Structural relationships between nascent apoA-I-containing particles that are extracellularly assembled in cell culture

Trudy M. Forte,[1] John K. Bielicki, Laura Knoff, and Mark R. McCall

Department of Molecular and Nuclear Medicine, Life Sciences Division, 1-213, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720

Abstract Apolipoprotein A-I (apoA-I) incubated with CHO cells assembles three major nascent lipid complexes with diameters of 7.3, 9, and 11 nm. Previous studies suggested that the smaller nascent particles were precursors for the larger nascent ones. To test this hypothesis, the 7.3, 9, and 11 nm apoA-I–lipid complexes formed by incubating CHO cells with lipid-free apoA-I were isolated and subsequently each subpopulation was re-incubated with cells in the absence of other subpopulations. The physical–chemical characteristics of each subpopulation were examined before and after re-incubation in an effort to understand relationships, if any, between the different nascent complexes. The 7.3, 9, and 11 nm complexes were unique in that each of the particles had pre-alpha mobility on agarose gels; this rapid migration was not altered by re-incubation with cells. Protein crosslinking studies indicated that the 7.3, 9, and 11 nm complexes possessed 2, 3, and 4 apoA-I molecules per complex, respectively; it is unlikely that the size of the particle and number of apoA-I molecules per particle played a role in the increased negative charge of the particles. The present study shows that smaller particles did not give rise to larger ones upon re-incubation with cells. Rather, the 11 and 9 nm particles both generated smaller discs (the 11 nm giving rise primarily to 9 nm discs and the 9 nm complex giving rise to 7.3 nm discs) suggesting that, during incubation with cells, the complexes are destabilized and remodeled into smaller, not larger, complexes. Surprisingly, the 7.3 nm complexes during re-incubation with cells were extremely stable and did not undergo size alteration. When the 7.3 nm particles were incubated with additional small quantities of lipid-free apoA-I (1–2 μg/ml), larger discoidal complexes were generated suggesting that the formation of larger particles may be driven by the availability of lipid-free apoA-I.—**Forte, T. M., J. K. Bielicki, L. Knoff, and M. R. McCall.** Structural relationships between nascent apoA-I-containing particles that are extracellularly assembled in cell culture. *J. Lipid Res.* 1996. **37:** 1076–1085.

Supplementary key words apoA-I • extracellular assembly of HDL • nascent HDL • pre-alpha HDL • Chinese hamster ovary cells

Apolipoprotein (apo) A-I, a 243 amino acid protein, is the major protein associated with HDL. Elevated plasma concentrations of apoA-I have been associated with decreased risk for atherosclerosis. HDL, particularly the subclass containing apoA-I without apoA-II (LpA-I), has been shown to stimulate cholesterol efflux and net transport from cells (1–4) thereby initiating the early events of the putative reverse cholesterol transport process. In the latter process, excess cell cholesterol is removed by the HDL, esterified by lecithin:cholesterol acyltransferase (LCAT), and returned to the liver for catabolism. The anti-atherogenic role of HDL is, in part, related to its contribution to the reverse cholesterol transport pathway.

We have previously shown that lipid-free recombinant apoA-I, as well as purified plasma apoA-I, incubated with Chinese hamster ovary (CHO) cells is able to assemble nascent HDL particles by recruiting phospholipid and cholesterol from the cell membrane (5, 6). Nascent products assembled by this mechanism consisted of three major subpopulations with diameters of approximately 7.3, 9.0, and 11.0 nm; moreover, time- and concentration-dependence studies indicated that the formation of particles proceeded in a step-wise fashion where the smallest particle, 7.3 nm, appeared to give rise to larger 9 and 11 nm complexes (5). Thus we postulated a precursor–product mechanism for the formation of larger complexes whereby increased lipidation, or fusion, of smaller particles generated larger ones. In the present study we investigated this hypothesis using isolated nascent subpopulations formed in CHO cell cultures and re-incubating such distinct nascent HDL with CHO cells in the absence of other subpopulations. As

Abbreviations: apoA-I, apolipoprotein A-I; CHO, Chinese hamster ovary; HDL, high density lipoprotein; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; UC, unesterified cholesterol; PL, phospholipid; rHDL, reconstituted HDL, POPC, palmitoyloleoylphosphatidylcholine.

[1]To whom correspondence should be addressed.

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little is known about the physical–chemical characteristics of the major subpopulations, these parameters were also examined in an effort to understand the relationship, if any, between different nascent subpopulations. Our results indicate that, rather than forming larger particles, the 9 and 11 nm complexes form smaller complexes upon reincubation with cells. The 7.3 nm particle is extremely stable and does not form larger particles unless additional lipid-free apoA-I is added to the medium, suggesting that, in this case, formation of larger particles is driven by the presence of lipid-free apoA-I.

MATERIALS AND METHODS

Isolation of apolipoprotein A-I

HDL (d 1.063–1.21 g/ml) was isolated from approximately 1 liter of fresh blood bank plasma. HDL were reisolated at a density of 1.21 g/ml to insure that the fraction was pure and then treated with 3 M guanidine hydrochloride at 37°C for 3 h as previously described (7). Isolated apoA-I was purified on a Sephacryl S-200 HR column and eluted fractions were assayed for purity by SDS-PAGE. Fractions containing only apoA-I were pooled; the isolated apoA-I showed only a single band on SDS-PAGE. Purified apoA-I was dialyzed into a buffer containing 150 mM NaCl, 20 mM Tris, and 0.27 mM EDTA, pH 8.0, and refrigerated until used.

