O-Linked Glycosylation Modifies the Association of Apolipoprotein A-II to High Density Lipoproteins*

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O-Linked glycosylation is a common post-translational modification of apolipoproteins, but no structural or functional role for it has been identified. We examined the biosynthesis of apolipoprotein (apo) A-II in Hep G2 cells and in glycosylation-defective Chinese hamster ovary (CHO) cell mutants transfected with apoA-II cDNA. Three monomeric isoforms of apoA-II with an apparent molecular mass of 8.3, 9.8, and 11.4 kDa were synthesized by Hep G2 cells and transfected wild-type CHO cells. The 9.8- and 11.4-kDa isoforms were sialylated but not the 8.5-kDa isoform. Transfected IdlD cells, which are defective in the biosynthesis of galactose and N-acetylgalactosamine, only produced the 8.5-kDa isoform; however, when grown in media supplemented with these sugars, IdlD cells produced all three isoforms of apoA-II. Pulse-chase analysis of IdlD cells showed that glycosylation was not necessary for secretion of apoA-II. Glycosylation did modify the association of apoA-II with nascent high density lipoprotein (HDL) secreted by Hep G2 cells. The sialylated isoforms were lipid-poor and were present in the lipoprotein-deficient density range, whereas the nonsialylated 8.5-kDa isoform was associated with LpA-I, A-II lipoprotein particles in the HDL density range. ApoA-II from transfected IdlD cells, regardless of glycosylation, were lipid-poor. When preincubated with HDL from serum, however, sialylated apoA-II from both IdlD cells and Hep G2 cells associated with lipoprotein particles within the HDL density range, whereas nonsialylated apoA-II was found throughout the HDL density range. In summary, O-linked glycosylation is not necessary for the secretion of apoA-II but does modify the association of apoA-II to HDL and may, therefore, play an important role in the metabolism of HDL.

Apolipoprotein (apo) A-II is the second most abundant protein on high density lipoproteins (HDL) (1, 2) and is important in the metabolism and function of HDL (3–5). It exists as a 17.4-kDa homodimer and has no consensus sequence for N-linked glycosylation (1, 2). Plasma apoA-II is not significantly glycosylated (1); however, in lymph (6) and in Hep G2 cell media (7), approximately half of apoA-II is O-linked glycosylated. The function of the glycosylation of apoA-II and the metabolic fate of glycosylated apoA-II is not known.

Besides apoA-II, several other apolipoproteins are O-linked glycosylated (8, 9). ApoC-III exist in the serum as nonsialylated, monosialylated, and disialylated isoforms (10, 11), and the proportion of disialylated apoC-III has been observed to be increased in newborns (12), abetalipoproteinemia (13), and hypertriglyceridemia (14). ApoE also exist in the plasma in several sialylated isoforms (15–17), and the degree of sialylation was observed to be increased in diabetes (18). Studies of natural mutants (19), site-directed mutants (20, 21), and transfected cell lines defective in glycosylation (22), however, have failed to reveal a role for the O-linked glycosylation of apoC-III and apoE.

In the case of apoA-II, the presence of glycosylated isoforms in lymph (6) and Hep G2 cell media (7), but not in plasma, suggests that glycosylated apoA-II is either deglycosylated or undergoes increased catabolism in the plasma, which raises a possible role for glycosylation in the metabolism of apoA-II. For many proteins, glycosylation is known to have widespread effects on both structure and function of proteins (23, 24) and is often particularly important during biosynthesis of proteins (23–25). In order to evaluate the role of glycosylation in the biosynthesis of apoA-II, we examined the secretion of apoA-II in Hep G2 cells and in transfected Chinese hamster ovary (CHO) cells defective in glycosylation. We established that O-linked glycosylation is not necessary for the secretion of apoA-II but does alter the association of apoA-II with lipid and its distribution within HDL subfractions. These results provide evidence for a novel function for glycosylation of proteins and represent the first potential role for post-translational modification of apolipoproteins.

