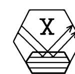

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PROTEINS AND THEIR CRYSTALS

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Abstract
Non-membrane proteins such as the pokeweed antiviral protein from *Phytolacca acinosa* (PAP-S*aci*) and the tryptophan (W)-repressor binding protein *A* (WrbA) and also membrane protein, the five-chlorophyll reaction center of photosystem II from *Pisum sativum*, have been crystallized in our laboratory.

A. Non-membrane proteins

The antiviral protein, PAP-S*aci*

The antiviral protein, PAP-S*aci*, isolated from seeds of the Chinese pokeweed plant, *Phytolacca acinosa*, was crystallized. Interestingly, of two bands seen close to one another in the SDS-PAGE (molecular masses of approximately 29 kDa and 30 kDa), only one, the 30 kDa form, was retrieved from re-dissolved PAP-S*aci* crystals. The diffraction data colorless PAP-S*aci* crystals with dimensions of about 0.5 0.2 0.2 mm were collected using synchrotron radiation at the IMB Jena - University of Hamburg - EMBL Beamline X13, DESY (Hamburg) to a resolution of 1.7 A. The crystal structure of PAP-S*aci* was solved by molecular replacement, using the atomic coordinates of *Phytolacca americana* PAP-I (PDB ID: 1PAF [1]) as a search model [2]. The excellent map quality allowed for an 'X-ray sequencing' approach (with the exception of the Asn/Asp and Gln/Glu ambiguities) as several amino acid exchanges with respect to the sequence of PAP-I from *Ph. americana* were clearly evident. The full sequence of PAP-S*aci* was determinated using MALDI-MS and tandem mass spectrometry techniques. According to the known sequence of PAP-S*aci* the protein structure was rebuild. The refined structure includes 261 residues, one N-acetyl-D-glucosamine monosaccharide (GlcNAc) moiety and 383 water molecules, yielding an R factor of 18.1% and free *R* factor of 22.3%. PAP-S*aci* contains a canonical RIP fold consisting of eight -helices and a six-stranded -sheet. One GlcNAc residue was found to play a critical role in crystal lattice formation, forming a packing interface across a crystallographic two-fold with the identical sequon of an adjacent monomer [3, 4].

Tryptophan (W)-repressor binding protein *A*, WrbA

Sequence analysis and homology modeling identified the tryptophan (W)-repressor binding protein A (WrbA), the polypeptide that specifically binds tryptophan repressor protein (TrpR) [5], as a member of the new class of flavodoxin-like proteins with typical / twisted open- sheet fold. The protein binds flavinemononucleotide (FMN) specifically and weaker than many flavodoxins. The WrbA has no influence on the affinity or form of DNA binding by the TrpR; its physiological role is still unclear [6]. The protein WrbA was overexpressed in *E. coli* and purified. 5-mg/ml WrbA protein has been used for crystallization experiments. Crystallization trials were performed in "Cryschem" plates (Hampton Research, Laguna Niguel, CA, USA) for sitting drops, in capillary tubes and in dialysis button at room temperature. Within 6 weeks, colorless WrbA crystals with dimensions of about 0.3 0.2 0.1 mm were grown in capillaries and in dialysis button from reservoir solution containing 3.0 M ammonium sulfate and 0.1 M Tris pH 7.50. Other crystals of WrbA were grown in sitting drops from the B5 solution of JBScreen Crystal Screening Kit 5 (JenaBioscience GmbH, Jena, Germany). The WrbA protein crystals grown in capillary were measured directly in the capillary at the EMBL Beamline X13, DESY (Hamburg) to a resolution of 2.2 Å. Structure solution of the WrbA apo-protein is in the progress.

B. Membrane proteins

Five-chlorophyll reaction center of photosystem II

Photosystem II (PSII) is a multisubunit pigment-protein complex located in the photosynthetic membranes of green plants, algae and cyanobacteria. It contains many cofactors, which together trap, transfer and modulate the utilization of solar energy to drive the water splitting reaction. These reactions are being responsible for the production of atmospheric oxygen and indirectly for almost all the biomass on the planet [7]. For the central role of PSII in bio-energetics, PSII has been studied using different experimental techniques [8, 9].

The higher plant's photosystem II consists of the reaction center proteins D1 and D2, - and -subunits of cytochrome b-559, two chlorophyll-binding internal antenna proteins CP43 and CP47 and the complex of manganese-stabilizing proteins of 33, 23, and 16 kDa sizes. The five-chlorophyll reaction center of photosystem II was isolated from green pea (*Pisum sativum*) and purified according to Vacha [10]. 15-mg/ml (1.3-mg/ml chlorophyll a) protein has been used for crystallization experiments. JBScreen Crystal Screening Kits (JenaBioscience GmbH, Jena, Germany), MembFac™ crystallization screen for membrane proteins (Hampton Research, Laguna Niguel, CA, USA) and MemStart™ sparce matrix (Molecular Dimensions Limited, Soham, UK) were used as a starting point for screening and optimizing crystallization conditions for the five-chlorophyll reaction center of photosystem II using vapour diffusion methods. Crystallization solutions prepared in-house were used as well. Different types of precipitants and detergents and different pH values were tested experimentally. Optimal values (pH around 7.00 and PEG4-6K as a precipitant) have been already found. N-dodecyl- -D-maltoside (DM) was found as acceptable detergent. It was found that amphiphile 1, 2, 3 -heptanetriol [11] does not promote protein denaturation, small pH changes have no effect on protein crystallization. Crystallization experiments on the PSII membrane proteins are still in the progress.

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