Cell incubations and isolation of apoA-I lipid complexes

ApoA-I, at a concentration of 10 μg/ml, was incubated with confluent CHO Cl 9 cell cultures as previously described except that serum-free McCoy's 5A medium was used (5). Cells were grown in Corning 375 cm^2 flasks. Conditioned medium was harvested after 24 h and immediately placed on ice; gentamicin sulfate (0.1 mg/ml), EDTA (1 mg/ml), and phenylmethylsulfonylfluoride (0.5 mM) were added and cell debris was removed by filtration. The medium was concentrated by ultrafiltration with a Minitan (Millipore) concentrator using 10,000 molecular weight cutoff, low binding regenerated cellulose plates; recovery was typically 70% of the original apoA-I added to the cells. ApoA-I from concentrated medium did not appear to undergo major changes because it was identical on SDS-PAGE to starting apoA-I, as previously reported (6) and lipid-free apoA-I isolated from the medium had the same prebeta mobility on agarose gels as starting apoA-I.

To isolate specific apoA-I–lipid subpopulations, i.e., 7.3, 9, and 11 nm complexes, formed during incubation with cells, we first attempted chromatographic separations utilizing FPLC with a Superose 12 column. This procedure did not separate the 9 and 11 nm discoidal components and also resulted in considerable loss of material. More effective separation of apoA-I–lipid complexes was accomplished by first ultracentrifugally sepa-

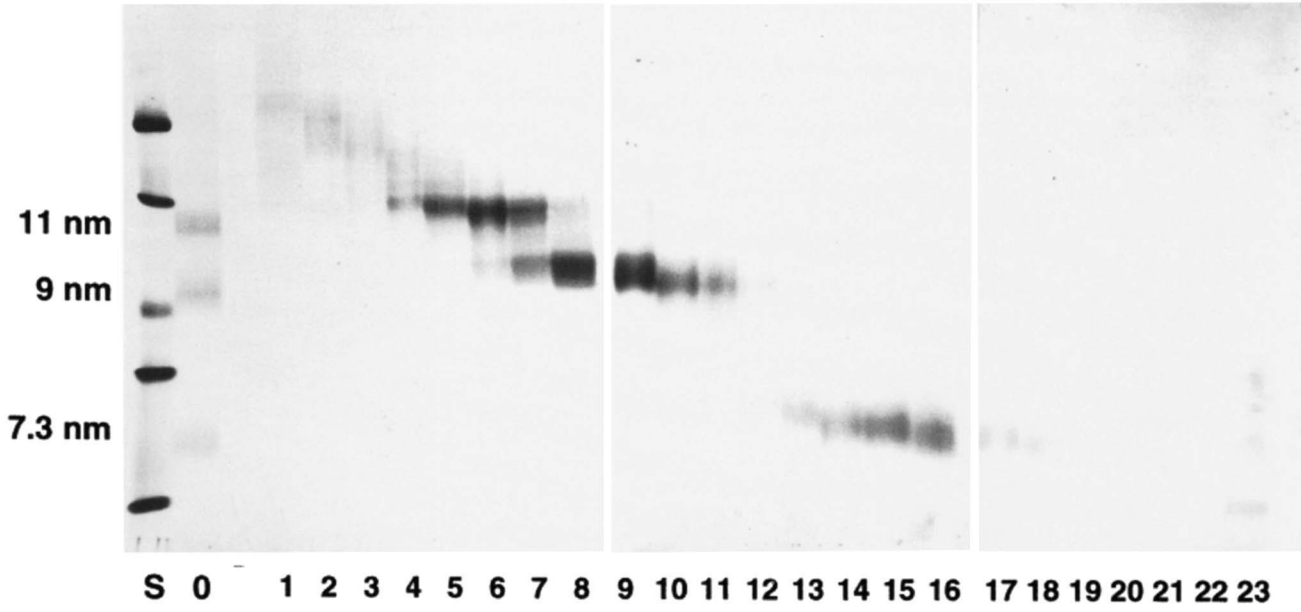

Fig. 1. Density gradient fractions electrophoresed on 4–30% nondenaturing gradient gels. Lane "S" shows standards which from top to bottom represent thyroglobulin, 17.0 nm; ferritin, 12.2 nm; catalase, 10.4 nm; lactic dehydrogenase, 8.1 nm; and albumin, 7.1 nm. Lane "O" is the unfractionated d < 1.235 g/ml sample showing major bands at 11, 9, and 7.3 nm. Lanes 1–23 correspond to the 23 0.5-ml fractions pipetted from the density gradient; 40 μl of each fraction was electrophoresed. Fractions 4–6 were pooled for 11 nm complexes, 9–11 were pooled for 9 nm complexes, and 13–17 were pooled for 7.3 nm complexes.

