Contribution of betaine lipids to the architecture and interaction of algal membranes

Abstract: Algal cell membranes are mainly constituted of glycerolipids, which can be classified in three groups: (i) phospholipids containing phosphate, mainly synthesized in the endoplasmic reticulum (ER), (ii) galactolipids without phosphate, synthesized in chloroplasts, and (iii) betaine lipids, also without phosphate, but synthesized in the ER. In previous studies based on neutron diffraction, we have shown that the physicochemical properties of galactolipids are very different from those of phospholipids. Here, we intend to determine the physicochemical properties of betaine lipids and whether they are similar to those of phosphatidylcholine lipid, a phospholipid they replace when algae are grown in phosphate deprivation.

Background

Microalgae are considered as a novel feedstock to produce a variety of biomolecules, most prominently triacylglycerol (TAG, containing three fatty acids) and essential ω3 fatty acids (FAs). The exertion of stress conditions, like phosphate (Pi) starvation, is a common route to trigger TAG accumulation in microalgae. A better understanding of why and how microalgae induce the biosynthesis of FAs and TAG under such stress conditions is required to rationalize the development of genetically engineered strains to produce biofuels or high-value products.

Under Pi starvation, phospholipids known to be replaced by betaine lipids (BLs) in extraplastidial membranes in microalgae. BLs, which are glycerolipids with two FAs, are hypothesized to be analogues of the main eukaryote phospholipid phosphatidylcholine (PC) (Figure 1) and they often contain a high proportion of ω3 fatty acids. In higher plants, the synthesis of BLs is lost, driving plants to other strategies to cope with phosphate starvation: they replace their phospholipids by glycolipids and do not accumulate TAG as much as microalgae.

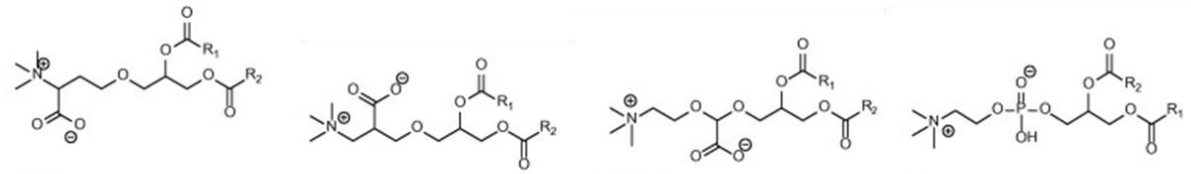

Figure 1: Chemical structure of three types of betaine lipids (DGTS, DGTA, and DGCC) and of a common phosphatidylcholine (PC) lipid. Adapted from [1].

The objective of this beamtime proposal is to evaluate to what extent BLs and PCs share physicochemical properties, and to what extent betaine lipids are suited as surrogates for PC lipids. Key aspects for the understanding of membrane properties in vivo are area per lipid, membrane bending rigidity, and the strength and range of the hydration repulsion.

The headgroups of both PCsand BLs feature hydrophobically capped dipoles (see Figure 2), which are known to repel each other in aqueous environments [2]. From a fundamental viewpoint, BLs thus appear to be promising candidates as phosphorous-free PC substitutes. Closer inspection, however, reveals that the dipolar-hydrophobic headgroups are attached to the central glycerol moiety differently in betaine lipids than in PC lipids (Figure 1). To what extent the branched configuration in BLs alters the physicochemical properties in terms of bending rigidity and hydration repulsion is difficult to predict. Moreover, the three known BLs diacylglyceryltrimethylhomoserine (DGTS), diacylglycerylhydroxy-methyltrimethylalanine (DGTA) and diacylglycerylcarboxyhydroxymethylcholine (DGCC) have a systematically increasing linker length between the two charges of their dipole (Figures 1 and 2), and it can be expected that these differences affect the mutual interaction between the dipolar/hydrophobic motifs.

Results of previous experiments [8-02-585 and 8-02-645]

During Pi starvation, higher plants replace their membrane phospholipid by the glycolipid DGDG. In previous studies based on neutron diffraction, we have shown that the physicochemical properties of galactolipids are very different from those of phospholipids [3, 4]. This concerns their 3-dimensional

architecture [3] as well as the inter-membrane hydration repulsion, which has been shown to result in a much shorter decay length [3, 4].

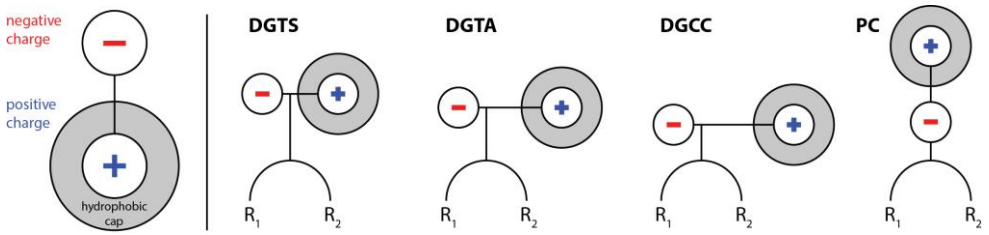

Figure 2: (left) Schematic representation of a dipolar/hydrophobic chemical motif featuring a positive charge shielded with hydrophobic groups (here: methyl). (right) Schematic representation of the dipolar/hydrophobic headgroup structures of the three betaine lipids DGTS, DGTA, and DGCC, and of a phosphatidylcholine (PC) lipid.

The authors have documented expertise with the determination of the key physicochemical aspects of lipid membranes. The basic approach is to study oriented, solid-supported lipid multibilayers formed on silicon wafers for use in a humidity chamber The high-precision new generation of humidity chambers now available on D16 will be used to extract the pressure–distance curves of each lipid by measuring the lamellar spacing [3-5] and the Bragg sheets shape vs. the osmotic pressure in the multilayer stack. From the resulting pressure-distance curves we will extract the decay and the extent of the membrane interactions at short-ranges (below 30Å), and from the Bragg sheet shape we will extract the membrane bending rigidity (from the analysis of the off-specular signal) [5-7].

Estimated time

We propose to investigate the following samples:
- PC 16:0/16:0 and DGTS 16:0/16:0 to see the impact of polar head with saturated fatty acid chains. Both lipids can be purchased at Avanti Polar Lipids.
- PC 22:6/22:6, natural DGTS and natural DGTA that are enriched in the 20:5/20:5 fatty acids to compare the impact of polar head with unsaturated fatty acid chains.

We would like to analyse each of the samples under 8 humidity conditions (1 run) in order to determine the phase (hexagonal II or lamellar), measure their swelling limit in D_2O and determine the corresponding pressure-distance curves as done in [2, 3]. In the previous project we could analyse 4 samples in 5 days, we therefore apply for 6 days on D16 to continue this work. The choice of D_2O contrast is motivated by the need to maximize the weak diffuse scattering signal (along $q_{//}$) and to reduce the incoherent signal from the water. This is essential for a successful analysis of the Bragg sheets shape [5-7].

Why ILL/D16?

Because of the great humidity control setup (NMI3) and because high flux is required for the off-specular analysis (diffuse scattering is much less intense).

References.

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