Vesicle formation of nucleolipids consisting of hydroxy fatty acids and nucleoside monophosphates

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Abstract

Background

A protocell is a dynamic vesicle characterized by a fatty acid bilayer that is subjected to a fast exchange of monomers and micelles between the solution and the vesicle membrane. The basic principles to reach the level that a protocell could multiply by dividing shell (lipid membrane) and core (genomic information) have been formulated and, to a certain extent, experimentally demonstrated. It has been demonstrated that lipid-based membranes containing a (replicating) genome are amenable to growth and division. Small unilamellar vesicles divide after micelle addition. Autocatalytic self-replicating micelles are formed from amphiphiles generated from the alkaline hydrolysis of ethyl caprylate (shell replication). Besides transportation of bioactive molecules, vesicles can be used to compartmentalize reactions. However, most lipids studied in this context have a passive function, i.e. forming the boundaries of the vesicle or helping in membrane transport.

Results

A series of α/β-hydroxy fatty acids and α-amino fatty acids, covalently bound to nucleoside-5'-monophosphates *via* a hydroxyl or amino group on the fatty acid was examined for spontaneous self-assembly in spherical aggregates and their stability towards intramolecular cleavage. Staining the resulting hydrophobic aggregates with BODIPY-dyes followed by fluorescent microscopy; gave several distinct, dilution dependent, images of vesicles varying from small, nanoscale spheres to higher order aggregates and giant, micrometer sized particles. Other observations include rod-like vesicle precursors and, by mixing two complementary nucleolipids, multilamellar constructions. NMR was used to assess the stability of a representative sample of nucleolipids. 1D ^{31}P NMR revealed that β-hydroxy fatty acids containing nucleotides were pH-stable while the α-analogs are acid labile. Degradation products identified by [^{1}H-^{31}P] heteroTOCSY revealed that phosphoesters are cleaved between sugar and phosphate, while phosphoamidates are also cleaved at the lipid-phosphate bond. For the latter compounds, the ratio between both degradation pathways is influenced by the nucleobase moiety. However no oligomerization of nucleotides was observed; nor the formation of cyclic phosphonucleotides, possible intermediates for oligonucleotide synthesis.

Conclusions

The nucleolipids with a deoxyribose sugar moiety form small or large vesicles, rod-like structures, vesicle aggregates, giant vesicles or multilamellar structures. Some of these aggregates can be considered as intermediate forms in vesicle formation or division.

However, we could not observe nucleotide polymerization or cyclic nucleotide function of these nucleolipids, regardless of the sugar moiety that is investigated (deoxyribose, ribose, xylose). To unravel this observation, the chemical stability of the constructs was studied. While the nucleolipids containing β-hydroxy fatty acids are stable as well in base as in acid circumstances, others degraded in acidic conditions. Phosphoramidate nucleolipids

hydrolyzed by P-N as well as P-O bond cleavage where the ratio between both pathways depends on the nucleobase. Diester constructs with an α-hydroxy stearic acid degraded exclusively by hydrolysis of the phosphorus to 5'-O-nucleoside ester.

As the compounds are too stable and harsh conditions would destruct the material itself, more reactive species such as lipid imidazolates of nucleotides (L. Orgel's research) need to be synthesized to further analyze the potential polymerization process.

Keywords

Nucleolipid, vesicles, hydroxy fatty acids, protocell, chemical stability, supramolecular assembly, Intramolecular catalysis, Fluorescence microscopy, BODIPY, NMR stability study