review

Plasma lipoproteins: apolipoprotein structure and function

Robert W. Mahley, Thomas L. Innerarity, Stanley C. Rall, Jr., and Karl H. Weisgraber

The Gladstone Foundation Laboratories for Cardiovascular Disease, The Cardiovascular Research Institute, Departments of Pathology and Medicine, University of California, San Francisco, CA 94140

Abstract Plasma lipoprotein metabolism is regulated and controlled by the specific apolipoprotein (apo-) constituents of the various lipoprotein classes. The major apolipoproteins include apoE, apoB, apoA-I, apoA-II, apoA-IV, apoC-I, apoC-II, and apoC-III. Specific apolipoproteins function in the regulation of lipoprotein metabolism through their involvement in the transport and redistribution of lipids among various cells and tissues, through their role as cofactors for enzymes of lipid metabolism, or through their maintenance of the structure of the lipoprotein particles. The primary structures of most of the apolipoproteins are now known, and various functional domains of these proteins are being mapped using selective chemical modification, synthetic peptides, and monoclonal antibodies. Furthermore, the establishment of structure-function relationships has been greatly advanced by the identification of genetically determined variants of specific apolipoproteins that are associated with a disorder of lipoprotein metabolism. Future studies will rely heavily on the use of recombinant DNA technology and site-specific mutagenesis to elucidate further the correlations between structure and function and the role of specific apolipoproteins in lipoprotein metabolism.—**Mahley, R. W., T. L. Innerarity, S. C. Rall, Jr., and K. H. Weisgraber.** Plasma lipoproteins: apolipoprotein structure and function. *J. Lipid Res.* 1984. **25:** 1277–1294.

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The plasma lipoproteins are water-soluble macromolecules (pseudomicellar particles) representing complexes of lipids (triglycerides, cholesterol, and phospholipids) and one or more specific proteins, referred to as apolipoproteins. The lipoproteins are separated into various classes based on the density at which they float by ultracentrifugation. They are further classified on the basis of particle size, electrophoretic mobility, or affinity chromatography. The specific classes have unique metabolic functions (for review see Refs. 1–3).

I. THE MAJOR PLASMA LIPOPROTEINS

Human plasma lipoproteins are commonly divided into six major classes. *Chylomicrons* are the largest lipoproteins (>100 nm in diameter). They are synthesized by the intestine to transport dietary triglyceride and cholesterol from the site of absorption in the intestinal epithelium to various cells of the body. The triglycerides of these particles are hydrolyzed within the plasma compartment by the action of lipoprotein lipase, which is attached to endothelial surfaces. The fatty acids liberated during hydrolysis are used as an energy source by various cells or are taken up by adipocytes and stored as triglycerides. The lipoprotein particles generated by the action of lipoprotein lipase on chylomicrons are referred to as *chylomicron remnants;* they are enriched in cholesterol and are rapidly cleared by the liver. Chylomicron remnants that accumulate in the plasma of animals after consuming diets high in fat and cholesterol and in the plasma of patients with type III hyperlipoproteinemia are β-migrating very low density lipoproteins (β-VLDL) (4).

Very low density lipoproteins (VLDL, d < 1.006 g/ml) are 30- to 90-nm particles that transport triglycerides and cholesterol from the liver for redistribution to various tissues. Within the plasma compartment, the triglycerides of VLDL are hydrolyzed to free fatty acids by lipoprotein lipase (and hepatic lipoprotein lipase) generating a series of smaller, cholesterol-enriched lipoproteins including *intermediate density lipoproteins* (IDL, d = 1.006–1.019 g/ml) and *low density lipoproteins* (LDL,

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins: LDL, low density lipoproteins; apo-, apolipoprotein; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; SDS, sodium dodecyl sulfate.

d = 1.019–1.063 g/ml). The LDL represent the end product of VLDL catabolism (~20-nm particles) and are the major cholesterol-transporting lipoproteins in the plasma. As a result of defective apolipoprotein (apo-) B,E(LDL) receptors in patients with familial hypercholesterolemia, LDL accumulate in the plasma, and their levels correlate with the existence of accelerated coronary artery disease (5).

High density lipoproteins (HDL, d = 1.063–1.21 g/ml) appear to arise from several sources, including the liver and intestine. In addition, HDL or HDL precursors appear to be produced within the plasma compartment during lipolytic processing of chylomicrons by the generation of phospholipid-protein disks arising from the surface of the lipolyzed chylomicron (6). The HDL, the smallest (~8–12 nm in diameter) of the lipoproteins, are involved in a process referred to as reverse cholesterol transport, a postulated pathway whereby HDL acquire cholesterol from peripheral tissues and transport the cholesterol, directly or indirectly, to the liver for excretion (for review see Ref. 7). The recent observations suggesting that a negative correlation exists between HDL and accelerated vascular disease in man have focused attention on this lipoprotein class and its role in cholesterol metabolism (for review see Ref. 8). In man, much of the cholesteryl esters present in the lipoproteins appears to be formed in association with HDL by the enzyme lecithin:cholesterol acyltransferase (LCAT). This enzyme catalyzes the transfer of a fatty acid (usually linoleic acid) from the β-position of lecithin (phosphatidylcholine) to the 3-β-hydroxy position of cholesterol. The cholesteryl esters formed by this reaction are transferred to other lipoproteins by lipid transfer or exchange proteins in the plasma. In the discussion to follow, the roles of the various apolipoproteins in regulating the complex interrelationships among the several lipoprotein classes will be considered.

II. APOLIPOPROTEINS AND THEIR ROLES IN METABOLISM

The apolipoprotein constituents of the major plasma lipoproteins can be visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as shown in **Fig. 1.** It is now established that the apolipoproteins of the various lipoproteins regulate lipoprotein metabolism and determine the unique roles of these lipoproteins in lipid metabolism. Several major functions have thus far been ascribed to specific apolipoproteins. One well-established function is their involvement in the *transport and redistribution of lipids among various tissues.* The delivery of lipids to specific cells involves the recognition of specific apolipoproteins by cell surface lipoprotein receptors. Apolipoproteins B-100 and E have been shown to mediate the interaction of lipoproteins with apoB,E(LDL) receptors of the liver and extrahepatic tissues and with apoE receptors (possibly representing remnant receptors) of the liver (for review see Ref. 3). These receptors mediate the uptake of apoB-100- and apoE-containing lipoproteins and regulate their levels in the plasma. In addition, they are responsible for the redistribution of cholesterol among cells for use in membrane biosynthesis, for use as a precursor for steroid production, e.g., in the adrenal, ovary, or testes, or for cholesterol elimination from the body (as occurs through the liver). Regulation of lipid transport by specific apolipoproteins is exemplified by the roles of hepatic apoB-100 in VLDL biosynthesis and secretion and of intestinal apoB-48 in chylomicron biosynthesis and secretion. Patients with defective apoB production or assembly of apoB-containing lipoproteins lack the ability to form VLDL and/or chylomicrons (for review see Ref. 9). These abnormalities disrupt the lipid transport functions of the intestine and/or liver in the synthesis of VLDL and chylomicrons.

