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# COMMUNICATIONS

## The Structure of Plasma Low Density Lipoproteins: Experimental Facts and Interpretations—A Minireview[1]

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**ABSTRACT**

From data on size and chemical composition, low density lipoprotein (LDL) can be described as a spherical particle having cholesteryl esters and triglycerides contained in a spherical core covered by the closely packed hydrophobic ends of phospholipids and unesterified cholesterol, while the head groups of the phospholipids, together with protein, occupy the surface. Such a model is compatible with early small angle X-ray and neutron scattering studies which, by postulating spherical symmetry, assigned the LDL constituents to locations predicted from the radial electron density distribution. However, the concept of spherical symmetry, as applied to LDL structure, was recently challenged by results obtained from freeze-etching electron microscopy and small angle X-ray scattering experiments. Novel interpretations of these data suggest that the surface of LDL contains 4 electron-dense globules, located at tetrahedral positions, which have a capacity for structural remodeling at least as a function of the 2 temperatures studied (21C and 41C). It is reasonable to presume that the LDL protein (apo LDL) plays a role in the organization of the surface and overall LDL structure. However, until the chemical properties of apo LDL, and its behavior in solution and at the water-lipid interface are better understood, the validity of the proposed models cannot be assessed.

The interest in the study of the structure of the plasma lipoproteins, which by buoyant density criteria are commonly referred to as low density lipoproteins (LDL), has been recently heightened with the discovery that by interacting with specific membrane receptors, these lipoproteins exhibit regulatory functions in cell metabolism (1). Attempts to elucidate the LDL structure have been numerous and were mainly focused on the intact lipoproteins (2-4). Only recently, promising reassembly techniques have been developed and are expected to provide important new approaches to structural research (5). A fundamental limitation in the study of its structure is that LDL, like the other plasma lipoproteins, has a dynamic, fluid structure which may not be amenable to a static, rigid description. The structural flexibility of the LDL particle has been recognized (6), but the ranges of such adaptibility and the molecular events attending it have not been clarified. The assumption in any structural approach is that the definition of a basic structural pattern is compatible, both with physico-chemical data and thermodynamic principles. Once such a basic structure is understood, it should be possible to evaluate permissible structural perturbations and correlate them to LDL function(s).

On such premises, we will attempt to provide a brief overview on the field of LDL structure using old and new information.

### Earlier Concepts

A dominant concept relative to the overall geometry of all plasma lipoproteins is that they are spherical or quasispherical particles having an apolar core surrounded by polar surface components (2-4). As an example, $LDL_2$, a lipoprotein which is separated between d 1.019 and 1.063 g/ml, is 220 Å in diameter and has a molecular weight (MW) of $2.75 \times 10^6$. The conclusion that this particle is quasispherical in shape is based on data obtained from the techniques of electron microscopy (7), small angle X-ray scattering (8-11) and analytical ultracentrifugation (12-14), but is equivocal in that none of these methods provide direct evidence of the actual shape of the LDL molecule. Electron microscopy conducted mainly on negative stained samples has important limitations since analyses were performed on dried specimens, which are conditions conducive to particle deformation (7). Similarly, the interpretation of early small angle X-ray scattering

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studies have relied on the assumption, which has been challenged by more recent ones (15) (see following text), that LDL is a particle having spherical symmetry. The ultracentrifugal studies are also in question because the hydrodynamic frictional ratio calculated from sedimentation and diffusion measurements in the analytical ultracentrifuge departs from unity, i.e., $f/f_0$ = 1.1, (12) a reflection of either deviation from spherical shape, particle hydration, or both. At this time, there is no way to clearly distinguish between these 2 contributions to the frictional ratio. However, LDL asymmetry, at least intended as a particle which departs from a perfect sphere, cannot be ruled out.

Attention should be given to the LDL core. Important information has been gathered from small angle X-ray (3,4,9,15,16) and neutron (17) scattering studies as well as from thermal (18) and spectroscopic (19-20) analyses. The scattering studies have been interpreted as showing that LDL has a well defined low electron density region compatible with the existence of a core containing most of the cholesteryl esters and triglycerides. A most important contribution, however, has come from the thermal analyses (18), which indicate that LDL undergoes a broad reversible thermal transition between 20 and 45 C. This cooperative transition, which is associated with the disappearance of the 36-Å fringe in the X-ray scattering curve, has been attributed to an order → disorder phase transition of the cholesteryl esters. When in the ordered phase (10 C), the cholesteryl esters have been viewed as arranged in concentric layers with a 36-Å periodicity, whereas at higher temperatures the periodicity is lost, although the radial arrangement is retained. These observations imply that some degree of organizational constraint is present, a concept which also appears to be supported by $^{13}$C-NMR studies (19,20). Therefore, it would seem that, above the thermal transition, the cholesterol ring system and fatty acyl chains in LDL have a lower degree of rotational mobility compared to model systems. In this context, measurements of fluorescence depolarization using 1,6-diphenyl-1,3,5-hexatriene (DPH) and perylene have shown that the hydrophobic environments in LDL have microviscosity values which are higher than those obtained when the lipids are studied free of protein (21).

