CHAPTER 18

Lipoprotein structure

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1. Introduction

Lipoproteins are soluble complexes of proteins (apolipoproteins) and lipids that transport lipids in the circulation of all vertebrates and even insects. Lipoproteins are synthesized in the liver, in the intestines, arise from metabolic changes of precursor lipoproteins, or are assembled at the cell membranes from cellular lipids and exogenous lipoproteins or apolipoproteins. In the circulation, lipoproteins are highly dynamic. They undergo enzymatic reactions of their lipid components, facilitated and spontaneous lipid transfers, transfers of soluble apolipoproteins, and conformational changes of the apolipoproteins in response to the compositional changes. Finally, lipoproteins are taken up and catabolized in the liver, kidney, and peripheral tissues via receptor-mediated and other mechanisms. This chapter deals almost exclusively with the human lipoproteins.

1.1. Main lipoprotein classes

Although the assembly, structure, metabolism, and receptor interactions of lipoproteins are determined by their apolipoprotein components, the most common classifications of lipoproteins are based on their hydrated density or mobility on agarose gel-electrophoresis.

The classification into chylomicrons (CM), very low-density (VLDL), low-density (LDL), and high-density (HDL) lipoproteins is based on their relative contents of protein and lipid that determine the densities of these lipoprotein classes. Chylomicrons have only 1-2% protein while HDL have about 50% protein by weight. The diameters of lipoproteins are inversely correlated with their densities and range from about 6000 Å for CM down to 70 Å for the smallest HDL (Fig. 1).

The general structural organization is similar for all the lipoprotein classes: the apolipoproteins and amphipathic lipids (mostly phospholipids and unesterified cholesterol) form a 20-Å shell on the surface of spherical particles. This shell encloses a core of neutral lipids (triacylglycerols, cholesteryl esters, and small amounts of unesterified cholesterol and other dissolved lipids, e.g., lipid-soluble vitamins). The main protein components are characteristic of each lipoprotein class; they are indicated in Fig. 1, and will be described in detail in Section 3 of this chapter.

The principal functions of the lipoprotein classes are determined by their apolipoprotein (apo) and lipid components. The CM are synthesized in the intestines for the transport of dietary triacylglycerols to various tissues. VLDL are synthesized in the



Fig. 1. Major lipoprotein classes (CM, VLDL, LDL, HDL) based on their density. Lipoprotein diameters range from about 6000 Å for CM to 70 Å for HDL. The outer shell (\sim 20 Å) of all lipoproteins consists of apolipoproteins, unesterified cholesterol, and phospholipids; the spherical core contains triacylglycerols and cholesteryl esters. CM and VLDL have the highest contents of triacylglycerols, and 1–10% apolipoproteins by weight; LDL and HDL contain mostly cholesteryl esters in their cores, and 20–50% of apolipoproteins. The major apolipoprotein components of the various classes of lipoproteins are indicated with the solid lines; secondary or minor apolipoprotein components are indicated with the dashed lines. In this figure, 'cholesterol' refers to both esterified and unesterified cholesterol; triglycerides = triacylglycerols.

liver for the export of endogenous triacylglycerols, while LDL arise from the metabolic transformation of VLDL in circulation. The function of LDL is to deliver cholesteryl esters to peripheral tissues and to the liver. HDL are synthesized and assembled in the liver and intestine or are formed from metabolic transformations of other lipoproteins in circulation, and from cellular lipids at the cell membranes (see Chapter 20). HDL remove excess cholesterol from cells and transport it to liver and steroidogenic tissues for metabolism and excretion.

Lipoproteins are also classified by their electrophoretic mobility on agarose gels into α , pre β , and β lipoproteins, corresponding to HDL, VLDL, and LDL density classes respectively; CM, when present, remain at the electrophoretic origin.

Although lipoprotein concentrations in blood plasma are highly variable, depending on age, sex, feeding state, metabolic/hormonal state, and disease state of individuals, a representative lipoprotein distribution for a fasting, healthy, adult male in plasma is approximately 0 mg/dl for CM, 150 mg/dl for VLDL, 410 mg/dl for LDL and 280 mg/dl for HDL [1].

1.2. Lipoprotein subclasses

The lipoproteins within each class are heterogeneous in terms of their density, size, and lipid and apolipoprotein contents and compositions, as well as in their functional

properties. They can be separated into subclasses by ultracentrifugation, gel filtration, electrophoresis, or affinity chromatography methods.

Based on ultracentrifugal and gel-filtration separations, HDL have been subdivided into HDL₁, HDL₂, and HDL₃ subclasses, from the largest and least dense to the smallest and most dense particles. The HDL₁ subclass is enriched in apo E and is least abundant and often disregarded. Non-denaturing gel electrophoresis has further separated the main HDL₂ and HDL₃ subclasses into HDL_{2a}, HDL_{2b}, HDL_{3a}, HDL_{3b}, and HDL_{3c} species spanning a density range from 1.085 to 1.171 g/ml and size (diameter) range from 106 to 76 Å, respectively [2].

Using anti-apolipoprotein immunoaffinity columns two major subclasses of HDL have been separated: one containing apo A1 but no apo A2 (LpA-I) and another containing both apo A1 and apo A2 (LpA-I/A-II). Minor proteins (apo E, apo Cs) may or may not be present in significant amounts in these HDL subclasses [3]. On average, human HDL contain about 70% by weight of apo A1, 20% of apo A2, and 10% of the minor apolipoproteins.

Two-dimensional separations of HDL (agarose gel-electrophoresis in one dimension and non-denaturing polyacrylamide gel-electrophoresis in the second dimension) have yielded α -migrating, pre β -migrating, and γ -migrating HDL subclasses of various sizes and compositions. The pre β -migrating subclasses are present in low concentrations in plasma (in contrast to the abundant α species), and in somewhat higher concentrations in interstitial fluid, but are metabolically very important as they represent the nascent forms of HDL that are especially active in cholesterol uptake from cells (pre β_1 -HDL) and cholesterol esterification by lecithin cholesterol acyltransferase (LCAT) (pre β_{2} - and pre β_3 -HDL) [4]. Because of their key role in cholesterol uptake from cells, the pre β_1 -HDL have been studied quite intensively (J.P. Kane, 1985). They contain 2 molecules of apo A1 per particle, and no apo A2 nor other proteins. Their mass ranges from 60 to 80 kDa, with a content of about 90% protein, 7% phospholipids, 0.3% unesterified cholesterol, and 1.8% esterified cholesterol. The pre β_1 -HDL represent only 7% of total apo A1 in plasma and have an apo A1 conformation distinct from apo A1 in α -migrating HDL. Much less is known about the other pre β -HDL subclasses, except that they are discoidal in shape, contain large proportions of phospholipid, and have masses around 300 kDa.

