CRYSTALLOGRAPHIC STUDY OF Escherichia coli FLAVOPROTEIN WRBA, A NEW NAD(P)H-DEPENDENT QUINONE OXIDOREDUCTASE

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Abstract

The flavoprotein WrbA from Escherichia coli represents a new family of multimeric flavodoxin-like proteins implicated in cell protection against oxidative stress. The recently revealed NAD(P)H-dependent quinone oxidoreductase activity stimulated determination of crystal structures of E. coli WrbA and the following search for structural features characterizing the new family of reoxidative proteins. Two different crystal forms were obtained for E. coli WrbA in complex with its flavin cofactor (FMN) and the crystal structures were determined to resolutions of 2.6 and 2.0 Å. In order to investigate influence of FMN binding on the protein structure, WrbA apoprotein (without FMN bound) was crystallized and the diffraction data were recorded to a resolution of 1.85 Å.

Introduction

The protein WrbA from Escherichia coli was identified as the founding member of a new family of flavodoxin-like proteins, based on sequence analysis and homology modeling [1]. Following biochemical characterization [2] confirmed the E. coli WrbA to be flavoprotein binding flavin mononucleotide (FMN) as physiological cofactor. According to recent report [3], E. coli WrbA and its homologue in Archaeoglobus fulgidus use FMN as redox-active cofactor for quinone oxidoreductase activity, when transferring electrons from NADH (the preferred electron donor) to the quinone acceptor. Enzymes that assume with NAD(P)H:quinone reductase activity protect cells against oxidative stress via the two-electron reduction of quinones [4-7] implicate the WrbA family in cell oxidative-stress response. Consistent with this role E. coli WrbA was reported to transfer two electrons at a time [8] and to be expressed under the control of the stress response factor rpoS [9].

Experimental work on E. coli WrbA [2] revealed unique features distinguishing the WrbA family from flavodoxins. The major differences consist in multimer formation and weaker FMN binding. These differences embedded in the protein structure imply probably the functional diversity between 1) monomeric flavodoxins being involved in one-electron transport processes and 2) multimeric WrbA proteins executing the two-electron reduction of quinones. Since the crystal structures of WrbA homologues [10] reported to date did not provide the sufficient structural interpretation of the biochemical data, determination of crystal structure of the E. coli WrbA was pursued. Here we report crystals and diffraction analysis achieved to date for E. coli WrbA and its complex with flavin cofactor.

Materials and methods used for crystallization

Recombinant WrbA protein, with molecular weight of about 21 kDa, was expressed in E. coli CY1507(lDE3) cells and purified according to previously reported procedure [2]. Pure WrbA protein was obtained without bound cofactor FMN. The protein complex with FMN was prepared by incubating pure WrbA apoprotein with equimolar concentration of FMN (Sigma), affording 96% occupancy of the WrbA-FMN complex [11].

WrbA protein at 0.25 mM (5 mg/ml) in 20 mM Tris-HCl, pH 7.5, was used for crystallization trials (for WrbA apoprotein, as well as for the WrbA complex with FMN). Screening of different temperatures (ranging from 4 to 25 °C) and precipitants was carried out by the standard sitting-drop vapor-diffusion technique, using the crystallization screening kits (Hampton Research, Sigma) and homemade solutions. For a broad screening of crystallization conditions for WrbA-FMN complex 96-well Intelliplates and the Phoenix microdispenser (Art Robbins Instruments) were used, other crystallization experiments were carried out using 24-well Cryschem plates (Hampton Research).
Results