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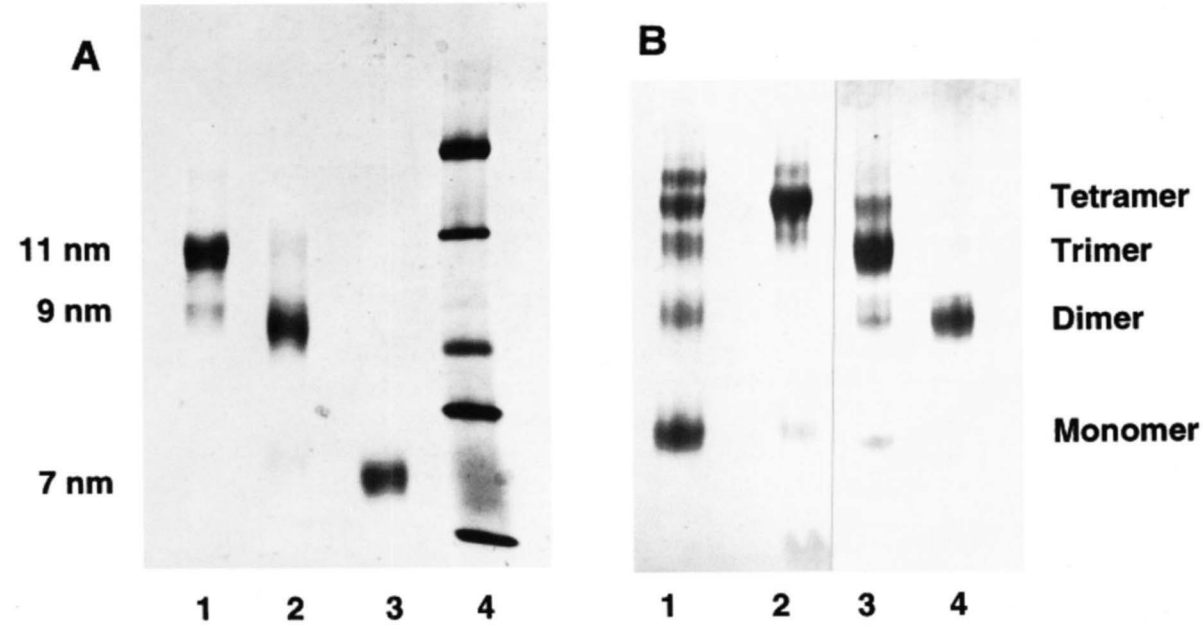

Fig. 2. Determination of homogeneity of isolated complexes and the number of apoA-I molecules associated with each particle. A: Nondenaturing gradient gel profiles of isolated complexes. Lane 1 is the 11 nm pool which is slightly contaminated with 9 nm particles; lane 2 is the 9 nm pool that contains traces of the 11 nm component; lane 3 is the 7.3 nm particle pool which appears not to be contaminated with other subpopulations. The standard, lane 4, is the same as that described in Fig. 1. B: SDS-PAGE gels of purified apoA-I and complexes crosslinked with dimethylsuberimidate; lane 1 shows the crosslinked apoA-I standard; lane 2, 11 nm complex showing mainly 4 apoA-Is per particle; lane 3, 9 nm complex showing mainly 3 apoA-Is per particle; and lane 4, 7.3 nm complex showing 2 apoA-Is per particle.

rating a d < 1.235 g/ml fraction from the concentrated medium and then applying this fraction to the density gradient previously described by McCall, Forte, and Shore (8). The gradient yielded 23 0.5-ml fractions; each fraction was subjected to nondenaturing gradient gel electrophoresis to assess its particle size distribution. Fractions showing a predominance of 11, 9, or 7.3 nm particles with little contamination from other subpopulations were pooled; these enriched subpopulation pools were used for re-incubation with CHO cells.

Re-incubation studies

Pools containing predominantly 11, 9, or 7.3 nm particles were re-incubated at a concentration of 10 μg apoA-I/ml with CHO cell monolayers for 24 h in serum-free McCoy's medium. Such incubations were carried out either in 175 cm^2 or in 75 cm^2 flasks. After incubation, the reisolated medium was concentrated by ultrafiltration and the d < 1.235 g/ml fraction was separated ultracentrifugally. Recovery of apoA-I after reincubation with cells was 75 ± 21%. Control incubations were carried out with precursor complexes (10 μg apoA-I/ml) incubated in McCoy's medium in the absence of cells. The complexes were reisolated in the same manner as those from cells; recovery of apoA-I in control incubations was 101 ± 23%.

Compositional and electrophoretic analyses

Protein was determined by the Markwell et al. (9) modified method of Lowry. Phospholipid was determined by the method of Chen, Toribara, and Warner (10) and cholesterol by the method of Omodeo Salè et al. (11). Free fatty acid was assayed by the NEFA kit (Wako Chemical, Richmond, VA).

The size distribution of particles was determined by nondenaturing gradient gel electrophoresis as described by Blanche et al. (12). To assess changes in distribution of particles, the gels were scanned and the area under the curves for each component was calculated. In some experiments, proteins were transferred to nitrocellulose and bands were probed with apoA-I specific antibody.

SDS-polyacrylamide gel electrophoresis was carried out, according to the procedure of Laemmli (13), on 4–20% gels obtained from Schleicher and Schuell (Keene, NH).

Agarose gel electrophoresis was carried out on Beckman Paragon gels. Proteins were transferred to nitrocellulose by diffusion and bands were probed with antibody to apoA-I as previously reported (6).

Electron microscopy

Samples to be evaluated by electron microscopy were dialyzed against ammonium acetate buffer, pH 7.4.

