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Softness of Atherogenic Lipoproteins: A Comparison of Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) Using Elastic Incoherent Neutron Scattering (EINS)

Christian Mikl[†], **Judith Peters**[‡,||,#], **Marcus Trapp**[‡], **Karin Kornmueller**[†], **Wolfgang J. Schneider**[§], and **Ruth Prassl**[*,†]

[†]Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, Graz, Austria

[‡]Institut de Biologie Structurale, Grenoble, France

[||]Institut Laue Langevin, Grenoble, France

[#]Université Joseph Fourier Grenoble I, France

[§]Department of Medical Biochemistry, Medical University Vienna, Max F. Perutz Laboratories, Vienna, Austria

Abstract

Apolipoprotein B100 (apoB100)-containing plasma lipoproteins (LDL and VLDL) supply tissues and cells with cholesterol and fat. During lipolytic conversion from VLDL to LDL the size and chemical composition of the particles change, but the apoB100 molecule remains bound to the lipids and regulates the receptor mediated uptake. The molecular physical parameters which control lipoprotein remodeling and enable particle stabilization by apoB100 are largely unknown. Here, we have compared the molecular dynamics and elasticities of VLDL and LDL derived by elastic neutron scattering temperature scans. We have determined thermal motions, dynamical transitions, and molecular fluctuations, which reflect the temperature-dependent motional coupling between lipid and protein. Our results revealed that lipoprotein particles are extremely soft and flexible. We found substantial differences in the molecular resiliences of lipoproteins, especially at higher temperatures. These discrepancies not only can be explained in terms of lipid composition and mobility but also suggest that apoB100 displays different dynamics dependent on the lipoprotein it is bound to. Hence, we suppose that the inherent conformational flexibility of apoB100 permits particle stabilization upon lipid exchange, whereas the dynamic coupling between protein and lipids might be a key determinant for lipoprotein conversion and atherogenicity.

Lipoproteins are naturally occurring globular nanoparticles, which harbor an apolar oily core filled with cholesteryl esters and triglycerides—the cargo of the particles—surrounded by an amphipathic shell of phospholipids, free cholesterol, and apolipoproteins. As major carriers of lipids in the blood they are assigned with the function to supply tissues and cells with cholesterol and energy-rich fat. These substances are essential for membrane synthesis and fuel storage in cells and are precursors for signaling molecules. However, lipoproteins are

[*]**Corresponding Author**ruth.prassl@oeaw.ac.at.

Supporting Information. Sample preparation, neutron scattering experiments, chemical and electrophoretic analysis, list of hydrogen fractions and effective mean force constants, and ESR measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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also associated with cardiovascular diseases such as atherosclerosis or stroke, which are among the most prevalent causes of death in developed countries.[1]

Among the pro-atherogenic lipoproteins there are two classes, low density lipoprotein (LDL) and very low density lipoprotein (VLDL), both of which contain one single molecule of apolipoprotein B100 (apoB100) wrapped around their surfaces. ApoB100 is one of the largest monomeric amphipathic glycoproteins known and mediates the binding to cellular lipoprotein receptors, thus being involved in the progression of cardiovascular disorders. In contrast to LDL, VLDL particles harbor some additional small exchangeable apolipoproteins at their surface. VLDL from chicken egg yolk is highly homologous to the human equivalent, except that apoB100 is proteolytically cleaved.[2]

During metabolism, triglyceride-rich VLDL is converted to cholesteryl ester-rich LDL. Although this conversion is accompanied by extensive shrinking of the particles from about 50 to 20 nm in diameter, corresponding to about one-sixth of its surface area, apoB100 remains bound to its carrier stabilizing the whole particle (Figure1A). Nonetheless, the release of triglycerides results in a rearrangement of apoB100, which is enabled by the intrinsic elasticity of apoB100 consisting of static domains connected by highly flexible regions.[3,4] For simple steric reasons apoB100 must partially penetrate into the surface lipid layer with different rates and depths, or even be immersed into the hydrophobic lipid core,[5] depending on the lipoprotein it is bound to. In line with this, we propose that compositional and particle size dependent differences in lipoprotein resiliences as well as the dynamic coupling between apoB100 and lipids are key determinants for lipoprotein remodeling. To study the molecular dynamics of apoB100-containing lipoproteins in terms of thermal molecular fluctuations, we have performed a comparative study using Elastic Incoherent Neutron Scattering (EINS). EINS is a method sensitive to atomic motions, in particular to hydrogen atoms due to their high incoherent scattering cross section. In a time scale of pico- to nanoseconds and a space window of a few angstroms, neutron scattering reflects thermal motions averaged over the whole investigated sample. Accordingly, EINS is a well-suited method to investigate the overall dynamics of complex biological systems like cells, membranes, membrane proteins,[6,7] or lipoproteins.