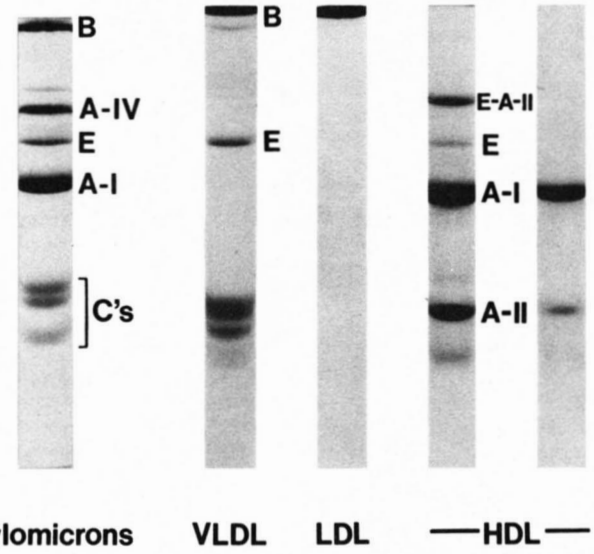

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gels of human plasma lipoprotein classes demonstrating the major apolipoproteins associated with each lipoprotein class. From Mahley and Innerarity (3); used with permission.

A second function of specific apolipoproteins involves their role as *cofactors for enzymes of lipid metabolism* (for review see Ref. 2). Lipoprotein lipase, which catalyzes the hydrolysis of chylomicrons and VLDL triglycerides, requires the presence of apoC-II. The LCAT reaction is activated by apoA-I, which many believe is the major activator in the plasma. However, it is now apparent that other apolipoproteins possess some degree of cofactor activity, including A-II, C-I, C-II, C-III (10), and A-IV (11). The cofactors appear to activate LCAT by providing a suitable lipid or liposome interface upon which the enzyme can work.

An additional function for specific apolipoproteins involves their role in the *maintenance of the structure of the lipoproteins.* Various apolipoproteins, e.g., apoB, A-I, and E, appear to stabilize the micellar structure of the lipoproteins and function, in association with phospholipids on the surface of the particles, to provide a hydrophilic surface. The structure and function of the major apolipoproteins will be discussed in the sections to follow.

A. Apolipoprotein E

Apolipoprotein E is a constituent of chylomicrons, chylomicron remnants, VLDL, and HDL-with apoE (HDL_1, HDL_c) (Fig. 1). The plasma concentration is 3–7 mg/dl in normolipidemic subjects, and can be as high as 20–60 mg/dl in subjects with certain types of hyperlipidemia, especially type III (1). This apolipoprotein displays a complex isoform pattern that is due to the presence of multiple, genetically determined alleles at a single locus and to the presence of post-translational sialylation (12–14). Six common phenotypes of apoE are revealed by isoelectric focusing: three homozygous (E4/4, E3/3, and E2/2) and three heterozygous (E4/3, E4/2, and E3/2) phenotypes (for review see Refs. 3, 13, and 15). The major isoforms of apoE have pI values ranging from 5.7 to 6.2. The minor isoforms represent the glycosylated forms of the major protein. The most common phenotype in the human population is E3/3, which is present in approximately 60% of the subjects studied (14, 16–20). The homozygous phenotype E2/2 (and occasionally heterozygosity associated with E2) has been shown to be predictive of the occurrence of dysbetalipoproteinemia (21) and necessary for the expression of type III hyperlipoproteinemia, as will be discussed later (for review see Refs. 15 and 22). Recently, a patient has been reported who expresses features of type III hyperlipoproteinemia, but apparently lacks detectable quantities of apoE (23).

Amino acid sequence analyses have demonstrated that apoE is a polypeptide composed of 299 amino acids (M_r = 34,200) (see **Fig. 2** for the sequence of apoE3) (24). The structure has been confirmed by cDNA analysis of apoE mRNA (25, 26). Apolipoproteins E2 and E4 differ from apoE3 by virture of single amino acid substitutions (24). Apolipoprotein E4 differs from apoE3 at residue 112 in the sequence. Whereas apoE3 possesses cysteine at this site (the only cysteine in E3 is at 112), apoE4 lacks cysteine within its structure and possesses the amino acid arginine at this site [which may be designated by the notation E4($Cys_{112} \rightarrow Arg$)]. This substitution of a basic residue for a neutral amino acid accounts for the relative +1 charge difference displayed by apoE4 compared to apoE3 (for review see Refs. 3, 15, and 22). It is now known that apoE2 is genotypically heterogeneous. Three forms of apoE2 have been described, and all have been found to be associated with the occurrence of type III hyperlipoproteinemia (24, 27–30) (**Table 1**). These involve the substitution of a neutral amino acid for either lysine or arginine within the molecule (this type of substitution accounts for the charge difference seen for E2 compared to E3). The variants are denoted by relating the structure of the mutant to the structure of apoE3 (Table 1). The most frequent variant of apoE2 possesses cysteine substituted for the normally occurring arginine at residue 158; thus, the notation is E2($Arg_{158} \rightarrow Cys$).

Apolipoprotein E has a high degree of ordered structure (as do other apolipoproteins), particularly α-helical structure, both as predicted (24) and as determined experimentally (31). A probable major lipid-binding domain has been predicted to occur in the carboxy-terminal third of the sequence (24). As demonstrated by analysis of its mRNA (25, 26), apoE is synthesized with an 18 amino acid signal peptide, which is co-translationally cleaved (25). Apolipoprotein E does not have a propeptide form (25, 26). The protein is secreted in a highly sialylated form (25, 32), but is then processed by an unknown mechanism to its circulating plasma state in which about 80% of the protein is not sialylated. Apolipoprotein E has been mapped to chromosome 19 in humans (33), as have apoC-II (34) and the apoB,E(LDL) receptor (35).

Other animal species also have an apoE homologue with a plasma lipoprotein distribution similar to that of human apoE. The complete structure of rat apoE has been predicted by cDNA analysis of its mRNA, and it demonstrates 70% homology with the human protein (36). Rabbit and canine apoE also demonstrate about this same degree of homology (Rall, S. C., Jr., K. H. Weisgraber, and R. W. Mahley, unpublished results) and share with the E apolipoproteins of other species the curious feature of almost complete lack of homology at both the amino- and carboxy-termini (37).

Apolipoprotein E is one of the most extensively studied of all the apolipoproteins and appears to have numerous functions. It is likely that as studies continue, more roles will be described, presumably even roles that are not directly related to lipid metabolism. The major functions are described in the following sections.