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Samples were stained with 2% sodium phosphotungstate and examined in the JEOL 100C electron microscope as previously described (14).

Crosslinking of proteins

Chemical crosslinking of apoA-I was achieved by the dimethylsuberimidate dihydrochloride method of Swaney and O'Brien (15). Crosslinked complexes were delipidated and electrophoresed on 4–30% SDS-polyacrylamide gels using crosslinked purified apoA-I as a standard.

RESULTS

Isolation and identification of precursor complexes

The apoA-I–lipid complexes isolated by density gradient ultracentrifugation were evaluated by nondenaturing gradient gel electrophoresis and a representative gel profile is shown in **Fig. 1.** Typically, fractions 4–6 (enriched in 11 nm complexes), fractions 9–11 (enriched in 9 nm complexes), and fractions 13–17 (enriched in 7.3 nm complexes) were pooled; mean densities of the pools were 1.089, 1.112, and 1.159 g/ml, respectively. The most dense fraction, 23, is essentially lipid-free apoA-I; this fraction was assayed for the presence of phospholipid and was found to contain less than 1 molecule phospholipid per molecule apoA-I. The top of the gradient contained complexes larger than 11 nm but, because these were heterogeneous in size, no further studies were carried out with these complexes.

The isolated pools were subjected to nondenaturing gradient gels to ascertain the degree of purity of each of the fractions. As seen in **Fig. 2A,** the 11 and 9 nm particles are contaminated with minor amounts of other complexes but the 7.3 nm particle is extremely clean without traces of additional complexes. The 11, 9, and 7.3 nm complexes were subjected to crosslinking to determine the number of apoA-Is associated with each complex. As shown in Fig. 2B, the number of apoA-Is increases with increasing size where the 7.3 nm complexes contain 2 apoA-Is and the 9 and 11 nm complexes contain, 3 and 4 molecules of apoA-I, respectively.

Morphology and charge properties of the isolated complexes

The morphology of the isolated complexes was determined by electron microscopy and, as revealed in **Fig. 3,** the 11, 9, and 7.3 nm complexes consist of discoidal particles where those in the 11 nm fraction are clearly larger than the others. The 7.3 nm particles consist mainly of small, stubby discs.

Isolated complexes were electrophoresed on agarose gels to determine whether the overall charge differed in complexes of different size and numbers of apoA-I. The electrophoretic pattern is shown in **Fig. 4.** Compared with purified apoA-I (pre-beta mobility) and plasma HDL (alpha mobility), the complexes have a distinct faster, pre-alpha migration; migration of complexes is 30% greater than that of alpha particles. There is no apparent difference in migration between particles of different size suggesting that particle size and number of apoA-Is on a given particle are not determinants of the fast migration of precursor particles. The precursor particles were assayed for the presence of free fatty acids and none could be detected, thus ruling out the contri-

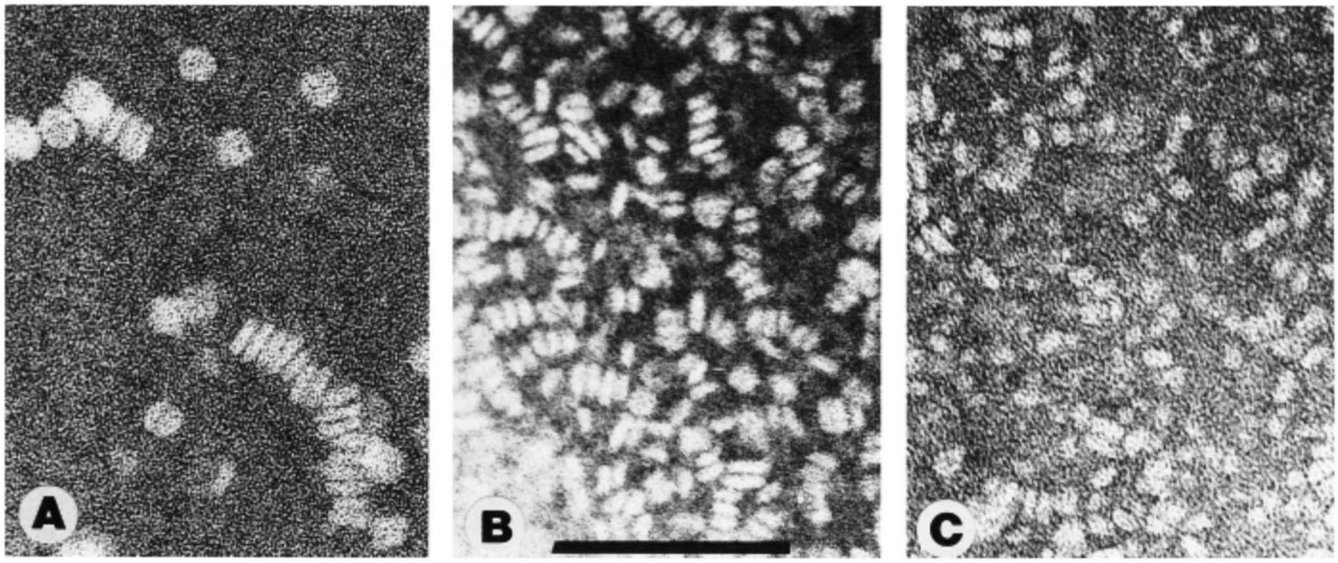

Fig. 3. Electron micrographs of negatively stained complexes: (A) 11 nm pool, (B) 9 nm pool, and (C) 7.3 nm pool. The bar marker represents 100 nm and is the same for all micrographs.