Other lipoprotein classes can also be separated into subclasses of varying density and size by the same separation methods. Subclasses of LDL in the density range from 1.027 to 1.060 g/ml and size range from 270 to 210 Å have been obtained and shown to have different metabolic properties [5]. Small dense LDL, containing high amounts of triacylglycerols, appear to be the most proatherogenic LDL species. Chylomicrons and VLDL undergo continuous density, size, and composition changes due to the hydrolysis of their triacylglycerols by lipoprotein lipase and exchanges of soluble apolipoproteins; therefore, these lipoprotein classes consist of a continuous spectrum of particles.

2. Lipid components

2.1. Lipid composition

The lipid content and composition of the major lipoprotein classes are listed in Table 1 [6], which shows that the total lipid content is inversely correlated with the density of the lipoproteins. Glycerolipids, mainly triacylglycerols, are the major lipid components of CM and VLDL, but constitute less than 11% of the lipids of LDL and HDL, which are enriched in cholesteryl esters (24–51%). Unesterified cholesterol is found in all the lipoprotein classes in relatively low proportions because it is actively esterified by LCAT on HDL and then redistributed to LDL and VLDL by cholesterol ester transfer protein. The total phospholipid content of lipoproteins increases with increasing density and is directly related to the surface area of the lipoprotein particles, as the surface of lipoproteins is covered by a monolayer of phospholipids (PL) and apolipoproteins. By far, the main phospholipid constituents are phosphatidylcholines (PC) (57–81% wt PL) followed by sphingomyelins (12–26% wt PL). Lyso PC and other phospholipids (phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol) constitute 5–15% wt PL. Glycolipids are present only in trace amounts and free fatty acids contribute less than 3% to the total mass of lipids.

2.2. Fatty acid composition

In the fasted state, the fatty acid composition of lipoprotein lipids reflects their biosynthetic origins and metabolic transformations in circulation (Table 2) [6].

The glycerolipids (predominantly triacylglycerols) have high proportions of 18:1 and 16:0 fatty acids, reflecting synthesis in the liver. The bulk of cholesteryl esters of human lipoproteins is formed in circulation by the action of LCAT. This enzyme acts on HDL

Lipid composition of lipoprotein classes "										
	CM ^b	VLDL ^c	LDL °	HDL °						
Density (g/ml)	< 0.94	0.94-1.006	1.006-1.063	1.063-1.210						
Total lipid (% wt)	98–99	90-92	75-80	40-48						
Glycerolipids (% wt lipid) ^d	81-89	50-58	7-11	6-7						
Cholesteryl esters (% wt lipid)	2–4	15-23	47-51	24-45						
Unesterified cholesterol (% wt lipid)	1–3	4–9	10-12	6-8						
Phospholipids (% wt lipid) ^e	7–9	19-21	28-30	42-51						
PC (% wt PL)	57-80	60-74	64-69	7081						
SM (% wt PL)	12-26	15-23	25-26	12-14						
Lyso PC (% wt PL)	4-10	~5	3-4	~ 3						
Other (% wt PL)	6–7	6-10	2-10	5-10						

Table 1
Lipid composition of lipoprotein classes a

^a Adapted from Skipski [6].

^b Chylomicrons (CM) were isolated during absorption of fat meals from plasma or lymph.

^e VLDL, LDL, and HDL were isolated from fasting plasma or serum.

^d Most glycerolipids are triacylglycerols, only about 4% are diacylglycerols or monoacylglycerols.

^e Phospholipid (PL), phosphatidylcholine (PC), sphingomyelin (SM).

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Fatty acid	VLDL	LDL	HDL	
Glycerolipids:				
16:0	26.9	23.1	23.4	
18:0	2.9	3.3	3.5	
18:1	45.4	46.6	43.8	
18:2	15.7	15.7	15.9	
20:4	2.5	5.4	7.6	
Cholesteryl este	ers:			
16:0	11.5	11.3	11.4	
18:0	1.4	1.0	1.1	
18:1	25.6	22.4	22.4	
18:2	51.8	59.9	54.8	
20:4	6.1	6.9	6.3	
Phospholipids:				
16:0	33.8	36.0	31.8	
18:0	14.7	14.3	14.5	
18:1	12.2	11.6	12.3	
18:2	20.3	18.9	20.6	
20:4	13.6	13.2	15.7	

 Table 2

 Fatty acid composition of glycerolipids, cholesteryl esters and phospholipids present in lipoprotein classes ^a

^a Adapted from Skipski [6]. Lipoproteins were isolated from fasting plasma. Composition is in wt% of total fatty acids.

phosphatidylcholines and unesterified cholesterol to form lyso PC and the cholesteryl ester products. In this reaction, LCAT uses preferentially PC species with 18:2 or 18:1 fatty acids in the *sn*-2 position, thus enriching cholesteryl esters in these fatty acids. In contrast, PC containing 18:0 or 20:4 fatty acids is a poor substrate for LCAT, explaining the decreased contents of these fatty acids in the cholesteryl esters. The fatty acid composition of the phospholipids found in lipoproteins is similar to the phospholipid fatty acid composition of cell membranes, especially of hepatic and intestinal cells, including relatively high contents of the essential fatty acids 18:2 and 20:4.

In general, in the fasting state, the fatty acid compositions across the lipoprotein classes for specific types of lipids are fairly similar due to the transfers of lipids among all the lipoproteins by cholesterol ester transfer protein and the phospholipid transfer protein. Postprandially the fatty acid composition of VLDL glycerolipids reflects to some extent the fatty acid composition of dietary fat, but the fatty acid compositions do reflect the fatty acid composition of the meal, especially 8–10 h after the meal when CM concentrations in lymph and plasma are maximal.

