Eur Biophys J (2009) 38:145–158
DOI 10.1007/s00249-008-0368-y

REVIEW

Molecular structure of low density lipoprotein: current status and future challenges

Ruth Prassl · Peter Laggner

Received: 13 June 2008 / Accepted: 28 August 2008 / Published online: 17 September 2008

Abstract This review highlights recent advances in structural studies on low density lipoprotein (LDL) with particular emphasis on the apolipoprotein moiety of LDL, apolipoprotein B100 (apoB100). Various molecular aspects of LDL are outlined and obstacles to structure determination are addressed. In this context, the prevailing conceptions of the molecular assembly of LDL and how the synergy of complementary biochemical, biophysical and molecular simulation approaches has lead to the current structural model of LDL are discussed. Evidence is presented that structural heterogeneity and the intrinsic dynamics of LDL are key determinants of the functionality of LDL in both health and disease. Some key research directions, remaining open questions and rapidly emerging new concepts for medical applications of LDL, are furthermore outlined. The article concludes by providing an outlook concerning promising future strategies for the clarification of the molecular details of LDL, in particular of apoB100, combining recent advances in molecular modeling with developments of novel experimental techniques. Although new insights into the molecular organization of LDL are forthcoming, many open questions remain unanswered. The major challenge of the next decade will certainly be the elucidation of the molecular structural and dynamic features of apoB100.

Keywords Low density lipoprotein · Apolipoprotein B100 · Molecular structure

R. Prassl (✉) · P. Laggner
Institute of Biophysics and Nanosystems Research,
Austrian Academy of Sciences, Schmiedlstr. 6,
8042 Graz, Austria
e-mail: ruth.prassl@oeaw.ac.at

Abbreviations

LDL	Low density lipoprotein
apoB100	Apolipoprotein B100
SAXS	Small angle X-ray scattering
SANS	Small angle neutron scattering
MRI	Magnetic resonance imaging
VLDL	Very low density lipoprotein
IDL	Intermediate density lipoprotein

Introduction

Low density lipoprotein (LDL) is one of the most fascinating macromolecular assemblies ever evolved by nature and has consequently attracted the attention of scientists for decades. As the principal transporter for cholesterol in the blood, circulating LDL guarantees a constant supply of cholesterol for tissues and cells and is an essential constituent of human life. The cellular uptake of LDL is mediated by specific receptors, either the classical B/E receptor or the scavenger receptor pathway (Brown and Goldstein 1976; Steinberg et al. 1989), and is strongly dependent on the structure of LDL particles and on the proper conformational orientation of apolipoprotein B100 (apoB100) located on the surface of LDL. The specific interactions of apoB100 with cellular receptors are the main focus of interest because these recognition mechanisms are intimately involved in the emergence and progression of various diseases, such as hypercholesterolemia, hyperlipidemia and atherogenesis. In particular, raised plasma levels of LDL are linked to an increased risk for the progression of atherosclerosis and cardiovascular diseases (Lusis 2000). Moreover, dysregulations of LDL due to abnormalities in LDL structure have been identified as independent predictors of risk for coronary heart diseases (Packard et al. 2000; Packard 2006).

LDL is highly heterogeneous in nature, varying in buoyant density, size, surface charge and chemical composition (Chapman et al. 1998; McNamara et al. 1996). These distinct biophysical characteristics play an important role in determining the fate of LDL in the subendothelial space (Pentikainen et al. 2002; Skalen et al. 2002). Small, dense LDL subclasses are, for example, more atherogenic than their light counterparts, which are more susceptible to modification (Hurt-Camejo et al. 2000; Packard 2006). Such modifications of LDL, principally through oxidation, enzymatic degradation or lipolysis, cause the formation of early atherosclerotic lesions by the deposition of lipids in the arterial wall. The primary event in atherosclerosis is hence the accumulation and retention of LDL in the arterial subendothelium, where the binding of proatherogenic LDL to proteoglycans is one of the key factors for the initiation of atherogenesis (Skalen et al. 2002). Once this occurs, LDL is prone to aggregation and fusion and is rapidly taken up by macrophages to form foam cells (Kruth 1997; Williams and Tabas 2005).

The way in which lipopoproteins, especially LDL, interact with arterial walls leading to the formation of foam cells and consequently to the progression of atherosclerotic plaques, and the nature of the structural, mostly oxidative, modifications of LDL involved in these processes are, however, not the focus of this review, and the reader is instead referred to special literature (Beisiegel and St Clair 1996; Binder et al. 2002; Fraley and Tsimikas 2006; Lusis 2000). In fact, it turned out from many studies that atherosclerosis is a chronic inflammatory event strongly depending on the metabolism of apoB100-containing lipoproteins, which in turn is predominantly triggered by structural features.

The focus of this review is the molecular structure of LDL, with special emphasis on its protein moiety, apoB100. Recent advances in, obstacles to and challenges in LDL structure determination and, finally, the prospects for future research activities to solve the structural features of LDL are presented and discussed.

Overview of LDL

There is a general consensus in the literature that LDL particles are organized into two major compartments, namely an apolar core, comprised primarily of cholesteryl esters, minor amounts of triglycerides and some free unesterified cholesterol surrounded by an amphipathic shell. This outer shell is composed of a phospholipid monolayer containing most of the free unesterified cholesterol and one single molecule of apoB100 [for reviews see (Kostner and Laggner 1989; Laggner 1995)]. An illustrative diagram of an LDL particle is provided in Fig. 1.

