STRUCTURE *and* FUNCTION *of* APOLIPOPROTEINS

Edited by

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Structure and Function of Apolipoproteins

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PREFACE

Plasma apolipoproteins were first identified and characterized in 1960, and researchers since have been fascinated to discern both the homology and diversity within this protein family. When we look at these amphiphilic proteins — able to interact with hydrophobic lipids while remaining in an aqueous environment — we may recall the Roman god Janus: one of his faces oriented toward the future, the other looking to the past. Just as Janus watched the gates of the city, the apolipoproteins determine the fates of the plasma lipids, either carrying them inside the lipoproteins or releasing them by opening the receptor gates to the cells.

This book compiles current knowledge about the structures and functions of the apolipoproteins from leading researchers around the world. Its major aim is to see beyond the specific features of the different apolipoproteins to their common properties that derive from the mode of assembly of amino acid constituents. That assembly generates homologous primary and scondary structures within this class of proteins.

Since the apolipoproteins are intrinsically involved in the development (and regression) of atherosclerosis, a review of their physiological properties should contribute to a better understanding of this disease and help advance strategies for its control.

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She received the Merck, Sharp and Dohme Award for Lipoprotein Research and has received several research grants from the Belgian Research Council, the European Community, and private industry. Her current research interests are in the field of atherosclerosis, and more specifically, on the quantitation, structure, and functional aspects of plasma apolipoproteins.

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Chapter 1

HUMAN PLASMA APOLIPOPROTEINS IN BIOLOGY AND MEDICINE

Henry J. Pownall and Antonio M. Gotto, Jr.

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I. INTRODUCTION

The major lipid species of human plasma are the phospholipids, cholesterol, triglycerides, and cholesteryl esters. These insoluble species are transported in blood as macromolecular complexes of lipids and proteins known as plasma lipoproteins. The five major plasma lipoproteins are the high-, low-, intermediate-, and very-low-density lipoproteins (HDL, LDL, IDL, and VLDL, classified according to their hydrated densities) and chylomicrons. Chylomicrons are transient lipoproteins; secreted by the intestine following an oral fat load, they cause postprandial plasma to appear cloudy. Apolipoproteins play a key role in the metabolism of lipoproteins. Some, for example, activate enzymes that are important in the covalent modification of lipids and in the remodeling of lipoprotein subfractions. Others contain receptor ligands that target remodeled lipoproteins to specific tissue sites where their respective lipid complements are stored or used.

Modern techniques in molecular and cellular biology, physical biochemistry, and enzymology have provided improved models of the dynamics of apolipoproteins: their synthesis, assembly with lipids, secretion into the plasma compartment, and ultimate turnover at apolipoprotein-dependent tissue sites. This chapter focuses on human apolipoprotein genetics and the pivotal roles of the apolipoproteins in lipoprotein structure, metabolism, and the development of premature cardiovascular disease. The techniques currently used to determine apolipoprotein and lipoprotein structure have been reviewed elsewhere,[1,2] as have apolipoprotein structure[3-6] and metabolism.[7]

The associations of apolipoproteins with the major nontransient lipoproteins are shown in Table 1. It might be noted that the letter names apo A, apo B, apo C, and so on should be considered to some extent arbitrary; their original intent — to denote distinct families of lipoproteins with shared structures and functions — is now known to be an oversimplification.

II. ENZYME ACTIVATION

Of the plasma proteins that affect the structure and subsequent catabolism of lipoproteins, four appear to be of major importance: cholesteryl ester transfer protein (CETP) and the enzymes lecithin-cholesterol acyltransferase (LCAT), lipoprotein lipase (LPL), and hepatic lipase. Of these, LCAT and LPL require apolipoprotein cofactors. LCAT catalyzes the formation of nearly all plasma cholesteryl esters. In the absence of free-cholesterol, LCAT has phospholipase A_2 activity. Both of these reactions are activated by apo A-I,[10] the most abundant protein of human plasma HDL. There is always an ample supply of free cholesterol *in vivo*, with most of the LCAT destined for acyltransferase rather than phospholipase activity.