2.3. Lipid organization

The surface of all lipoproteins consists of a lipid monolayer containing all the phospholipids, and about 2/3 of all unesterified cholesterol, plus the corresponding apolipoproteins.

The dynamic properties of the lipid monolayers depend largely on the nature of the constituent lipids. For example, the surface lipids of LDL are more condensed and rigid than those of HDL due to the presence of more saturated fatty acids in the phospholipids of LDL, a higher sphingomyelin-to-PC ratio, and a higher unesterified cholesterol-to-phospholipid ratio in LDL (M.C. Phillips, 1989). The surface monolayer of VLDL is even more fluid than that of HDL due to differences in the monolayer lipids. Apolipoproteins exert little or no effect on the average dynamic properties of the surface lipids especially in LDL and VLDL. In fact, isolated surface lipids, reconstituted into vesicles or microemulsions, have similar fluidity, diffusion rates, and mobility as the lipid monolayer components in the intact lipoproteins. The core lipids (triacylglycerols and cholesteryl esters) partition poorly into the surface lipid monolayer, accounting for approximately 3 and 1 mol%, respectively, of the surface lipids [7].

Under physiological conditions, the interior of lipoproteins is a fluid spherical droplet of the neutral lipids, including small amounts of dissolved unesterified cholesterol (about 1/3 of the total) and other lipophilic molecules. In HDL and VLDL, the core lipids do not appear to be organized because of the small volume available in HDL, and because of the high content of fluid triacylglycerols in VLDL. However, the core of LDL particles is known to have the cholesteryl esters organized into two interdigitated concentric layers that may undergo cooperative phase transitions at temperatures between $19-32^{\circ}$ C. This organization is optimal at a specific cholesteryl ester/triacylglycerol ratio of 7/1 where a separate fluid phase of triacylglycerol is present in the center. At different core lipid ratios there is mixing of the neutral lipids [8]. Phase transitions in the core lipids of LDL apparently result in changes in the secondary structures of apolipoprotein B100 on the surface. This indicates some coupling of hydrophobic regions of the apolipoprotein with adjacent neutral lipids.

3. Apolipoproteins

3.1. Classes and general properties

The apolipoproteins found in plasma are classified into two broad types: the nonexchangeable and the exchangeable (or soluble) apolipoproteins. Apolipoprotein B100 (apo B100) and apo B48, the principal protein components of LDL, VLDL, Lp(a) and CM are non-exchangeable apolipoproteins. They are very large and water-insoluble proteins that are assembled with lipids at their site of synthesis in the endoplasmic reticulum of liver or intestinal cells (Chapter 19). These non-exchangeable apolipoproteins circulate bound to the same lipoprotein particle through various metabolic transformations in plasma, until they are cleared, as lipoproteins, via specific receptors (Chapter 21). In contrast, the exchangeable apolipoproteins (e.g., apo A1, apo A2, apo Cs, apo E) have much smaller molecular masses than apo B100 or apo B48, are more or less soluble in water in their delipidated states, can transfer between lipoprotein particles, and can acquire lipids while in circulation (see Table 3) [9].

The common function of all apolipoproteins is to help solubilize neutral lipids in the circulation. The apolipoproteins bind readily to phospholipid/water interfaces

Apolipoprotein ^a	Molecular weight ^h	Lipoprotein class ^e	Concentration in plasma (mg/dl)
Apo A1	28,100	HDL, CM	130
Apo A2 ^d	17,400	HDL	40
Apo A4 °	44,500	СМ	15
Apo(a)	$3-8 \times 10^{5}$	Lp(a)	0.1-40
Apo B100 ^g	512,000	LDL, VLDL	250
Apo B48 ٤	242,000	СМ	
Apo C1	6,600	VLDL, CM, HDL	3
Apo C2	9,000	VLDL, CM, HDL	12
Apo C3	9,000	VLDL, CM, HDL	12
Apo D ^h	22,000	HDL	12
Apo E ¹	34,200	VLDL, CM, HDL	7

Table 3 Major human apolipoproteins

^a Other, minor apolipoproteins isolated from lipoprotein fractions include apo F, apo H, apo L, and apo M. They are present in plasma in low concentrations and do not have well-defined functions in lipoprotein metabolism.

^b Polypeptide molecular weights do not include carbohydrate contributions.

^c In bold are the lipoprotein classes containing the highest proportion of the apolipoprotein; in italics are secondary lipoprotein classes.

^d Human apo A2 is a disulfide-linked dimer of two identical monomers of 8.7 kDa; other mammalian apo A2s are monomeric.

^e Apo A4 is a glycoprotein found in lymph CM and is mostly (90%) lipid-free in plasma. Exists in several polymorphic states.

^f Apo(a) is a highly polymorphic protein, containing variable numbers of kringle structural units and glycan chains. Homologous to plasminogen, apo(a) is bound to apo B100 in LDL by a disulfide linkage forming Lp(a).

^g Apo B48 contains 48% of the N-terminal sequence of apo B100. Both are glycoproteins. Apo B48 is present in variable amounts depending on feeding state and CM concentration.

^h Apo D belongs to the lipocalin protein family, it may bind progesterone, but its role in lipoprotein metabolism is unknown.

ⁱ Apo E is glycosylated and consists of three polymorphic forms (apo E2, E3, and E4).

and, under appropriate conditions, can spontaneously form discrete particles with phospholipids. In vivo, the assembly of apolipoproteins with lipids to form lipoproteins may require the assistance of cell proteins such as the microsomal lipid transfer protein or the ABC1 transporter.

Intimately related to their ability to bind phospholipids, and to solubilize neutral lipids within lipoprotein particles, the apolipoproteins have the ability to change conformation to adjust to changing lipid contents, compositions, and metabolic states of the lipoproteins. In apo A1, the adaptable structural regions have been called the mobile or hinge domains. Analogous domains likely exist in other apolipoproteins, as epitope recognition by specific antibodies and exposure to proteolytic digestion change with changing lipid contents and compositions of various lipoproteins.