Over time, a broad arsenal of physico-chemical techniques has been employed to study various aspects of LDL

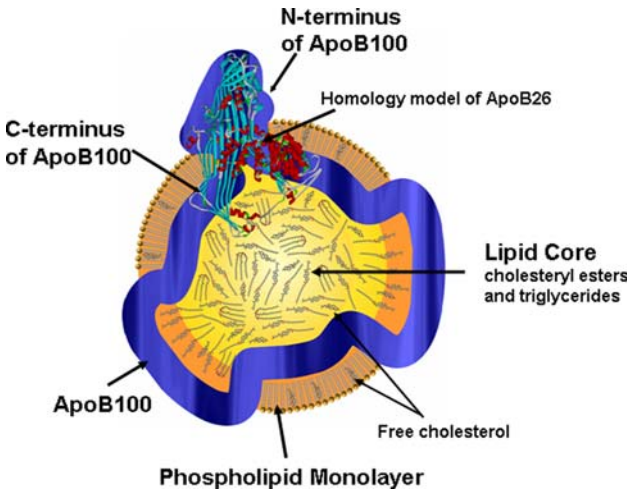

Fig. 1 Schematic model of an LDL particle. The lipid core is depicted in the fluid phase above the phase transition temperature. ApoB100 is shown as a blue string wrapped around the surface of the LDL particle, partially penetrating the phospholipid monolayer and the inner core. A 3D-homology model of the N-terminal region, corresponding to about 26% of the amino acid sequence, is superimposed

structure [for reviews see refs. (Hevonoja et al. 2000; Kostner and Laggner 1989; Schumaker et al. 1994)]. These techniques have included electron microscopy, X-ray and neutron small angle scattering, nuclear magnetic resonance spectroscopy, differential scanning calorimetry, circular dichroism-, infrared- and fluorescence spectroscopy. Most of them have been extensively used to characterize the structure of LDL, and several complementary but also controversial ideas about LDL structure were derived, which are discussed in the following sections.

In general, LDL particles assume a globular shape with an average particle diameter of about 22 nm. One must, however, keep in mind that LDL particles are highly heterogeneous with respect to composition and, range in size from 18 to 25 nm. Thus, LDL can be easily subdivided into distinct highly homogeneous LDL subspecies, which are identified on the basis of their hydrated densities and physicochemical characteristics (Chapman et al. 1988). These subspecies also differ in their receptor-binding affinity (Nigon et al. 1991), susceptibility to oxidative modifications (Dejager et al. 1993; Schuster et al. 1995) and in their atherogenic behavior. Small, dense LDL show lower affinity to the LDL-receptor, but increased unspecific binding to cell surfaces (Galeano et al. 1998). Indeed, high concentrations of small, dense LDL have been identified as an additional risk factor for atherosclerosis (Austin et al. 1990; Austin and Edwards 1996; Galeano et al. 1998), and are associated with atherogenic conditions such as hyperbetalipoproteinemia (Teng et al. 1985) or familial combined hyperlipidemia (Hokanson et al. 1995). Thus,

subtle changes in the particle diameter might alter the local conformation of apoB100, thereby inducing changes in the net surface charge of LDL. This higher negative net charge in turn results in a lower affinity for the cellular LDL-receptor, resulting in reduced clearance rates of LDL and enhanced atherogenicity (Lund-Katz et al. 1998). Based on these types of findings, both LDL particle size and shape can be considered to be important physiological determinants of LDL function.

LDL core structure

Of all circulating macromolecules in blood, LDL is the only one presently known to undergo a structural transition strikingly close to body temperature (Deckelbaum et al. 1975). The physiological role of this temperature-induced transition is still a matter of debate since no clear-cut evidence for its function has so far been found. It has, however, been hypothesized that the transition might play a role in the progression of atherosclerosis through its effects on cellular pathways of LDL recognition. Below the thermal transition temperature, the core-located lipids are arranged in an ordered liquid-crystalline phase. Above the transition temperature, however, the neutral lipids are organized in a fluid, oil-like, randomly distributed state as demonstrated by X-ray and neutron small angle (Deckelbaum et al. 1977; Laggner and Müller 1978; Laggner et al. 1984). The actual transition temperature varies between extremes of 15 and 35°C, depending on the individual donor and correlates well with the ratio of cholesteryl esters to triglycerides, being lower for higher triglyceride content (Deckelbaum et al. 1975, 1977; Laggner et al. 1977; Pownall et al. 1980; Pregetter et al. 1999). LDL from hypertriglyceridemic patients, which is rich on triglycerides, consistently does not show a thermal transition above 0°C. Thus, the core lipids remain in their fluid phase, independent of temperature (Sherman et al. 2003). Such LDL particles show a lower binding affinity for the LDL-receptor than normolipidemic LDL. Based on this, one can hypothesize that impaired LDL-receptor binding is associated with an increased triglyceride content and linked to changes in the core structure.

In contrast, for low triglyceride concentrations, a microphase separation was postulated, in which the triglyceride molecules separate in an oily innermost core compartment (Pregetter et al. 1999). At the same time, the surrounding cholesteryl esters are strongly immobilized and the core retains a higher viscosity. Under these conditions, the intracellular degradation of LDL is decelerated (Lusa and Somerharju 1998) and the activity of lipid transfer proteins is lower (Morton and Parks 1996; Zechner et al. 1984).

Core-shell model of LDL

The physical state of the core lipids might induce changes in the local molecular dynamics of the surface monolayer (Aviram et al. 1988; Coronado-Gray and Van Antwerpen 2003; Laggner et al. 1978; Mckeone et al. 1993; Morrisett et al. 1984). According to this model, the interactions of core lipids with the surrounding phospholipid monolayer in conjunction with changes in the lipid-protein interactions are responsible for structural modifications, which in turn influence the recognition and binding of LDL by receptors (Aviram et al. 1988; Mckeone et al. 1993). This view is consistent with studies reporting that apoB100 differs in structure according to the core lipid composition. More clearly, the conformation of apoB100 is an intimate feature of its interaction with LDL lipids (Banuelos et al. 1995; Laggner et al. 1978).

By this means, not only the arrangements of the phospholipid molecules and of apoB100 are changed, but also the overall size and shape of the particle are affected. Accordingly, controversial views about a deviation from the spherical structure of LDL have been discussed on the basis of small angle X-ray scattering (SAXS) data (Luzzati et al. 1979) and negative stain electron microscopy (Gulik-Krzywicki et al. 1979; Spin and Atkinson 1995). Later on, Orlova et al. (1999) could definitively show that snap-frozen LDL with the core lipids already in the liquid-crystalline state can reveal an ellipsoidal particle shape. From these cryo-EM images, Orlova et al. elaborated a low-resolution three-dimensional map of LDL. The map was reconstructed from thousands of different images grouped into distinct classes and was used to derive overall dimensions of $25 \times 21 \times 17.5$ nm for the class of ellipsoidal particles.

