Self-association of cholesterol molecules near the membrane oversaturated with cholesterol modelling initial stages of processes leading to atherosclerotic plaque formation: computer simulation and experimental studies

Cholesterol (Chol) is the most prevailing sterol in the animal kingdom. It is an essential component of mammalian cell membranes – over 90% of cellular Chol is associated with plasma membranes. Chol content in the membrane is strictly controlled; however degenerative processes like oxidation of phospholipids and Chol lead to an increased relative content of membrane cholesterol. This Chol excess results in formation of pure Chol microdomains in the lipid matrix of the biomembrane. It is known from experimental studies on model phosphatidylcholine-cholesterol (PC-Chol) membranes, that when the Chol:PC molar ratio exceeds 1, Chol bilayer domains are formed in the membrane and when it exceeds 2, also Chol crystals are detected outside the membrane. Extra-membrane Chol crystals are harmful as they participate in pathologic processes leading among others to formation of gallstones and atherosclerotic plaques. The origin of Chol crystals in the arterial wall is not clear but it has been shown in *in vitro* studies that microdomains in membranes of vascular smooth muscle cells precede and contribute to the development of atherosclerotic plaques. One of the possible ways initiating Chol crystallisation there might be detachment of individual Chol molecules from microdomains; once outside the membrane, Chol molecules due to their very low monomeric solubility and amphiphilicity, self-associate into ordered structures.

Formation of Chol crystals is a long macroscopic process, which has many intermediate states. However, even though Chol plays crucial biological roles, and Chol crystals are harmful, very little is known about these intermediate states and particularly, about early forms of Chol crystallisation. This is mainly due to experimental difficulties caused predominantly by very low monomeric solubility of Chol. What is more, completely unknown and unstudied is the effect of physiologic conditions on Chol crystallisation, which takes place in pathologic processes.

In this project, molecular modelling methodology will be used to reveal subsequent early steps of Chol self-association process, and biophysical experimental methods will be used to study Chol aggregates on the macroscopic scale. Using computational methods of molecular dynamics simulation, free energy perturbation and umbrella sampling, systems consisting of Chol molecules, water, ions and Chol oxidation products (oxysterols) will be studied, to get a complete picture of the early stages of Chol self-association and self-organisation in water and in physiologic conditions that includes both energetic and temporal aspects.

In the next stage of the project implementation, the biophysical properties self-associated aggregates of 200 Chol molecules doped with oxysterols will be determined.

The same structural and dynamical properties of self-associated macroscopic Chol aggregates will be studied using experimental spin-labelling electron paramagnetic resonance (SL-EPR) and differential scanning calorimetry (DSC) methods. Also, their long-range structural order will be determined using X-ray diffraction. This way, it will be possible to mutually verify the results obtained using these different methodological approaches. Positive verification will significantly strengthen the impact of the project.

Implementation of the project will enable us to obtain detailed information about Chol self-association on the atomic and molecular level. This will help in better understanding of the nature of this process, which in the human body has adverse effects leading to formation of gallstones and atherosclerotic plaques. This understanding might help in designing agents able to suppress further growth of Chol aggregates in pathologic processes that constitute significant health as well as economic problems.

Computer modelling will be performed at the Department of Computational Biophysics and Bioinformatics, Jagiellonian University, Poland; X-ray diffraction measurements at the Department of Crystal Chemistry and Crystal Physics, Jagiellonian University, Poland; and SL-EPR and DSC experiments at the Department of Biophysics, Medical College of Wisconsin, USA.