

POLYUNSATURATED LIPIDS IN MEMBRANE FUSION EVENTS

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It has been reported that lipids with ethanolamine headgroups and polyunsaturated hydrocarbon chains facilitate membrane fusion [1]. Model experiments suggested existence of a link between the rates of liposome fusion and the degree of membrane curvature elastic stress imparted on the lipid matrix by unsaturated lipids with ethanolamine headgroups [2,3]. Recently, a more accurate theoretical description of the free energy of intermediate structures of membrane fusion was reported [4,5]. The likelihood of fusion depends critically on the elastic energy of monolayer bending and the membrane deformations to fill the so-called packing voids of fusion intermediates.

We investigated the influence of the six-fold unsaturated docosahexaenoic acid (22:6n3, DHA) in 18:0_{d35}-22:6 phosphatidylethanolamine (SDPE) on the coefficient of membrane monolayer curvature elasticity and the spontaneous radius of curvature. The influence of polyunsaturation on void energy was evaluated qualitatively by following lamellar-to-inverse hexagonal phase (H_{II}) phase transitions in SDPC/water dispersions as a function of water content and temperature. We altered the membrane curvature of purified SDPE by dehydrating the inverted micelles of the H_{II} phase under thermodynamically well-defined conditions using osmotic stress. The high sensitivity of polyunsaturated PE to oxidation was experimentally challenging. We developed a method for rapidly equilibrating SDPE with PEG/water solutions resulting in less than 1% oxidation. The water content of samples, the lipid phase state as a function of water content and temperature, and the order parameter profiles of the perdeuterated sn-1 chains of SDPE were recorded by NMR. The radius of curvature of inverted micelles was determined by x-ray diffraction. Our results indicate that spontaneous curvature and membrane bending elasticity of polyunsaturated SDPE are close to values determined for 18:1-18:1 phosphatidylethanolamine (DOPE), the "golden standard" for lipid monolayers under curvature elastic stress [6,7]. However, in contrast to DOPE, the polyunsaturated SDPE converted to an H_{II} phase immediately at the gel-to-liquid crystalline phase transition temperature independent of the level of osmotic stress, suggesting that SDPE lowers the energy of packing voids that tend to destabilize non-lamellar lipid phases. This lowering of void energy could be explained by the surprising flexibility of polyunsaturated hydrocarbon chains that allow for large changes of effective chain length without energy penalties,

therefore making it easier to fill the voids. The flexibility of DHA chains in membranes was confirmed by NMR and x-ray diffraction studies. Quantum mechanical- and molecular dynamics simulations related DHA flexibility to low energetic barriers for rotations about vinyl-methylene bonds of the polyunsaturated chain [8].

Neuronal tissue and, in particular, synaptosomal membranes contain high concentrations of DHA. In synaptosomal membranes of mammals typically 30-50 mol% of all fatty acids are DHA [9]. A significant fraction of DHA is located in diacyl phospholipids and plasmalogens with ethanolamine headgroups. Polyunsaturated ethanolamines are major constituents of synaptic vesicles and may facilitate membrane fusion events in the synaptic cleft [1]. The membranes of sperm contain very high concentrations of DHA as well. Several studies have demonstrated a positive correlation between the ability of sperm to fertilize eggs and the DHA content of their membranes [10]. It is possible that factors other than membrane biophysical properties enhance fusion rates in the presence of high DHA concentrations. Nevertheless, the thought that DHA enables fusion events by lowering the free energy of fusion intermediates is appealing.

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