1. Cholesterol transport and metabolism

The E apolipoproteins are increased in concentration in the plasma of many animals after feeding diets high in fat and cholesterol and become a major protein constituent of two cholesterol-rich lipoproteins, β-VLDL (intestinal and hepatic remnants) and HDL_c (cholesterol-rich HDL-with apoE) (for review see Refs. 3, 7, and 38). Available data now suggest that the β-VLDL may

Apolipoprotein E

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1          10         20         30         40         50         60
KVEQAVETEP EPELRQQTEW QSGQRWELAL GRFWDYLRWV QTLSEQVQEE LLSSQVTQEL

           70         80         90         100        110        120
RALMDETMKE LKAYKSELEE QLTPVAEETR ARLSKELQAA QARLGADMED VCGRLVQYRG

           130        140        150        160        170        180
EVQAMLGQST EELRVRLASH LRKLRKRLLR DADDLQKRLA VYQAGAREGA ERGLSAIRER

           190        200        210        220        230        240
LGPLVEQGRV RAATVGSLAG QPLQERAQAW GERLRARMEE MGSRTRDRLD EVKEQVAEVR

           250        260        270        280        290        299
AKLEEQAQQI RLQAEAFQAR LKSWFEPLVE DMQRQWAGLV EKVQAAVGTS AAPVPSDNH
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Apolipoprotein A-I

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1          10         20         30         40         50         60
DEPPQSPWDR VKDLATVYVD VLKDSGRDYV SQFEGSALGK QLNLKLLDNW DSVTSTFSKL

           70         80         90         100        110        120
REQLGPVTQE FWDNLEKETE GLRQEMSKDL EEVKAKVQPY LDDFQKKWQE EMELYRQKVE

           130        140        150        160        170        180
PLRAELQEGA RQKLHELQEK LSPLGEEMRD RARAHVDALR THLAPYSDEL RQRLAARLEA

           190        200        210        220        230        243
LKENGGARLA EYHAKATEHL STLSEKAKPA LEDLRQGLLP VLESFKVSFL SALEEYTKKLNTQ
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Apolipoprotein A-II

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1          10         20         30         40         50         60
QAKEPCVESL VSQYFQTVTD YGKDLMEKVK SPELQAEAKS YFEKSKEQLT PLIKKAGTEL

           70         77
VNFLSYFVEL GTQPATQ
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Apolipoprotein C-I

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1          10         20         30         40         50      57
TPDVSSALDK LKEFGNTLED KARELISRIK QSELSAKMRE WFSETFQKVK EKLKIDS
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Apolipoprotein C-II

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1          10         20         30         40         50         60
TQQPQQDEMP SPTFLTQVKE SLSSYWESAK TAAQNLYEKT YLPAVDEKLR DLYSKSTAAM

           70         79
STYTGIFTDQ VLSVLKGEE
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Apolipoprotein C-III

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1          10         20         30         40         50         60
SEAEDASLLS FMQGYMKHAT KTAKDALSSV QESQVAQQAR GWVTDGFSSL KDYWSTVKDK

           70         79
FSEFWDLDPE VRPTSAVAA
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Fig. 2. Amino acid sequences of human plasma apolipoproteins. One letter amino acid code: A = Ala, C = Cys, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, K = Lys, L = Leu, M = Met, N = Asn, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, V = Val, W = Trp, and Y = Tyr. In cases where there are discrepancies between the protein sequence and the cDNA sequence predictions, the cDNA predictions have been used as they are less prone to errors (see text).

TABLE 1. Human apolipoprotein E variants[a]

Isoelectric Focusing Position	Charge Relative to ApoE3	Substitution(s)	Receptor Binding Activity Relative to ApoE3	Reference
E4	+1	$Cys_{112} \rightarrow Arg$	100%	24, 27
E2	−1	$Arg_{158} \rightarrow Cys$	<2%	24, 27
E2	−1	$Arg_{145} \rightarrow Cys$	45%	28
E2	−1	$Lys_{146} \rightarrow Gln$	40%	29
E3	0	$Cys_{112} \rightarrow Arg$, $Arg_{142} \rightarrow Cys$	<20%	unpublished
E3	0	$Ala_{99} \rightarrow Thr$, $Ala_{152} \rightarrow Pro$	unknown	26
E1	−2	$Gly_{127} \rightarrow Asp$, $Arg_{158} \rightarrow Cys$	4%	30

[a] The electrophoretic charge, structure, and apoB,E(LDL) receptor binding activities of the variants are compared to normal apoE3, the most frequently occurring apoE structure, the sequence of which is given in Fig. 2.

be atherogenic lipoproteins by virtue of their ability to cause massive cholesteryl ester accumulation in macrophages (for review see Refs. 3, 7, 39, and 40). On the other hand, HDL_c may be formed in cholesterol-fed animals in response to the deposition of cholesterol in peripheral tissues and the need to transport the cholesterol from these tissues to the liver for elimination of the cholesterol from the body (3, 7, 39, 40). The HDL_c would thus be considered protective or anti-atherogenic lipoproteins that participate in redistribution of cholesterol among various cells and in the delivery, either directly or indirectly, of cholesterol to the liver. Apolipoprotein E is the determinant responsible for the cellular uptake of both the HDL_c and β-VLDL (3).

Apolipoprotein E-containing lipoproteins, specifically the HDL-with apoE (HDL_1, HDL_c), play a major role in cholesterol transport in subjects with abetalipoproteinemia (41, 42). These subjects, who lack apoB-containing lipoproteins (9), possess significant quantities of the HDL-with apoE, which are capable of delivering cholesterol to cells via the apoB,E(LDL) receptors (42). In addition, it is of interest that the HDL-with apoE are a major class of lipoproteins in the plasma of human neonates (42). Both neonates and abetalipoproteinemic subjects may depend, to various degrees, upon lipoproteins containing apoE to deliver cholesterol to various tissues.

2. *Receptor-mediated uptake of specific lipoproteins*

Shortly after the description of the LDL receptors on fibroblasts by the now classic studies of Goldstein, Brown, and associates (for review see Refs. 5 and 43), it was observed that not only apoB-containing lipoproteins, e.g., LDL, but also apoE-containing lipoproteins, e.g., HDL_c, interacted with these receptors (for review see Ref. 3). The apoB,E(LDL) receptors are present in both extrahepatic and hepatic tissues. In addition, a unique receptor that interacts with apoE-containing lipoproteins (but not normal LDL) has been described in the liver (44, 45). These apoE receptors may represent the chylomicron remnant receptor of the liver.

Apolipoprotein E binding to the apoB,E(LDL) and apoE receptors is characterized by a much higher affinity compared to the binding of LDL (apoE HDL_c possessing a 20- to 25-fold greater affinity). The higher affinity of apoE binding to the apoB,E(LDL) receptor apparently results from the potential of apoE-containing particles to form multiple interactions with several sites on these receptors (46–48). The apoE also binds with a similar high affinity to the apoE receptors from the liver (44). The high affinity binding of apoE to both the apoB,E(LDL) and apoE receptors correlates with the very rapid rate of plasma clearance of the apoE-containing lipoproteins (clearance in minutes to hours) compared to a much slower clearance of apoB-containing LDL (2 to 3 days). It has now been established that apoE is the major determinant responsible for mediating receptor binding of HDL-with apoE (HDL_1, HDL_c), VLDL, chylomicron remnants, and β-VLDL from cholesterol-fed animals and from type III hyperlipoproteinemic subjects (49).

Much has been learned about the receptor binding activity of apoE by studying the structure and function of the abnormal forms of apoE2 that occur in patients with type III hyperlipoproteinemia (for review see Refs. 3 and 22). Type III hyperlipoproteinemia is a familial disorder characterized by hypertriglyceridemia and hypercholesterolemia that results from defective clearance of remnant lipoproteins (β-VLDL) secondary to the occurrence of apoE2. The various mutant forms of apoE2 all display an impaired ability to interact with both apoB,E(LDL) and apoE receptors (binding activity ranging from <2% to ~45% of normal apoE3 binding) (27–29, 50) (Table 1).