MATERIALS AND METHODS

Cell Culture—The CHO cell lines Pro-5 (26), IdlD (25), Lec 2 (26), Lec 8 (26) (American Type Culture Collection), and the hepatic Hep G2 cell line (American Type Culture Collection) were grown in α-modified minimum essential medium (GIBCO-BRL) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and 0.35 μg/ml of amphotericin B. To deplete intracellular pools of galactose (Gal) and N-acetylgalactosamine (GalNAc), cells were grown for 36–48 h without serum in a 1:1 mix of α-modified minimum essential medium and Ham's F-12 medium with the above supplements and 6.25 μg/ml insulin, 6.25 μg/ml transferrin, 6.25 ng/ml selenious acid, 5.35 μg/ml linoleic acid, and 1.25 mg/ml bovine serum albumin. When IdlD cells were grown in the above serum-free media supplemented with 20 μM of Gal and 200 μM of GalNAc, they are designated as +IdlD cells and as −IdlD cells when grown without these sugars. Stably transfected cell lines were

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The abbreviations used are: apo, apolipoprotein; HDL, high density lipoprotein; CHO, Chinese hamster ovary; +IdlD cells, IdlD cells grown in the presence of Gal and GalNAc; −IdlD cells, IdlD cells grown in the absence of Gal and GalNAc; PAGE, polyacrylamide gel electrophoresis; LPDS, lipoprotein-deficient serum; LpA-I, A-II, apoA-I- and apoA-II-containing lipoprotein particles; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.
maintained in media supplemented with 0.125 mg/ml of active G-418 (GIBCO-BRL).

**Plasmid Construction and Transfection**—A partial cDNA for apoA-II (2) was extended by the polymerase chain reaction with an oligonucleotide that coded for the missing amino acid residues from -1 to -12. The sequence was confirmed by DNA sequencing, and the cDNA was ligated into the unique BamHI site of the pLen expression vector (27) to produce the plasmid designated pLenA-II. CHO cells were cotransfected with 25 pg of pLenA-II and 1 pg of pSV2neo (28) by calcium phosphate precipitation (29). At 48 h the medium was replaced with fresh medium containing 1 mg/ml of active G-418, and thereafter, the medium was replaced every 2 days with fresh medium containing G-418. At 14 days, G-418-resistant colonies were isolated, recultured, and screened for expression of apoA-II by immunoprecipitation as described below. Clonal cell lines were isolated by limiting dilution.

**Immunoprecipitation of Apolipoproteins**—Logarithmically growing cells were metabolically labeled for approximately 12 h with 100 μCi/ml of Trans35S-label (ICN) in methionine and cysteine-free Dulbecco’s modified Eagle’s medium (GIBCO-BRL), supplemented with 0.4 mg/ml proline, 2 mM glutamine, 100 IU/ml of penicillin, 100 μg/ml of streptomycin, 0.25 μg/ml of amphotericin B, and 50 μM ZnCl. For pulse-chase studies, the cells were labeled with 100 μCi/ml of Trans35S-label for 30 min followed by a chase in media supplemented with 10 mM of methionine and 10 mM of cysteine. Medium or cells were solubilized in lysis buffer (30) and immunoprecipitated with polyclonal sheep anti-apoA-II antibody (Boehringer Mannheim) and Pansorbin (Calbiochem) as described previously (30). Co-immunoprecipitation of apoA-I and apoA-II was performed by first immunoprecipitating apoA-I in cell-culture media with polyclonal sheep anti-apoA-I antibody (Boehringer Mannheim) and Pansorbin. After centrifugation, the immunoprecipitate was washed with phosphate-buffered saline and resuspended in lysis buffer containing detergent (30). After the addition of polyclonal sheep anti-apoA-II antibody, the samples were processed as described previously (30). Immunoprecipitated apolipoproteins were either analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10–20% gradient Tricine gel (Novex) or by two-dimensional gel electrophoresis (6). Autoradiography was performed after treatment with Amplify (Amer sham Corp.). The half-life of secretion was calculated from quantitation of intracellular apoA-II by densitometry, and the results are reported as the mean of three experiments plus and minus one standard error of the mean. Neuraminidase treatment was performed with 0.7 unit of Clostridium perfringens neuraminidase (Calbiochem) and phenylmethylsulfonyl fluoride (100 μg/ml) for 12 h at 37 °C on cell-culture media that was dialyzed in 100 mM sodium acetate (pH 5.1) and 1 mM CaCl2.

