Experimental report

17/02/2020

Proposal: 8-04-870 **Council:** 4/2019

Title: Dynamics of isolated apolipoprotein B-100 (apoB-100) as a function oftemperature

Research area: Other...

This proposal is a new proposal

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Samples: Protein apo-B100 in presence or not of peptides

Instrument	Requested days	Allocated days	From	To
IN13	4	3	17/01/2020	20/01/2020

Abstract:

Low-density lipoproteins (LDL) are macromolecular assemblies of phospholipids, cholesterol and fat, stabilized by a glycoprotein. We propose to investigate here the molecular dynamics of the protein moiety of LDL; apolipoprotein B-100 (apoB-100), under temperature, lipid-free in the presence of detergents or stabilized by amphiphilic peptides. ApoB-100 is involved in the cellular uptake of LDL, thus deeply linked to LDL biological role. This project aims to provide new information about the molecular dynamics of lipid-free apoB-100, which has never been measured before and that will be combined with future structure determination from cryo-electron microscopy (cryo-EM). In cryo-EM, phospholipid mimicking peptides are added to stabilize the highly flexible structure of apoB-100 : comparison between dynamics of apoB-100 with and without peptides by the means of incoherent neutron scattering is then needed to quantify the stabilisation effect. By gathering all data, we expect to obtain a more comprehensive picture of the structure and dynamics of apoB-100, to relate with LDL functionality.

Exp. Report Inter 8-04-870 (17 - 20 January 2020)

Dynamics of isolated apolipoprotein B-100 (apoB-100) as a function of temperature

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Introduction :

Low-density lipoproteins (LDL) are macromolecular complexes, naturally found in human plasma. They can be described as a hydrophobic core, containing triglycerides, cholesterol and cholesteryl esters, surrounded by an amphipathic surface, constituted by a phospholipid monolayer and a large glycoprotein : apolipoprotein B-100 (apoB-100). The major role of LDL is to transport cholesterol in blood circulation, from the liver to peripheral tissue. However, LDL are also known to be involved in the development of cardiovascular diseases, like atherosclerosis.

A detailed understanding of the structure and dynamics of both LDL and apoB-100 protein could then help in the development of new concepts for therapy of atherosclerosis, that remains one of the major causes of morbidity and mortality in the Western world [1]. Until then, studies about healthy normolipidemic LDL (N-LDL), and comparisons with a triglyceride-rich form (TG-LDL) mimicking their pathological version, were conducted using various techniques, including neutron scattering ([2],[3],[4]).

However, studies of apoB-100 protein remain equally crucial, as it is involved in the functionality of LDL, and directly contributes to the structure of the whole LDL. First SANS experiments were conducted on lipid-free apoB-100 combined with contrast matching to develop a first low resolution model [5], but since then, no other studies on the sole protein were performed. In link with a cryo-EM experiment conducted in February 2019 (IECB, Bordeaux, France), this project aims at gaining new dynamics information about lipid-free apoB-100 protein.

Sample preparation and measurements :

About 180 mg of apoB-100 protein were prepared by Karin Kornmueller and her group at the Medical University of Graz (Austria). However and despite repeated and huge efforts, after the protein extraction procedure, the final sample still contains remaining Nonidet-40 detergent (NP-40) that could not be removed from the solution, and must have been accounted in our experiments.

The whole solution (apoB-100 protein + remaining NP-40) was lyophilized. Then, about 450 mg of the dry sample was rehydrated from D_2O vapor at a hydration level of 0.4 mg D_2O/mg sample, in a flat sample holder. The cell was then sealed with a lid of 2.6 mm height. Besides, a solution of NP-40 detergent alone, in the same proportions as in the whole sample of interest, was also prepared in Graz, and lyophilized. It was also mounted on a flat sample holder, and sealed with a 2.9 mm height lid.

Elastic incoherent neutron scattering (EINS) measurements were performed on the spectrometer IN13, for a temperature range of [20 K : 315 K]. The dynamics of the NP-40 detergent alone was also checked, through measurements in the same temperature range. In addition, correction measurements of the empty cell were carried out at 300 K.

Data analysis and results :

The elastic data were analyzed using LAMP software. They were first corrected by the empty cell alone, and in a second time by the empty cell and the detergent contribution. The data were then normalized by the points at 20 K. Finally, the Mean-Square Displacements (MSD) were retrieved assuming the Gaussian approximation, and shown in Fig. 1.

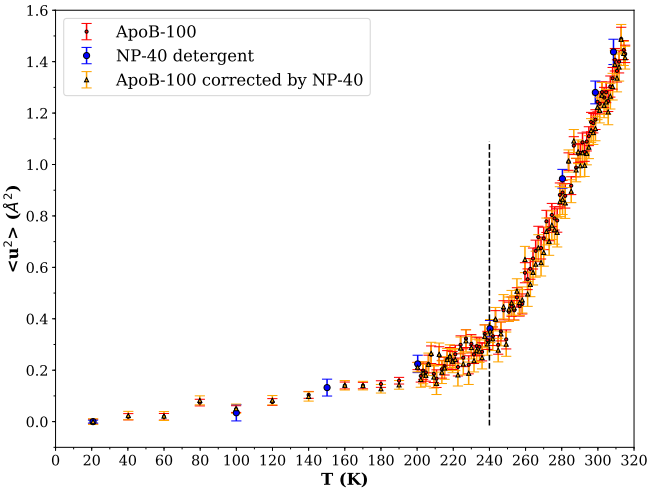

Figure 1: Comparison between the average dynamics of apoB-100 protein and detergent NP-40 in D_2O-hydrated powder form. The red circle points represent the sample (apoB-100 + NP-40) only corrected by the empty cell. Whereas the orange triangle points show the sample corrected by both the empty cell and detergent contributions. The blue bullet points come from the NP-40 detergent measurements.

Fig. 1 shows that all the MSD are superimposed. It means that the average dynamics of the whole sample, of the apoB-100 protein alone (correction by the NP-40) and of the NP-40 detergent seem to be comparable. However, some corrections due to the various masses are still missing and under progress.

MSD as high as 1.5 $Å^{-2}$ at 310 K are very high compared to other known proteins [6], however it was observed in staining electron microscopy that the apoB-100 protein can adopt many different conformations (see Fig. 2), which suggested an important dynamics.

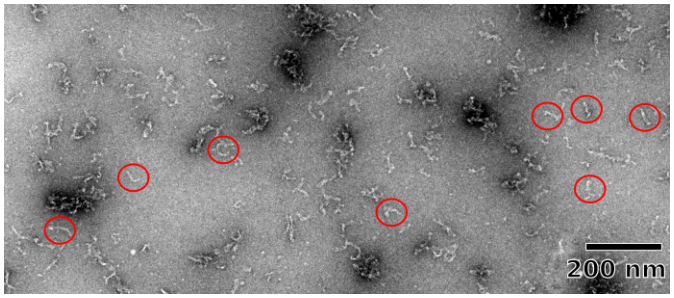

Figure 2: Picture of apoB-100 protein from negative-staining electron microscopy. Exemples of different conformations are shown in the red circles.
Credits : Karin Kornmueller, Gerd Leitinger (Medical University of Graz).

To conclude, these first results tend to show that the apoB-100 protein and the detergent NP-40, left from protein extraction procedure, are very similar. If the observed huge dynamics stay relevant with the known properties of apoB-100, it remains difficult to separate both contributions and focus only on the protein.

To do so, quasi-elastic neutron scattering could be a solution, by enabling the separation of each contribution, and would give more insight into the details of apoB-100 dynamics. For that, a proposal for continuing this experiment will be submitted for the next cycle.

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