Several of the exchangeable apolipoproteins in their respective lipoproteins are known to activate or inhibit plasma enzymes. Apo A1, and to a lesser extent apo E, apo A4, and apo C1, activate LCAT, apo C2 activates lipoprotein lipase, and apo C3 and apo A2 may act as inhibitors of hepatic lipase.

Apolipoproteins also have roles in receptor recognition. The best characterized interaction is the recognition of the LDL receptor by apo B100- and apo E-containing lipoproteins. Recently, a receptor for HDL (SR-BI) has been described which binds various apolipoproteins, including apo A1 and apo A2.

In addition to the well-known functions of apolipoproteins in lipid binding and solubilization, modulation of enzymatic activities, and receptor recognition, other functions have been described for apolipoproteins. For example, apo E has been implicated in in situ nerve repair and regeneration, as well as in plaque formation in Alzheimer's disease. Apo(a) may have a function in the clotting process, while apo A4, produced in the intestine and the hypothalamus, may have a role in signaling satiety in the fed state.

Evidently, the multiple functions of apolipoproteins are determined by their unique, modular structures encoded by families of genes.

3.2. Gene organization

The gene structures of the major exchangeable apolipoproteins are very similar to each other, whereas the apo B gene is distinct, as are the gene structures of apo(a) and apo D. Most of the genes of the exchangeable apolipoproteins contain four exons and three introns, with similar locations of intron/exon boundaries, and similar intron and exon lengths for the first three exons (see Fig. 2) [10]. The differences in the total length of the mRNA are due to the differences in the length of the 4th exon. The mRNAs encompass a 5'-untranslated region, a region encoding the signal sequence, a short pro-segment, the mature sequence of the protein, and a 3'-untranslated region. Exons 3 and 4 of the genes encode the entire mature sequence. For the apo A4 gene, the only difference from the other exchangeable apolipoprotein genes is the absence of the first exon and intron in the 5'-untranslated region. The homologies in the gene and protein sequences of the exchangeable apolipoproteins indicate that these genes evolved by gene duplication from a primordial gene resembling the gene of apo C1.



Fig. 2. Organization of the genes of the exchangeable apolipoproteins [10]. The boxes represent the exons joined by introns (broken lines). The open portions of the boxes correspond to the 5'- and 3'-untranslated regions, the hatched portions are the signal peptide regions, and the filled parts represent the regions that code the mature protein sequences. The narrow open portions in exon 3 of the apo A1 and apo A2 genes represent their pro-segments. Numbers above the exons indicate the number of nucleotides in each exon.

In contrast to the genes of the exchangeable apolipoproteins, the apo B gene is short with respect to the length of its 29 exons. It is also quite asymmetric: 19 introns are concentrated within the first 1000 codons, and two very long exons (exons 26 and 29) occur in the 3'-third of the gene sequence. No homology is found between the apo B gene and other known genes [9]. It encodes one of the longest known polypeptide chains containing 4536 amino acid residues. Apo B48 is encoded by the same gene as apo B100, but the mRNA is edited by a specific enzyme in human intestine. The enzyme changes codon 2153 from a Gln to a stop codon, resulting in the production of apo B48, a protein of 242 kDa, lacking the LDL receptor-binding region of apo B100 (D. Driscoll, 1990).

3.3. Primary sequences

The exchangeable apolipoproteins, in addition to having similar genes, have similar primary amino acid sequences. Their sequences contain 11 and 22 amino acid homologous repeats, the latter consisting of two 11-mers, as shown in Fig. 3 [11]. The last 33 amino acids encoded by exon 3 can be aligned into three 11-mers, while the sequences encoded by exon 4 fit, in general, into 22-mer segments that often start with

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Fig. 3. Amino acid sequences of human apolipoproteins arranged in 11-mer and 22-mer repeats [11]. (A) The last 33 amino acids encoded by exon 3 are divided into three (A, B, C) 11-mer repeats. (B) The sequences encoded by exon 4 contain 22-mer repeats each consisting of two 11-mers (A and B), and some 11-mer repeats. If there are 10 or more amino acids in the same column with equivalent physical properties (i.e., hydrophobic, basic, acidic), they are boxed. Numbers at the beginning and end of a sequence give the amino acid locations in the mature apolipoprotein.

a Pro (J.I. Gordon, 1986). The significance of these repeated sequences is that they are predicted to form amphipathic α -helices. These helices in apolipoproteins and synthetic peptides have been shown experimentally to bind to phospholipid surfaces and to be effective in solubilizing lipids. Thus, the 22 amino acid repeats in α -helical organization are the lipid-binding units of the exchangeable apolipoproteins. Furthermore, specific repeats in the sequences encoded by exon 4 have other functional roles such as LCAT activation by the 143–165 repeat of apo A1, LDL receptor-binding by the 136–150 region of apo E, and lipoprotein lipase activation by residues 44–79 of apo C2.

In contrast, the apo B100 sequence contains many internally repeated sequences that bear little resemblance to the sequences of the exchangeable apolipoproteins [12]. There are unique, Pro-rich repeats of 25 and 52 residues that contain high proportions of hydrophobic amino acids. These regions could be in contact with the core lipids in LDL and VLDL. Other features of the apo B100 sequence include (1) 25 cysteine residues (16 in disulfide linkages), (2) at least 4 heparin-binding regions, and (3) 19 potential glycosylation sites (16 of which are occupied by glycan chains, contributing about 9% of the molecular mass of apo B100). Most of the disulfide linkages and free Cys residues cluster in the amino-terminal region of the apolipoprotein. The receptor-binding domain of apo B100, between residues 3353 and 3371, does have some homology with the corresponding receptor-binding region of apo E.

Apo(a) is a distinct, highly polymorphic glycoprotein that is covalently linked to apo B100 of LDL by a disulfide linkage, to form Lp(a). Apo(a) is homologous to plasminogen, containing variable kinds and numbers of kringle sequences. Each kringle repeat contains about 80 amino acids and three internal disulfide bridges. While apo(a) has sequences homologous to those forming the plasminogen catalytic, serine protease site, the peptide sequence corresponding to the cleavage site required for activation of plasminogen is modified in apo(a), so that the serine protease activity is not expressed. In fact, the function of apo(a) in plasma is not known in the 30% of the population that expresses this apolipoprotein in significant levels (A.M. Scanu, 1988).