Based on these results, it seems reasonable to speculate that temperature might trigger a conversion from a roughly spherical to a more elliptical particle shape. Thus, an oblate ellipsoid shape is postulated for a subset of LDL particles, provided that the core lipids are in the liquid-crystalline state (Orlova et al. 1999; Van Antwerpen et al. 1997; Van Antwerpen 2004).

Simultaneously, the provocative idea arose that not only the overall shape of LDL particles might deviate from spherical, but also that the internal frozen lipid core is not necessarily centered radially. Indeed, some of the EM images presented by Orlova et al. (1999) clearly show an organized three-layer internal structure with a distance between the layers of about 3.6 nm. This distance corresponds well to the length of cholesteryl esters and is consistent with the characteristic periodicity observed in SAXS patterns for LDL below the transition temperature. Originally, the key evidence for the most widely accepted concept of the concentric spherical shell model of the core lipids was derived from different methodological

approaches including negative stain electron microscopy, SAXS and small angle neutron scattering (SANS) (Atkinson et al. 1977; Baumstark et al. 1990; Laggner et al. 1977; Laggner and Kostner 1978), and certainly this model is still accurate for quasi-spherical LDL species, in particular, if we take into account the morphological heterogeneity seen in the EM images. Moreover, this heterogeneity excellently reflects the inter-individual variances of LDL species and the marked impact of the chemical composition on the structural arrangement of lipids.

In order to examine this new model suggested by cryo-EM, we performed a simulation study, in which we composed a hypothetical cylindrical LDL particle with the overall dimensions suggested by cryo-EM and with internal lamellar repeat units with high and low electron density. From this simple structural model, we calculated the theoretical SAXS curve shown in Fig. 2. This curve strikingly resembles the experimental SAXS curve obtained for LDL particles below the transition temperature. As a matter of fact, we can say that SAXS cannot clearly distinguish between quasi-spherical and cylindrical particles, if the lengths of the axes are not distinctly different (i.e., less than 15%). Nevertheless, SAXS can definitively identify repeat units but not strictly differentiate between concentric layers or cylindrical lamellas. Following these lines of argument, the structural features—elliptical particle shapes with internal layers—observed in some cryo-EM images on LDL species with a "frozen" lipid core, are also reflected in simulated SAXS curves. Indeed, this new view is also consistent with low-resolution X-ray diffraction patterns of LDL crystals, grown from LDL particles with lipids in the liquid-crystalline state, which exhibit a pronounced orientation along one axis (Lunin et al. 2001; Prassl et al. 1996). This preferential orientation could well originate from a lamellar arrangement of cholesteryl esters within the crystal (Fig. 3).

Moreover, the division of the core into compartments separated by walls composed of ordered cholesteryl esters equally supports the notion that, at low triglyceride content, a microphase separation occurs within the core of LDL

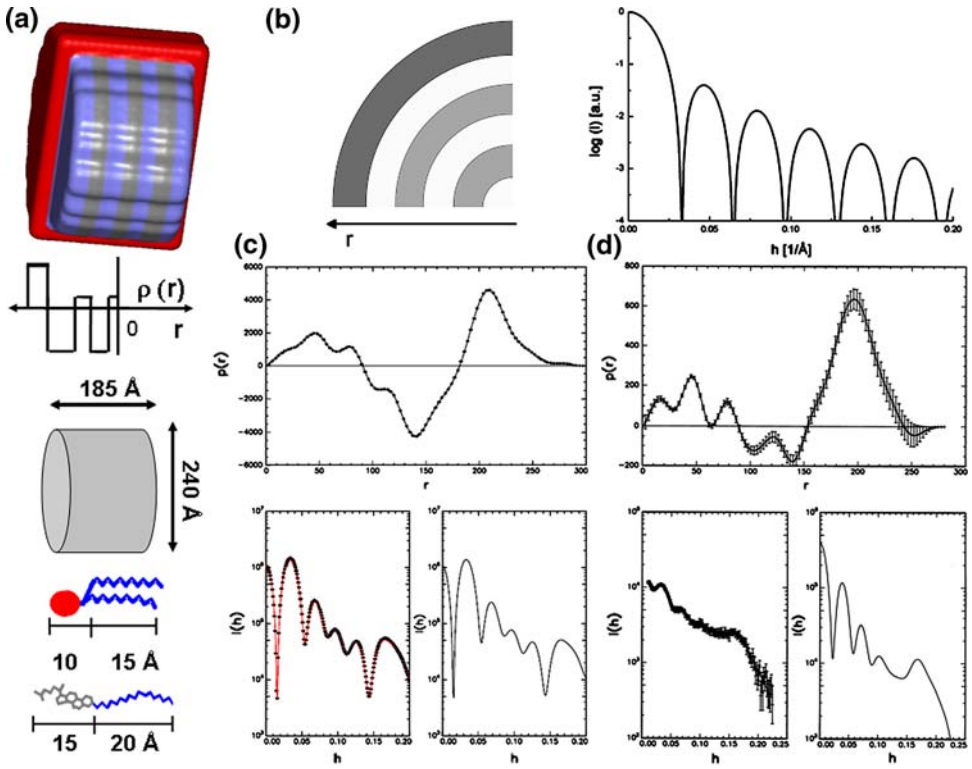

Fig. 2 Cylindrical core-shell model for LDL particles. The simulated model is based on a lamellar-layered molecular architecture of LDL as suggested by Orlova et al. (1999). **a** A cylindrical shell with dimensions of 185 × 240 Å and a centrosymmetric inhomogeneous core is assumed. A highly electron dense outer layer (*red*) representing the protein and the phospholipid headgroups, low electron density layers (*blue*) representing the acyl chains of phospholipids and cholesteryl esters as well as cholesterol parts (*gray*) of intermediate electron density are taken to calculate a theoretical electron density distribution function. The dimensions used for the cholesteryl ester and phospholipid molecules are indicated. **b** For comparison, a quadrant sector electron density distribution for the spherical core-shell model of LDL and the corresponding theoretical scattering curve are shown. **c** The simulated SAXS curve derived from the cylindrical model, together with the calculated real space electron-pair distance distribution function are shown in comparison to the experimental SAXS data derived from LDL below the phase transition (**d**)

