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LIPOPROTEIN-APOPROTEIN EXCHANGE IN AQUEOUS SYSTEMS: RELEVANCE TO THE OCCURRENCE OF APOA-I AND APOC PROTEINS IN A COMMON PARTICLE

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SUMMARY. Human plasma high density lipoproteins (HDL) were labeled in vitro with [^{125}I]apoA-I. Chromatography of the [^{125}I]HDL on Sephacryl S-200 revealed that a certain fraction of [^{125}I]apoA-I readily dissociates from the intact particle over a wide range of HDL concentrations. The relatively constant value of the dissociated apoA-I concentration observed at various HDL concentrations suggests that a "critical monomer concentration" of apoA-I is in equilibrium with the parent lipoprotein. Addition of [^{131}I]apoC proteins to HDL induces additional dissociation of oligomeric apoA-I with the concomitant incorporation of apoC into a new particle of about 460,000 daltons.

INTRODUCTION

The plasma lipoproteins are lipid-protein complexes which can be isolated as quasi-discrete particles having characteristic densities, sizes, and compositions (1). Alaupovic (2) has hypothesized that within the plasma high density lipoproteins (HDL) two families of HDL containing exclusively either apoC proteins or apoA proteins exist. These two families designated as LP-A and LP-C respectively, also have similar dimensions and therefore cannot be separated on the basis of size or density. The apolipoproteins are water-soluble whereas the extracted lipids of plasma lipoproteins are practically insoluble. Because the plasma apolipoproteins are water-soluble, a finite number of these molecules should exist as a free monomer or oligomer in equilibrium with the intact lipoprotein; moreover, the dissociation of apolipoproteins from lipoproteins provides a plausible mechanism for the putative transfer or exchange of apolipoproteins between lipoproteins classes (3-4). Herein we present physicochemical evidence for the in vitro movement of the apolipoproteins of HDL.

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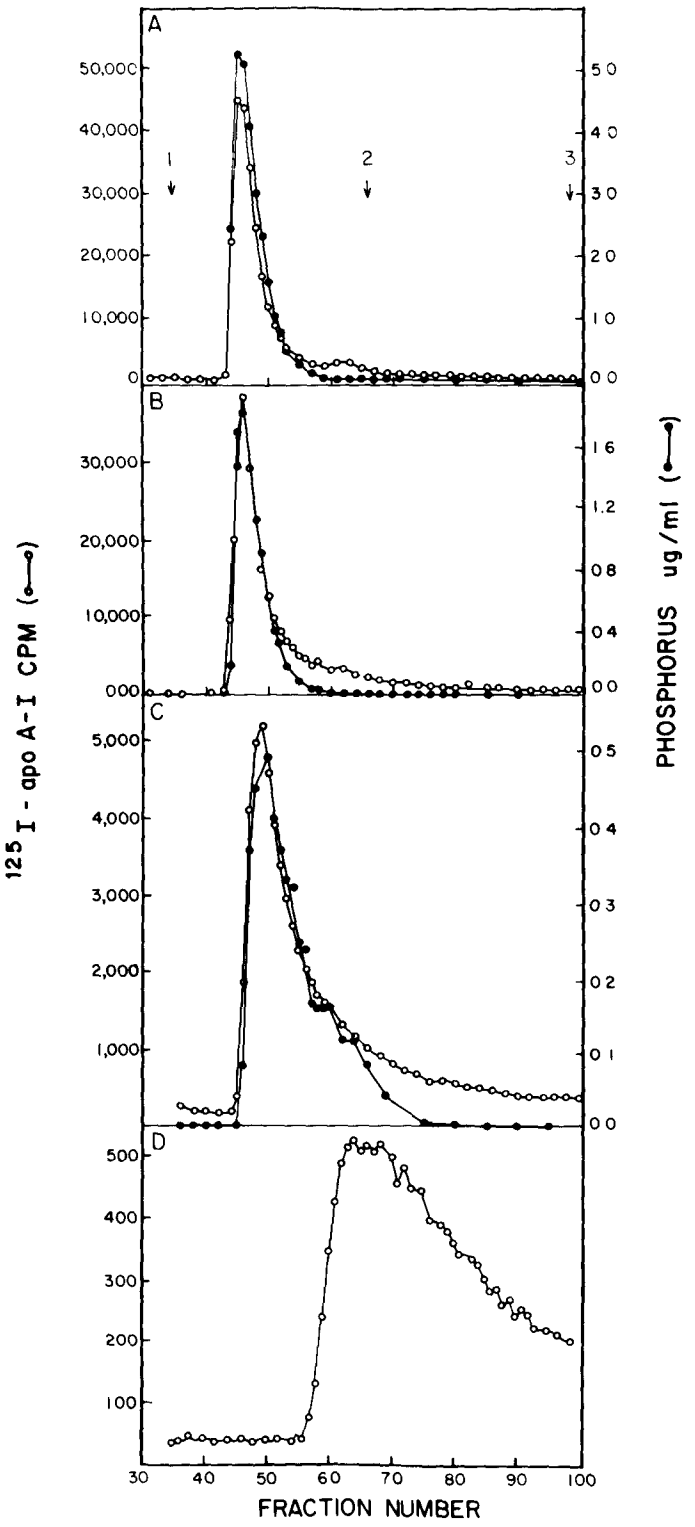
EXPERIMENTAL

