Structure and Dynamics of Apoptotic Model Membranes

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Life is based on cell division, proliferation and cell death (apoptosis). Amongst the three phases of apoptosis (initiator, effector- and execution phase), the effector phase is of particular interest, because its detailed understanding may be fundamental for novel therapeutic approaches for various diseases, including atherosclerosis, cancer, or Alzheimer to name but a few. During the effector phase the bilayer looses its asymmetric lipid distribution and neutral sphingomyelinase (nSMase) hydrolyses bulk sphingomyelin (SM) to Cer, which in turn may lead to apoptotic body formation. The structural changes occurring on the supramolecular level and the involved time scales are, however, largely unknown.

Therefore, we focussed on the structural changes in membranes during the SM conversion to Cer using the well characterized nSMase from *Bac cereus*. Mammalian membranes were mimicked by an equimolar mixture of palmitoyl oleoyl phosphatidylcholine (POPC)/SM. The structural reorganization occurring within the membranes during the enzyme reaction was followed by time-resolved small- and wide-angle X-ray-scattering (SWAXS). Cer levels and reaction kinetics were determined by high performance thin layer chromatography. The kinetic results were correlated to POPC/SM model membranes containing defined SM/Cer ratios, using biophysical techniques, such as differential scanning calorimetry, infrared spectroscopy, SWAXS, fluorescence microscopy and dynamic light scattering experiments.

We found, that the enzyme hydrolysed more than 50 % of the total SM-amount of the membrane to Cer and water soluble phosphocholine after approximately 100 min of reaction time. Interestingly however, the membrane structural reorganization takes about two to three times longer than the enzymatic formation of Cer. The reason is most likely hindered lateral diffusion due to Cer induced phase separation into gel-like Cer/SM-rich and fluid-like POPC-rich lipid domains.