Double Electron-Electron Resonance of Molecular Clusters in Biological Membranes

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Double electron-electron resonance spectroscopy (DEER, also known as PELDOR) is sensitive to spin-spin dipolar interactions between spin labels, at the nanoscale range of distances. Most often, DEER is applied to study distances between two spin labels attached to the same molecules. Here, we report results of DEER studies of nano-clusters, containing large number of molecules; such clusters naturally appear in biological membranes.

Plasma membrane is known to be highly compartmentalized, with lipids and proteins organized in specific domains, the so-called lipid rafts, with cholesterol (Chol) as an important constituent. Lipid rafts are assumed to be involved in different cellular processes, such as signal transduction, membrane trafficking, and protein activity. DEER was applied here to spin-labeled cholestane 3β-doxyl-5α-cholestane (DChl), which structure resembles that of Chol. DChl was diluted in bilayers comprised of saturated dipalmitoyl-glycero-phosphocholine (DPPC) and unsaturated dioleoyl-glycero-phosphocholine (DOPC) phospholipids, with Chol added in different proportions. The DEER data were described in terms of enhanced local concentrations which allowed to detect clustering of DChl molecules. The lateral distribution in the clusters was found to change drastically with Chol content: in absence of Chol the DChl molecules are randomly distributed in the clusters while in presence of Chol the distribution becomes quasi-regular. The found superlattice parameter was 3.7 nm. The found regularity of DChl lateral distribution was interpreted by raft sub-structuring, with DChl molecules embedded between the substructures.

Free fatty acids play various roles in biological membranes. Their functioning depends on intermolecular interactions. DEER was applied to study spin-labeled stearic acids in gel-phase phospholipid bilayers composed of DPPC/DOPC mixtures or of palmitoyl-oleoyl-glycero-phosphocholine (POPC) lipids. The results showed that in all cases the stearic acids are assembled into lipid-mediated lateral clusters, with a characteristic intermolecular distance of ~2 nm. On presence of antimicrobial peptides (AMP) the lateral distribution in clusters was found to change, which indicates the AMP impact on the lateral lipid organization in the membranes.

This work was supported by Russian Science Foundation, Grant # 21-13-00025.