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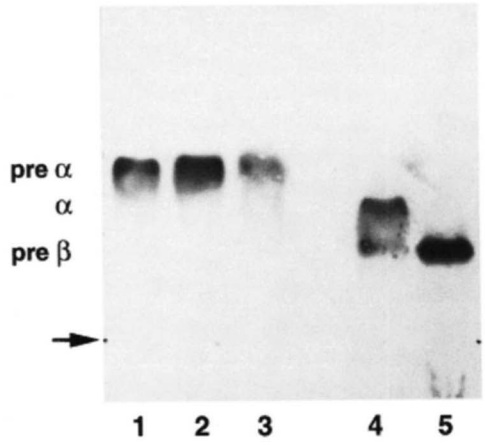

Fig. 4. Charge properties of complexes electrophoresed on agarose gels. The protein bands were transferred to nitrocellulose and Western blots were carried out using apoA-I-specific monoclonal antibodies. Lane 1, 11 nm complex; lane 2, 9 nm complex; lane 3, 7.3 nm complex; lane 4, plasma HDL; and lane 5, lipid-free apoA-I. The nascent complexes all exhibit pre-α migration compared with the α migration of isolated plasma HDL and the pre-β migration of lipid-free apoA-I.

bution of negatively charged fatty acids. In addition, albumin incubated with the precursors did not alter their charge properties (data not shown).

Changes in size distribution and lipid composition of isolated complexes after re-incubation with CHO cells

Each of the isolated apoA-I–lipid complexes was re-incubated in either the presence or absence of CHO cells and after 24 h the complexes were reisolated from the medium and evaluated for change in size distribution by nondenaturing gradient gel electrophoresis. Scans from a representative experiment showing the major changes in particle distribution during incubation are shown in **Fig. 5A;** the percent change in distribution of precursors after incubation with cells is depicted in Fig. 5B. As noted in the scans, the 11 nm complexes, after incubation with cells, gave rise to primarily 9 nm particles (change from 6% to 28% after incubation) and a small quantity of 7.3 nm particles. The 9 nm particles generated mainly 7.3 nm particles (change from 4% to 26% after incubation). Surprisingly, the 7.3 nm precursor was not altered in size during re-incubation suggesting that it is a thermodynamically stable particle that is not readily transformed to larger particles. All apoA-I–lipid complexes isolated from the medium after re-incubation with cells maintained their pre-alpha mobility (data not shown).

To ascertain whether changes in the lipid composition of complexes occurred after re-incubation with cells, the cholesterol:phospholipid molar ratio of the complexes was determined (**Table 1**). Compared with controls, there is only a small decrease (8%) in the unesterified cholesterol:phospholipid (UC:PL) ratio of the 11 nm complex after re-incubation. This is consistent with the observation that the major subpopulations after re-incubation are 9 and 11 nm particles that typically have a high UC:PL ratios. The 7.3 nm complex, on the other hand, appears to have a somewhat increased (20%) UC:PL ratio. However, the ratio does not approach that seen for either the 9 and 11 nm particles incubated in the absence of cells and thus is consistent with the nondenaturing gradient gel data demonstrating that the 7.3 nm complex does not give rise to appreciably larger particles after incubation with cells. On the other hand, the 9 nm nascent complexes re-incubated with cells show a significant decrease in the UC:PL ratio compared with controls. This decrease is consistent with the appearance of 7.3 nm particles (Fig. 5) which have a low UC:PL ratio.

To generate extracellularly assembled apoA-I–lipid complexes, we normally incubate 10 μg apoA-I/ml with the CHO cells; however, approximately 20–30% of the added apoA-I forms lipid complexes while the remainder stays in the lipid-free form. This suggested to us that the presence of lipid-free apoA-I may be required to drive the assembly of the larger particles from the 7.3 nm complexes. To test this premise we incubated the 7.3 nm complexes (10 μg apoA-I/ml) with cells in the presence of 1 or 2 μg/ml of lipid-free apoA-I. As shown in **Fig. 6,** additional larger complexes are formed when lipid-free apoA-I is present in the medium with the 7.3 nm particles suggesting that apoA-I may be a driving force in the formation of larger complexes.

DISCUSSION

Structural relationships between extracellularly formed apoA-I–lipid complexes

Several laboratories including our own have demonstrated that apoA-I incubated with cells including CHO cells (5), fibroblasts (16), endothelial cells (17), and macrophages (18, 19) was able to recruit phospholipid and cholesterol from the cells to form protein–lipid complexes. Our previous studies indicated that the apoA-I–lipid complexes thus formed represented a spectrum of particles with distinct particle diameters including 7.3, 9, and 11 nm species. The interrelationship between these particles was unclear; however, previous time-dependence studies indicated that the 7.3 nmm particle was the first to appear (within 1 h) and that larger complexes appeared sequentially with increasing time of incubation (5). These earlier studies suggested that there may be a precursor–product relationship between the various particles wherein the smaller 7.3 and 9 nm particles were likely to give rise to larger complexes. Surprisingly, the present study showed that

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these smaller complexes when re-incubated with cells were not direct precursors for larger ones. Rather, it appeared that the larger complexes; i.e., 11 and 9 nm discoidal particles, were more likely to form smaller discs when re-incubated with CHO cells. The 11 nm complexes gave rise primarily to 9 nm complexes and a small amount of 7.3 nm particles while 9 nm complexes gave rise mainly to 7.3 nm particles. This suggests that in our cell culture system reorganization and remodeling of larger discs favors the formation of smaller particles.

