Phase separation in giant vesicles: photo-oxidation and toxins interaction

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Using giant unilamellar vesicles (GUVs) made from POPC, DPPC, cholesterol and a small amount of a porphyrin-based photosensitizer that we name PE-porph, we investigated the response of the lipid bilayer under visible light, focusing in the formation of domains during the lipid oxidation induced by singlet oxygen. This reactive species is generated by light excitation of PE-porf in the vicinity of the membrane, and thus promotes formation of hydroperoxideswhen unsaturated lipids and cholesterol are present. Using optical microscopy we determined the lipid compositions under which GUVs initially in the homogeneous phase displayed Lo–Ld phase separation following irradiation. Such an effect is attributed to the in situ formation of both hydroperoxized POPC and cholesterol. The boundary line separating homogeneous Lo phase and phase coexistence regions in the phase diagram is displaced vertically towards the higher cholesterol content in respect to ternary diagram of POPC:DPPC:cholesterol mixtures in the absence of oxidized species. Phase separated domains emerge from sub-micrometer initial sizes to evolve over hours into large Lo–Ld domains completely separated in the lipid membrane. This study provides not only a new tool to explore the kinetics of domain formation in mixtures of lipid membranes, but may also have implications in biological signaling of redox misbalance.Further, we also conduct experiments on the response of the oxidized membranes under the action of actinoporins.

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