Membrane interactions of ternary phospholipid/cholesterol bilayers with viscumin

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Small unilamellar vesicles (SUV) are produced by repetitive extrusion cycles through polycarbonate membranes with 80 nm pore size. Ternary mixtures of dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC) and cholesterol are used, yielding liposomes with final mean diameters of 144 nm (for DPPC/DOPC/cholesterol = 1:6:3) or 166 nm (for DPPC/DOPC/cholesterol = 3:4:3). Viscumin, also named mistletoe lectin, is a ribosome inactivating protein of class II (RIP II protein). When viscumin is dissolved in the PBS buffer in which the SUV are formed, it distributes homogeneously inside and around the SUV with no elevated membrane adsorption, as proved by ELISA.

Cryo transmission electron microscopy (CTEM) shows that with an increase of the saturated phospholipid DPPC the number of deformed discoidal SUV augments and that these discoidal SUV tend to aggregate in piles, see figure. Aggregation is proportional to the number of viscumin molecules per vesicle for the same lipid mixture. For 30 mol% DPPC only about one viscumin molecule is needed to induce adherence of two vesicles. Aggregation is reversible as is disappears upon dilution and can also occur without proteins, e.g. for membranes with DSPC/cholesterol = 6:4. It is therefore assumed that aggregation is produced by hydrophobic interaction of the bilayer membrane.

Figure. DPPC/DOPC/cholesterol vesicles containing viscumin.