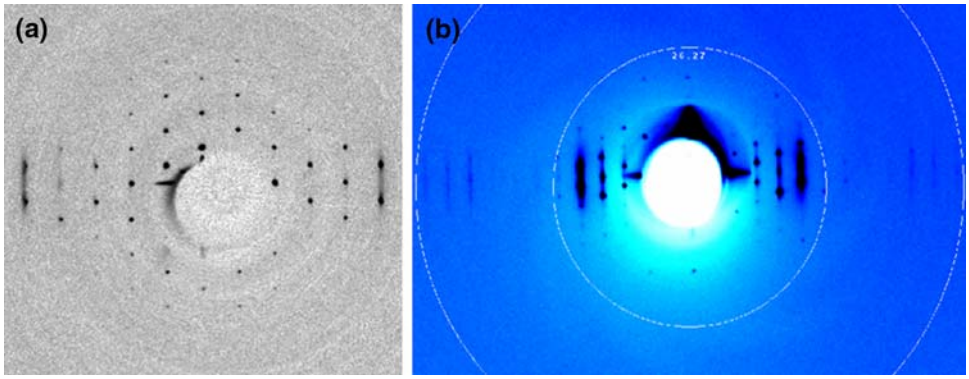

Fig. 3 X-ray diffraction images obtained for crystals grown from highly homogeneous LDL subspecies are shown. The diffraction data indicate a colloidal type of crystal form, with a one dimensional long range order along the short axis. Reflections up to the 12th order (15 Å) are seen. The prominent 5th order at 35 Å, corresponds well to the length of a cholesteryl ester molecule. The diffraction data were collected at the X-ray Source BW7B, EMBL, Hamburg (**a**) and at the SAXS beamline ELETTRA, Trieste (**b**). The preliminary crystallographic data are published Prassl et al. (1995)

(Pregetter et al. 1999) whereby, the cholesteryl ester and triglyceride molecules separate into distinct microenvironments. With increasing triglyceride content, however, this ordered state condensation of lipids diminishes with a concomitant decrease in melting transition temperature (Pregetter et al. 1999). Consistent with this, predominantly spherical shaped particles were found for LDL with a high triglyceride content (Sherman et al. 2003).

Taken together, the overall shape of LDL as well as the internal core lipid arrangement is characterized by distinct conformational deviations dependent on the chemical composition, the actual lipid phase transition behavior and the lipid-to-protein ratio. In this regard, however, the temporary formation of local molecular microenvironments such as triglyceride segregation must be considered.

Beyond the immediate impact of core structure on particle organization as outlined above, important questions concerning the lipid core still remain to be answered. An interesting question, for instance, relates to whether the lipid phase transition occurs rapidly enough to allow a reorganization of the core lipids during blood circulation? Also of interest is whether a compartment enriched in triglycerides serves as a physiological reservoir for lipophilic minor constituents, such as vitamins or antioxidants. Extrapolating from this, could a transient increase in local core concentrations of lipophilic constituents and their subsequent periodic redistribution be harnessed for the delivery of lipophilic drugs within a confined core of LDL? The latter is an attractive idea for the exploitation of a natural occurring nanoparticle as a reservoir for therapeutic agents. Indeed, several basic studies have already underlined the feasibility of such an approach (Firestone 1994; Hammel et al. 2003; Rensen et al. 2001), whereas final success is primarily expected for targeting growing malignant tumor cells. The rationale behind these approaches is that tumor cells have a high requirement for cholesterol and often express an elevated level of LDL-receptors, which could serve as molecular targets for drug delivery. Promising results have, for example, been recently achieved with the targeting of glioblastoma multiforme with paxlitacel-oleate-loaded synthetic LDL nanoparticles (Nikanjam et al. 2007a, b).

LDL surface layer

The oily lipid core is surrounded by a monolayer of phospholipids composed mainly of phosphatidylcholine and sphingomyelin. Interestingly, the lipid molecules within the surface layer of LDL have been shown to separate into local molecular environments enriched either in cholines or in sphingomyelin and free cholesterol (Sommer et al. 1992; Vauhkonen and Somerharju 1989). Most likely, this phospholipid microphase separation facilitates the diffusion of core lipids towards the surface, which is an important point for the action of hydrophilic lipid transfer proteins and lipid hydrolyzing enzymes. Nevertheless, one should keep in mind that the phospholipid microdomains are not static or confined in size and numbers. Rather, due to the absence of stringent packing constraints they confer structural dynamics to the moieties of apoB100 penetrating the phospholipid monolayer. Detailed SANS studies have indicated that apoB100 is located by ~0.8 nm "further out" than the phospholipid headgroups (Laggner et al. 1981). Based on simple geometrical considerations, and assuming about 700 phospholipid molecules per LDL particle, it becomes obvious that large parts of the surface layer must be occupied by apoB100 (Laggner et al. 1978). Nevertheless, details on phospholipid–protein arrangements in LDL are still missing, since the poor solubility of apoB100 makes reassembly experiments with defined lipid components extremely

difficult. Experimentally, however, it was shown by 31P-NMR that about 20% of the polar headgroups are immobilized, presumably by phospholipid–protein interactions (Yeagle et al. 1977). Similar results were found by Finer et al. (1975) and Lund-Katz and Phillips (1986) using 1H-NMR and 13C-NMR, respectively. In this regard, evidence was obtained that phosphatidylcholine is more closely associated to apoB100 than sphingomyelin (Sommer et al. 1992).