Unlike the larger complexes, the 7.3 nm particles were remarkably stable and did not form additional complexes upon re-incubation with cells. Part of the difference in behavior of the 7.3 nm complexes, compared with 9 and 11 nm complexes, may be related to the protein–lipid stoichiometry of the extracellularly assembled particles (summary in **Table 2**). It is estimated that six of the potential seven 22 amino acid amphipathic helices in apoA-I are associated with the edge of the 7.3 nm disc (Table 2); this dense packing of apoA-I at the edge of the disc may provide an extremely stable protein–lipid complex. Our data strongly suggest that a small quantity of lipid-free apoA-I may drive the formation of larger particles from the 7.3 nm complexes because our previous studies demonstrated that generation of larger complexes is both time- and apoA-I concentration-dependent where 7.3 nm particles first predominated followed by the generation of 9 and 11 nm particles (5). In such incubations, lipid-free apoA-I was always available. We cannot, however, completely rule out the possibility that the larger complexes are the result of direct production from lipid-free apoA-I. Although speculative, lipid-free apoA-I may destabilize the 7.3 nm particles perhaps through protein–protein interactions thus permitting remodeling of the particle.

The unesterified cholesterol to phospholipid mole ratio decreases during incubation of 11 or 9 nm complexes with CHO cells. This can be accounted for either by loss of cholesterol from, or addition of phospholipid to, the complexes. Although one cannot discount the ability of the larger complexes to donate cholesterol to cells, it is less likely to occur in the non-dividing confluent cultures. However, phospholipid addition to the complexes may occur because, as suggested in Table 2, there are two amphiphatic helices per apoA-I molecule

