INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Micro-Crystallization and Time-Resolved Diffraction Studies of a Bacterial Photosynthetic Reaction Center

Petra Båth
Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

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Abstract

Photosynthesis is one of the most important set of chemical reactions in nature as they can convert sunlight into hydrocarbons and chemical energy. The proteins responsible for this are two general types of reaction centers that can be found in a wide variety of living organisms capable of photosynthesis, from bacteria to algae and plants. Despite the range of host cells the reaction centers themselves have fairly conserved structure and function where the absorption of light leads to an electron transfer process and eventually the production of energy. The work in this thesis is focused on the bacterial reaction center from *Blastochloris viridis*, which is an analogue to photosystem II in plants. Our studies aimed to further examine exactly what happens in the protein as light is absorbed.

X-ray crystallography has been an important tool for determining the atomic structure of proteins for several decades. This technique requires that the protein in question is in a crystalline form or else no structural data can be obtained. The development of a new generation of X-ray sources, X-ray free-electron lasers, makes new types of experiments possible but it also requires new ways of preparing crystals for the highly specialized delivery systems used. This thesis presents new ways of preparing membrane protein microcrystals for different types of delivery media. A new way to make crystals in lipidic cubic phase is presented based on setting up crystallization trials in deep-well plates and vials rather than the standard gas-tight syringes. This basic protocol has been developed to add crystal seeds as well as making crystals in an oxygen-free environment. Using this method a 2.3 Å resolution X-ray structure of reaction center was obtained from seeded crystals measuring only 20 μm. For crystals growing in vapour diffusion several techniques of generating crystals are presented depending on how far the screening protocols have been developed; initial crystals can simply be crushed into the size required and more homogeneous microcrystals can be produced by a seeding protocol. These crystals were then used in a time resolved study at an XFEL showing the structural movements of the cofactors in the protein picoseconds after photon absorption.