Experimental Report

Proposal: **8-04-694** **Council:** 10/2012

Title: Dynamics of lipoprotein particles under high pressure

This proposal is a new proposal

Researh Area: Biology

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Samples: Low density lipoprotein LDL

Instrument	Req. Days	All. Days	From	To
IN13	9	7	19/03/2013	26/03/2013

Abstract:

We propose to investigate molecular dynamics of native and biochemically modified low density lipoprotein (LDL) nanoparticles, which are macromolecular assemblies of phospholipids, cholesterol and fat stabilized by a protein moiety, under high hydrostatic pressure. The conception of a dynamic landscape similar to natural membranes seems to be reasonable for lipoprotein species, which we assume to be highly sensitive to pressure. Thus this project aims to study the impact of specific modifications of LDL on the dynamics, organization and interplay of lipids and protein under high pressure conditions. By combining all data we expect to obtain a more comprehensive picture of the structure and dynamics of LDL to be discussed in relation to its biological role.

Report 8-04-694 – IN13

Dynamics of lipoprotein particles under high pressure

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Low density lipoproteins (LDL) are naturally occurring macromolecular assemblies of lipids, fat and a single protein component, termed apolipoprotein B100 (apo-B100). Apo-B100 stabilizes the structure of the lipid nano-assembly and triggers the function of LDL in human circulation. The major role of LDL in humans is the transport of cholesterol and fat to tissues and cells. Apart from its vital role in physiology, LDL is intimately involved in the progression of cardiovascular diseases, in particular atherosclerosis. Chemical modifications of LDL e.g. by lipid oxidation cause an accumulation and retention of oxidized LDL in the subendothelial space for ingestion by macrophages to be transformed into foam cells. In combination with inflammatory reactions LDL retention in the arterial wall constitutes the first stage of atherosclerosis. Atherosclerosis dramatically increases the risk for myocardial infarction and stroke, which are amongst the major causes of morbidity and mortality in Western civilization resulting in substantial economic burden imposed on health care systems (1).

To date specific information on molecular motions, intrinsic flexibility or conformational stability is missing, especially with respect to the structural integrity of modified LDL. Ultimately, it is primarily the combination of above mentioned physicochemical parameters that determine the physiological function of LDL in humans. Lipoproteins, which are macromolecular assemblies of lipids and proteins, have shown a distinct dynamical behavior as function of temperature (2), which is very similar to biological membranes, and we assume lipoproteins to be highly sensitive to pressure as well. Thus, the conception of a dynamic landscape for lipoprotein species similar to natural membranes seems to be reasonable; however, this has never been shown experimentally before. By using neutron spectrometers with different energy resolutions, we will directly get access to molecular motions corresponding to different time scales.

Within the framework of the LTP 8-4 attributed to high pressure experiments in 2012, we did a first measurement of native LDL under hydrostatic pressure up to 600 bar to test the feasibility and the conditions for such investigations. Preliminary results are described in (3). Between 20 and 300 bar, we only found a shift of the summed intensities to higher temperature, but at 600 bar the slope of the curve changed as well, indicating a lower stability of the sample against temperature under these pressure conditions. To confirm these surprising findings and to better understand them, we repeated the experiment on native LDL, by scanning a sample in solution (D_2O) on IN13 at temperatures of 292, 303 and 313 K, and at pressures of 0, 300, 600 and 1200 bar. The sample was checked before the neutron experiment by DSC (see figure 1) and a core-melting transition temperature of LDL of 27 °C (301 K) and a protein denaturation temperature of 81 °C (354 K) were determined. We thus measured atomic mean square displacements (MSD) $\langle u^2 \rangle$ at a temperature below, just on and above the transition temperature as function of pressure (see figure 2).

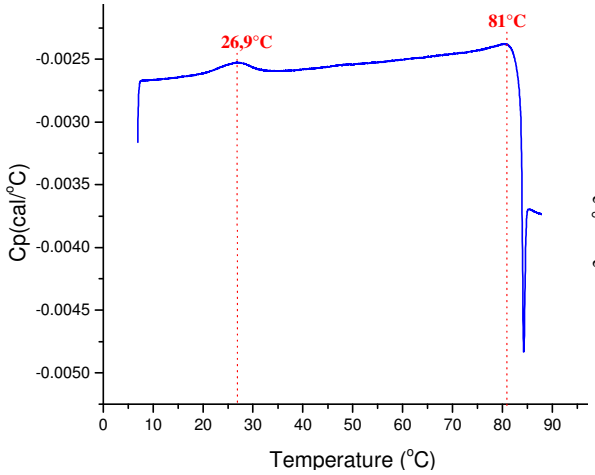

Figure 1: DSC measurement of native LDL.

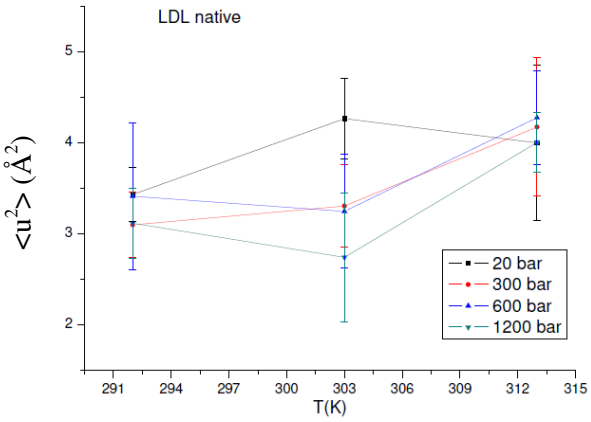

Figure 2: Mean square displacements $\langle u^2 \rangle$ of LDL as function of temperature and pressure.

Data analysis clearly indicated that the MSD for different pressures almost overlapped within error bars below and above the transition temperature, but MSD were much more disperse close to the transition point around 300 K. The error bars were, however, quite big, as the pressure cell was highly absorbing and thus the statistics were rather poor. The MSD were generally highly confirming earlier results described in (2).

On the basis of these results we obtained beam time at the PSI/Switzerland and investigated recently LDL under high pressure by SANS measurements. The results showed structural changes in LDL morphology under elevated pressure as well. In the future, we would like to do a more precise pressure scan of molecular dynamics in native LDL and modified forms.

References

1. V. L. Roger et al., Circulation (2011) **123,** e18-e209.
2. Mikl, C., Peters, J., Trapp, M., Kornmüller, K., Schneider, W. and Prassl, R., Journal of the American Chemical Society 133 (2011), 13213 - 13215.
3. J. Peters et al., ILL report LTP 8-4.