Structural study of a novel flavodoxin-like protein, WrbA: Effect of flavin cofactor on crystallization

J. Wolfova,1,2 J. Brynda,1,3 R. Grandori,4 J. Carey,5 J. Mesters6 and I. Kuta Smatanova1,2

1Institute of Physical Biology, University of South Bohemia Ceske Budejovice, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic
2Institute of Systems Biology and Ecology, Academy of Science of the Czech Republic, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic
3 Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16637 Prague 6, Czech Republic
4 Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy
5Chemistry Department, Princeton University, Washington Rd and William St, NJ-08544-1009 Princeton, USA
6Institut fuer Biochemie, Universitaet zu Luebeck, Ratzeburger Allee 160, D-23538 Luebeck, Germany

The structural characterization of flavoprotein WrbA (tryptophan repressor binding protein A) from Escherichia coli is motivated by the unique properties that distinguish the protein from other flavodoxin-like proteins. The WrbA protein is considered the prototype of a new family of multimeric flavoproteins [1,2], that contain flavin mononucleotide (FMN) as a physiological cofactor. According to the latest observations the members of WrbA family exhibit the quinone oxidoreductase activity, which is proposed to be implicated in protection mechanisms against oxidative stress [2].

The WrbA protein was expressed in E. coli CY15071(λDE3) cells and purified as described previously [1]. Due to the loss of FMN cofactor during purification, the WrbA apoprotein was crystallized first. The multicrystals were obtained by using standard vapor diffusion methods as well as advanced crystallization techniques based on counter diffusion. Optimization of crystallization conditions by application of additives, especially Cd-chloride and Li-citrate, led to getting single crystals suitable for diffraction measurements [3]. The WrbA apoprotein crystals (Fig.1A) diffracted to a resolution of 2.2 Å at synchrotron DESY, beamline X13 (EMBL). The space group was found to be P2 with unit cell parameters a = 58.2, b = 201.7, c = 120.6 Å, α = 90, β = 91, γ = 90°. Unfortunately the crystals couldn’t be used for data collection due to difficulties with finding appropriate cryoprotectant and due to inconvenient unit-cell parameters. Search for better crystallization conditions is in progress.

Further crystallization experiments were done on the reconstituted holoprotein. The complex of WrbA apoprotein with the FMN cofactor was prepared by incubation of pure WrbA apoprotein with equimolar concentration of FMN (Sigma). Crystallization was carried out at 285 K by the sitting-drop vapor-diffusion technique, with droplets containing equal parts of protein and precipitant solution. The flavin cofactor improved the crystallizability of protein significantly, probably through the stabilization of the protein framework. Yellow, well-shaped crystals of WrbA holoprotein grew even from several crystallization conditions. Complete data sets of crystals grown from 2 different conditions were collected at 100 K at synchrotron DESY, beamline X13 (EMBL) with an X-ray wavelength of 0.805 Å using a MAR CCD detector. The programs Denzo and Scalepack [4] were used for processing, indexing and scaling the data. The WrbA holoprotein crystals crystallized in primitive tetragonal system, with 2 molecules per assymmetric unit.
The crystals grown from different conditions had different space groups (P4_3212; P4_1212) and different unit-cell dimensions (a = b = 94.351 Å, c = 175.371 Å; a = b = 61.125 Å, c = 168.375 Å; respectively). The resolutions of 2.60 Å and 1.99 Å, respectively, were reached. Solving of the structures from both data sets is in progress.

Aknowledgements

This work is supported by grant of the Ministry of Education of the CR (KONTAKT ME640) to I.K.S. and by NSF Grant INT-03-09049 to J.C. Grants MSM6007665808 and AVOZ60870520 are also acknowledged.

Some data sets were collected with the kind help of Jeroen Mesters, who cooperates on the project concerning the flavoprotein WrbA.

References