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Possibilities of obtaining swollen lipid cubic phases using various ionogenic surfactants

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Membrane proteins (MP) play a significant role in cell physiology, participating in various processes such as signal transmission, ion transport, and others. In this regard, MP is the target of about 60% of currently used drugs. Obtaining atomic structures of MP is of fundamental importance for understanding the relevant molecular mechanisms and developing new therapeutic agents. One of the two main methods for determining the structure of proteins is X-ray diffraction analysis, which involves crystallization followed by diffraction measurements. Crystallization in lipid cubic phases (LCP) is widely used to obtain MP crystals. However, due to the limited size of water channels in LCP (30-60 Å), this approach is difficult to use for MP with large extracellular domains. Thus, the development of methods for swelling the water channels of lipid cubic phases becomes important.

Previously, a method for swelling the water channels of lipid mesophases through electrostatic repulsion (using ionogenic surfactants) was proposed. The article [1] shows that the addition of diacylphosphatidylglycerols makes it possible to reach water channel diameters up to 240 Å. Successful crystallization of a membrane protein with an extracellular domain size of 75 Å has also been described for such systems [2]. The resolution of the obtained structure turned out to be low (~6 Å), and other examples of successful application of this approach for protein crystallization are unknown.

As an alternative example of an ionic surfactant, we selected the common detergent sodium lauryl sarcosinate (LS). This substance is significantly cheaper than expensive diacylphosphatidylglycerols, which is crucial for conducting large-scale screening tests. It has been shown that when LS is added to monoolein or monopalmitin in a mass ratio of 2 to 6% (w/w), swollen LCPs with water channel diameters of 100-150 Å can be obtained. At higher concentrations of LS, swollen lamellar phases with a lattice parameter of 60-200 Å are observed (lattice parameter increases as the water content increases and decreases as the concentration of LS increases).

In order to approximate the real crystallization conditions, the effect of charge screening under the action of a precipitant was studied. The addition of a precipitant with a low salt content (Tris buffer solution, ionic strength 0.3M) to the swollen LCPs reduces the diameter of the water channel to 60-90 Å. In the case of using a precipitant with a high salt content (1M ammonium sulfate, ionic strength 3M), the diameters of the water channel decrease to 35-60 Å, which no longer exceeds the usual values. On the other hand, statistical analysis of membrane protein structures [3] in relation to crystallization conditions shows that more than 70% of MP structures were obtained using precipitants containing polyethylene glycol (PEG). Thus, using LS and PEG-based precipitants with a low salt content, it is possible to obtain systems potentially suitable for MP crystallization with an extracellular domain size of at least up to 90 Å.

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