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The FNR-like domain of the *Escherichia coli* sulfite reductase flavoprotein component: crystallization and preliminary X-ray analysis

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Abstract

The FNR-like domain of the *Escherichia coli* sulfite reductase flavoprotein subunit was crystallized using the hanging-drop technique, with PEG 4000 as precipitant. The crystals belong to space group $P3_112$ or enantiomorph, with unit-cell parameters $a = b = 171.0$, $c = 152.1$ Å. A solvent content of 75% was determined by a calibrated tetrachloromethane/toluene gradient which corresponds to three monomers per asymmetric unit. A 3 Å resolution native data set was collected at beamline W32 of LURE, Orsay, France.

1. Introduction

Escherichia coli or *Salmonella typhimurium* NADPH-sulfite reductase (SiR) is a multimeric and soluble hemoflavoprotein that participates in the cell sulfate assimilation pathway to produce L-cysteine and organic sulfur derivatives (Kredich, 1971). In its native form SiR is composed of eight α-chains (SiR-FP) and four β-chains (SiR-HP). As in microsomal cytochrome P_{450} reductase and NO synthase, SiR-FP contains two distinct flavin-containing domains (Correll *et al.*, 1993): a N-terminal flavodoxin-like domain that binds one FMN, and a C-terminal ferredoxin-NADP$^+$ reductase-like FAD-containing domain (Ostrowski *et al.*, 1989; Eschenbrenner *et al.*, 1995*a*). Each β-chain contains one [4Fe–4S] cluster and one siroheme (Crane *et al.*, 1995).

The electron flow through SiR has been studied in detail (Siegel *et al.*, 1974): two electrons are transferred from NADPH first to FAD and subsequently to FMN before being transferred one by one to SiR-HP, where the six-electron reduction of sulfite takes place. SiR-FP also has diaphorase activity and is able to transfer electrons either from FMN site to other acceptors, such as cytochrome *c* or ferricyanide, or directly from the FAD to AcPyADP$^+$ or free exogenous flavin (Eschenbrenner *et al.*, 1995*b*).

It has been shown that the two SiR-FP flavinic domains can be separated by proteolytic cleavage between Ser217 and Thr220 (Eschenbrenner *et al.*, 1995*b*). From the properties of the resulting fragments it can be concluded that the N-terminal domain (SiR-FP23) is responsible for the polymerization of the α-chains (Eschenbrenner *et al.*, 1995*b*) and that the C-terminal monomeric domain (SiR-FP43) is able to catalyze efficient NADPH-dependent reductions of free flavins (Eschenbrenner *et al.*, 1995*a*). SiR-FP43 belongs to the ferredoxin NADP$^+$ reductase (FNR) structural family which includes FNR's, phthalate dioxygenase reductase (PDR) (Correll *et al.*, 1992), neutrophil NADPH oxidase and flavin reductases. The crystal structures of PDR and of two FNR's, one from spinach (Karplus *et al.*, 1991) and the other from the cyanobacteria *Anabaena* (Serre *et al.*, 1996) are now available.

Here we report the crystallization and preliminary crystallographic study of SiR-FP43, defined as the FNR-like domain of SiR. Knowledge of its three-dimensional structure will allow us to perform detailed comparisons with the known structures of spinach and *Anabaena* FNR's, and provide new insights into SiR-FP functional properties.

2. Methods and results

Purification of SiR-FP43 issued from limited proteolysis of native SiR-FP was carried out as previously described (Eschenbrenner *et al.*, 1995*a*). All the crystallization experiments were carried out at 293 K using the hanging-drop vapor-diffusion technique (Wlodawer & Hodgson, 1975). Preliminary crystallization trials were conducted using the sparse-matrix sampling method of Jancarik & Kim (1991). Long fine needles, unsuitable for a crystallographic study, were obtained within one week for many conditions containing polyethylene glycol as precipitant. In order to identify the critical factors for the crystallization of the FNR-like fragment, we resorted to the incomplete factorial approach (INFAC) (Carter & Carter, 1979). This procedure allows the sampling of a large number of crystallization conditions using a limited number of experiments. An incomplete factorial experiment using 96 hanging drops was set up to test (i) several pH values (between 5.5 and 7.5), (ii) several PEG concentrations and molecular weights and (iii) various additives (such as salts NaCl, KCl and sodium citrate) and cofactors (FAD, FMN, NADP$^+$). After one week, small usable trigonal crystals appeared in one drop [SiR-FP43 solution (10 mg ml^{-1}), 35%(*w*/*v*) PEG 4000, 10 m*M* sodium citrate, 100 m*M* Tris–HCl pH 7 and 0.1%(*w*/*v*) sodium azide]. In order to optimize the initial conditions, a new series of experiments was then performed using a systematic approach to search in the vicinity of the conditions found in the preliminary experiments. The optimal crystallization droplets were obtained by mixing 2 µl of a SiR-FP43 solution (10 mg ml^{-1}) with 2 µl of reservoir solution containing 23%(*w*/*v*) PEG 4000, 100 m*M* sodium citrate, 100 m*M* Tris–HCl pH 7.5 and 0.1%(*w*/*v*) sodium azide, equilibrated against 1 ml of reservoir. Large crystals (0.9 × 0.4 × 0.4 mm) grew in about two weeks.