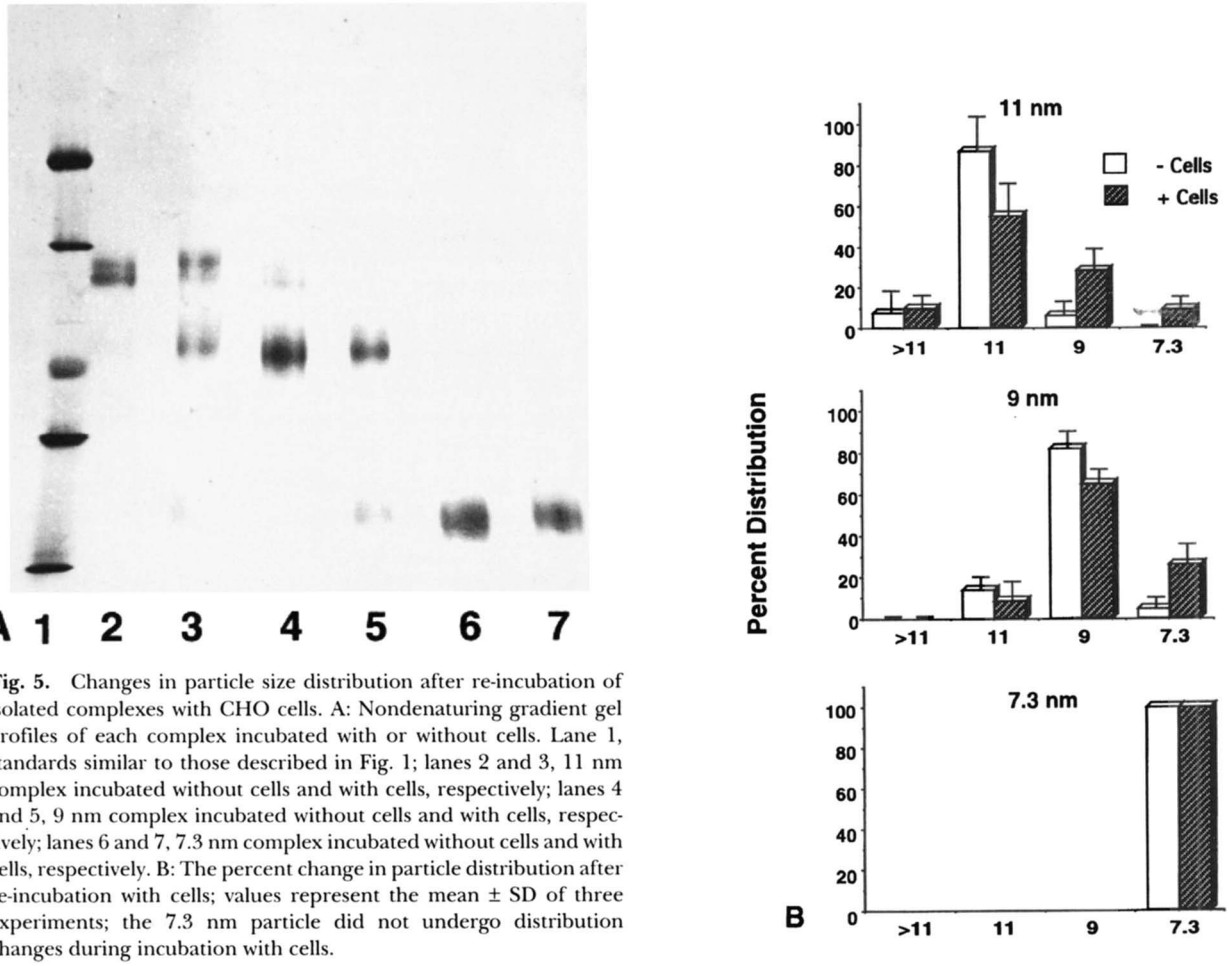

Fig. 5. Changes in particle size distribution after re-incubation of isolated complexes with CHO cells. A: Nondenaturing gradient gel profiles of each complex incubated with or without cells. Lane 1, standards similar to those described in Fig. 1; lanes 2 and 3, 11 nm complex incubated without cells and with cells, respectively; lanes 4 and 5, 9 nm complex incubated without cells and with cells, respectively; lanes 6 and 7, 7.3 nm complex incubated without cells and with cells, respectively. B: The percent change in particle distribution after re-incubation with cells; values represent the mean ± SD of three experiments; the 7.3 nm particle did not undergo distribution changes during incubation with cells.

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TABLE 1. UC:PL mole ratio of complexes re-incubated in the presence and absence of CHO cells

Particle (nm)	UC/PL Mole Ratio No Cells	UC/PL Mole Ratio With Cells
7.3	0.40 ± 0.03	0.47 ± 0.04
9	0.84 ± 0.02	0.62 ± 0.04[a]
11	0.92 ± 0.05	0.85 ± 0.03

Each value represents the mean ± SD from three experiments.
[a]P = 0.001 compared with no cells.

not associated with the edge of the 11 and 9 nm discs that may be capable of binding additional phospholipid. Addition of phospholipid to the 11 nm complex (contains 4 apoA-Is) may destablize the particle with the generation of 9 nm complexes containing 3 apoA-Is and the release of one molecule of apoA-I that may be associated with a small charge of lipid or be lipid-free. Fusion of two apoA-Is containing a small quantity of phospholipid and cholesterol could give rise to additional 7.3 nm particles. Alternatively, apoA-I in the lipid-free form could recruit additional phospholipid and cholesterol from cells thus forming 7.3 nm particles. The same process probably occurs during the re-incubation of 9 nm particles (contain 3 apoA-Is) with cells except that the 3 apoA-I-containing complex is remodeled primarily into 7.3 nm complexes containing two molecules of apoA-I.

Complexes formed in CHO cell cultures are different in composition and apoA-I–lipid stoichiometry from reconstituted high density lipoprotein (rHDL) formed in vitro by cholate dialysis using palmitoyloleoylphosphatidylcholine (POPC) or egg yolk lecithin and cholesterol. Under cholate dialysis conditions, complexes have been generated that are similar in size to those formed by incubation of apoA-I with CHO cells; i.e., small particles ranging in size from 7.7 to 8.4 nm have been reported (20–22) while larger particles including two distinct complexes, one approximately 9.6–9.7 nm and the other 10.8–13.6 nm, have also been reported (20–23). In size, these rHDL are similar to the 7.3, 9, and 11 nm complexes isolated from CHO cell medium, respectively. The 7.3 nm particles in the present study have the same number of apoA-I molecules (two) per particle as the small rHDL complex. However, the 9 nm and 11 nm particles in the present study possess 3 and 4 apoA-Is per particle, respectively, whereas rHDL of similar size possess only 2 and 3 apoA-Is, respectively. The difference in the number of apoA-I molecules in 9 and 11 nm CHO cell complexes compared to rHDL may be related to differences in phospholipid in the two types of complexes; we have previously shown that, in addition to phosphatidylcholine, CHO cell complexes are relatively enriched in sphingomyelin (6). The CHO cell complexes have higher unesterified cholesterol to phospholipid ratios than the rHDL complexes, suggesting that the phospholipid composition of such complexes favors cholesterol recruitment more so than rHDL complexes. It has been demonstrated that sphingomyelin has a greater capacity for sequestering cholesterol than phosphatidylcholine (24), thus part of the cholesterol enrichment in the CHO cell complexes may be due to its relatively high content of sphingomyelin.

Surface charge properties of the precursors

In the present study we have demonstrated that the isolated 11, 9, and 7.3 nm particles have a greater negative surface charge than plasma HDL, hence the extracellular complexes have pre-alpha mobility. Pre-alpha HDL was first described for small molecular weight HDL isolated from LCAT-deficient subjects by Norum et al. (25) and more recently Asztalos et al. (26) described pre-alpha migrating HDL in normal human plasma. The relationship between the pre-alpha mobility of our complexes and that previously described for plasma apoA-I-containing lipoproteins is unknown; it is provocative, however, that in the case of LCAT deficiency the small molecular weight HDL are rich in