The receptor-defective mutants have helped to define the domain of the apoE molecule responsible for mediating receptor binding (for review see Ref. 3). The sites of substitution associated with defective receptor binding involve residues 142, 145, 146, and 158. These

substitutions involve an interchange of neutral amino acids for either lysine or arginine. Previously, the use of selective chemical modifications of both arginine and lysine had established the importance of these two amino acid residues in mediating receptor binding of apoE, as well as apoB (51, 52). In addition to the mutants focusing attention on the mid-portion of the apoE molecule, a region enriched in arginine and lysine residues, studies using a monoclonal antibody directed to the binding epitope and studies using fragments of the apoE molecule all indicate that the binding domain resides in the vicinity of residues 140 to 160 of the molecule (53–55).

3. Heparin binding

Apolipoprotein E-containing lipoproteins bind to heparin (56), as is also true for the apoB-containing LDL. The same region of apoE that mediates interaction with the lipoprotein receptors has recently been shown to mediate binding to heparin (Weisgraber, K. H., T. L. Innerarity, S. C. Rall, Jr., and R. W. Mahley, unpublished results). It is possible that the binding of these lipoproteins to heparin (or to other glycosaminoglycans) may represent an important physiological mechanism for lipoprotein binding to endothelial surfaces (in association with lipolytic processing) or to the ground substance of the arterial wall (in association with atherogenesis).

4. Formation of cholesteryl ester-rich particles

Cholesterol can be acquired from cholesterol-loaded cells or from an inert support (Celite) by HDL that lack apoE (57). Under experimental conditions that provide a source of active LCAT, the small HDL-without apoE (~10 nm) are increased in size by a parallel increase in the cholesteryl ester and apoE content of the particles. As particle size increases progressively, HDL-with apoE of three distinct sizes are formed: small HDL_1 (~15 nm); large HDL_1 (~20 nm); and HDL_c (~25 nm) (57). These quantum increases in size appear to correlate with the formation of one, two, or three layers of cholesteryl ester within the core of the HDL-with apoE. Apolipoprotein E is required for the formation of these large, cholesteryl ester-rich particles, and it apparently functions to stabilize the surface or to allow for an increase in core size (Koo, C., T. L. Innerarity, and R. W. Mahley, manuscript submitted for publication). In the absence of a source of apoE, the large, cholesterol-rich HDL particles cannot be formed. The presence of apoE on these cholesterol-rich particles would target these lipoproteins to cells with apoB,E(LDL) or apoE receptors.

5. Lipolytic processing of type III β-VLDL

Ehnholm et al. (58) have shown that hepatic β-VLDL from patients with type III hyperlipoproteinemia are not converted to LDL-like particles following incubation with lipoprotein lipase in vitro. Addition of apoC-II and d > 1.21 g/ml lipoprotein-deficient plasma results in conversion of the β-VLDL to "IDL" (but not "LDL"). However, the addition of apoE3 and d > 1.21 g/ml lipoprotein-deficient plasma allows for lipolytic processing of the hepatic β-VLDL to "LDL." Apolipoprotein E2 plus d > 1.21 g/ml lipoprotein-deficient plasma does not mediate this conversion. It has been postulated that apoE3 (but not apoE2) can interact with a factor in the d > 1.21 g/ml lipoprotein-deficient fraction, possibly a lipid transfer or exchange protein, which modifies the chemical composition and allows lipolytic processing to occur. Precisely how apoE3 mediates the change in the hepatic β-VLDL, allowing the further processing of "IDL" to "LDL" by lipoprotein lipase, remains to be determined. The inability of the apoE2-containing β-VLDL to be converted normally to LDL may be relevant to the in vivo observation that type III hyperlipoproteinemic subjects have low levels of plasma LDL (22).

6. Inhibition of mitogenic stimulation of lymphocytes

An immunoregulatory receptor capable of binding apoE has been demonstrated on the surface of lymphocytes (59–62). The binding of apoE-containing lipoproteins to the immunoregulatory receptors renders the lymphocytes resistant to mitogenic stimulation. It appears that the binding of these lipoproteins to the receptors inhibits early transformation events required for lymphocyte activation, e.g., calcium uptake, phosphatidylinositol turnover, and cyclic nucleotide metabolism (for review see Ref. 3). These observations suggest that apoE may have a broader role in immunology than is now understood.

7. Expanded role for apolipoprotein E in metabolism suggested by unique tissue distribution

A major site of synthesis of apoE is the liver (for review see Ref. 2). In addition, Basu et al. (63) demonstrated that macrophages produce large quantities of this protein. Apolipoprotein E is released from these cells independently of the release of cholesterol. However, apoE is available to combine with cholesterol acceptors, such as HDL, in the extracellular fluid and to participate in reverse cholesterol transport (for review see Refs. 3 and 7). The HDL-with apoE have been shown to be formed in vitro (57) and have been shown to be principal cholesterol-transporting lipoproteins in peripheral lymph (64, 65). In addition to the possible participation of the macrophage apoE in lipid transport in interstitial fluid of peripheral tissues, apoE synthesized and secreted by macrophages may function as a regulator of cellular activity in the environment of macrophages by conveying to these cells the state of activation or

inactivation of the macrophage system. Werb and Chin (66) have shown that during various stages of activation, e.g., endotoxin stimulation, the macrophages markedly decrease production of apoE, whereas unstimulated (resting) macrophages produce large quantities of this protein.

More recently, numerous other tissues have been shown to possess significant quantities of the mRNA for apoE. These tissues include the brain, adrenal, spleen, ovary, kidney, and muscle (67–70). It has been estimated that 10 to 20% of the circulating apoE could be derived from synthesis by peripheral tissues (70). The synthesis of apoE by peripheral tissues could provide a mechanism whereby cholesterol could be redistributed among cells within that tissue or to cells of other tissues. The presence of apoE would provide a high affinity ligand that mediates uptake by lipoprotein receptors.

Most surprising was the presence of apoE mRNA in the brain of the rat, marmoset, and human at concentrations approximately one-third of that observed in the liver (70). By immunocytochemistry, apoE within the brain has been localized almost exclusively to a specific cell type, the astrocytic glia (71). Apolipoprotein E has previously been shown to be present in the cerebral spinal fluid at concentrations exceeding those expected for a plasma-derived protein (72). It is reasonable to speculate that apoE may function in the transport of lipids within the brain (apoB appears to be absent in spinal fluid). In addition, apoE may play an expanded role within tissues of the nervous system, but this remains to be proven.

B. Apolipoprotein B

Apolipoprotein B, a primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL (Fig. 1), is heterogeneous and exists primarily as two forms: apoB-100 and apoB-48. Apolipoprotein B-100 is synthesized by the liver and is an obligatory constituent of VLDL, IDL, and LDL. In man, apoB-48 is synthesized by the intestine and is found in chylomicrons and chylomicron remnants (for review see Ref. 73).

