Optimization of Crystallization of the Flavoprotein WrbA by using Additives

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Tryptophan (W)-repressor binding protein A, WrbA, is an *Escherichia coli* stationary-phase protein. Its predicted influence on the binding interaction between DNA and the tryptophan repressor (TrpR) wasn't proved [1] and thus its physiological function remains unclear. According to sequence analysis and homology modelling, WrbA was identified as the founding member of a new protein family, sharing the open, twisted α/β fold typical for flavodoxins [2]. The biochemical and biophysical studies of purified WrbA apoprotein [1] revealed some unique properties of the WrbA family: lower affinity for its cofactor - the flavin mononucleotide (FMN) - and the multimeric character of protein in solution. WrbA protein is apparently the first characterized case in which multimerization is associated directly with the flavodoxin-like domain itself. In all other multimeric flavodoxins the flavodoxin-like domain is fused to a multimerization domain [3]. WrbA protein and its homologs thus present a unique family among the typical flavodoxin-like proteins. Structural analysis may aid in understanding these unique properties and may reveal the physiological role of WrbA in the living organisms. This was a motivation for searching of diffraction-quality crystals.

WrbA apoprotein crystals grown by standard and advanced crystallization techniques consisted of twinned plates. The quality of crystals was successfully improved by using additives and gelling protein solution for crystallization. Crystals suitable for X-ray diffraction measurement were measured at synchrotron DESY, beamline X13 (Hamburg), at cryotemperature. Crystals diffracted to 2.2 Å. Solving of protein structure is in progress.

Limited proteolysis [4] of WrbA apoprotein led to preliminary identification of folded substructures and flexible parts of protein structure.

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