Another important parameter is the cholesterol content, as cholesterol is known to modulate lipid fluidity. Fluidity is a thermodynamic property that determines molecular dynamics within a confined volume, directly influencing the rate of chemical interactions. From this mechanistic point of view, it is plausible to assume that control of cholesterol content serves to maintain a fluidity optimum in lipoproteins, which balances the requirements of thermodynamic stability for lipid transport in blood with availability for controlled lipid discharge in metabolism. Subtle differences in cholesterol-controlled fluidity between lipoprotein particles may have profound effects on their individual residence time in circulating blood, on their interaction with vascular wall tissue and on their intracellular enzymatic degradation. The fluidity optimum may be impaired in pathophysiological conditions of lipid disorders (Dachet et al. 1990; Klein et al. 1991), whereas a correlation between fluidity, enzyme activity, oxidisability and LDL metabolism was shown to exist (Aviram et al. 1992; Foucher et al. 1996; Parks et al. 2000; Schuster et al. 1995). Hence, it seems reasonable, that the anti-atherogenic effect of cholesterol-lowering drugs, which is biochemically reflected in a reduction in the amount of cholesterol-rich lipoproteins, could be causally related to an altered fluidity of the lipoproteins upon drug treatment in a way that cholesterol reduction coincides with an increase in lipid fluidity. These changes in lipid mobility and in the microviscosity of the lipid environment may thwart accumulation of lipoprotein particles in the subendothelial matrix and diminish their retention in the vessel wall. The measurement of lipoprotein fluidity could therefore potentially serve as a complementary diagnostic tool to conventional lipid analysis (Caslake et al. 2003).

Apart from these considerations, another attractive idea concerns the functionalization of LDL particles for molecular imaging of diseased tissues which overexpress LDL-receptors. Possible targets would include atherosclerotic plaques and tumors. Besides the radioisotope-labeling of LDL for nuclear imaging, a rather novel approach suggests the modification of LDL with gadolinium-carrying chelator molecules, which are intercalated in the phospholipid monolayer, for magnetic resonance imaging (MRI) (Corbin et al. 2006; Frias et al. 2007). The use of LDL in this way as a targeted contrast imaging agent for medical diagnosis by MRI may allow for an early assessment of diseased sites at the molecular level and with a high spatial resolution.

In summary, besides its role in lipid metabolism and in lipid-associated metabolic disorders, LDL is becoming increasingly attractive as a molecular tool for medical applications, especially since LDL escapes recognition by the immune system and absorption by RES.

Protein moiety: apoB100

ApoB100, which constitutes approximately 20% of the LDL particle by weight, is the only protein component of LDL. Consisting of 4,536 amino acid residues (Chen et al. 1986; Knott et al. 1986) and with a molecular mass of about 550 kDa for the glycosylated form, it is among the largest monomeric glycoproteins known (Swaminathan and Aladjem 1976; Yang et al. 1986). Besides its large size, apoB100 is characterized by a moderate hydrophobicity of 3.82 kJ/residue, a value intermediate between intrinsic membrane proteins and exchangeable apolipoproteins (Chen et al. 1986). Thus, apoB100 is insoluble in aqueous solution and once secreted on a lipoprotein remains bound to the particle throughout lipid metabolism.

It is now generally accepted that apo B100 is wrapped around the surface of the LDL particle and thus stabilizes the structure of the protein–lipid complex (Laggner et al. 1981; Atkinson and Small 1986; Chatterton et al. 1991, 1995a). Spin and Atkinson (1995) have shown by electron microscopy using LDL in the frozen hydrated state that the particle is surrounded by a dense halo of protein, while Phillips and Schumaker (1989) found an irregular ring shaped structure in a roughly equatorial form surrounding LDL. From mapping studies by immuno-electron microscopy using monoclonal antibodies Chatterton et al. (1991) reported that the mapped epitopes are distributed over one hemisphere of the LDL particle. Thereby, the topology of apoB100 in LDL is characterized by the fact that the NH_2-terminal and the COOH-terminal end of the protein are in close proximity to each other and that the NH_2-terminal region forms a protrusion with a compact globular structure.

Concomitantly, it was suggested that apoB100 is composed of globular domains connected by flexible chains surrounding the LDL particle in a belt-like manner. Accordingly, a multi-domain composition for apoB100 was indicated by different experimental approaches. For instance, three domains were identified by proteolysis (Chen et al. 1989) and five domains were found by trypsin releasibility (Yang et al. 1989).That said however, the detailed configuration of apoB100 on the surface of LDL is still unknown and a matter of debate.

Nevertheless, a consensus has been reached that amphipathic α-helices and β-sheets represent the major conformational motifs of apoB100. In a more recent computational model based on a secondary structure analysis of apoB100,

a so called "pentapartite" structure was elaborated, proposing five consecutive domains, termed NH_2-$\beta\alpha_1$-β_1-α_2-β_2-α_3-COOH (Segrest et al. 1994, 2001) and, to date, this concept serves as the basis for the structure of apoB100 (Fig. 4). However, by looking more closely at the distinct domains of apoB100, it becomes immediately evident that, except for the N-terminal domain, little information is available on the molecular features of the structural motifs.

The NH_2-terminal domain of apoB100 was found to be highly trypsin-releasable since it is not tightly associated with lipids. It contains most of the Cys residues of apoB100 in the form of disulfide bonds, which results in the very compact folding of this region. As this region shows a high homology to lamprey lipovitellin (Mann et al. 1999) for which the atomic crystal structure is known [PDB:1LSH (Anderson et al. 1998)], different homology modeling approaches have been performed (Jiang et al. 2005; Mann et al. 1999; Segrest et al. 1999). A representative three-dimensional model, based on sequence alignment and subsequent homology modeling, is shown in Fig. 5 and it is also overlayed in the sketch of apoB100 on LDL shown in Fig. 1. The homology model shown here, represents the first 1,150 amino acids from the *N*-terminus of apoB100 and corresponds to apoB26 (i.e., 26% of the apoB100 sequence). The structure contains a characteristic β-barrel followed by an α-helical region and a triangular-shaped lipid-binding pocket formed by three amphipathic β-sheet structures, which have been proposed to accommodate some lipid molecules (Dashti et al. 2002). While the amphipathic antiparallel β-strands continuously extend up to residue 2,045 in human apoB100, the bottom of this triangular lipid pocket (160 residues up to residue 1,150) showed the best similarity to the amphipathic antiparallel β-sheets (residues 1,343–1,513) of lipovitellin and were modeled accordingly (Fig. 5). Only recently, Jiang et al. (2007) have elaborated structural constraints for this domain within a reconstituted lipoprotein particle by direct imaging of a nanogold-labeled apoB-fragment using scanning transmission electron microscopy. This technique promises to provide viable future strategies for the examination of single protein–lipid interactions at the molecular level. Accordingly, detailed information on the tertiary structure of this domain within a lipid assembly was presented (Jiang et al. 2007). Jiang et al. (2005) could, moreover, show that the domain is composed of different folding units, which unfold independently, indicating differences in chemical stability. Essential functional roles of the N-terminus include the modulation of the interactions of apoB100 with lipases (Sivaram et al. 1994) and with scavenger receptors on macrophages (Kreuzer et al. 1997). Equally important, the N-terminal 22–29% of apoB100 is essential for the co-translational assembly of lipoprotein particles in the liver (Burch and Herscovitz 2000; Ingram and Shelness 1997; Tran et al. 1998). The proper formation of intramolecular disulfide linkages in particular is essential for lipoprotein assembly (Tran et al. 1998), in which the microsomal triglyceride transfer protein (MTP) plays a critical role by triggering the proper folding and lipid transfer to the nascent apoB (Hussain et al. 2003; Segrest et al. 1999).