Crystals and diffraction data of Wrba protein in complex with FMN

First crystals leading to structure solution were obtained for the Wrba-FMN complex. Deep yellow, well-formed tetragonal crystals (Fig. 1A) were obtained at 12°C from several different crystallization conditions tested in the 96-well sitting-drop vapor-diffusion experiments. X-ray diffraction data collected at 100 K at synchrotron DESY, beamline X13 (EMBL) revealed two forms of tetragonal crystals differing in space group and unit-cell parameters. Resolution of 2.6 Å was reached for crystal form with space group P4_2_2_2 and unit-cell dimensions: a = b = 94.35 Å, c = 175.38 Å. The second crystal form diffracting to a resolution of 2.0 Å belongs to space group P4_1_2_1_2 and has unit-cell dimensions: a = b = 61.13 Å, c = 168.38 Å. Based on the data sets obtained both structures were solved. Structure solution of the Wrba-FMN complexes revealed that the protein forms tetramers assembled as dimer of dimers with 222 symmetry. We reported the detailed crystallization and diffraction analysis of the E. coli Wrba in complex with FMN recently [11]. The two structures were refined and their detailed interpretation is under way.

Crystals and diffraction data of Wrba apoprotein

Unlike the Wrba-FMN complex, the pure Wrba apoprotein turned out to be much more difficult to crystallize. The first Wrba apoprotein crystals grew as multocrystalline clusters of thin plates in several crystallization conditions, notably from precipitant containing 2.0 M ammonium sulfate in 0.1 M Tris-HCl, pH 8.5. Optimization of standard vapor-diffusion experiments by using additives (CdCl₂ or lithium citrate) was required to get single crystals (Fig. 1B; [13]). Nevertheless, the apoprotein structure could not be solved due to high crystal mosaicity and anisotropy accompanied by the large unit cell (see diffraction pattern in Fig. 2A).

Apparently better crystallizability of the Wrba-FMN complex indicated the positive effect of FMN on crystallization of Wrba protein. FMN was suggested to promote crystallization through specific binding to protein leading to favorable intermolecular interactions and/or reducing the flexibility of the part of polypeptide chain involved in crystal contacts [11]. In order to investigate the prospective effect of FMN binding on formation of stable crystal contacts, crystal structure of Wrba apoprotein was further pursued. Following crystallization trials were carried out based on conditions yielding crystals of the Wrba-FMN complex. Growth of Wrba apoprotein crystals was observed in one of the tested conditions only, namely with precipitant containing 30% PEG 4000, 0.1 M Tris-HCl, pH 8.5, 0.2 M MgCl₂. However, change of temperature during crystallization was required to obtain good-quality crystals. At the beginning, when nucleation occurs, crystallization experiments were carried out at 12°C. After 1 or 2 weeks the temperature was increased to 25°C, the optimal temperature for crystal growth. Well-formed colorless crystals (Fig. 1C,D) appeared approximately a week later. Diffraction data of the new Wrba apoprotein crystals reported here (Fig. 2B) were collected at 100 K at synchrotron DESY, beamline X12 (EMBL), equipped with a large Mar CCD (225 mm) detector. Crystals diffracted to a resolution of 1.85 Å. Although the Wrba apoprotein crystals and the crystals of the Wrba-FMN complex were obtained from the same precipitant, Wrba apoprotein crystallized with different space group (orthorhombic P2₁2₁2) and unit cell than previously determined for the Wrba-FMN complex. The change of crystal symmetry upon FMN binding supported the role for FMN to influence formation of crystal contacts. Finding of the new crystallization conditions for Wrba apoprotein led to improvement of diffraction parameters of the Wrba apoprotein crystals (Fig. 2) such that the structure could be solved and compared to crystal structure of the Wrba-FMN complex. The suggested effect for FMN in favoring crystal lattice formation through the specific interaction with protein will be investigated.

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Conclusions

Crystal structures of *E. coli* WrbA in complex with FMN were determined to characterize the new protein family. The following structural analysis is focused mainly on unique features of WrbA proteins among flavoproteins, tetramerization and special organization of FMN-binding site, that were proposed to be associated with the oxidoreductase activity via the two-electron transport. New crystallization conditions were found for WrbA apoprotein, resulting in crystal structure affording the investigation of the effect of FMN binding on the structure of WrbA protein.

References


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