Although both forms of apoB have been the object of many structural studies, the structure of both remains elusive. Upon removal of lipids, apoB-100 and apoB-48 are insoluble in aqueous buffers except in the presence of detergents or denaturants such as sodium dodecyl sulfate (SDS), urea, or guanidine hydrochloride (73). Delipidated apoB-100 is also highly insoluble and forms aggregates, although recently water-soluble apoB has been prepared, which is, however, probably still aggregated (74, 75). Even in the presence of SDS, a stable dimer of apoB has been reported (76). Both apoB in LDL or delipidated apoB are very sensitive to oxidation and cleavage by proteases (77, 78). Handicapped by these obstacles, progress in determining the primary and secondary structure of apoB has been slow, and even the molecular weight of the protein is uncertain. A number of studies have suggested that apoB-100 is composed of multiple subunits of $M_r < 100,000$ (73, 78) while other investigators, using sedimentation equilibrium or gel permeation chromatography, have estimated the molecular weight to be 250,000–275,000 (76, 79). The most recent investigations using sedimentation equilibrium in guanidine-HCl have demonstrated that apoB-100 from humans has a $M_r \simeq 400,000$ (Schumaker, V. N., J. Elovson, J. Jacobs, and D. L. Puppione, personal communication).

Apolipoprotein B-100 is not distinguished by an unusual amino acid composition (80). It contains 14 mol of half-cystine residues/250,000 g of protein. In LDL, two of the apoB sulfhydryl groups are free, while twelve of these residues form six intramolecular disulfide bonds (74). Apolipoprotein B-100 contains carbohydrate as 8–10% of its protein mass (81). Both high mannose chains and complex oligosaccharides that contain N-acetylglucosamine, galactose, mannose, and N-acetylneuraminic acid are found in apoB-100 (82).

Because of the difficulties of working with this protein, it is doubtful that it will be sequenced completely, although small fragments have been sequenced (83, 84). The protein structure will most likely be determined by sequencing the apoB-100 cDNA; such studies are currently in progress in a number of laboratories.

In addition to apoB-100, two other species of apoB are usually observed on 4% SDS-polyacrylamide gels. They have molecular weights of approximately 300,000, and 100,000 and have been designated apoB-74 and apoB-26 (80). Amino acid composition data have suggested that apoB-74 and apoB-26 are fragments of apoB-100 (80). Recent studies have shown that these fragments can be produced by the incubation of LDL with the proteolytic coagulation enzyme kallikrein (85). Apolipoprotein B-48, however, does not appear to be a fragment of apoB-100. Metabolic, biosynthetic, and genetic evidence all indicate that apoB-48 is a distinct protein that is closely related structurally to apoB-100 (73, 86). The two proteins are most likely products of different genes (73).

Apolipoprotein B-100 interacts with heparin and other sulfated glycosaminoglycans and is thought to play a role in atherosclerosis (87, 88). Immunochemical studies have located apoB in the arterial wall (89), and LDL-like lipoproteins have been extracted from both grossly normal intima and fatty-fibrous plaques from the human aorta (90).

Apolipoprotein B-100 is the protein determinant on LDL that recognizes the apoB,E(LDL) receptor; this recognition of the receptor is the first step in the

receptor-mediated catabolism of LDL (43). Three-fourths to two-thirds of the LDL is catabolized by the apoB,E(LDL) receptor pathway, chiefly by the liver (91). Modification of the lysine residues by a procedure that neutralizes the positive charge (acetoacetylation and carbamylation) on the ϵ-amino group or modification of the arginine residues by the reagent 1,2-cyclohexanedione abolishes the binding of apoB-100 to both the apoB,E(LDL) receptor and to heparin (52, 56). These results indicate that the arginine and lysine residues of apoB-100 are involved in the interaction with heparin and the apoB,E(LDL) receptors. As stated in the previous section, the region of apoE responsible for receptor and heparin binding has been shown to be enriched in lysine and arginine residues. It is reasonable to assume that the region of apoB-100 that binds to the receptor is also rich in arginine and lysine residues. However, the monoclonal antibody that binds to the receptor binding domain of apoE does not cross-react with apoB (49). Apolipoprotein B-48 does not bind to the apoB,E(LDL) or the apoE receptor (92) and is not the determinant responsible for uptake of β-VLDL by macrophages (Milne, R. W., Y. L. Marcel, T. L. Innerarity, and R. W. Mahley, unpublished results).

Recently, monoclonal antibodies have been used to probe the structure of apoB. Marcel et al. (93) have prepared six monoclonal antibodies to apoB-100. An examination of the stoichiometric relationship between the binding of three of these antibodies with human LDL indicated that a single Fab fragment reacts with one LDL particle. These results suggest that there is a single molecule of apoB per LDL particle (94). Four of the six antibodies block LDL binding to the apoB,E(LDL) receptor. These four antibodies are conformation dependent and will recognize apoB-100 when it is incorporated into cholesteryl ester · phospholipid microemulsions (95). Co-titration experiments have shown that the four inhibiting antibodies recognize epitopes grouped in a similar region of apoB-74 (96). These antibodies do not react with apoB-26 or apoB-48 (93). These results suggest that the apoB-74 fragment contains the recognition site that binds to the apoB,E(LDL) receptor.

Limited digestion of LDL by proteolytic enzymes has also been used to gain information about the structure and function of apoB-100 (97, 98). The LDL digested with trypsin retain about one-third of their receptor binding activity (99); LDL treated with protease from the *S. aureus* V8 strain retain complete binding activity (100). One possible explanation for the full retention of receptor binding activity after partial digestion is that apoB contains repetitive sequences (100), although other explanations have been suggested (101). Recently, four laboratories have used partial digestion of apoB combined with apoB monoclonal antibodies to probe the structure of apoB-100 (97, 101–103). Sodium dodecyl sulfate-polyacrylamide gels of the tryptic fragments of apoB-100 revealed over 45 fragments (101). Most antibodies identified a number of fragments while other antibodies only reacted with one or two. The results from these studies revealed that three antibodies that abolish receptor binding react strongest with a 68-kDa fragment (97), a 70-kDa fragment (100), or a 22-kDa fragment (Innerarity, T. L., unpublished results).

Most studies of the biosynthesis of apoB have been performed using the rat as a model. In contrast to man, both apoB-48 and apoB-100 are synthesized in the liver of the rat (104). As shown by a cultured human hepatoma cell line (HEP G2) and cultured primary rat hepatocytes, apoB is biosynthesized by the hepatocytes (105). These studies in rat hepatocytes also indicate that apoB-48 and apoB-100 are distinct proteins and that apoB-48 is neither the precursor nor the product of apoB-100. Most of the apoB synthesized by the intestinal jejunum is apoB-48 (106). The apoB-48 is synthesized in the rough endoplasmic reticulum and transfers to the smooth endoplasmic reticulum and Golgi vesicles where it is associated with chylomicron-sized particles (107). The apoB-48, but not the apoB-100, is phosphorylated intracellularly, but the functional significance of this observation is not known (108).