**Density Gradient Ultracentrifugation**—Density gradient ultracentrifugation was performed on media (4 ml) from cells labeled with Trans35S-label and supplemented with bovine serum albumin (2 mg/ml), phenylmethyisulfon fluoride (100 μg/ml), pepstatin (1 μg/ml), leupeptin (1 μg/ml), and aprotinin (1 μg/ml). When indicated either 0.2 ml of fasting human serum or 10 mg of protein of lipoprotein-deficient serum (LPDS, d > 1.21 g/ml) (31) was preincubated with cell-culture media for 1 h at 37 °C prior to ultracentrifugation. Lecithin:cholesterol acyltransferase (LCAT) in serum was inactivated with 1 mM dithiothreitol (32). Cell-culture media was adjusted to a density of 1.35 g/ml with KBr, and continuous density gradient ultracentrifugation was performed (33). Fractions of 1.2 ml were collected, and density was determined gravimetrically. Apolipoproteins were immunoprecipitated from the fractions after dialysis in phosphate-buffered saline.

**RESULTS**

**Biochemical Characterization of Recombinant ApoA-II**—To examine the role of O-linked glycosylation of apoA-II, we expressed apoA-II in CHO cell lines that have well-characterized defects in glycosylation (Fig. 1). ApoA-II produced by transfected CHO cells and Hep G2 cells was labeled with Trans35S-label, immunoprecipitated, and analyzed by SDS-PAGE, followed by autoradiography (Fig. 2). ApoA-II purified from plasma and radioiodinated migrated as a single band of 8.5 kDa (Fig. 2, lane 1), whereas apoA-II from Hep G2 cells migrated as a triplet of 8.5, 9.8, and 11.4 kDa (Fig. 2, lane 2). Excess unlabeled apoA-II purified from plasma effectively competed with radioiodinated apoA-II secreted by Hep G2 cells (Fig. 2, lane 9), and no co-migrating bands were detected by immunoprecipitation with preimmune serum (data not shown), thus confirming the specificity of the immunoprecipitation. Neuraminidase treatment of apoA-II from Hep G2 cells converted the 9.8- and 11.4-kDa isoforms to the 8.5-kDa isoform (Fig. 2, lane 10). Compared with Hep G2 cells, Pro-5 cells produced relatively more of the 9.8- and 11.4-kDa isoforms of apoA-II. The IdID cell line (25), which is deficient in UDP-Glc/Gal 4-epimerase and cannot synthesize oligosaccharides containing GalNAc and Gal (Fig. 1), produced all three isoforms of apoA-II only if grown in media supplemented with these sugars (+IdID cells; Fig. 2, lane 4). In the absence of these sugars, only the 8.5-kDa isoform of apoA-II was observed (−IdID cells; Fig. 2, lane 5). In addition, a minor lower molecular mass band was sometimes observed (Fig. 2, lane 5), which may be a proteolytic fragment of apoA-II because of the increased susceptibility of nonglycosylated proteins to proteases (25, 34). The Lec 2 and the Lec 8 cell lines (26), which are defective in the addition of sialic acid and galactose, respectively (Fig. 1), only produced the 8.5-kDa isoform of apoA-II (Fig. 2, lanes 6 and 7). Based on its migration, apoA-II produced by transfected Lec 8 cells apparently did not contain sialic acid, most likely because the preferred substrate for the sialyltransferases is the disaccharide core (23, 24, 35).
We next analyzed apoA-II synthesized by Hep G2 cells and transfected CHO cells by two-dimensional gel electrophoresis. ApoA-II from Hep G2 cells existed in at least six major isoforms (Fig. 3A), as has been described previously (7). The 0 and 1 isoforms are the major isoforms found in plasma and co-migrated with the 8.5-kDa band observed by SDS-PAGE. The more acidic and higher molecular mass isoforms were converted to the 0 and 1 isoform after neuraminidase treatment (Fig. 3B). Based on their migration, the doublet appearing just to the right and above the 0 isoform corresponds to the 9.8-kDa isoform observed by SDS-PAGE (Fig. 2), whereas the most acidic and highest molecular mass doublet corresponds to the 11.4-kDa isoform. The 0 and 1 isoform doublet and the other two doublets were shown previously to differ from each other by only a single positive charge due to cyclization of the N-terminal glutamine of apoA-II (7). Transfected +lID cells produced the same isoforms as Hep G2 cells but contained relatively more glycosylated isoforms than did Hep G2 cells (Fig. 3C). The remaining minor bands appearing to the left and above the first member of each doublet is the pro-form of apoA-II (7). LdID cells grown without sugar supplements (Fig. 3D), Lec 2 cells (Fig. 3E) and Lec 8 cells (Fig. 3F), only produced the 0 and 1 isoform, which is consistent with what was observed by SDS-PAGE (Fig. 2). The additional minor bands, which were lower in molecular mass and more basic, may be proteolytic fragments of apoA-II, which form because of the increased susceptibility of nonglycosylated proteins to proteases (25, 24). We did not observe a consistent difference in the migration of the isoforms of apoA-II produced by the mutant CHO cell lines, although they would be predicted to differ in their content of core oligosaccharides. This may be due to the inability to resolve these minor differences by electrophoresis and because of the disproportionate effect of sialylation on the migration of proteins by SDS-PAGE (7, 20, 22, 24). In summary, three isoforms of apoA-II are detected by SDS-PAGE but by two-dimensional gel electrophoresis at least six major isoforms of apoA-II were observed. Although SDS-PAGE did not separate the charge isoforms of apoA-II, it was sufficient for the separation of the sialylated isoforms of 11.4 and 9.8 kDa from each other and from the 8.5-kDa nonsialylated isoform of apoA-II.