3.4. Secondary structures

The exchangeable apolipoproteins, devoid of lipids, have substantial amounts of α -helical structure in physiological aqueous solutions and their α -helix content increases markedly upon lipid binding. For example, apo A1 is about 50% helical in the lipid-free state, and becomes 60–85% helical when bound to lipids, as measured by circular dichroism spectroscopy.

Various computer algorithms have predicted the existence of amphipathic α -helix segments, coinciding quite well with the 22-mer repeats of the exchangeable apolipoproteins. The predicted helical structures have a non-polar face, and a larger polar face. Depending on the relative distribution of the charged residues on the polar face, the amphipathic helices of the exchangeable apolipoproteins fall into three classes: A, G^{*}, and Y helices [13].

In the A-type helices (see Fig. 4) the basic residues are found at the boundary between the polar and non-polar helix faces. The hydrophobic chains of the basic amino acids, in fact, contribute to the hydrophobicity of the non-polar face of the helix. The



Ala • Leu • Asp • Lys • Leu • Lys • Giu • Phe • Gly • Asn • Thr • Leu • Giu • Asp • Lys • Ala • Arg • Giu 11 16 21

Fig. 4. Type-A amphipathic helix corresponding to residues 7–24 of apo C1. (Left) Helical wheel representation with the α -helix axis in the center of the wheel and consecutive amino acids at 100° from each other. The non-polar residues are at the top of the wheel, basic residues occur at the interface between the non-polar and polar sides of the helix, and negatively charged residues appear in the middle of the polar face (at the bottom of the wheel). (Right) The helix is represented as a flattened cylinder cut along the center of the polar face.

acidic residues, on the other hand, are located along the middle ridge of the polar face and are fully exposed to solvent. Such A helices in specifically designed synthetic peptides bind effectively to phospholipids and lipoproteins, and readily solubilize lipids. The A-type helices are indeed the fundamental lipid-binding units of apolipoproteins. Two 22 amino acid synthetic A helices in tandem, joined by a Pro, are even more effective in binding lipids, suggesting inter-helix cooperativity.

The G^* helices are amphipathic helices typically found in globular proteins. Their basic and acidic residues are randomly distributed on the polar face of the helix. The G^* helices usually participate in protein–protein interactions rather than protein–lipid interactions. G^* -type helices are mostly found in the N-terminal regions of exchangeable apolipoproteins, encompassing the 11-mer repeats encoded by exon 3.

Amphipathic helices of the Y-type have alternating clusters of basic and acidic residues on the polar face. Their distinction from the A-type helices in terms of lipid binding is not clear, as synthetic peptides representing A- and Y-types of helical segments of apo A1 have comparable lipid-binding properties.

Because of the large size and insolubility in water of apo B100, secondary structure measurements have been conducted on intact LDL particles and proteolytic fragments solubilized in detergents or reassembled with lipids. Reported α -helix contents in LDL range from 20 to 43%, and for β -structure from 12 to 41%, as determined by circular dichroism and infrared spectroscopic measurements. Computer analysis of the sequence of apo B100 suggests the presence of five regions of clustered secondary structures: (1) an N-terminal sequence containing G-type helices (residues 58–476); followed by

(2), an amphipathic β -strand region (residues 827–1961); (3) a region with α -helices resembling A- and Y-type amphipathic helices and including other kinds of helices (residues 2103–2560); (4) a second β -strand region (residues 2611–3867); and (5) a C-terminal, third region of amphipathic α -helices (residues 4061–4338) [14]. The N-terminal region of apo B100, containing the G-helices and high contents of Cys, consists of one or more globular domains (residues 22–303 and 512–721) that are involved in interactions with the microsomal triglyceride transfer protein, a protein required for the assembly of nascent apo B100 with lipids. The central and C-terminal regions of α -helices may be involved in reversible lipid binding by apo B100, and the β -strand regions may participate in irreversible interactions with lipids, possibly involving core lipids.

3.5. Three-dimensional structures in solution

While most of the known functions of apolipoproteins are associated with their lipidbound states, lipid-free or lipid-poor exchangeable apolipoproteins do exist in plasma and interstitial fluid, and have important metabolic roles in lipid uptake from cells, transfers between lipoproteins, structural remodeling of lipoproteins, and apolipoprotein catabolism.

Fragments of two apolipoproteins, apo E (22 kDa N-terminal fragment) and apo A1 (C-terminal fragment missing 43 N-terminal amino acid residues) have been crystallized and their structures determined by X-ray methods (Fig. 5). The apo E structure [15] (Fig. 5A) consists of an elongated (65 Å) four-helix bundle and a connecting short helix. The structure is stabilized by hydrophobic interactions, salt-bridges, and leucine-zipper



Fig. 5. (A) Ribbon X-ray structure of the apo E 22 kDa N-terminal fragment, showing the elongated 4-helix bundle of the lipid-free protein monomer [15]. The LDL receptor-binding region is boxed. (B) X-ray structure of a tetramer of the apo A-I fragment lacking the N-terminal 43 amino acids [16].

interactions. The receptor-binding region contains a cluster of basic residues on the surface of one long helix. This structure confirms the importance of amphipathic α -helices as a fundamental structural motif of apolipoproteins, even in the lipid-free state. Indeed, the four main helices contain 19, 28, 36, and 35 amino acids and encompass several of the predicted 11-mer and 22-mer repeats. The first two helices and their connecting short helix consist of the three 11-mer repeats encoded by exon 3 plus the first 22-mer repeat of exon 4, while the last two helices each consist of two 22-mer repeats. However, as important as this partial structure of apo E is for our understanding of apolipoprotein folding in solution, it does not represent accurately the configuration of the receptor-binding region because the lipid-free form of this apo E fragment binds to the LDL receptor with very poor affinity. Only in the lipidated form does apo E adopt the correct structure for high-affinity binding to the receptor.

It is hypothesized that when apo E binds to lipid surfaces the four-helix bundle opens at a hinge region to expose the hydrophobic faces of the helices to the lipid. In addition, the C-terminal 10-kDa fragment, missing from the crystal structure, is a major lipid-binding domain of apo E, but its contribution to the intact structure of apo E is not known.