HDL was isolated from the plasma of normal fasting subjects by precipitation with phosphotungstate and ultracentrifugation (5). ApoA-I was isolated by gel filtration of apoHDL in 3 M guanidine-HCl. ApoC proteins were isolated from the plasma of type V hyperlipidemic subjects by the method of Brown et al. (6). ApoA-I and total apoC proteins were labeled with [^{125}I] and [^{131}I], respectively, by the IC1 method (7). The specific activity of [^{131}I]apoC was based on amino acid analysis. [^{125}I]labeled apoA-I was incorporated into HDL by the method of Shepherd et al. (8). The labeled HDL was purified by gel filtration on Sephacryl S-200. The specific activity of the [^{125}I]apoA-I labeled HDL was determined from a phosphorus analysis and the assumption that phosphorus composes 1% of the HDL weight. The specific activity of apoC proteins was based upon an amino acid analysis. In double counting experiments, a correction was made for a 27% spillover of [^{131}I]counts into the [^{125}I]channel. Phosphorus content was measured by the method of Bartlett (9). Cholesterol analyses were performed with a kit from Boehringen-Mannheim. Molecular weights were determined by analytical ultracentrifugation and analytical gel filtration chromatography on a calibrated column (1.6 x 90 cm) of Sephacryl S-200. All experiments were conducted in a buffer comoposed of 0.15 M NaCl, 0.01 M Tris pH 7.4 and 0.01% azide.

RESULTS AND DISCUSSION

Varied amounts of HDL were chromatographed on Sephacryl S-200. At concentrations >6.5 mg in 1 ml, [^{125}I]apoA-I and phosphorus eluted together in fractions 44 and 45 (Figure 1A). An additional small peak of [^{125}I]apoA-I containing no phosphorus appeared in fraction 62. Thus, apoA-I can be dissociated from HDL and even at relatively high lipoprotein concentrations there is little or no alteration of the size and composition of HDL. As the amount of HDL applied to the column was reduced, the amount of apoA-I which was transferred from the lipoprotein to the aqueous phase remained relatively constant. The effect at lower HDL concentrations is shown in Figure 1 B-D. The profile of the [^{125}I]apoA-I is shifted to progressively larger elution volumes and its coincidence with the profile of phosphorus concentration diminished when lower concentrations of HDL were chromatographed. It appears that this is accomplished by the dissociation of apoA-I from HDL. This is supported by the observation that at very low HDL concentrations, apoA-I elutes at a position similar to that observed when a similar portion of free apoA-I is chromatographed (fractions 62 and 66, respectively). The concentration of apoA-I which is dissociated from HDL (Figure 1 A-C) is


A
B
C
D
1
2
3
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40,000
30,000
20,000
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0
5 0
4 0
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0 5
0 4
0 3
0 2
0 1
0 0
500
400
300
200
100
30 40 50 60 70 80 90 100
FRACTION NUMBER
125 I - apo A-I CPM (—o—)
PHOSPHORUS ug/ml (—•—)

constant at 0.003 ± 0.0006 mg/ml, a quantity that is similar to the reported monomer concentration of apoA-I (10).

Chromatography of HDL produced at least a five-fold dilution of all components. In Figure 1 D, the amount of apoA-I contained in the 0.65 mg of HDL that was applied to the column was insufficient to give, after column dilution, a concentration greater than 0.0006 mg/ml. All of the apoA-I in this experiment eluted as a broad peak whose maximum coincided with that of free apoA-I. The phosphorus concentration was too small to quantitate but was positive in fractions 40-45 with no well-defined peak. The elution of apoA-I extended beyond the salt peak suggesting that there was some partitioning of apoA-I between the gel matrix and the aqueous phase. These findings demonstrate that at low HDL concentrations, apoA-I dissociates from HDL as a monomer. In the absence of more sensitive lipid analyses, it is impossible to determine whether the elution volume of the apoA-I-deficient HDL is different from that of HDL.