Effect of Glycosylation on the Secretion of ApoA-II—In order to examine the role of O-linked glycosylation in the secretion of apoA-II, we performed pulse-chase studies on Hep G2 cells (Fig. 4A) and transfected lID cells grown in the presence (+lID cells; Fig. 4B) and in the absence of Gal and GalNAc (−lID cells; Fig. 4C). After 4 h of chase of Hep G2 cells, the majority of intracellular apoA-II was secreted out of the cell and appeared in the medium. Similar kinetics of secretion were observed for secretion of glycosylated apoA-II produced by +lID cells. Compared with Hep G2 cells, however, only trace amounts of intracellular glycosylated apoA-II was observed for +lID cells. This is similar to what has been observed previously for apoE when expressed in CHO cells versus Hep G2 cells (20, 36). When grown without Gal and GalNAc, −lID cells only produced the 8.5-kDa isoform, but it was also readily secreted out of the cells by 4 h of chase. We consistently observed, however, that for the early time points the rate of secretion from −lID cells (Fig. 4C, lanes 1–3) appeared to be slightly slower relative to +lID cells or Hep G2 cells. From a mean of three experiments, we calculated the half-life of secretion of apoA-II from these cells to be 52 ± 2 min for Hep G2 cells, 50 ± 6 min for +lID cells, and 78 ± 2 min for −lID cells. Overall these results suggest that unlike other glycoproteins, glycosylation is not necessary for the secretion of apoA-II; however, the slightly longer half-life of secretion of apoA-II by −lID cells suggest that glycosylation may facilitate the secretion of apoA-II.

Effect of Glycosylation on the Association of ApoA-II with HDL—The effect of glycosylation on the association of apoA-II with HDL was analyzed by density gradient ultracentrifugation of cell-culture media from Hep G2 cells (Fig. 5). In the lipoprotein-poor deficient serum (LPDS) density range (d > 1.21 g/ml), four bands were observed (Fig. 5A, lanes 1–3). The top band is a nonspecific protein that could be removed after repeated preclearing of the samples (Figs. 2 and 3) and was also detected when immune serum other than anti-apoA-II was used for immunoprecipitation (Fig. 5D). The remaining three lower molecular mass bands are the three isoforms of apoA-II. The sialylated 9.8- and 11.4-kDa isoforms were primarily detected in the LPDS density range. In contrast, the majority of nonsialylated 8.5-kDa isoform of apoA-II (Fig. 5A, lanes 4–7) was associated with lipid and present throughout the entire HDL density range (d = 1.063–1.21 g/ml). ApoA-II dimers were detected in the samples with a density of HDL and when analyzed under reducing conditions only appeared to consist of the 8.5-kDa isoform (Fig. 5B, lanes 3–7). When media from Hep G2 cells was preincubated for 1 h at 37 °C prior to ultracentrifugation with fasting serum (Fig. 5C), in order to provide an exogenous source of HDL, the sialylated 9.8- and 11.4-kDa isoforms shifted to the HDL density range (d = 1.125–1.21 g/ml). Preincubation with serum resulted in