Various cryoprotective solvents (glycerol, sucrose, 3-methyl 2,4-pentanediol and polyethyleneglycol 400), and combinations of such solvents were tested in order to collect X-ray data at cryogenic temperatures. Unfortunately, all these attempts resulted in crystals that did not diffract under the cold nitrogen stream. Consequently, the native X-ray intensities were

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Table 1. *Data-collection statistics*

Crystal data			
Space group	$P3_112$ or $P3_212$		
Cell dimensions (Å)	171 × 171 × 152.1, 90 × 90 × 120		
Solvent (%)	75		
V_m (Å^3 Da^{-1})	4.97		
Data collection			
	Crystal 1	Crystal 2	Final set
Resolution (Å)	3.0	3.0	3.0
Number of observations	65790	73844	71209
Number of reflections	34049	37193	46053
R_{sym} (%)	8.2	10.6	
R_{merge} (%)			12.1 (26.6)†
Completeness (%)	69.2	58.7	92.3 (79.8)†
Redundancy			1.9
$I/\sigma(I)$	4.6	4.1	4.5 (1.8)†

† Data in last resolution shell (3.2–3 Å).

collected at room temperature on two different crystals using the W32 beamline and a MAR Research image plate at 293 K at the LURE (Orsay, France) synchrotron source. The 3 Å data sets were processed with *MOSFLM* and scaled with the *CCP*4 crystallographic package (Collaborative Computational Project, Number 4, 1994). The space group was determined according to R_{sym}values and systematic extinctions. Further details concerning the resulting complete data set are given in Table 1. Crystals belong to the $P3_112$ space group or its enantiomorph with $a = b = 171.0$ and $c = 152.1$ Å. They diffract to 2.7 Å resolution.

Introduction of crystals and solvent drops in a calibrated carbon tetrachloromethane/toluene gradient leads to densities of

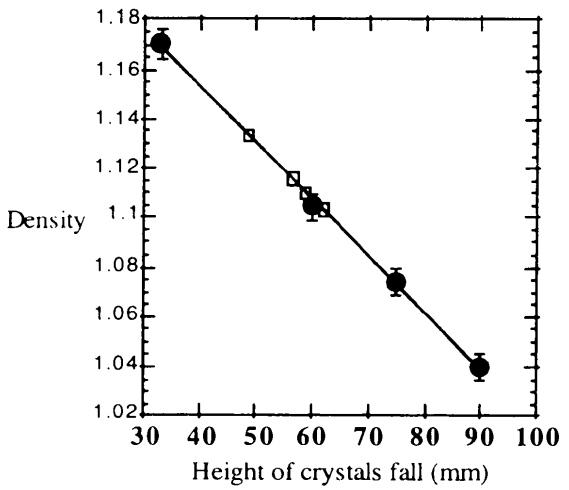

Fig. 1. Crystal density determination by a tetrachloromethane/toluene gradient. Solution drops of known density are depicted by •, whereas crystal positions are shown as □.

$\rho_c = 1.12 \pm 0.015$ and $\rho_s = 1.0618$, respectively (Fig. 1). For a partial specific volume of the protein approximated as $\gamma_p = 0.737$ cm^3 g^{-1}, the number n of protomers per unit cell of volume V (cm^3) can be derived as $NV(\rho_c - \rho_s)/[(M(1 - \gamma p \rho_s)]$, where N is Avogadro's number and M the molar weight of one protomer (Matthews, 1968). According to the experimental densities, the crystals contain 15 ± 3 molecules in the unit cell, corresponding to three molecules in the asymmetric unit and 75% solvent.

Neither the spinach (Karplus *et al.*, 1991) nor the *Anabaena* (Serre *et al.*, 1996) FNR's (either complete or polyalanine models) gave clear solutions with a molecular replacement procedure using the program *AMoRe* (Navaza, 1994). This is probably explained by the low amino-acid sequence homology (~23%).

The orientation of the NADP binding domain with FAD binding domain often differs in this protein family. In consequence, we applied the molecular replacement procedure using each domain as a separate search model. As all these approaches failed, a search for heavy-atom derivatives is under way.

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