As briefly mentioned above, because the binding and retention of LDL by proteoglycans in the intima is the initiating process in early atherosclerosis, the identification of the glycan-binding sites on apoB100 is of general interest. In this context, eight binding sites to glycosaminoglycan have been found, most of them close to the C-terminus of the molecule (Camejo et al. 1998). The site identified as being physiological active is nevertheless located in the NH_2-terminal end of apoB100 (Goldberg et al. 1998).

The second domain, encompassing residues 1,000–2,000, being irreversibly lipid-associated, is predominantly composed of amphipathic β-strands. It seems reasonable to suggest that this region is involved in the initiation of triglyceride assembly in nascent apoB (Shelness et al. 2003). Together with the next domain (α2-domain), both are most

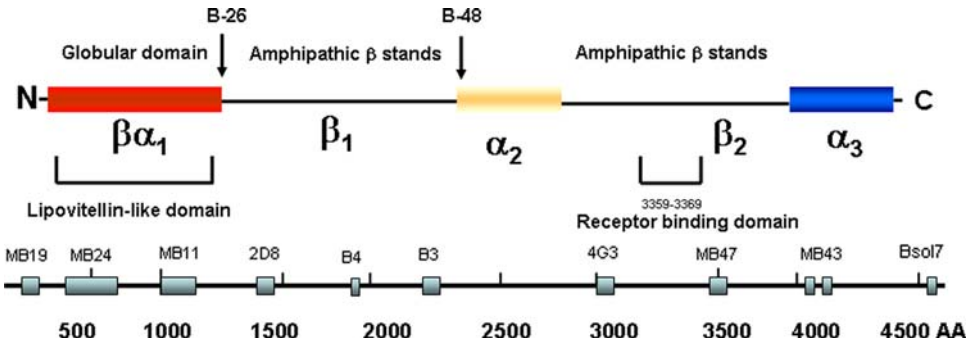

Fig. 4 Schematic diagram of the pentapartite structural model adapted from Segrest et al. (Segrest et al. 1994, 2001) (*upper panel*). The N-terminal globular lipovitellin-like domain corresponds to about 26% of the amino acid sequence, followed by amphipathic β-strands containing the receptor-binding domain. The C-terminal region is mainly α-helical. The lower panel shows the surface accessibility of sequences of apoB100 to monoclonal antibodies, which were used by Chatterton et al. (1995b) to map apoB100 on the surface of LDL by means of immuno EM

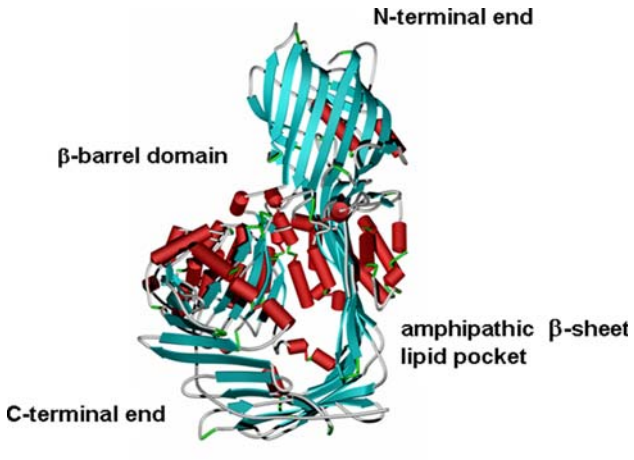

Fig. 5 Homology model of apoB26. The three dimensional homology model was calculated using the program Modeller V7.7 (Sali and Blundell 1993). Input coordinates were taken form the crystal structure of lipovitellin [PDB-ID: 1LSH (Anderson et al. 1998)]. The resulting structural model represents 1,150 amino acids from the N-terminal domain of apoB100

likely responsible for the accommodation of apoB100 on the particle surface and for the intimate association with the lipid environment. Moreover, the conformational flexibility of these parts most probably allows apoB100 to accommodate to changes in lipoprotein particle size. Apart from this, little information is available on the functional role or on structural features of these sequences.

It is now generally accepted that a cluster of basic amino acids (residues 3,359–3,369) within the β_2-domain preceding the COOH-terminal end forms the receptor-binding motif (Hospattankar et al. 1986; Yang et al. 1986). There is less consensus concerning a second receptor-binding site located within residues 3,147–3,157 (Knott et al. 1986; Law and Scott 1990). Both sites, however, are rich in cationic amino acids with Lys residues bearing active ε-amino groups for specific receptor-targeting (Lund-Katz et al. 1988). Based on this, the clusters of surface exposed basic amino acids are suggested to interact with the anionic sites of the LDL-receptor. Consistent with this, chemical modification of more than 20% of the active Lys residues essentially abolishes receptor binding (Lund-Katz et al. 1988). Interestingly, inserting acidic amino acid residues in sequences of basic residues within the receptor-binding region did not inhibit receptor binding but instead diminished proteoglycan binding. This behavior suggests that the mechanisms of interaction are different in that proteoglycan binding is based on ionic interactions, while receptor-binding activity is more likely associated with conformational changes of apoB100 (Boren et al. 1998). It cannot, however, be excluded that glycan binding to basic amino acids additionally induces a rearrangement of the tertiary structure of apoB100 that facilitates receptor binding.