The metabolism of apoB-48 and apoB-100 is markedly different (86, 109). In man, apoB-100 produced by the liver is secreted as a nascent particle of VLDL. Hydrolysis by lipoprotein lipase first produces IDL and finally LDL. A small part of the VLDL and IDL is taken up by the liver, but most of the VLDL is converted to LDL, which are slowly catabolized by the liver (approximately 45% of the LDL pool per day) (110). The plasma concentration of apoB-100 in normal subjects is 80–100 mg/dl (1). Apolipoprotein B-48 produced by the intestine is secreted as chylomicrons. The triglycerides in the chylomicrons are partially hydrolyzed and the resulting remnants are rapidly taken up by the liver (half-life less than 1 hr) (2). Although apoE (and not apoB-48) mediates the uptake of the chylomicron remnants, VLDL (which also contain appreciable quantites of apoE but not apoB-48) are not avidly taken up by the liver (91, 109). Although not normally found in the plasma of fasting individuals, apoB-48 has been identified in the plasma of fasting cholesterol-fed dogs and in fasted individuals with dysbetalipoproteinemia (4). The plasma of an individual deficient in apoE has also been found to contain large quantities of apoB-48 (23).

C. Apolipoprotein A-I

Human apoA-I circulates in plasma primarily as a component of HDL. It is also present on chylomicrons but is rarely found in significant amounts on chylomicron remnants, VLDL or their remnants, or LDL (Fig. 1).

Apolipoprotein A-I has two major sites of synthesis: the intestine and the liver. The intestinally derived apoA-I enters the circulation associated with chylomicrons but is rapidly transferred to HDL particles during lipase hydrolysis of chylomicrons. Hepatic apoA-I enters the circulation probably associated with nascent HDL particles having little or no core of cholesteryl ester. The concentration of apoA-I in plasma is about 100–150 mg/dl (1), and apoA-I has a plasma half-life of about 4 days (111).

Apolipoprotein A-I is a single polypeptide of 243 amino acids (calculated M_r = 28,100) of known sequence, and the amino acids cysteine and isoleucine are characteristically absent (112). The sequence of human apoA-I described by Brewer et al. (112) is given in Fig. 2, except that residues 34, 146, and 147 are Glu rather than Gln. The occurrence of Glu at these positions has been demonstrated by cDNA analysis of apoA-I mRNA (113, 114). The mRNA for apoA-I specifies a translation product that includes a co-translationally cleaved 18 amino acid signal peptide and a 6 amino acid propeptide (115, 116) with a sequence of Arg-His-Phe-Trp-Gln-Gln- in humans (113, 114). Both newly secreted intestinal (32, 117) and hepatic (32) apoA-I appear in plasma as the 249 amino acid proprotein, which is then processed slowly to the major plasma form (118, 119). The COOH-terminal sequence of the propeptide is atypical and may indicate a processing pathway that is unique to apoA-I (120, 121).

As determined by isoelectric focusing, apoA-I demonstrates several isoforms. The major isoprotein has a pI of 5.6–5.7 with one and sometimes several acidic isoforms apparent. Fresh plasma or serum generally reveals only the major isoform (A-I-1) and one minor, more acidic isoform (A-I-2) (122, 123). The generation of the more acidic isoforms occurs by an as yet unknown process that is probably related to manipulation and storage but is unlikely to represent a true genetic polymorphism (124). Apolipoprotein A-I is known not to undergo any post-translational modifications such as glycosylation, phosphorylation, etc. In both serum and HDL, the propeptide form of apoA-I (relative charge of +2 compared to A-I-1) can be demonstrated by isoelectric focusing (1–2% of the total apoA-I isoforms) (32, 117, 125). True genetic variants of human apoA-I have been demonstrated, however, primarily as a result of screening studies using isoelectric focusing as the identifying method (122, 123, 126). Although infrequent, ten distinct apoA-I variants have been characterized as to their primary structural difference from normal apoA-I (**Table 2**).

From a number of studies in other animals, A-I apolipoproteins from a variety of species appear to be highly homologous to human apoA-I (for review see Ref. 130). Besides the human protein, only the complete covalent structure of canine apoA-I is known, and it demonstrates over 80% homology with its human counterpart (131). A feature that distinguishes apoA-I of primates from that of other species is the presence of paired proline residues at positions 3 and 4. The apoA-I of lower species has only one of these prolines; the significance of this, if any, is unknown (130).

Apolipoprotein A-I has an additional, important structural feature. A large portion of the protein structure is a series of tandemly repeated 22 amino acid segments, punctuated almost exclusively by proline residues, that are predicted to be helical and amphiphilic in character (132). The amphiphilic helix has been implicated as a crucial structural element in the lipid-binding property not only for apoA-I, but also for other apolipoproteins as well (133). Lipid-binding domains have been identified in apoA-I (for review see Ref. 134), and the lipid-binding property has been thoroughly investigated. The tandemly repeated amphiphilic helix is a property shared by only apoA-I and apoA-IV (see section on apoA-IV).

Because apoA-I binds lipid and is the major protein constituent of HDL, it is clear that it must be an important structural component of lipoproteins. Its other major function is to serve as a cofactor (activator) for LCAT (135). In vitro, there appears to be an absolute requirement for apoA-I for LCAT activity. However, it is not known at present whether this is also the case in vivo. Current information suggests that apoA-I is not rate limiting for LCAT activity in vivo. The mechanism by which apoA-I activates LCAT is not entirely understood, but it seems likely that the amphiphilic helical

TABLE 2. Human apolipoprotein A-I variants[a]

ApoA-I	Charge Relative to Normal A-I	Substitution	Reference
Münster-3(A)	+1	$Asp_{103} \rightarrow Asn$	125
Münster-3(B)	+1	$Pro_{4} \rightarrow Arg$	125
Münster-3(C)	+1	$Pro_{3} \rightarrow His$	125
Münster-3(D)	+1	$Asp_{213} \rightarrow Gly$	unpublished
Giessen	+1	$Pro_{143} \rightarrow Arg$	127[b,c]
Milano	−1	$Arg_{173} \rightarrow Cys$	128[d]
Marburg(Münster-2(A))	−1	$Lys_{107} \rightarrow 0$	129[b]
Münster-2(B)	−1	$Ala_{158} \rightarrow Glu$	unpublished[c]
Münster-4	+2	$Glu_{198} \rightarrow Lys$	unpublished
Norway	+2	$Glu_{136} \rightarrow Lys$	unpublished[e]

[a] Frequencies range from 0.01 to 0.1% of the population studied.
[b] Deficient in the activation of LCAT in vitro, about 60% of normal. All others, with the possible exception of Münster-2(B), fall within the normal range (coefficient of variation ±17%).
[c] Heterozygous individuals have reduced ratio of variant/normal A-I.
[d] Both A-I/A-I dimers and A-I/A-II dimers are present; these dimers are nearly inactive in stimulating LCAT in vitro, but the reduced A-I monomer is normal in this regard.
[e] One homozygous individual identified (Ref. 126).

structures are involved. Synthetic peptides of apoA-I that encompass the amphiphilic repeats can serve as LCAT activators (134), as can model amphiphilic peptides that have no sequence homology to apoA-I (134, 136). Furthermore, certain of the human apoA-I variants are deficient in their ability to activate LCAT in vitro (Table 2, footnote *b*), and in these cases the primary structural abnormality can be predicted to perturb the structure of the amphiphilic region (127, 129). Proapolipoprotein A-I is as efficient as apoA-I in activating LCAT in vitro (129), and it also binds well to lipid. Therefore, the function of the propeptide, if any, remains obscure.

