Interactions of Opposing Lipid Leaflets and Nucleation of Cholesterol Crystals.

R. Ziblat, K. Kjaer[1,2] L. Leiserowitz[3] and L. Addadi

Department of Structural Biology, Weizmann Institute of Science, 76100 Rehovot, Israel

[1] Max-Planck Institute of Colloids and Interfaces, D-14476 Potsdam / Golm, Germany

[2] Niels Bohr Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark

[3] Department of Materials and Interfaces, Weizmann Institute of Science, 76100 Rehovot, Israel,

Cell membrane lipid bilayers are currently thought to consist of different phases and different domains at the nanometer scale. These domains (also known to some as 'lipid rafts') differ in composition, structure, stability, and consequently in properties and function. They selectively incorporate or exclude specific proteins, and thereby fulfill an important function in cell activity and signaling. Understanding the rules that govern membrane dynamics and structure is thus crucial to understanding cell biology.

Cholesterol is an essential component in the lipid membrane composition, generally found in all animals. In the plasma membrane, cholesterol concentrations were reported to be as high as 25-45%.[1] Cholesterol crystals are found in large quantities in gall stones and atherosclerosis. The latter is considered to be the main killer in western countries, leading to either heart failure or stroke. The mechanism and conditions at which these crystals are formed are unclear. We show here that such crystals can nucleate in lipid membranes and that the concentrations at which the cholesterol phase separates to form crystals are close to the actual concentrations in the plasma membrane.

Grazing incidence X-ray diffraction (GIXD) has been used for studying the structure of two-dimensional (2D) lipid monolayers at the air-water interface. Membranes are, however, composed of two opposing leaflets, i.e. of two juxtaposed monolayers, sandwiched between water. We have recently developed a method which enables GIXD measurements on single hydrated lipid bilayers, sandwiched between nanometric thick water layers.[2, Annual Report 2008] Using this method we are comparing the structure and phase behavior in bilayers to monolayers and by that study the interactions between the opposing leaflets of the bilayer.

Glycerol lipids are by far the most ubundant lipids in the cell membrane. Among those the largest group in the plasme membrane are the phosphocholines. As a representative of this group we chose 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC).[1] Bilayers composed of DPPC and cholesterol at different ratios were studied and compared to their corresponding monolayers. (Figure 1) Data shows that at cholesterol concentrations of 36%-54% a phase separation occurs in the bilayer samples. In the monolayer only one crystalline phase exists, which is a DPPC:Cholesterol mixed phase. The bilayer with the same lipid composition as in the monolayer has two crystalline phases: At Cholesterol=36% a DPPC:Chol mixed phase exists along with a pure DPPC phase. At Cholesterol=54% a similar DPPC:Chol mixed phase exists but this time along with a pure Cholesterol crystal one bilayer thick, which has a structure the same as that of the 10×7.5 $Å^2$ motif.[3]

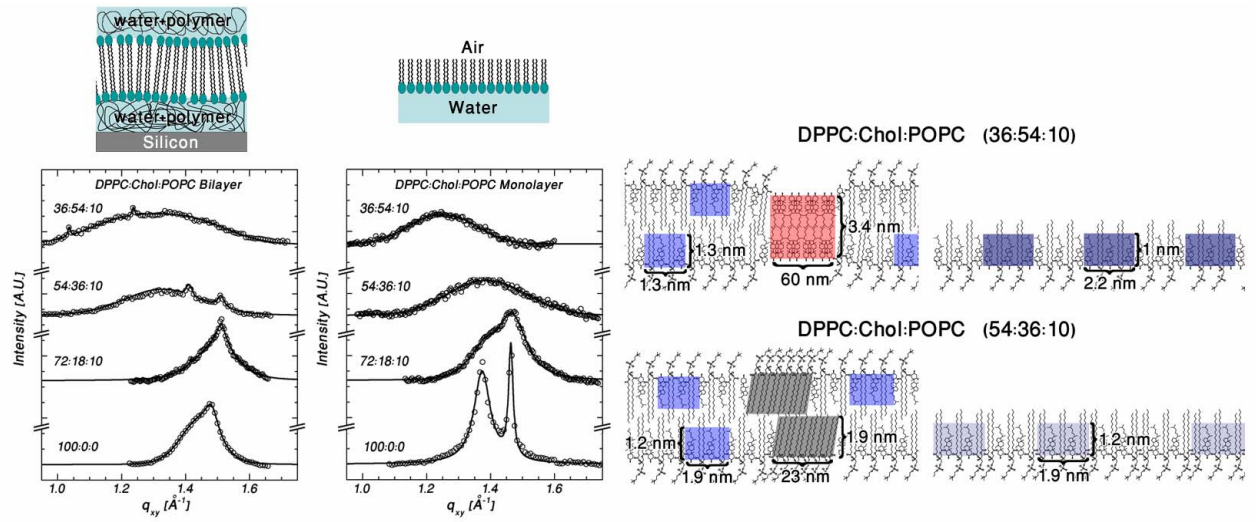

Figure 1: (Left) Bragg peaks of DPPC:Cholesterol:POPC at different ratios for bilayers and their corresponding monolayers. Bilayers were measured using the humidity chamber and the monolayers were measured on a Langmuir trough. (Right) Bilayer and monolayer schemes showing that at cholesterol concentrations of 36%-54% the bilayer has two crystalline phases whereas in the monolayer only one mixed crystalline phase exists. Coherence lengths, crystal thickness and phases were all derived directly from the diffraction data.

Differences in phase behaviour at high cholesterol concentration were also visible in Sphingomyelin:Cholesterol samples.[2] Sphingomyelin (SM) and Cholesterol are considered to be the main components of lipid rafts in cells. Our data show the concentration point from which cholesterol phase separates and form cholesterol crystals. These crystals are bilayer thick, formed by partial interdigitation of the exocyclic chains. Therefore, these crystals cannot be formed in monolayer systems and can only be studied in bilayers. The concentration point for cholesterol nucleation in SM:Cholesterol mixtures is 34% whereas in DPPC:Cholesterol it is 54%. We believe that these crystal domains act as nucleating sites for the formation of 3D cholesterol crystals, which in turn lead to atherosclerosis.

References

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