Here, we report on the first characterization of lipoprotein dynamics with EINS, comparing the mean-square displacements (MSD) and the mean environmental force constants $\langle k \rangle$ of human LDL (hLDL), human VLDL (hVLDL), and yolk VLDL (yVLDL). MSD serve as a measure for the flexibility of the particles, and $\langle k \rangle$-values quantify the structural resilience. EINS measurements were complemented with spin-label electron spin resonance (ESR).

For EINS measurements all lipoprotein samples were lyophilized and rehydrated with D_2O to a level consistent with about one hydration layer covering the particle (see Supporting Information, Figure S1 and Table S1, S2). EINS scans in the temperature range from 20 to 310 K were performed on the backscattering spectrometer IN13 (energy resolution of 8 μeV) at the Institut Laue Langevin (ILL), Grenoble, France.[8] For calculation of the MSD (Figure 1B), a scattering vector (Q) range of 0.52 to 2.06 $Å^{-1}$ was chosen (Figure S2). Mean environmental force constants $\langle k \rangle$ were calculated from the slope in the scan MSD versus T.[9] The results are summarized in Table S3.

In the low-temperature region, the atoms are trapped in a conformational substate, and motion is reduced to harmonic vibrations around equilibrium positions.[9] In that range, the dynamics of the lipoproteins $\langle k \rangle$ lies between 0.77 and 1.21 N/m for hVLDL and hLDL, respectively. Compared to well-studied models like hydrated purple membrane[9,10] (~1.7 N/m) or myoglobin[11] (~2 N/m), lipoproteins are softer, however. This behavior correlates well with a high contribution of lipids[7] to the global dynamics of lipoproteins. Once the particles

enter the anharmonic temperature region at about 180–200 K, a dynamical transition occurs in all lipoprotein species and the MSD and <*k*'> values begin to differ.

In the range 260 to 270 K a noticeable second dynamic transition was observed, which was also reported to occur in the same temperature regime for purple membrane.[9] Above this transition, distinctions in the dynamics between LDL and VLDL become clearly visible: at physiological temperature less structural resilience and higher MSD of VLDL underline the extreme softness and flexibility of VLDL samples compared with those of LDL without significant differences between VLDL samples of different origin (i.e., human and egg yolk). It is not unlikely that the pronounced softness of VLDL is dominated by the higher amount of lipids (Table S1). Interestingly, we could not detect a motional contribution of core lipids attributable to the well-known core melting transition, which occurs between 290 and 310 K in LDL,[12] but not in VLDL.

To gain information about the contributions of surface, outer shell, and inner core components to global lipoprotein dynamics, EINS and ESR measurements were performed in the presence of sucrose.

Sucrose is a widely used cryoprotectant for biological macro-molecules, in particular preventing damage during freezing processes. This stabilizing effect can be described as trapping the material in a hard, harmonic substate over the entire temperature range;[13] however, the mechanism is still under discussion. There is evidence that disaccharides act as kosmotropes[14] and strongly influence the dynamics of a protein, which is dependent on external solvent relaxations.[15]