Fig. 3. Two-dimensional gel electrophoresis of apoA-II produced by CHO cells. Media from the following cell lines labeled with Trans3'S-label was immunoprecipitated for apoA-II and analyzed by two-dimensional gel electrophoresis, followed by autoradiography. A, Hep G2 cells; B, neuraminidase-treated Hep G2 cell media; C, transfected +lID cells; D, transfected −lID cells; E, transfected Lec 2 cells; F, transfected Lec 8 cells. The major 0 and 1 apoA-II isoforms of plasma are circled.

Fig. 4. Pulse-chase analysis of apoA-II secreted by lID cells. Hep G2 cells (row A), transfected +lID cells (row B), and transfected −lID cells (row C) were labeled for 0.5 h with Trans3'S-label followed by a chase, indicated in hours above each lane. Media and cell extracts from each time point were immunoprecipitated for apoA-II and analyzed by SDS-PAGE, followed by autoradiography.
Based on the density of each fraction, the cells.

Each lane indicate the approximate location of the major lipoprotein fractions. Based on the density of each fraction, the arrows above each lane indicate the approximate location of the major lipoprotein fractions. M and D refer to the position of the monomer and dimer forms of apoA-II, respectively.

Fig. 5. Density distribution of apoA-II secreted by Hep G2 cells. Media from Hep G2 cell labeled with Trans35S-label was separated by continuous density gradient ultracentrifugation, immunoprecipitated for the following apolipoproteins, and analyzed by SDS-PAGE, followed by autoradiography. A, nonreduced apoA-II; B, reduced apoA-II; C, reduced apoA-II preincubated with serum; and D, apoC-II. Based on the density of each fraction, the arrows above each lane indicate the approximate location of the major lipoprotein fractions. M and D refer to the position of the monomer and dimer forms of apoA-II, respectively.

Fig. 6. Co-immunoprecipitation of apoA-I and apoA-II. Media from Hep G2 cells labeled with Trans35S-label was co-immunoprecipitated with the following immune serum and analyzed by SDS-PAGE, followed by autoradiography. Lane 1, anti-apoA-I; lane 2, anti-apoA-II; lane 3, anti-apoA-I followed by anti-apoA-II; and lane 4, no immune serum. The top and bottom arrows indicate the position of apoA-I and apoA-II, respectively.

Fig. 7. Density distribution of apoA-II secreted by +IdlD cells. Media from transfected ldlD cells labeled with Trans35S-label was separated by continuous density gradient ultracentrifugation, immunoprecipitated, and analyzed by SDS-PAGE, followed by autoradiography for the following conditions. A, nonreduced apoA-II from +IdlD cells; B, nonreduced apoA-II from −IdlD cells; C, nonreduced apoA-II from +IdlD cells preincubated with serum; and D, nonreduced apoA-II from −IdlD cells preincubated with serum. Based on the density of each fraction, the arrows above each lane indicate the approximate location of the major lipoprotein fractions. M and D refer to the position of the monomer and dimer forms of apoA-II, respectively.

and 2), thus establishing the specificity of the co-immunoprecipitation. Immunoprecipitation in the absence of any immune serum and without any washing also did not result in any bands co-migrating with apoA-I or apoA-II (Fig. 6, lane 4). When apoA-I and apoA-II was sequentially co-immunoprecipitated, we detected both apoA-I and apoA-II (Fig. 6, lane 3), suggesting that they are present on the same lipoprotein particles. However, compared with the direct immunoprecipitation of apoA-II (Fig. 6, lane 2), in which we observed all three isoforms of apoA-II, only the non-sialylated 8.5-kDa isoform of apoA-II was associated with LpA-I, A-II (Fig. 6, lane 3). This supports our previous observation that the sialylated isoforms of apoA-II secreted by Hep G2 cell media are lipid-poor, which could account for their lack of association with any other apolipoproteins.