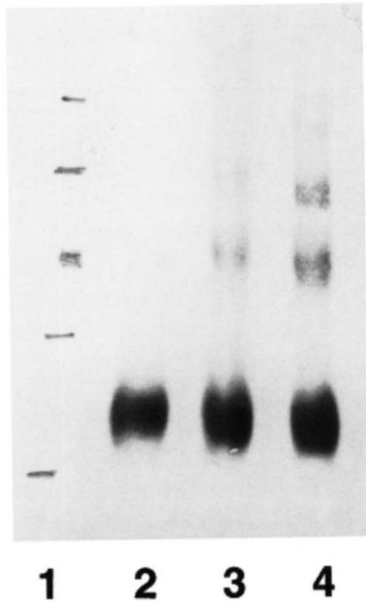

Fig. 6. Effects of lipid-free apoA-I on 7.3 nm particle redistribution in the presence of CHO cells. The 7.3 nm particles were incubated with cells at a concentration of 10 μg apoA-I/ml and either 1 or 2 μg/ml lipid-free apoA-I was added to the flask. Samples were electrophoresed on nondenaturing gradient gels, transferred to nitrocellulose, and Western blotted with apoA-I-specific antibody. Standards in the first lane are the same as those in Fig. 1. Lane 2, 7.3 nm complexes incubated alone with cells; lane 3, 7.3 nm complexes incubated with 1 μg/ml apoA-I plus cells; lane 4, 7.3 nm complexes incubated with 2 μg/ml apoA-I plus cells.

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TABLE 2. Physical/chemical parameters of extracellularly formed apoA-I–lipid complexes

Particle Size (nm)	Estimated Mol. Wt. (Daltons)[a]	# A-I Molecules Per Particle[b]	# PL Molecules Per Particle	PL/A-I	# UC Molecules	UC/A-I	# Helical Segments per A-I Associated with Disc Perimeter[c]
7.3	84,000	2	30	15	12	6	6
9	206,000	3	112	34	91	30	5
11	330,000	4	194	47	175	44	5

[a]Estimated from nondenaturing gradient gels.
[b]Obtained from apoA-I crosslinking data in Fig. 2B.
[c]Calculated as described by Jonas et al. (21).

apoA-I-containing particles that, in their physical and chemical properties, are not unlike the complexes formed in CHO cell cultures, suggesting that LCAT may play a role in determining surface charge. Although speculative, LCAT activity may be required to normalize the charge of the pre-alpha particles; disc to sphere transformation may alter apoA-I folding thus diminishing exposed negatively charged amino acids or increasing positively charged ones, and/or LCAT activity may alter phospholipid composition in such a way as to decrease negatively charged groups. These mechanisms for altering charge properties of the complexes are presently under investigation.

Previous reports from several laboratories using rHDL formed with POPC demonstrated that such apoA-I–lipid complexes generated by cholate dialysis had pre-beta mobility (27, 28). The studies by Agnani and Marcel (27) indicated that rHDL charge was related to the complex size and number of apoA-I molecules associated with the complex; thus larger complexes with greater numbers of apoA-I molecules/particle had a greater negative surface charge. It was suggested by these authors that the charge properties of the rHDL may be related to the conformation of apoA-I on the complex. Sparks et al. (29) also demonstrated that rHDL had pre-beta mobility and that the addition of cholesterol increased the negative charge of the complexes in addition to increasing the size of the particle, suggesting that the incorporation of cholesterol affected apoA-I conformation. A recent study on the effects of phospholipid acyl chain length and degree of unsaturation on rHDL properties suggested that both these parameters, as well, induce changes in the conformation of apoA-I which is reflected by changes in charge of the particles (30). In the present study there appear to be differences in the packing (Table 2) of apoA-I helices in the 7.3 nm particle versus those of the 9 and 11 nm particles yet they all have similar charge properties. This suggests that the increased charge of our complexes is not be solely related to changes in protein conformation associated with an increase in apoA-I number and particle size.

Physiological significance

Studies on the incubation of lipid-free apoA-I with various types of cells indicate that apoA-I is efficient in recruiting cholesterol from cells but that phospholipid is a required participant (5, 19). Not all cells, however, are donors of cell cholesterol as demonstrated by Komaba et al. (31) who discovered that apoA-I could not readily recruit cholesterol from smooth muscle cells. This suggests that recruitment of cholesterol from cells by lipid-free apoA-I is likely dependent on cell type as well as factors such as membrane phospholipid species, cholesterol turnover in cells, and the packing of cholesterol in membranes.

Lipid-free apoA-I has been shown to be present in plasma (32, 33) and interstitial fluid (34). In addition to direct secretion of lipid-free apoA-I, non-lipidated apoA-I may be generated in plasma during hydrolysis of VLDL in the presence of HDL, hepatic lipase, and CETP as demonstrated by the in vitro incubations of Clay et al. (35). Because of its small size, lipid-free apoA-I would readily diffuse into the interstitial space and its diffusion rate would be greater than that of mature HDL with larger radii. HDL, particularly those without apoA-II, are effective in stimulating cholesterol efflux from peripheral cells (1–4); moreover, as recently demonstrated by Yancey et al. (19), lipid-free apoA-I is twice as effective as mature HDL in recruiting cholesterol from lipid-laden mouse macrophages. The above studies suggest that the extracellular pathway for HDL assembly may be a mechanism for regulating cellular cholesterol accumulation. Furthermore, our present study suggests that extracellular assembly of HDL is a dynamic process that is dependent on the availability of free apoA-I.

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