In regard to the LDL surface, current concepts confine its components, i.e., apoprotein(s), phospholipids and unesterified cholesterol, to a monolayer surrounding the apolar core. The surface location of the phospholipids receives support from kinetic studies using phospholipase $A_2$ as a probe (22). Although the results of these studies are compatible with an equivalent phospholipid pool, nuclear magnetic resonance (NMR) (23,24) and electron spin resonance (ESR) (25,26) data favor the existence of at least 2 distinct populations whose relative rotational motions are influenced by the extent of their interaction with proteins. Similarly unsettled is the location of unesterified cholesterol. Structural information deduced from chemical analyses has assigned this sterol to a position at the surface monolayer farther away from the aqueous environment when compared to the polar head group of phospholipids (27). Moreover, fluorescence studies have provided evidence that the unesterified cholesterol molecules of LDL are in closer proximity to the protein than to the cholesteryl esters (28). Studies using Filipin III as a probe have suggested a surface location for unesterified cholesterol although its actual position in the monolayer was not defined (R. Bittman, personal communication). Equally unsettled is the location of the LDL protein, apo B. In this case, the extent of covalent modification by succinic anhydride has been used to support the concept that this protein is predominantly located at the surface (6) but reservations against this proposal have been raised (2). The results obtained from the enzymatic digestion of LDL by proteolytic enzymes have been of little help regarding this question (2,6); the extent of hydrolysis has been limited and this may be the consequence of the intrinsic properties of the LDL protein at the LDL surface, its extent of interaction with lipids, or both. Conflicting results have also been reported on the number and nature of the peptides released after proteolysis which renders the interpretation of the results more difficult. In addition, the establishment of the secondary structure of the apoprotein at the LDL surface has not proven to be straightforward (2,5). The estimates of the relative proportion of $\alpha$-helix, random coil and $\beta$-structure have varied from laboratory to laboratory and a dependence of the protein conformation on temperature and amount of lipid has been observed. Early studies support the idea of a structurally flexible apo B (29,30). The temperature dependence of the conformational changes in the apoprotein (29,30) is particularly important since it raises the question of the relationship between changes in protein conformation and lipid organization within the LDL core. Quantitative information is needed on the actual fraction of apo B exposed to the aqueous environment and that facing core lipids.

**Current Concepts**

It is recognized that the size and density of LDL particles vary among different normolipemic individuals and in patients with hyperlipoproteinemia (31). This microheterogeneity has been attributed to either differences in amount of lipids, particularly triglycerides (32), or to the composition of the LDL protein (33). The interest in the microheterogeneity of human LDL was heightened by the recent observations based on equilibrium density gradient ultracentrifugations (34) and on the combination of isopycnic and rate zonal density gradient ultracentrifugations (unpublished observations), indicating that the LDL class of d 1.019 to 1.063 g/ml is heterogeneous even within a single individual. Further structural studies on various LDL species should provide information of great interest.

Recently, we have extended these studies to LDL from rhesus monkeys fed a normal purina chow diet (14). The normolipidemic animals were found to contain 3 major LDL species, LDL-I, LDL-II and LDL-III, separable by a combination of isopycnic and rate zonal density gradient ultracentrifugations. Important structural differences were found, particularly between LDL-III and the other 2 LDL species. LDL-III had a mean buoyant density of 1.050 g/ml and a larger MW ($3.47 \times 10^6$) than LDL-I ($3.32 \times 10^6$) and LDL-II ($2.75 \times 10^6$), which floated at d 1.027 and 1.036 g/ml, respectively; an apoprotein having the same amino acid composition as the other 2 LDL but a higher content in galactose and sialic acid was also found. LDL-III with the higher glycosylated apoprotein was immunologically less reactive to anti-LDL-II antisera than LDL-I and LDL-II and also exhibited spectroscopic differences by circular dichroism. Moreover, we found that LDL-III crossreacts with antisera directed to human Lp(a) lipoprotein (35). Thus, LDL microheterogeneity extends not only to size and hydrated density, but also to the type of protein moiety. This heterogeneity may be a reflection of differences in metabolic pathways and function of the various LDL species.

Recently, the concept of LDL as a centrosymmetric perfect sphere has been challenged by the studies of Luzzati and coworkers (15). A recent interpretation of their small angle X-ray scattering experiments conducted on LDL solutions at variable solvent electron densities suggests that the surface of LDL has 4 protein globules located at tetrahedral positions capable of thermally dependent structural changes. This arrangement appears to be supported by freeze-etching electron microscopic studies (36) using a novel, rapid freezing technique. Moreover, the independence of the electron density distribution of the LDL particle from that of the solvent was experimentally determined (37). Very recently, Ohtzuki et al. (38) have examined unstained preparations of human serum LDL by dark field imaging with a scanning transmission electron microscope at 2 $\times 10^6$ magnification. Surface irregularities were noted, although no attempts were made to establish their geometry. More studies in this direction are highly desirable.

**Conclusions**

It is evident from the preceding discussion that the structural organization of LDL is still an unsettled question. Although one could adopt the general concept of an apolar core surrounded by a polar monolayer, this idea does not explain the molecular basis for the microheterogeneity of LDL. The structure of LDL protein has not been resolved; thus, its chemical and solution properties as well as its behavior at interfaces remain, to a large degree, unknown. Without a better knowledge of apo B, it is unlikely that the fluid-like structure of LDL and the extent of its structural flexibility to conform to lipid content and temperature can be defined. Many questions lie ahead. Among them is the establishment of the structural correlation among the various LDL species, the interrelationship between core and surface components, as well as the surface organization of the surface components. As the functional properties of LDL continue to receive attention, the need for complementary structural information will increase. This structural information, however, may not only derive from direct physico-chemical studies of the intact particles but also from the investigation of the mechanisms of their biogenesis, mode of interaction with the other plasma lipoproteins and cells and from the analyses of genetic variants. The pursuit of recent promising observations on the reassembly of LDL (5) should also prove highly informative.

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