A fragment of apo A1 lacking 43 amino acids from the N-terminus has been crystallized into 2 or 3 distinct conformations. The best studied one is a tetramer where each monomer polypeptide chain forms continuous amphipathic α -helices that curve into a horseshoe with a diameter of about 100 Å [16]. The monomers are organized into dimer pairs of antiparallel overlapping sequences giving a closed, distorted circular structure (Fig. 5B). This structure again illustrates the importance of amphipathic α helices, shows that they can form long continuous structures, and suggests models for interaction with lipids; however, it does not agree with known hydrodynamic and spectroscopic properties of lipid-free apo A1 monomers under physiologic conditions. The best current information for intact monomeric apo A1 in solution indicates that it has an elongated and rather rigid structure $(125 \times 25 \text{ Å})$ about two helices thick. The N-terminal Trp residues cluster near each other, and the compact and structured N- and C-terminal regions of the protein are within 35 Å of each other (A. Jonas, 2002). In fact, one of the crystallized forms of the apo A1 fragment suggests a monomeric protein in a hair-pin fold of extended helices, that is quite compatible with the proposed fold of the intact monomeric apo A1.

The three-dimensional structures of two insect apolipoproteins, apolipophorin-III from locust and Sphinx moth, have been solved by X-ray and NMR methods (M.A. Wells, 1991; R.O. Ryan, 1997). Both of these proteins bind reversibly to insect lipoprotein (lipophorin) surfaces and undergo dramatic structural changes in the process. The structures of the insect apolipophorins, similar to that of the apo E fragment, consist of elongated helix bundles of 5 amphipathic helices (Fig. 6).

It is important to note that exchangeable apolipoproteins have an extraordinary capacity for structural adaptation in response to solution conditions or to different lipid environments. In solution, high salt conditions, pH changes, or inclusion of organic solvents can lead to distinct structural states, with abnormally high α -helix contents, and specific degrees of oligomerization, as seen for the crystallized tetramer of apo A1. Under physiologic conditions, the transformation from a lipid-free to a lipid-bound state often involves a marked increase in α -helix content, and transfer of hydrophobic amino



Fig. 6. Ribbon diagrams of the structure of moth apolipophorin III: (A) the helix-bundle configuration determined by X-ray analysis of the lipid-free protein; (B) proposed 'open' configuration of the apolipophorin as it binds to the surface of the insect lipoproteins. The helices are numbered 1 through 5, starting at the N-terminus (D.R. Breiter, 1991).

acid residues from a buried protein environment to a lipid environment. In lipid-bound states, changes in the content and composition of the lipid — surface phospholipids, cholesterol, or core lipids — can induce structural changes in the apolipoproteins. Also, binding of other apolipoproteins to the lipoprotein surface can affect the conformation of the resident apolipoproteins. Thus, there are potentially many distinct conformational states accessible to each exchangeable apolipoprotein. This is likely the case also for lipid-bound apo B100 and apo B48.

4. Complexes of apolipoproteins with lipids

4.1. Binding of apolipoproteins to phospholipid surfaces

Interactions of apolipoproteins with phospholipids are essential for the assembly of lipoproteins, stabilization of lipoprotein structures, and expression and modulation of

apolipoprotein functions. The main experimental approaches for the study of apolipoprotein interactions with phospholipids have used isolated, exchangeable apolipoproteins in conjunction with aggregated lipids dispersed in water or spread at the air/water interface. The aggregated lipid states include lipid monolayers, various types of liposomes (small unilamellar vesicles, large unilamellar vesicles, multilamellar vesicles), and emulsions. All these lipid systems consist of or include phospholipids, especially phosphatidylcholines.

Apolipoproteins bind or adsorb readily to the phospholipid surfaces [17]. For fluid egg PC surfaces, in vesicle or emulsion form, the binding affinities (K_{ds}) for exchangeable apolipoproteins range from 0.2 to 9 μ M, depending on the apolipoprotein, presence or absence of cholesterol in the surface, and curvature of the surface. Apo A4 has the lowest affinity for egg PC surfaces, in agreement with the observation that most of the apo A4 in plasma is lipid-free. The other exchangeable apolipoproteins have affinities comparable to one another, and similar calculated stoichiometries of amino acids bound per PC, averaging 0.70 ± 0.35 . The percent of phospholipid surface occupied by the apolipoproteins, at saturation, is in the range from 8 to 12%. In the absence of cholesterol, this corresponds to the maximal compression of the surface PC, required to accommodate the amphipathic helices of the apolipoproteins. Cholesterol added to the surface initially increases the available surface area for apolipoprotein binding, but then decreases it at higher cholesterol contents (e.g., 30 mol%) [17].

The rates of interaction with phospholipid surfaces are highest for lower molecular weight apolipoproteins or peptide analogs that are monomeric and unfolded [18]. However, upon binding to phospholipid surfaces most apolipoproteins and peptides become highly α -helical. In fact, helix formation largely accounts for the negative enthalpy of binding. At the phospholipid surface, the amphipathic helices lie with their axes parallel to the surface. The polar and charged residues of the α -helices are at the level of the polar head groups of the phospholipids, but do not appear to interact electrostatically with them. The prevalence and importance of intra or intermolecular helix-to-helix interactions is not yet elucidated.

Desorption of exchangeable apolipoproteins from phospholipid surfaces occurs in two steps: unfolding and loss of α -helical structure, followed by a slow desorption step whose rate depends on the length of the apolipoprotein and its affinity constant for the surface. Generally, the smaller molecular weight apolipoproteins desorb more rapidly and exchange between phospholipid surfaces more readily than larger apolipoproteins. One exception is apo A2, which has a higher affinity for PC than apo A1, and can displace apo A1 from model PC surfaces and from native lipoproteins.

As already noted, delipidated apo B100 and apo B48 are not soluble in water; therefore, their investigation has been limited to the proteins solubilized with detergent or reconstituted with selected lipids.

The properties of lipoprotein-like particles reconstituted with defined lipids will be described in the next section.

