2-hydroxylated fatty acid derivatives promote and stabilize inverted hexagonal phases in phosphatidylethanolamine membranes

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Cellular functions are usually associated with the activity of proteins and nucleic acids. Consequently, human pathologies are classically viewed as malfunctions of these macromolecules. However, recent studies have shown that some lipids can regulate the localization and the activity of membrane proteins (1). 2-hydroxyoleic acid (2-OHOA) also known as Minerval, is a synthetic monounsaturated fatty acid, used as medicinal drugs that act through the regulation of the composition and structure of the cell membrane. The aim of membrane-lipid therapy is to develop drugs that are capable of influencing lipid organization through principles of structure-function, inducing a concomitant modulation of membrane-protein localization and activity. This type of regulation can have a final effect in cell signaling and gene expression, which might serve to reverse the pathological state.

Lipids can organize in different ways when they are dispersed in aqueous solutions depending on the lipid structure, water concentration, pH, ionic strength or system pressure (2,3). This lipid polymorphism is important in cell processes such as membrane fusion and fission, vesicular trafficking, the transport of macromolecules throughout the membrane and the stabilization of protein complexes in the lipid bilayer (4,5). Moreover, the way in which lipids are organized affects the interactions with membrane proteins, thus modulating their activity (6,7).

The aim of this project is to study the effect of different hydroxylated fatty acids (see Figure 1) on the structure properties of the membranes and compare them with the natural non-hydroxylated counterparts. To describe the exact non-lamellar phases that are formed (cubic, hexagonal or micellar) and at which temperature they are formed.

Figure 1. Hydroxylated fatty acids. From left to right: 2-hydroxyoleic (Minerval), 2-hydroxylinoleic, 2-hydroxy-α-linolenic, 2-hydroxy-γ-linoleic, 2-hydroxyarachidonic, 2-hydroxyeicosapentanoic and 2-hydroxydocosahexanoic.

Details of the experiments

The synthetic 2-hydroxylated and natural non-hydroxylated fatty acids were mixed with palmitoyl-oleoyl-phosphatidylethanolamine (POPE) (1:10, mol:mol). The same concentrations were also assayed with the natural, non-hydroxylated fatty acids as controls. The dispersions in a buffer solution were stored at 4 °C and equilibrated at room temperature for several hours prior to the X-ray measurements. The samples were loaded into 1-mm X-ray capillaries and flame sealed before their measurement. Temperature protocols were executed directly on samples mounted on the beam line and the phase conversions taking place in these dispersions will be followed in real time. Small- and wide-angle (SAXS and WAXS) synchrotron radiation X-ray data were collected simultaneously on the Soft Condensed Matter beamline A2 of Hasylab at the storage ring DORIS III of the Deutsches Elektronen Synchroton (DESY) in collaboration with Dr. Sergio Funari from the Hasylab. During data collection, samples were heated from 20ºC to 80ºC at a scan rate of 1ºC/min. Positions of the observed peaks were converted into distances, *d*, after calibration with the standards rat tendon tail and poly-(ethylene therephtalate) for SAXS and WAXS regions, respectively. Interplanar distances, d_{hkl}, were be calculated according to the following equation:

$$s=1/d_{hkl}=(2\sin\theta)/\lambda$$

where s is the scattering vector, 2θ is the scattering angle, λ (0.154 nm) is the X-ray wavelength, and *hkl*s are the Miller indexes of the scattering planes.

Results and their scientific relevance

The transition temperatures between gel (L_β) and fluid (L_α) phases were evaluated by x-ray diffraction techniques. No significant changes were observed when any of the fatty acid and fatty acid derivatives was used. However, both natural and synthetic molecules decreased the lamellar-to-hexagonal transition temperature of POPE membranes. Figure 2 shows the small angle x-ray diffraction spectra of two of the fatty acids, namely oleic acid (OA) and 2-hydroxyoleic acid (Minerval). All the 2-hydroxylated fatty acid derivatives also showed a capacity to stabilize inverted hexagonal phases (data not shown). The so-called Lipid Membrane Therapy is a new strategy to solve a cellular dysfunction through the modification of the lipid membrane properties. Thus, a compound would affect a cellular function by interacting with the plasma membrane and subsequently modifying its structure and downstream signaling. The changes induced in the membrane by a molecule provoke a change in the activity of certain proteins which will lead to the cell cycle arrest, cell differentiation or apoptosis (1). This new strategy may be applied in different fields such as in cancer treatments, cardiovascular pathologies, neurodegenerative processes, obesity, metabolic disorders, inflammation and autoimmune diseases. It has been observed that Minerval (2-hydroxyoleic acid) induces the differentiation and apoptosis of cancer cells and it has been postulated that this occurs due to its capacity to increase the propensity of nonlamellar-prone-lipids (e.g. phophatidylethanolamine) to form nonlamellar structures. The relevance of these hydroxylated fatty acids resides in their low hydrolysis rate in comparison with the natural, non-hydroxylated counterparts and also their low toxicity. The changes in the lamellar-to-nonlamellar transition temperature of POPE membranes observed in the present project definitely show the capacity of the mentioned synthetic hydroxylated fatty acids to promote and stabilize nonlamellar phases. These data will give an idea of the molecules that might possibly be used in therapies against different pathologies by means of the Lipid Membrane Therapy.

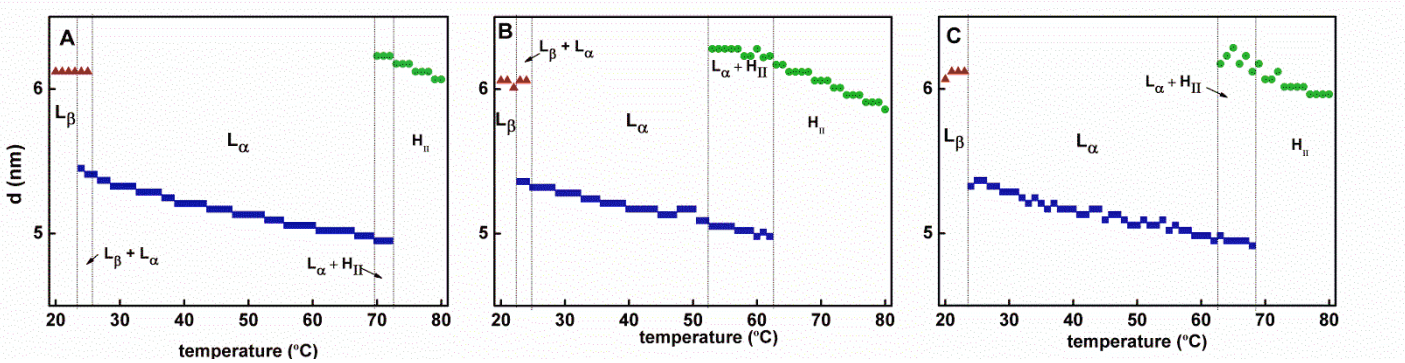

Figure 2. Small-angle X-ray diffraction spectra of **(A)** POPE, **(B)** POPE + 10 mol% OA and **(C)** POPE + 10 mol% 2OHOA. The transition temperature between gel (Lβ) and fluid or liquid-crystilline phases (Lα) was not affected by neither oleic acid nor 2-hydroxyoleic acid. The transition temperature between fluid and inverted hexagonal (H_{II}) phases decreased notably in the presence of the fatty acids.

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