Noteworthy, is that the highly conserved receptor-binding site is stabilized by the interaction of Arg3500 with Trp4369, indeed a single point amino acid mutation (Arg3500 to Glu3500) completely abolishes receptor recognition (Boren et al. 1998; Innerarity et al. 1987). This mutation is identical to the one identified in the common genetic disorder, familial defective apoB100 (Boren et al. 1998).

Only recently, the attractive idea arose to specifically block receptor binding by modification of Lys residues and, at the same time, to redirect LDL by apoB100-attached targeting sequences to alternate tumor-specific receptors (Zheng et al. 2005). This is an interesting strategy as it could potentially enable the exploitation of LDL as a natural endogenous carrier for targeted delivery of diagnostic and therapeutic agents to tumor cells.

Another important structural feature of apoB100 is its low conformational stability, which contributes significantly to the structural complexity of the molecule. Clearly, apoB100 can be viewed as a dynamic, flexible molecular string, able to adopt different conformations according to the size of the lipid droplet to which it is attached. It seems reasonable to speculate that this intrinsic flexibility of apoB100 allows the conversion of large very low density lipoprotein (VLDL) particles to smaller and denser intermediate density lipoprotein (IDL) and LDL particles without protein exchange. In support of this hypothesis, conformational changes in apoB100 during the metabolism of VLDL to LDL were detected either through the exposure of different epitopes (Krul et al. 1988) or through changes in accessibility to limited proteolysis, in the course of which, different enzymatic cleavage sites were identified for apoB100 in VLDL and LDL (Chen et al. 1989, 1994). Following these lines of thought, one could speculate that the protein accommodates to the surface of the shrinking particle with different rates and depths of penetration into the surface lipid layer. Indeed, molecular calculations based on particle size variations, molecular mass and surface lipid composition support experimental evidence that large parts of apoB100 are involved in the coverage of the core interface, occupying large surface areas in light LDL and less in dense LDL (McNamara et al. 1996). In this regard, it was suggested that the hydrophobic β-sheet domains are elastic and tightly anchored to neutral lipids, whereas the amphipathic α-helical domains respond rapidly to changes in surface pressure, becoming partially released and readsorbed during particle conversion (Wang et al. 2006). Hence, the binding of apoB100 to the phospholipid monolayer and partial penetration in the lipid core is accompanied by structural rearrangements of domains and changes in the orientation of the domains relative to each other.

A key take home message from the range of studies discussed here is that not only LDL particles but equally

apoB100 should be viewed as highly dynamic structures and that structural variations significantly alter the functional activity of LDL in vivo. The latter fact clearly underlines the importance of the increased efforts to elucidate the molecular structure of apoB100 in order to understand the pathobiochemical function of LDL. However, the intrinsic conformational flexibility and variability of apoB100 imposes serious technical complications in structural studies, as structural homogeneity is the key requirement for most structural techniques, in particular for X-ray crystallography or electron microscopic reconstructions. Unfortunately, these drawbacks have, so far, hampered successful three-dimensional structure analysis of apoB100, although preliminary success has been achieved in the growing of well-ordered, three-dimensional crystals of intact LDL (Prassl et al. 1996; Ritter et al. 1997). Although the resolution of the resultant X-ray crystallographic model was limited to 2.7 nm (Lunin et al. 2001), the feasibility of such an approach was nonetheless proven.

Lipid-free apoB100

A quite different approach to the elucidation of the structural motifs of apoB100 is to investigate the morphology of apoB100 in its lipid-free form after solubilization with detergents. At present, most information on full-length lipid-free apoB100 has been deduced from electron microscopy studies, which have revealed distinct differences according to the detergent and on the preparation or imaging technique used. Anyhow, the most striking common feature in all reports is that lipid-free apoB100 adopts an elongated conformation, regardless of whether it is described as a horseshoe, a bent thread or a long string of varying widths (Gantz et al. 2000; Ikai 1980; Phillips and Schumaker 1989; Zampighi et al. 1980). Accordingly, an elongated morphology with an overall length of about 55 nm became visible in the low-resolution model reconstructed from SANS patterns (Johs et al. 2006). This 3D-model, additionally, clearly delineates the modular configuration of apoB100 (Fig. 6).

Importantly, the transfer of apoB100 to a lipid-mimetic environment, primarily in detergent micelles, did not significantly change the overall secondary structure of the molecule (Johs et al. 2006; Walsh and Atkinson 1983, 1986), suggesting that structural constraints preserve the internal domain structure. Moreover, the thermal unfolding characteristics, which exhibit a multi-stage melting behavior, demonstrate the conservation of domains. Nevertheless, it was shown that the domains are prone to temperature-dependent alterations in their tertiary structure (Walsh and Atkinson 1990). In support of this finding, the low-resolution SANS model (Fig. 6) provides evidence that the

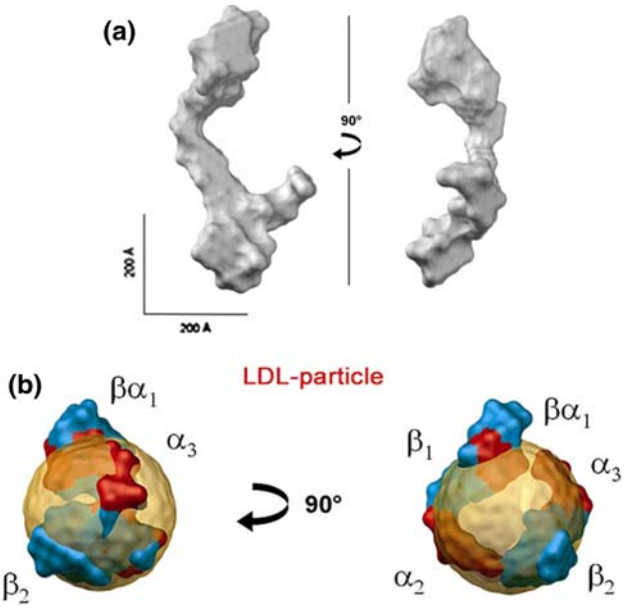

Fig. 6 Reconstructed three-dimensional model of lipid-free apoB100 derived from small angle neutron scattering data. **a** The averaged envelope shape of apoB100 is shown in surface representation [adopted from Johs et al. (2006)]. The shape on the right is rotated clockwise by 90° about the y-axis. The pronounced elongated particle morphology and the structured surface profile are clearly visible. **b** A secondary structure prediction based on the complete amino acid sequence of apoB100 was classified according to the pentapartite model proposed by Segrest et al. (1994) and mapped accordingly on the surface of the low-resolution model (α-helical structures are shown in red, *β*-sheet structures are shown in blue). Finally, the low-resolution model was wrapped around the surface of a sphere representing an LDL particle. By this means a hypothetical spatial arrangement of apoB100 on LDL was suggested [Johs et al. (2006)]