The LCAT reaction triggers the formation of mature HDL particles. As the enzyme forms additional cholesteryl esters, HDL particles are converted

TABLE 1
Distribution of Plasma Lipoproteins in Normal Fasting Human Plasma

	Plasma concentration			Lipoprotein class mol %				
Apolipoprotein	μ*M*	mol %	mg/dl	HDL	LDL	IDL	VLDL	mol wt
Apo A-I	43	41	130	100				28,400
Apo A-II	23	21	40	100				17,400
Apo B-100	5	4	250		92	8	2	512,000
Apo C-I	3	3	3	97		1	2	6,600
Apo C-II	13	12	12	60		10	30	9,000
Apo C-III	13	12	12	60	10	10	20	9,000
Apo D	6	5	12	>80				20,000
Apo E	2	2	7	50	10	20	20	35,000

Note: Based on the apolipoprotein data of Havel et al.[8] with the substitution of more recent data on apo D from Camato et al.[9]

from a nascent form rich in free cholesterol and phosphatidylcholine to a more mature form rich in cholesteryl esters.[11] This process may play an integral part in the inverse correlation between HDL levels and the frequency of coronary heart disease (CHD).[12]

In normal subjects, the major activator of LCAT is probably apo A-I, given its greater potency in assays *in vitro* and its higher plasma concentration (Table 1). Other activators of LCAT identified *in vitro* are apo A-IV,[13] apo E,[14] and apo C-I.[15]

LPL catalyzes the hydrolysis of triglycerides and phosphoglycerides associated with VLDL and chylomicrons. Apo C-II is required to activate this reaction, and the determinants responsible for the activation have been localized in experiments *in vitro* to the 24-amino-acid residues on the carboxyl end of the apolipoprotein.[16-18] Activation by the cyanogen bromide fragment in residues 60–78 is one half that of apo C-II, while activation by the synthetic fragment in residues 55–78 is equal to that of native apo C-II. *In vivo* data derived from human genetic studies in which the critical carboxyl region of apo C-II is missing or modified support the assignment of the activating determinant to this region (see below).

III. APOLIPOPROTEINS AS LIPOPROTEIN RECEPTOR LIGANDS

Many of the cells in contact with plasma contain surface receptors that bind to and internalize lipoproteins containing apo B and apo E. These lipoproteins are subsequently targeted to intracellular lysosomes, where they are enzymatically degraded. Through this process, cholesterol-rich lipoproteins are removed from plasma, and free cholesterol is liberated within the cell. The liberated free cholesterol then reduces the rate of cholesterol

biosynthesis[19,20] in the cell, downregulating the LDL receptor and stimulating cholesterol esterification by acylCoA-cholesterol acyltransferase (ACAT).

Two lines of evidence suggest that the liver is the major site of LDL removal from plasma. First, in animal experiments, approximately 70% of total-body uptake of radiolabeled LDL is found in the liver.[21-23] Second, a homozygote for familial hypercholesterolemia who received a liver transplant from a normal donor exhibited reduced levels of plasma cholesterol and an increased rate of clearance of labeled LDL following the surgery.[24] These observations have been used to explain the hypolipidemic action of drugs that act to upregulate B/E receptors in the liver. Specific site mutations in apo B or apo E can lead to decreased receptor-binding activity and subsequent hypercholesterolemia and premature atherosclerosis.[19,20]

IV. OTHER ROLES OF APOLIPOPROTEINS

Apo E has been demonstrated to have several roles linked to lipid metabolism, but not necessarily to lipoprotein metabolism, in relation to nerve regeneration[25-27] and modulation of immune response.[28,29] Three studies have implicated apo E in the traumatized nerve tissue repair mechanism.[25,30,31] These studies found a 100- to 200-fold increase in apo E concentration at the site of nerve damage caused by crushing a rat sciatic nerve. In these studies, apo E levels peaked after 7 to 10 days, and then declined to baseline levels within the 8 weeks required for nerve regeneration. Boyles et al.[25,26] have proposed a mechanism to explain this observation. They suggest that following the injury, resident and monocyte-derived macrophages migrate to the site of injury and begin secreting apo E. The apo E then binds to the lipid debris liberated by the destruction of myelin and delivers the debris to the macrophages. After a few days, the proximal stump of the nerve sends out neurites, one of which will survive and extend the new nerve into the neurolemmal tube. The tip of the regenerating neuron continues to express high B/E-receptor activity, presumably for transporting cholesterol and phospholipids for membrane biogenesis.[32,33]