4.2. Lipoprotein-like complexes

Although phospholipid liposomes are favored systems for the study of apolipoprotein binding to phospholipid surfaces, vesicle–apolipoprotein complexes are not ideal models for lipoproteins. Vesicles have an interior water compartment not present in lipoproteins, are incapable of solubilizing large amounts of neutral lipids within the phospholipid bilayer, and are too large to mimic the surface curvature of HDL. Thus, methods have been developed to prepare small, micellar complexes of exchangeable apolipoproteins (in particular apo A1) with lipids that mimic discoidal and spherical HDL in shape, composition, and functional properties. For LDL and VLDL, microemulsions and emulsions of lipids of selected diameter and composition, with added apo B100, make good models of the native lipoproteins.

Several methods are known for the reconstitution of HDL-like complexes from pure components: (1) spontaneous formation of HDL discs from dimyristoylphos-phatidylcholine liposomes; (2) detergent mediated reconstitution of HDL discs with various phospholipids; and (3) co-sonication of apolipoproteins and lipids to form either discoidal or spherical HDL analogs [19].

Dimyristoyl-PC liposomes can bind apolipoproteins reversibly, as described in the preceding section; however, at the transition temperature (T_m) of the lipid (24°C) and at sufficiently high proportions of apolipoprotein to dimyristoyl-PC (1/3 or greater, wt/wt), the apolipoproteins can lyse the liposomes to give rise to small discs analogous to nascent HDL. The rate of the liposome disruption and solubilization depends on the temperature of the reaction. It is highest at the onset of the main phase transition of dimyristoyl-PC and decreases a thousand-fold on either side of $T_{\rm m}$. While the same reaction does occur with dipalmitoyl-PC liposomes at the $T_{\rm m}$ of 41°C, the rate is much slower than for dimyristoyl-PC. For long-chain, unsaturated PC, such as palmitoyloleoyl-PC, the rates of reaction are too slow to be measured at accessible temperatures. Therefore, for practical purposes, under ordinary conditions, only dimyristoyl-PC can be used effectively to reconstitute HDL discs by this method. In this system, smaller apolipoproteins and peptide analogs disrupt dimyristoyl-PC liposomes at higher rates than larger apolipoproteins. Thus under some circumstances synthetic peptide mimics of A-type amphipathic helices may lyse even egg PC or palmitoyloleoyl-PC vesicles.

The mechanism of the disruption of dimyristoyl-PC liposomes into discoidal reconstituted HDL particles requires binding of the apolipoprotein to the liposome surface, followed by protein-protein interactions that lead to penetration of the bilayer by apolipoprotein, and breakdown of the liposome into bilayer discs. The discs are surrounded and stabilized on the periphery by the amphipathic helices of the apolipoproteins [20].

A more universal method for producing discoidal reconstituted HDL particles uses detergent (usually Na cholate) to solubilize the phospholipid into mixed micelles, followed by addition of exchangeable apolipoprotein and removal of detergent by dialysis or column chromatography. Depending on the proportions of PC to apolipoprotein, in the range from 1/1 to 4/1 (wt/wt), reconstituted HDL discs of different diameters containing different numbers of apolipoprotein molecules with varied conformations

can be produced. Cholesterol (up to 15 mol%) or small amounts of other lipids can be readily incorporated during the lipid solubilization step.

The third method for making HDL-like particles is extensive co-sonication of apolipoproteins with phospholipids, in the absence or presence of neutral lipids. Usually, the components are mixed in the proportions found in native HDL and yield discoidal or spherical analogs of HDL depending on the absence or presence of neutral lipids. While the ability to synthesize spherical reconstituted HDL particles by this method is very attractive, controlling particle homogeneity and yield is difficult.

To produce models of LDL, VLDL, and CM, lipid mixtures of PC and a neutral lipid are first sonicated to give metastable emulsion particles of the general size of the desired lipoproteins, and then the apo B component is added from a detergent dispersion, or exchangeable apolipoproteins are added in solution [17].

The advantages of reconstituted lipoproteins over the native lipoproteins are that the model particles can be made with a single apolipoprotein and one or a few defined lipid components to study each component individually. In addition, particles of uniform size can be isolated where high-resolution structural analysis is potentially possible. Also the reconstituted lipoproteins lend themselves for the study of structure–function relationships. In fact, reconstituted lipoproteins display all the known functions ascribed to native lipoproteins, including enzyme activation, receptor binding, and uptake and transfer of lipids.

4.3. Reconstituted HDL

The best studied reconstituted HDL are the discoidal particles containing apo A1 and a single type of PC, with or without added small amounts (<20 mol%) of other phospholipids or cholesterol [20]. The discoidal shape of the particles has been confirmed by negative-stain electron microscopy, small-angle X-ray scattering, and atomic force microscopy methods. All methods indicate a disc thickness of 45–55 Å, that corresponds to the phospholipid bilayer thickness, and diameters ranging from 70 to 180 Å. The main phase transition of the PC is preserved in the particles, but $T_{\rm m}$ is shifted about 3°C to higher temperatures, indicating a greater ordering and restriction of the PC in the particles than in corresponding liposomes.

Analyzed by non-denaturing gradient gel electrophoresis the particles display discrete diameters and size distributions that vary depending on the initial PC/apo A1 ratios of the preparations. Isolated particles of a specific size have reproducible, characteristic physical and functional properties. For example, some particles containing palmitoyloleoyl-PC (78 and 109 Å) are poor substrates for LCAT while others, particularly the 96 Å particles, are the best known LCAT substrates (A. Jonas, 1989). In addition, there is evidence of different binding affinities of the particles for SR-BI receptors (D.R. van der Westhuyzen, 2001). Thus reconstituted HDL subclasses, with different lipid contents, have different apolipoprotein conformations that result in dramatically altered functional properties. In vivo, this conformational adaptability of apolipoproteins probably leads to metabolic switching for the diverse functions of HDL subclasses. Apo A1 conformation is also regulated by the saturation or unsaturation of the PC acyl chains and by high contents of cholesterol (>15 mol %) or polyun-



Fig. 7. Diagrams of the proposed arrangement of two apo A1 molecules (residues 44–243) on the periphery of a discoidal reconstituted HDL particle (M.A. Tricerri, 2001). N, denotes the N-terminus and C, the C-terminus of each protein molecule. (A) 'Picket-fence' model in head-to-tail arrangement. (B) 'Picket-fence' in a head-to-head arrangement. (C) 'Belt' model. (D) 'Hair-pin' fold in a head-to-tail arrangement. (E) 'Hair-pin' fold in a head-to-head arrangement.

saturated PC. Other exchangeable apolipoproteins (apo A2, apo E, apo A4) also form discoidal reconstituted HDL with diameters that roughly correspond to their contents of amphipathic α -helices and molecular weights.