Analysis of the density distribution of apoA-II secreted by transfected ldlD cells revealed that the majority of apoA-II was not associated with lipid and appeared in the LPDS density range, regardless of its state of glycosylation (Fig. 7, A and B, lanes 1–3). Dimers of apoA-II were also detected in these lipid-poor density fractions. Preincubation with serum
shifted the sialylated apoA-II to primarily the HDL₃ density range and resulted in a staircase pattern of apoA-II distribution (Fig. 7C, lanes 4–6), as we observed for apoA-II secreted by Hep G2 cells. The majority of the 8.5-kDa isoform produced by −lID cells also shifted out of the LPDS density range when preincubated with serum, whereas the same isoform secreted by the nonsialylated apoA-II, the 8.5 kDa isofrom appeared throughout the HDL density range (Fig. 7D, lanes 4–7). ApoA-II dimers also shifted to the HDL density range when preincubated with serum (Fig. 7, C and D, lanes 4–6). Therefore, in contrast to Hep G2 cells, lID cells secreted all three isoforms of apoA-II without significant amounts of lipid, but like Hep G2 cells, in the presence of serum, the sialylated isoforms preferentially associated with lipoproteins of a density of HDL₃.

**DISCUSSION**

We found as reported previously (7) that apoA-II is secreted by Hep G2 cells in sialylated and nonsialylated isoforms. ApoA-II produced by transfected Pro-5 CHO cells had a similar distribution of isoforms as Hep G2 cells (Fig. 2), suggesting that the O-linked glycosylation of apoA-II is not strictly cell-dependent or due to aberrant post-translational processing by Hep G2 cells. This is supported by our previous observation of glycosylated apoA-II in thoracic duct lymph (6), which may be enriched in newly secreted apoA-II from the liver (38). The structure of the oligosaccharides on apoA-II are not known; however, a likely possibility is a disialylated type 1 core structure (Fig. 1), which is commonly found on secretory proteins (23, 24, 35). The 9.8-kDa isoform of apoA-II may contain one disialylated type 1 core oligosaccharide and the 11.4-kDa isoform two such oligosaccharides. This would be consistent with the molecular mass shift (Fig. 2) and the previously observed (7) negative charge shift of two and four for the 9.8- and 11.4-kDa isoforms, respectively. Although we were unable to resolve by SDS-PAGE or by two-dimensional gel electrophoresis the nonglycosylated apoA-II produced by −lID cells from apoA-II isoforms containing only the core oligosaccharides produced by Lec 2 and Lec 8 cells (Fig. 2 and 3), the 8.5-kDa isoform secreted by Hep G2 cells and Pro-5 cells is likely to be completely nonglycosylated. Nonsialylated isoforms of proteins commonly do not contain any core oligosaccharides due to incomplete utilization of potential glycosylation sites (11, 20, 35).

Because of the hydrophobic nature of apoA-II, we hypothesized that the addition of relatively large and hydrophilic oligosaccharides to apoA-II would alter its physicochemical properties and function. For many proteins, the addition of O-linked oligosaccharides is necessary for intracellular transport and secretion (23–25, 39); however, the nonglycosylated 8.5-kDa isoform produced by −lID cells was readily secreted (Fig. 4). The slightly longer half-life of secretion would, however, suggest that glycosylation may facilitate the secretion of apoA-II. Another minor difference that we observed was that in contrast to Hep G2 cells, only trace amounts of intracellular glycosylated apoA-II was detected. This has been found previously for other proteins expressed in CHO cells (20) and may indicate a short residence time of the protein in the Golgi(36).

