INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Lipidic Cubic Phase Microcrystallization and its Application in Serial Crystallography

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Abstract

Every living organism contain a cell membrane which is an important cell structure with a vast variety of different functions such as cell signaling, transportation and energy production. One of the most important functions is to produce energy for the cell to thrive. In humans and other organisms, oxygen is used as the final electron acceptor to drive reactions that pump protons across the membrane to create an electrochemical proton gradient. This electrochemical proton gradient is then harvested for the production of ATP, the currency of life. Even though the membrane proteins that are responsible for the electrochemical proton gradient belong to one of the most well-studied membrane protein families, there are still mechanisms to be revealed. Two of these mechanisms are proton pumping across the membrane and the route of oxygen to the active site of cytochrome *c* oxidase, the final enzyme in the respiratory chain that reduces oxygen to water. By using X-ray serial crystallography these mechanisms can be revealed.

Previous research has found that membrane protein crystallization is greatly improved if the environment of the protein mimics the native environment. Reconstituting the membrane proteins in a lipidic cubic phase, a membrane mimicking lipid bilayer, increases membrane protein stability and crystal packing. As a result, large volumes of good quality microcrystals for X-ray serial crystallography can be obtained. Our studies present a method that allows for better visualization of the crystallization process of microcrystals in lipidic cubic phase. The method was then used to produce microcrystals of a ba_3-type cytochrome *c* oxidase which resulted in the first room temperature structure at 2.3 Å resolution. This work was extended by a procedure to bind CO to the active site of the protein crystals, a first step for revealing the mechanisms of proton pumping and oxygen migration within the enzyme.

The method was also successfully used for other proteins where new crystallization hits were found and optimized. These include sensory rhodopsin II from halophilic archea and reaction centre from *Blastochloris viridis*. For reaction centre, the method was also used in combination with crystal seeding to create a new procedure for microcrystallization. The work presented in this thesis provides a foundation for further development of serial crystallography and for producing microcrystals for time resolved studies.