Other possible functions for apoA-I are as yet undefined. Although it appears that HDL functions in the process of reverse cholesterol transport (for review see Ref. 7), it is not known whether apoA-I is a causative or passive agent in this process. Many times, functions are discovered as a result of studies of abnormal and/or diseased states. An apoA-I abnormality has been implicated in lipoprotein disorders such as Tangier disease (120, 137–139) and A-I_{Milano} (128, 140), but the precise involvement has not been elucidated. One case of combined apoA-I and apoC-III deficiency associated with premature atherosclerosis (141, 142) has been shown to be due to a rearrangement in the genes for apoA-I and apoC-III (143), which are closely linked on chromosome 11 (144).

D. Apolipoprotein A-II

Human apoA-II occurs primarily as the second most abundant protein component of HDL (Fig. 1) and may be associated with other lipoproteins in much smaller amounts. The major site of synthesis of apoA-II is the liver. Its approximate concentration in plasma is 30–40 mg/dl (1), and it has an average half-life of about 4 days (111).

In humans, apoA-II is a dimer (M_r = 17,400) of identical subunits having 77 amino acids of known sequence (Fig. 2) covalently linked by a disulfide bridge at residue 6 (145). The human protein lacks the amino acids arginine, histidine, and tryptophan. The sequence has been confirmed by cDNA analysis of the apoA-II mRNA, except that it predicts Glu for residue 37 rather than Gln (146). The primary translation product of apoA-II mRNA includes an 18 amino acid signal peptide, which is co-translationally cleaved, and a 5 amino acid propeptide (with the sequence Ala-Leu-Val-Arg-Arg-), which is processed during and following export from the cell (147). The apoA-II propeptide has COOH-terminal paired basic residues, which is typical of other secreted proproteins. Its processing would therefore appear to be distinct from that of apoA-I (147).

The reduced (monomer) form of apoA-II demonstrates one major isoform with a pI of 4.9–5.0. It has also been shown that apoA-II has several minor isoforms, both more alkaline and more acidic than the major isoform, but it is not certain whether these represent either a true polymorphism or a post-translational modification (148). It is not known whether apoA-II is glycosylated, and no structural variants of apoA-II have yet been reported.

Other animal species also have apoA-II homologues. It is noteworthy that of all species studied, only the chimpanzee and man have dimeric apoA-II (149). In other primates, apoA-II lacks cysteine and therefore exists only as a monomer (130). The significance of this difference is unknown. Besides human apoA-II, two other complete apoA-II structures are known. The rhesus monkey apoA-II (also 77 amino acids) has six differences from human apoA-II (i.e., >90% homology), including a serine rather than cysteine at position 6 (150); marmoset apoA-II (77 amino acids) has 13 differences from human A-II (>80% homology), including serine at position 6 (Crook, D. H., K. H. Weisgraber, S. C. Rall, Jr., and R. W. Mahley, unpublished results). Both the rhesus and marmoset apoA-II have Gln at residue 37.

Apolipoprotein A-II, like other apolipoproteins, binds to lipid and has a high degree of ordered secondary structure, including amphiphilic regions. Lipid-binding domains have been identified in apoA-II (for review see Ref. 134). Apolipoprotein A-II is known to be able to completely displace apoA-I from HDL (151), but it is not known if this has any physiological significance. Except for its presence as a structural component of HDL, apoA-II is not known to have a specific function in HDL structure or metabolism. Several animal species in fact do not have a significant amount of apoA-II associated with their HDL (130). Human apoA-II can exist as a mixed disulfide dimer with apoE (152), and this complex does not exhibit the receptor binding activity characteristic of apoE itself (153). Whether this serves as a physiological modulator of apoE receptor binding is not known.

E. Apolipoprotein A-IV

Human apoA-IV is a prominent component of newly secreted chylomicrons but is not found in significant amounts associated with chylomicron remnants, VLDL, or LDL (Fig. 1). Although it is only a minor component of human HDL, it is a major apolipoprotein constituent of rat HDL (154). The significance of this difference is not known. Unlike most other apolipoproteins, a majority of apoA-IV is found in plasma in the lipoprotein-free rather than lipoprotein-bound fraction and redistributes readily between these two fractions (155). Apolipoprotein A-IV, like apoA-I, is synthesized almost exclusively by

the liver and the intestine and is present in human serum at a concentration of about 15 mg/dl (1).

Of the major human apolipoproteins, only the structures of apoB and apoA-IV are not known. Apolipoprotein A-IV lacks cysteine and is a single polypeptide of $M_r \simeq 46{,}000$ (156). It does not appear to be glycosylated or otherwise post-translationally modified. As determined by isoelectric focusing, apoA-IV generally shows one major isoform with a pI between that of apoA-I and apoA-II (122, 123). However, minor isoforms of apoA-IV have been demonstrated (157), and there is some evidence that apoA-IV exhibits genetic polymorphism in humans (122, 123).

Although the structure of the human protein is not known, a recent study of apoA-IV mRNA has elucidated the structure of this protein in rats (158). Apolipoprotein A-IV mRNA specifies a protein product that includes a 20 amino acid signal peptide that is co-translationally cleaved (121, 159) and a mature plasma polypeptide of 371 amino acids (158) with a calculated M_r = 42,500. Apolipoprotein A-IV has no propeptide. The structure of rat apoA-IV is revealing in that it also appears to have tandemly repeated amphiphilic helical structures very similar to those in apoA-I (158).

The presence of the tandem amphiphilic repeats in apoA-IV suggests that it might perform functions similar to apoA-I. In fact, apoA-IV has been shown to be a potent activator of LCAT in vitro (11). Furthermore, when LCAT is active, rat apoA-IV preferentially associates with the lipoproteins; inactivity of LCAT results in apoA-IV redistributing to the lipoprotein-free fraction (160). A question that remains to be resolved is why, with this large region of potential lipid binding, apoA-IV does not seem to bind to lipid as well as apoA-I, as evidenced by its susceptibility to displacement by other apolipoproteins and its occurrence in large amounts in the lipoprotein-free fraction of plasma.

F. The C Apolipoproteins

The C apolipoproteins are represented by three low molecular weight apolipoproteins, designated as apoC-I, C-II, and C-III, that are surface components of chylomicrons, VLDL, and HDL. They range in molecular weight from 6,600 to 8,800, and the complete amino acid sequence of each of the human proteins is known (Fig. 2). Although they appear to be diverse in their metabolic functions (to be discussed below), they share the common property of redistributing among lipoprotein classes (for review see Ref. 161). This redistribution may be related to the role that the C apolipoproteins play in the metabolism of lipoproteins. In the fasting state, the C apolipoproteins are mainly associated with HDL. During absorption of dietary fat by the intestine with the production of chylomicrons or during the active synthesis of VLDL by the liver, the C apolipoproteins preferentially redistribute to the surface of the triglyceride-rich chylomicrons and VLDL. In the reverse manner, as the triglyceride core of the VLDL and chylomicrons is hydrolyzed and depleted by the action of lipoprotein lipase, excess surface components (phospholipid, unesterified cholesterol, and apolipoproteins) are generated, and the C apolipoproteins along with the other excess surface components are transferred to HDL. Thus, the C apolipoproteins are associated with the equilibrium that occurs in the dynamic metabolic "remodeling" of plasma lipoproteins. Their roles in these metabolic processes will be discussed individually.