Reconstituted spherical HDL can be made by co-sonication of selected HDL components (e.g., apo A1, PC, cholesteryl ester) or by extensively reacting discoidal reconstituted HDL with LCAT in the presence of an exogenous source of cholesterol. The products are spheroidal and contain 2, 3, or 4 apo A1 molecules per particle, PC, cholesterol, and a cholesteryl ester core. The diameters of the particles range from 80 to 120 Å. Although not well studied, the conformation of apo A1 appears distinct from that in the discoidal particles and variable depending on the particle diameter (A. Jonas, 1990).

In reconstituted discoidal HDL, the apolipoproteins form a protective shell, one helix thick, around the periphery of the discs. According to current experimental evidence and models [21] (Segrest, 1999; Jonas, 2001), the helix axes are predominantly perpendicular to the acyl chains of the PC bilayer (see Fig. 7C,D). Earlier models, however, oriented the helical repeats almost parallel to the acyl chains, with the

antiparallel helical segments joined by tight β -turns (Fig. 7A,B) (Rosseneu, 1990; Schulten, 1997). The spatial organization of the amphipathic helices in spherical reconstituted HDL is not known and has not been modeled.

4.4. Structures of native lipoproteins

Electron microscopic images of lipoproteins show predominantly spherical shapes. Only nascent HDL appear as stacks of discs by negative-stain transmission electron microscopy. The stacks are artifacts of the method, since in solution nascent HDL and their reconstituted HDL analogs are free-standing discs. Regarding the structure of native HDL, their electron microscopic images do not have enough resolution to show any significant surface features, and other methods have not yet been successful in providing dependable structural information. The computer models generated for the reconstituted HDL discs are probably applicable for the larger nascent HDL particles, but no credible computer models exist for the spherical HDL. Based on the dimensions of HDL₃ particles, and the molecular volumes and surface areas of its component phospholipids, cholesterol, cholesteryl esters and amino acids, a space-filling model was constructed by Edelstein et al. in 1979 [22]. Their model is still valid today, with the exception that more apolipoprotein helix contacts are probably present, and the space under the helices is probably filled with cholesteryl ester (or triacylglycerol) molecules rather than with unesterified cholesterol, as shown in Fig. 8.

The N-terminal domain of apo B may have structural homology with lipovitellin. Lipovitellin is a soluble oocyte lipoprotein that contains 16% by weight of lipid and has sequence homology with the N-terminal 670 residues of apo B and with the microsomal lipid transfer protein. The X-ray structure [23] of lipovitellin contains a funnel-shaped cavity formed by two β -sheets lined with hydrophobic residues that are presumed to bind



Fig. 8. Structural model of human HDL₃ based on the composition and dimensions of the particles, and the molecular volumes and surface areas of the individual components. PL, phospholipid; FC, unesterified cholesterol; CE, cholesteryl ester; TG, triacylglycerol. Modified form of the model presented in Edelstein et al. [22] showing a cholesteryl ester molecule in the space under the apolipoprotein helices, and unesterified cholesterol (FC) in the surface monolayer.



Fig. 9. Individual images of LDL obtained by cryoelectron microscopy [22]. The box sizes are 270×270 Å. The unlabeled arrow indicates a region of high density that corresponds to a domain of apo B100; arrow with a 'p' indicates the pointed. N-terminal domain of apo B100; and an arrow with 'ld' points to a region of low density in apo B100.

phospholipids. A similar structure may be present in apo B and in the microsomal lipid transfer protein. Higher-resolution cryoelectron microscopy and negative-stain electron microscopy combined with monoclonal antibody tagging have revealed some structural features of apo B100 on LDL particles of relatively homogeneous sizes [24]. Images of LDL appear slightly ovoid and with average diameters of 233 Å. Individual LDL images show four or five high-density regions that correspond to the single molecule of apo B100. The dense regions are joined by lower-density connections. Although the entire protein appears in many images as a ring on the surface of the LDL (Fig. 9), the actual distribution of the protein domains is probably more dispersed and complex. The N-terminal domain of apo B100 can be identified as a high-density pointed end that protrudes more from the particle surface than any of the other domains. Some of the putative domains have approximately circular projections but the largest one, the one corresponding to residues 1700–3070, is elongated and is located at 50–140° latitude from the N-terminus.

5. Future directions

In spite of the great advances made during the last two decades in the elucidation of the structures and structure–function relationships of lipoproteins, much remains to be accomplished in this field of research.

Key areas of future research:

- (1) Structural and functional studies of the roles of minor lipid and apolipoprotein components of lipoproteins, including the products of oxidative reactions that occur in vivo and in vitro.
- (2) Investigation of the conformational adaptability of apolipoproteins during assembly

with lipids and during metabolic transformations of lipoproteins in circulation. Elucidation of the conformational changes at the atomic level will be a major challenge dependent on the success of high-resolution analysis of apolipoprotein and lipoprotein structures.

- (3) High-resolution determination of the three-dimensional structure of intact apolipoproteins and lipoproteins, under physiologic conditions. Accomplishing this goal will require not only major advances in the crystallization, X-ray, and NMR methodologies, but also the isolation or preparation of highly homogeneous reconstituted or native lipoproteins.
- (4) Further developments in computer modeling of reconstituted or native lipoproteins will require powerful molecular dynamics algorithms, and much more extensive experimental information on the folding and topology of the apolipoproteins. Especially promising are mass spectrometric methods combined with specific or random chemical cross-linking of apolipoproteins to establish spatial proximity.

Abbreviations

apolipoprotein
chylomicrons
high-density lipoproteins
equilibrium dissociation constant
lecithin cholesterol acyltransferase
low-density lipoproteins
lipoprotein(a)
phosphatidylcholine
phospholipid
scavenger receptor BI
main phase transition temperature
very low-density lipoproteins

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