Both apoC and apoA proteins are components of HDL. We have incubated total apoC proteins (5.5 mg) with HDL (6.5 mg) for 1 hr and chromatographed the mixture on Sephacryl S-200. As shown in the chromatogram of Figure 2, apoC is incorporated into the phosphorus-containing peak (fraction 41) with a concomitant loss of [^{125}I]apoA-I from HDL which elutes as a separate peak at fraction 52. Because its elution volume is smaller than that of monomeric apoA-I, the displaced apoA-I is probably oligomeric (concentration of peak tube = ∿0.1 mg/ml). The phosphorus-containing peak coelutes with maxima for [^{131}I]apoC and [^{125}I]apoA-I at fraction numbers 41 and 42. About 50% of the apoA-I (1.3 mg) was displaced by a smaller amount (0.7 mg) of apoC. Shoulders on the [^{131}I] and [^{125}I] profiles indicate heterogeneity in the composition of this peak. The smaller elution volume suggests that the

Figure 1: Concentration dependence of gel filtration profiles of HDL phosphorus and ^{125}I-apoA-I. A 1-ml sample containing, A, 6.5 mg; 3.25 mg; C, 1.6 mg; D, 0.65 mg; was applied to a column of Sephacryl S-200 as described in the experimental section. The arrows, 1-3, represent the respective elution positions of the void volume, apoA-I, and $^{22}Na^{+}$; 2 ml fractions were collected.

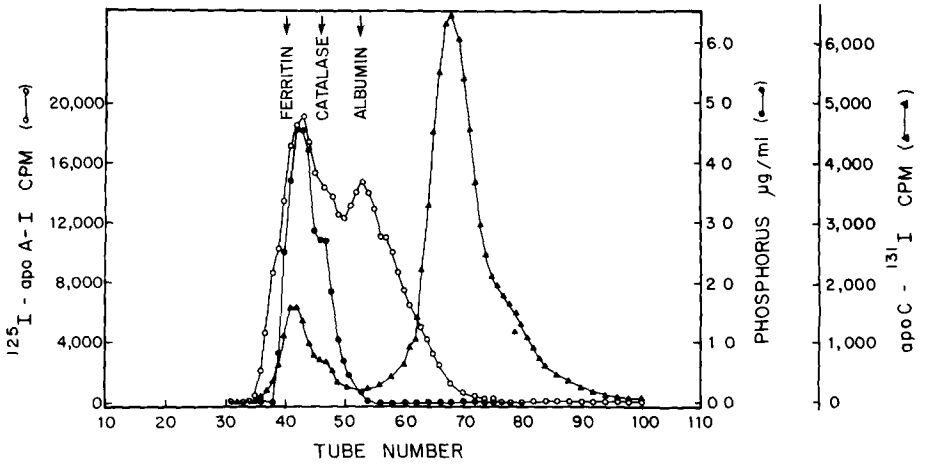

Figure 2: Gel filtration profile of the products formed by the interaction of 6.5 mg HDL (containing ^{125}I-apoA-I) and 5.5 mg total apoC proteins (^{131}I-labeled). Arrows indicate the molecular weight markers for ferritin (585,000), catalase (235,000) and bovine serum albumin (66,500). The profile of the absorbance at 280 nm was approximately equal to the sum of the ^{125}I and ^{131}I profiles. Column and fraction size are the same as in Figure 1.

molecular weight of the newly formed species is greater than that of HDL. We have measured the composition (Table 1) and molecular weight of fraction number 39. In the analytical ultracentrifuge only one species could be detected. Based upon a density calculated from composition and a sedimentation equilibrium measurement, we obtained a molecular weight of 433,000. From the calibrated column, a value of 485,000 daltons was obtained. Thus, a new lipoprotein particle having the composition and properties of LP-C (11) was generated by the addition of apoC to HDL with the displacement of apoA-I. This result, however would tend to refute rather than support the concept of lipoprotein families (2). This is borne out by our observation that apoC-proteins displace apoA-I (an A-apoprotein) from HDL. Therefore, the A- and C-proteins must associate with a similar structural entity. These data can be fitted to a general theory of micelle formation which has been elegantly described by Tanford (12). Specifically, we propose that one can view the HDL as a mixed micelle whose surface

TABLE 1

Composition of the Lipoprotein Produced by Exchange of ApoC-Proteins for HDL-ApoA-I[a]

Cholesterol (total)	12%
Phospholipid	56%
Protein	32%
[^{125}I]apoA-I/[^{131}I]apoC	4.4

a. This analysis was performed on fraction number 39 which contains the maximum peak for [^{131}I].

components exist in equilibrium with free monomeric or perhaps oligomeric species in the aqueous phase. The similarity of the reported monomer concentration of apoA-I and that of our dissociated apoA-I suggests that the dissociated form of the protein is monomeric except when displaced by other agents. We have designated this quantity as the "critical monomer concentration" of apoA-I in HDL which is analogous to the concept of critical micelle concentrations in micellar phenomena. Similar to mixed micelles, its value would vary with lipoprotein composition and concentration and the specific affinity, if any, of various components for each other.

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