We have performed measurements of hLDL and yVLDL with sucrose added before lyophilization. It is important to note that the lipoprotein composition, i.e. lipid to protein ratio, remained the same as without sugar (85% and 91% of the incoherent scattering stems from lipids in LDL and VLDL, respectively). As expected, with sucrose both lipoproteins are more rigid over the entire temperature range (Figure 1C). The most striking feature, however, was that the dynamics of VLDL became dramatically restricted and equaled that of LDL, in particular at higher temperatures. The dynamical transitions were still detectable, although less pronounced. This behavior is in contrast to proteins, such as myoglobin (trehalose)[13] and lysozyme (trehalose and sucrose),[16] in which the dynamical transition becomes totally suppressed by disaccharides, which affect the atomic fluctuations on the surface and the core of globular proteins.[15] In the case of lipoproteins, we speculate that sucrose predominantly impacts particle surfaces and does not impair the contribution of core lipids to global lipoprotein motion. Thus, sugar molecules surrounding the lipoprotein particle most likely interact with other hydrophilic groups or domains on the surface of the particle (besides water), i.e., of proteins and phospholipid headgroups.[17] To support this notion, we performed experiments with spin-label ESR (see Supporting Information). Two different spin labels were used to determine local mobilities in lipoproteins. One label probed the inner core (triglycerides and cholesteryl esters), and the other one the outer shell region (phospholipid acylchains). Both labels are highly sensitive to changes in the fluidity of their immediate environment. However, it has to be mentioned that the molecular motions probed by ESR spin labels are somewhat slower (rotational correlation times are in the ns time scale) than those obtained by EINS. Comparing the fluidity parameters from samples in the presence and absence of sucrose we did not find substantial differences for either the lipid core nor the outer shell, with VLDL being more fluid than LDL (Table 1).

This led us to the assumption that the rigidifying effects of sucrose, especially on VLDL samples, are limited to surface-exposed apolipoprotein domains and in part to phospholipid headgroups and cholesterol. Since the striking change in VLDL can not only be explained

by the higher amount of surface-located molecules, it appears likely that apoB100 displays distinct different mobilities in VLDL and LDL controlled by its direct molecular environment.

To summarize, EINS measurements of LDL and VLDL revealed the pronounced softness of pro-atherogenic lipoprotein classes and further indicated that the molcular dynamics of apoB100 is partially dependent on the lipoprotein it is bound to, i.e., with higher mobility in VLDL compared to LDL. The data support the notion that the lipolytic conversion of VLDL to LDL forces apoB100 into a more condensed and therefore rigid state, entirely compatible with the observed inhibition of relaxation caused by the presence of sugar.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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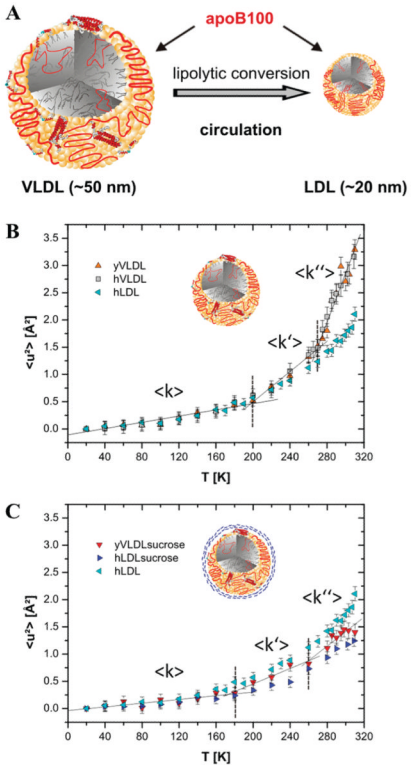

Figure 1.

(A) Scheme of the conversion of VLDL to LDL. (B) Mean square displacements (MSD) describing the dynamics of lipoproteins. hVLDL (gray squares), yVLDL (orange triangle upright), hLDL (blue triangle left). (C) With sucrose (10% w/v) added. yVLDLsucrose (red triangle down), hLDLsucrose (dark blue triangle right). For direct comparison the data of hLDL without sucrose are included. Sucrose surrounding the lipoprotein is mimicked by blue wavy lines. For yVLDL (−/+ sucrose), the temperatures of dynamical transitions (vertical dashed lines) and the regression lines used for evaluation of mean environmental force constants (solid lines) are indicated.

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Table 1

ESR Mobility Parameters[a]

	Sucrose	yVLDL	hVLDL	hLDL
Phospholipid	−	0.57	0.50	0.32
Acylchain Fluidity[b]	+	0.54	0.49	0.32
Core Fluidity[b]	−	0.81	0.77	0.44
	+	0.81	0.77	0.45
Rotational	−	2.3	2.9	6.9
Correlation Time[c]	+	2.2	2.8	7.3

[a] y, yolk; h, human; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

[b] (1-S_3), S_3 order parameter.

[c] τ_C rotational correlation time, [ns].