It appears that the liver is the major site of synthesis of the apoC proteins, with the intestine contributing a minor portion (162, 163). Apolipoproteins homologous to the human apoC proteins appear to be present in several animal models (130). The best characterized of these is the rat, which contains equivalents to apoC-I, C-II, and C-III (164). However, it is not clear in the other species that have been studied whether equivalents to all three human apoC proteins are present. Presently, amino acid sequence data are only available for the human apoC proteins.

1. Apolipoprotein C-I

Apolipoprotein C-I is the smallest of the C apolipoproteins and consists of 57 amino acids in a single polypeptide chain with a calculated M_r = 6,605 (Fig. 2). The plasma concentration of apoC-I in man is ~6 mg/dl (165). The amino acid sequence determined by protein sequencing (166, 167) has been confirmed by nucleotide sequencing of a full-length cDNA clone for apoC-I mRNA (168). In addition, the cDNA analysis indicates that apoC-I is synthesized with a 26 residue presegment that is co-translationally cleaved from the protein. Apolipoprotein C-I does not contain a propeptide (168).

Apolipoprotein C-I has a pI of 6.5 as determined by isoelectric focusing gels in the presence of 6 M urea (169). Based on the Chou-Fasman algorithm, it is predicted to have a high helical content. Residues 7 to 14, 18 to 29, and 33 to 53 represent amphiphilic helical structures, and it has been suggested that these regions are the lipid binding areas of apoC-I (133). The cyanogen bromide fragment representing residues 1 to 38 binds to egg yolk phosphatidylcholine, while the fragment representing residues 39 to 57 interacts weakly (170). In addition, studies of the lipid binding properties of synthetic apoC-I fragments have demonstrated that fragments 32 to 57 and 24 to 57 interact with dimyristoylphosphatidylcholine, while fragment 39 to 57 does not (171).

Apolipoprotein C-I has been shown to activate LCAT in vitro (172). Thus, it has the potential to participate in the esterification of the cholesterol that is transferred to HDL as a part of the excess surface components generated during lipolysis of VLDL and chylomicrons or that is transferred to HDL from cells. In this manner apoC-I may participate in the "remodeling" of HDL. The ability of apoC-I to activate LCAT may account for the normal plasma levels of esterified cholesterol in subjects with apoA-I deficiencies. Studies with synthetic peptides of apoC-I indicate that fragment 17 to 57 contains all of the structural features necessary to activate LCAT. This fragment is as active as the intact protein in activating LCAT (171).

2. *Apolipoprotein C-II*

Apolipoprotein C-II is a single polypeptide chain consisting of 79 amino acids with a calculated M_r = 8,824 (Fig. 2). The amino acid sequence of apoC-II that was initially reported (173) has been reexamined by protein sequencing (174) and by nucleotide sequencing of the cDNA for apoC-II mRNA (34, 146). The later analyses (34, 146, 174) agree with each other and reveal that apoC-II is composed of 79 amino acid residues, not 78 as initially reported. Other minor differences were also noted. The residue numbers given in the discussion that follows and the amino acid sequence in Fig. 2 correspond to the revised sequence of apoC-II (146, 174). As determined by isoelectric focusing gels in the presence of 6 M urea, apoC-II has a pI of 5.0 (169). The apoC-II gene has been mapped to chromosome 19 (34), and the protein is synthesized with a 22-residue presegment that is co-translationally cleaved (146). Apolipoprotein C-II does not have a prosegment (146).

Based on Chou-Fasman analysis, the apoC-II structure is predicted to contain three helical regions between residues 13 to 22, 29 to 40, and 43 to 52 (175). All three helical regions are amphiphilic in nature and, therefore, are considered potential lipid binding regions. Other structural features include β-turns at residues 9 to 12, 23 to 26, and 53 to 56 and a predicted β-structure between residues 61 and 75.

Apolipoprotein C-II is present in plasma at a concentration of ~4 mg/dl (161), and its prime metabolic function appears to be associated with its ability to act as a cofactor in activating lipoprotein lipase (176, 177). Patients with a familial apoC-II deficiency have severe hypertriglyceridemia and impaired plasma clearance of VLDL and chylomicrons, in spite of the presence of a functional lipase (178). Structure-function studies with cyanogen bromide fragments and synthetic fragments indicate two separate functional domains in apoC-II (179). The region responsible for activation of lipoprotein lipase is located between residues 56 to 79, while phospholipid binding appears to involve residues 44 to 52 (for review see Ref. 134). Apolipoprotein C-II has also been reported to activate LCAT (10).

3. *Apolipoprotein C-III*

Apolipoprotein C-III is the most abundant of the C apolipoproteins (~12 mg/dl plasma) (161). It consists of a single polypeptide chain of 79 amino acid residues (180) with a calculated M_r = 8,750 (Fig. 2). Apolipoprotein C-III occurs in plasma in three forms depending on the level of sialylation: C-III$_0$, C-III$_1$, and C-III$_2$. The subscript indicates the number of sialic acid residues that are present. The carbohydrate is O-linked and is attached to the threonine at residue 74 (180). The pI's of C-III$_0$, C-III$_1$, and C-III$_2$, as determined by isoelectric focusing gels in the presence of 6 M urea, are 5.1, 4.9, and 4.8, respectively (169). Nucleotide sequence analysis of a full-length cDNA clone for the apoC-III mRNA indicates that the protein is synthesized with a 20-residue presegment but no prosegment (146). The protein sequence inferred from the nucleotide sequence (146) is at variance with the previously determined protein sequence from residues 32 to 39 where the nucleotides predict ESQVAQQA, while the protein studies indicated SQQVAAQQ (180).

Chou-Fasman analysis of the amino acid sequence predicts that residues 1 to 39 and 54 to 69 would be α-helical and that β-turns are likely at residues 39 to 42 and 72 to 75. It has been proposed that residues 40 to 67 form an amphiphilic helix and, therefore, represent a major lipid binding domain. Lipid binding studies with synthetic fragments of apoC-III indicate that residues 48 to 79 contain the minimal amount of structure required to bind phospholipid (for complete review of data see Ref. 134).

The precise metabolic role of apoC-III and the significance of the sialic acid heterogeneity are unclear. It has been suggested that the presence of apoC-III may modulate the uptake of triglyceride-rich remnants by hepatic receptors (181, 182), and it has been demonstrated to activate LCAT (10). Apolipoprotein C-III has also been suggested to inhibit apoC-II activation of lipoprotein lipase (178), although a large excess is required, and the specificity of this inhibition has not been demonstrated.

Recently, the apoA-I and apoC-III genes have been shown to occur within 3 kilobases of each other (183), and this gene complex has been mapped to chromosome 11 (144). An interesting familial lipoprotein disorder associated with premature atherosclerosis and apoA-I and apoC-III deficiency has been described (141, 142). Genomic analyses in these patients indicate that these linked genes have undergone a rearrangement in their

structures (143), accounting for the absence of the proteins in plasma.

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