Lymphocytes express B/E receptors, and at low occupancy levels there is an enhanced responsiveness to mitogens that is apparently due to the delivery of fatty acids and other nutrients.[28,34,35] However, when lipoproteins bind to the immunosuppressive binding site, lymphocytes become resistant to mitogenic stimulation and to early transformation events that are obligatory to activation.[36] A subfraction of LDL that contains both apo B-100 and apo E suppresses lymphocyte proliferation.[37,38] Moreover, apo E appears to be an important factor in triggering the binding of lipoproteins to a specific low-affinity site on the cell surface of lymphocytes.[36] Lipoprotein internalization does not appear to be required for this process.[36] While it is known that the apo E receptor ligand responsible for binding to the immunosuppressive site on lymphocytes is distinct from that which binds to the B/E receptor, the

former has not been further identified.[39] Additional work with specific apo E mutants should better localize the factors that cause apo E-containing proteins to bind with the immunosuppressive receptor on lymphocytes.

The binding of HDL to cells may be important in "reverse cholesterol transport," a process that transfers cholesterol in peripheral tissues to the liver for degradation.[40-44] The protein specificity of this receptor has not been defined and no apolipoprotein mutants that are defective in this activity have been reported.[45] Moreover, cell-surface binding sites for HDL are not required for cholesterol efflux.[46]

The work of Oram and colleagues[42,47] lends credence to the hypothesis of a receptor on the surface of hepatic cells that interacts with apo A-I and possibly with apo A-II and apo A-IV. It is not clear whether this proposed receptor is exclusive to apo A, but the interaction with apo A-I appears to be prototypical. Binding of apo A-I to the receptor results in signal transduction and cholesterol translocation to the cell surface. The cholesterol then associates with the particle containing apo A-I and is removed from the cell. Oram and co-workers[42,47] report that the receptor may be a 110-kDa protein.

The distribution of apolipoproteins among individual lipoproteins is not uniform. Some lipoprotein particles do not contain all of the apolipoproteins that are isolated with that lipoprotein density fraction. The heterogeneity of lipoproteins as assessed by their apolipoprotein compositions has been extensively studied by Cheung,[48] who used a series of immunoaffinity columns to separate particles according to their reactivity with specific apolipoprotein antibodies. One subfraction, which is a very dense fraction of HDL, may circulate as an LCAT complex[49] that contains all of the machinery required for reverse cholesterol transport — LCAT, CETP, apo D, and apo A-I. More recent studies suggest these proteins exist in HDL precursors that have pre-β mobility on electrophoresis and that ultimately form the mature form of HDL, which has α mobility.[50]

V. STRUCTURE AND BEHAVIOR OF APOLIPOPROTEINS

Apolipoproteins are synthesized in the rough endoplasmic reticulum, and like all proteins that are destined for secretion, they contain hydrophobic leader sequences that are cleaved posttranslationally.

Apolipoproteins A-I, A-II, and C-II are secreted with short prosequences on their amino terminus. These sequences contain the essential elements commonly found in many other leader sequences, namely, an amino terminal methionine residue, a middle region that is rich in hydrophobic amino acids, and a carboxyl terminal region that contains many small neutral amino acids. However, the physiological roles of these sequences, which are cleaved in the plasma compartment, are not known.

The properties of the apolipoproteins are partially responsible for the mechanism that allows them to be transported among lipoprotein classes.

These properties also determine how the apolipoproteins perform their essential roles as lipid-associating proteins, enzyme activators, and receptor ligands. Since the three-dimensional structures of the apolipoproteins are not known, predictive algorithms that estimate the secondary structures from amino acid sequence data are used to compare apolipoproteins with one another and with other proteins.

Three such algorithms are the hydropathy plot of Kyte and Doolittle,[51] the probabilistic device of Chou and Fasman,[52] and the hydrophobic-moment plot of Eisenberg et al.[53] The hydropathy plot is a measure of the hydrophobicity of a protein as a function of its primary structure. The Chou-Fasman algorithm estimates the probability that a protein with a given sequence of amino acids will appear in an α-helix, a β-sheet, a turn, or a random coil. The hydrophobic moment is a numerical representation of the amphiphilicity of a protein in an α-helix. The higher the moment, the greater the amphiphilicity. Proteins in which one side of the helix is polar and the opposite side is nonpolar have the highest hydrophobic moment.

