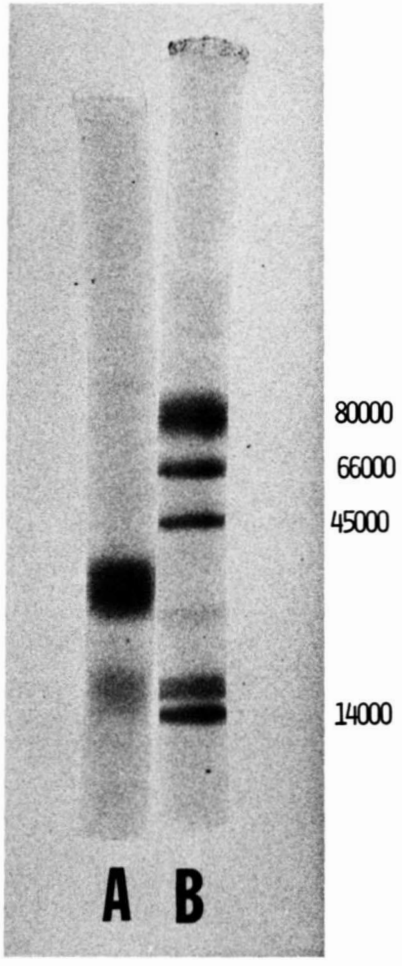

Fig. 6. SDS gradient gel electrophoresis. The apoproteins of the pre-beta subpopulation were resolved on SDS gradient gels (A). Marker proteins are displayed for comparison (B).

the pre-beta subpopulation could be visualized only after the sample was concentrated by ultracentrifugation.

More recently, in a study of the immunoreactivity of apoA-I, Børresen and Berg (25) noted immunoprecipitin arcs of both pre-beta and alpha mobility directly in serum. The pre-beta subpopulation, which they termed "free apoA-I," appeared to increase markedly with storage at 37°C, heating to 56°C, or delipidation by 1,1',3,3'-tetramethylurea. This implied that the pre-beta subpopulation might be artifactually generated. However, their analysis also revealed a shift in the migration of apoA-II, which can be interpreted to mean that apoA-I- and A-II-containing HDL of alpha mobility were changing mobility and that this change masked the pre-beta subpopulation which contained only apoA-I, not that more of the "free apoA-I" was being generated.

Like the pre-beta subpopulation found here, other subfractions of HDL have been reported to contain apoA-I but no apoA-II (3, 26–29). However, the lipoprotein subpopulation described here appears to be a different complex from those previously reported, in that the pre-beta subpopulation has a higher percentage of protein. Additional findings from our circular dichroic measurements point to a unique structure for the pre-beta migrating apoA-I-containing lipoprotein, in that the protein appears to be in a significantly different conformation. Recently, Cheung and Albers (13) have demonstrated that up to 50% of apoA-I exists on lipoproteins without apoA-II. We believe that the pre-beta subpopulation is a small subset of such a pool.

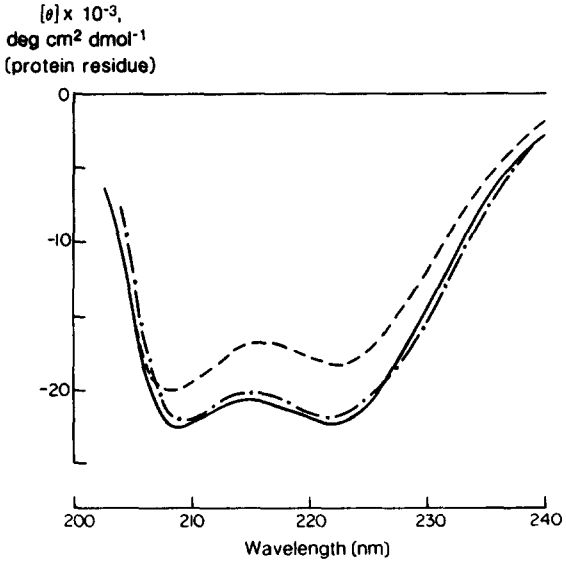

Fig. 7. Circular dichroic measurements of apoA-I-containing lipoproteins. The ellipticities of (A) ultracentrifugally isolated HDL (——), (B) alpha mobility subpopulation (— · —), and (C) pre-beta subpopulation (- - -) were measured from 240 to 200 nm. The helicity of each as determined from ellipticity at 222 nm was (A) 66.4%, (B) 64.3%, and (C) 52.0%.

The differences in lipid composition and in the secondary structure of the protein moiety of the pre-beta apoA-I-containing lipoprotein subpopulation that we have observed probably reflect important differences between the metabolic functions of this subpopulation and of the bulk of HDL. Such differences might include differential affinities for receptors and different capacities for association with lipids. The relatively lipid-poor pre-beta lipoproteins might acquire and stabilize surface lipid components derived from lipolysis of triglyceride-rich lipoproteins or perhaps might acquire cholesterol from peripheral cells. Conversely, the pre-beta species might be a transient intermediary in the formation of HDL from chylomicrons.

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