When we examined the lipid binding properties of the various isoforms of apoA-II secreted by Hep G2 cells, we observed a difference in the density distribution of sialylated and nonsialylated isoforms of apoA-II. Based on the their density, the sialylated isoforms of apoA-II were not associated with significant amount of lipid and appeared to be relatively lipid-poor (Fig. 5A). The nonsialylated isoform, in contrast, was associated with lipid and appeared in the HDL density range (Fig. 5). It was also the only isoform associated with LP-A-I, A-II lipoprotein particles (Fig. 6). After preincubation with serum, the sialylated isoform of apoA-II preferentially shifted to the HDL₃ density range (Figs. 5C and 7C). Inactivation of lecithin:cholesterol acyltransferase in the serum used for preincubation or the addition of LPDS to the cell culture media had no effect on the density distribution of apoA-II, indicating that it was the presence of HDL in serum and not lipoprotein-modifying enzymes that changed the distribution of apoA-II. Differences in the structure and composition of HDL may account for the association of sialylated apoA-II to serum HDL but not to HDL from Hep G2 cells. HDL isolated from Hep G2 cell media is low in core lipids and largely discoidal in shape (31, 40). The conformation of apolipoproteins on discoidal particles is different than that on spherical particles (41), and glycosylation may prevent apoA-II from assuming the proper conformation to bind discoidal particles. A similar steric mechanism may also explain why in the presence of HDL from serum that the sialylated apoA-II preferentially bound to the smaller diameter HDL₃ particles rather than the larger diameter HDL₄ particles (Fig. 5C). Alternatively, the glycosylated isoforms of apoA-II may bind to HDL₂ and HDL₃ equally well but after binding may increase the density of the lipoprotein particle to that of HDL₄.

In contrast to Hep G2 cells, transfected lID cells secreted apoA-II in the lipoprotein-poor density range, regardless of the state of glycosylation of apoA-II (Fig. 7). Other nonhepatic cell lines transfected with other apolipoproteins also secrete apolipoproteins without significant amounts of lipid (27, 42, 43), possibly due to a lower rate of lipid synthesis. We also observed that lID cells secreted apoA-II dimers in the lipoprotein-poor density region (Fig. 5, A and B), which indicates that lipid-association is not necessary for apoA-II dimerization.

The lack of difference in the density distribution of the sialylated and nonsialylated isoforms of apoC-II (Fig. 5D) and other apolipoproteins previously studied (21, 44) suggests that not only the addition but also the location of the oligosaccharide on apoA-II may be critical in altering its lipid binding properties. The location of the glycosylation sites on apoA-II are not known, but there are three structural domains on apoA-II, which contain serines or threonines, and that glycosylation could alter lipid binding: the hydrophobic face of the amphipathic helices, the hydrophilic face of the amphipathic helices, and the β-turns between the amphipathic helices. Addition of oligosaccharides on the hydrophobic face of an amphipathic helix could by changing the hydrophobicity of a protein or by steric interference decrease its affinity for lipid. Addition of negatively charged oligosaccharides to the hydrophilic surface of an amphipathic helix could decrease lipid affinity by interfering with the charge stabilization between positively charged groups of leucines on the hydrophilic side of the helix with the negative charge groups of phospholipids (45). If apoA-II is glycosylated at two adjacent sites on the hydrophilic face, this could destabilize secondary structure formation (34) and, therefore, lipid affinity. Although there is no consensus sequence for O-linked glycosylation, it most commonly occurs adjacent to β-turns (46). Glycosylation is known to alter the conformation of β-turns (47), and this could affect lipid binding of apoA-II by changing the angle between the amphipathic helices, which could explain the preferential association of sialylated apoA-II with the smaller diameter HDL₃ particles. Future studies of the site of glycosylation of apoA-II and its effect on structure will be useful in further exploring these possibilities.
The physiologic importance of the glycosylation of apoA-II is not known, but the results from this study suggest several possibilities. The absence of glycosylated isoforms of apoA-II in plasma could be due to increased renal clearance. If glycosylation reduces the affinity of apoA-II for lipoproteins, the free apoA-II because of its smaller size may be more readily removed by the kidney (48). If desialylation of apoA-II occurs in plasma, this could increase the affinity of apoA-II for the asialoglycoprotein receptor (49) and result in increased hepatic clearance. Because HDL particles that contain apoA-II are catabolized slower and result in increase hepatic clearance. Because HDL particles that contain apoA-II are catabolized slower and result in increased hepatic clearance.

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