As will be shown, the apolipoproteins can be divided into three groups based on their shared solution properties and on their structural homology at the level of both the apolipoprotein and the gene.

Group 1 apolipoproteins are A-I, A-II, A-IV, C-I, C-II, C-III, and E. All but apo A-II are composed of a single polypeptide chain. Apo A-II is a dimer that has a disulfide bridge at Cys.[6] This bridge is the exception among apolipoproteins and may have little function, since apo A-II in most species lacks cysteine. In general, group 1 apolipoproteins are small, water soluble, and contain little or no carbohydrate. They are so weakly associated with lipoproteins that they transfer from one lipoprotein surface to another via the aqueous phase.[54-57] Some early studies indicated that these proteins are prone to self-association. Other studies characterized the structure of the oligomers they form.[58] However, in the presence of comparable concentrations of phospholipids, no self-association is observed and it is doubtful this process is of any physiological importance. Moreover, lipid-to-protein interactions are apparently stronger than protein-to-protein interactions.[59]

Extensive evidence suggests that the group 1 proteins are partitioned between lipoproteins and the surrounding aqueous phase. Many studies have shown that the apolipoproteins are exchangeable and that proteins with a high affinity for HDL can displace those with a lower affinity.[60,61] Also, 30% of the apo A-I in rats is catabolized by the kidney in the absence of measurable renal uptake of HDL lipids.[62,63] This can only be due to the transfer of apo A-I from HDL to the surrounding aqueous phase, from where it is taken up by the kidney.

Animal studies with apo A-I and synthetic analogues of apolipoproteins have shown that there are at least two plasma pools that are in equilibrium with one another, but that are catabolized independently of one another.[62] One of these pools consists of the monomeric water-soluble proteins cata-

bolized by the kidney. The other consists of proteins bound to the HDL particles and catabolized largely by the liver. The affinity of the apolipoprotein for HDL determines the relative contribution of these two routes.

Most evidence suggests that the liver is the major organ that removes HDL particles from the blood. That the turnover of apo C proteins occurs at the same rate in HDL and VLDL, even though the turnover rates of other components in these lipoproteins are different, is further evidence that group 1 proteins partition between lipoproteins and the surrounding aqueous phase. Also, the specific activities of the labeled apo C proteins in HDL and VLDL remain identical, suggesting their rates of transfer between HDL and VLDL are much faster than their turnover rates.[64,65] This conclusion is consistent with the observation that apo C proteins transfer between lipoprotein surfaces with a half-time of less than 1 s.[56]

Circular dichroic (CD) measurements of native HDL and of model reassembled lipoproteins have provided some important clues on lipoprotein structure in solution and in association with lipids. In solutions of a denaturant such as 6 *M* guanidine hydrochloride, all the group 1 proteins are random coils. In a saline solution, CD measurements show an increased helical content that varies with the concentration and identity of the protein.[66] HDL has long been known to have a relatively high α-helical content.[67] When phospholipids are added to apolipoproteins of model HDL, the fraction of the α-helical structure substantially increases.[68] An important conclusion from these observations is that the α-helix is an essential structural unit of HDL apolipoproteins.

In 1974, Segrest et al.[69] proposed a model of the amphiphilic helical structure of lipid-binding proteins. The essential feature of this model, illustrated in Figure 1, is that the lipid-binding regions of an apolipoprotein are α-helices that contain both a polar and a nonpolar face. The nonpolar face penetrates into the hydrophobic region of the phospholipid monolayer that surrounds the neutral lipid core. The polar side faces the surrounding aqueous phase. Syntheses of native[70-72] and model peptides[73-75] having these minimal features strongly support the amphiphilic helical model. Some of these model peptides also activate LCAT, indicating that the activation of LCAT may occur through the association of the peptide with the substrate rather than with the enzyme.[73,74] According to one model,[76] full activation of LCAT requires a Gly at residue 13 of the 22-residue amphiphilic helical repeats of apo A-I. In aqueous solutions, the peptides exist largely as random coils, but the coils form a peptide-lipid complex that is highly helical in the presence of phospholipid.

Several predictive algorithms used to analyze the primary structure of group 1 proteins show other similarities. As with CD studies, the Chou-Fasman analysis predicts a large helical content for these proteins. Although the hydrophobicities of these proteins differ little from those of other globular proteins, the hydrophobic-moment algorithm identifies amphiphilic regions