domains are flexibly linked, allowing a certain degree of freedom in their spatial orientation. This intrinsic flexibility has most probably hampered successful crystallization of lipid-free apoB100 until now. To overcome this problem, attempts to render the protein in a more rigid conformation through structural stabilization are envisaged, and could be approached by co-crystallization with monoclonal antibody fragments or by the use of alternative lipid-mimetic substances for protein solubilization. Regarding the latter issue, amphiphilic polymers and lipid-like peptides appear to be the most promising candidates with which to achieve this goal. These substances have already proven their potential for solubilizing and stabilizing membrane proteins (Tribet et al. 1996, 1997; Yang and Zhang 2006; Zhao et al. 2006) and might pave the way to success in the case of apoB100.

Summary and outlook

As outlined above, the most conclusive experimental results on the structure of LDL have been generated by

single particle cryo-EM imaging and by model reconstruction using small angle scattering data. One must however bear in mind that these are low-resolution techniques, which, while highly suitable for large complexes, can only be conditionally adopted for the structure determination of apoB100 at the atomic level. SAXS, for example, is an excellent technique for the delineation of modular architectures and spatial arrangements of high-molecular complexes. SAXS can also provide reliable hints on the dynamics of proteins in solution (Prassl et al. 2006; Putnam et al. 2007). However, in the case of apoB100 or membrane proteins, when detergents might considerably contribute to the scattering profile, neutron scattering rather than SAXS should be used to render the detergent invisible by methods of contrast variation. The major advantage of these scattering techniques over 3D-electron microscopy (Sorzano et al. 2007) is that apoB100 or LDL can be directly investigated under near physiological conditions without further manipulations such as staining or freezing.

Certainly, X-ray crystallography is the technique of choice for determining protein structures at atomic level (i.e., at a resolution limit of below 3 Å). However, despite several attempts by different groups, no high resolution data on crystals of native LDL have yet been generated. Even solubilized apoB100 has, so far, failed to yield good quality crystals, most probably due to the intrinsic structural flexibility of the individual domains. Moreover, the preparation of sufficient pure, solubilized protein retaining native structure is not straightforward. Different solubilization agents and/or amphiphilic molecules for extraction, detergent exchange and purification have to be tested in a trial and error approach followed by biological assays to verify the functionality of the protein. Even then, it cannot be guaranteed that the most favorable surfactant is appropriate for nucleation and growth of single crystals.

A reasonable way ahead would be to proceed with well defined fragments. Specific truncated fragments of apoB100 are however, naturally expressed at a low level which is a problem for structural studies in which high protein concentrations with proper folding in the functional state of the protein are required. In an alternative approach, apoB100 could be specifically cleaved by the proteolytic enzyme kallikrein to generate two fragments, apoB26 (1,297 residues) and apoB74 (3,239 residues) with molecular masses of 150 and 400 kDa, respectively (Cardin et al. 1984). ApoB26, representing the N-terminal domain of apoB100 is a very promising candidate for crystallization trials as it is largely water soluble and it's homology to lamprey lipovitellin should be sufficient to solve the crystallographic phase problem by molecular replacement. If successful, the first crystal structure on a domain of apoB100 would be available and could yield valuable information on the mechanisms of lipoprotein assembly.

Beyond these considerations, a strong emphasis on protein crystallization and the overcoming of the experimental difficulties associated with the huge size and low solubility of the protein impeding high resolution crystallography are needed. In the future, high-throughput screening and optimization of crystallization conditions using random sparse matrix screening for precipitants and detergents will allow thousands of crystallization trials requiring only a few microliters of protein to be set-up within a few hours (Li et al. 2006). Such robotic-controlled approaches will significantly increase the overall success rate to grow well-ordered single crystals. Although these crystals will often be extremely small, new developments in X-ray optics combined with high brilliance synchrotron radiation will enable micro- and nano- diffraction patterns of such microcrystals to be obtained within a short time period (Nohammer et al. 2005). Along these lines, recent advances in the application of optical tweezers (Cojoc et al. 2007) towards the fixation of submicroscopic particle clusters for X-ray diffraction should be mentioned. In addition, fast data evaluation programmes will enable high resolution 3D-structure determinations to be performed within a reasonable time span.

Finally, valuable complementary strategies to obtain structural information could be provided by computational modeling and molecular dynamics simulation. A recent sequence homology search in combination with homology modeling and fold recognition by threading algorithms has for instance lead to the publication of a theoretical model of apoB100. This putative model is built of eight domains connected in a non-globular fashion forming a string (Krisko and Etchebest 2007). The model, however, awaits confirmation by experimental data.

Conclusions

The present understanding of the structure of LDL and of apoB100 has emerged from the concerted application of different physico-chemical techniques. Some of these methods, including neutron- or X-ray small angle scattering or various spectroscopic techniques have been challenged by the stringent demands associated with the highly complex nature of LDL.

The principal goal in the future, but also the most difficult goal to achieve, will be a classical description of the structure of apoB100 at atomic resolution, either within LDL or in lipid-free form. Advances in crystallization techniques and the application of new technical developments using synchrotron radiation sources provide at least moderate grounds for optimism.

Acknowledgments The work was supported by the Austrian Science Funds (P16479 and P20455 apart from Seegen Stiftung of the Austrian Acad Sci.). The author would like to thank Heinz Amenitsch and Manfred Kriechbaum for scientific support in SAXS experiments and Christopher Wrighton for text editing.

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