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Crystallization and X-ray diffraction studies of the fatty-acid responsive transcription factor FadR from *Escherichia coli*

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FadR, an acylCoA-dependent *Escherichia coli* transcription factor controlling the expression of genes involved in fatty-acid degradation and synthesis, has been crystallized. Crystals of two binary complexes were obtained. The FadR–CoA complex crystallized in space group $C222_1$, with unit-cell parameters a = 61.3, b = 102.0, c = 91.3 Å. The FadR–octanoyl-CoA complex crystallized in space group $P6_522$, with unit-cell parameters $a = b$ = 59.7, c = 296.2 Å. Both crystal forms diffracted to 3.5 Å on a rotating-anode generator. In both crystal forms, the asymmetric unit contains one subunit. The protein is known to be a homodimer; each subunit consists of two domains of unknown fold. For the acyl-CoA-binding domain, a previously undetected sequence homology to PAS domains, in particular the photoactive yellow protein, is reported.

1. Introduction

FadR is an *E. coli* transcription factor controlling the expression of genes important for the biosynthesis and degradation of fatty acids. FadR is a dimeric protein consisting of two identical subunits of 239 residues, with homology to other *E. coli* transcription factors containing helix–turn–helix motifs (DiRusso, 1988). Studies have revealed that FadR represses transcription of genes from the *fad* (fatty-acid degradation) family (DiRusso, 1988; DiRusso *et al.*, 1992) and that this repression is inhibited by the binding of long-chain fatty-acyl–CoA compounds to FadR (DiRusso *et al.*, 1992; Raman & DiRusso, 1995; DiRusso *et al.*, 1998). Characterization of the DNA and acyl-CoA-binding properties together with mutagenesis studies have shown that FadR consists of two domains (Raman & DiRusso, 1995; Raman *et al.*, 1997; DiRusso *et al.*, 1998). The DNA-binding function is contained within the N-terminal domain, where the remote homology to helix–turn–helix transcription factors also resides. The C-terminal domain contains the acyl-CoA-binding function and displays some homology to the CO dehydrogenase/acetyl-CoA synthase from *Clostridium thermoaceticum* (Raman & DiRusso, 1995). Thus, effector binding and subsequent loss of DNA binding occur in two separate domains and presumably

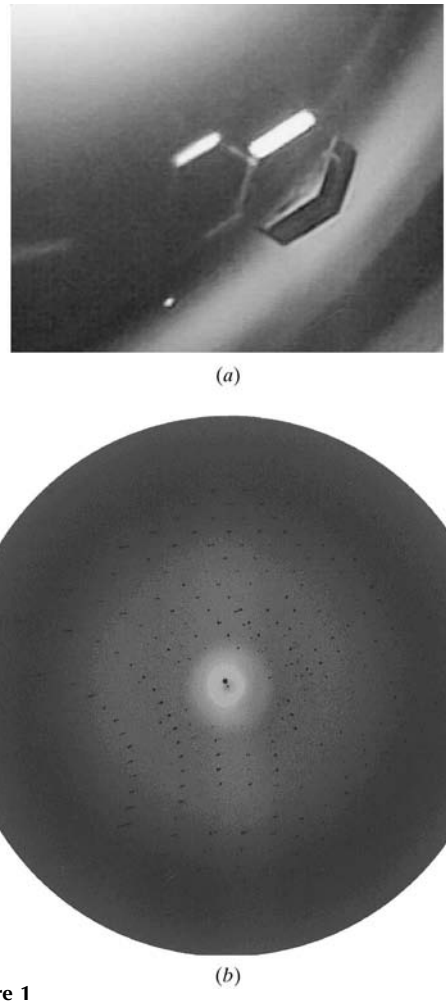

Figure 1
Crystals and X-ray diffraction pattern. (*a*) FadR–octanoyl-CoA crystals. (*b*) Diffraction image from a MAR345 image-plate detector collected from an FadR–octanoyl-CoA crystal; d = 300 mm, $\Delta\varphi = 1.0°$. The maximum resolution at the edge of the detector is 3.0 Å.

Table 1
Space group and data-collection statistics.

Values in parentheses are for the highest resolution shell.

	FadR–CoA	FadR–octanoyl-CoA
Temperature (K)	298	298
Wavelength (Å)	1.54	1.54
Space group	$C222_1$	$P6_522$
Unit-cell parameters (Å)	a = 61.3, b = 102.0, c = 91.3	$a = b$ = 59.7, c = 296.2
Resolution (Å)	15–3.7 (3.83–3.7)	15–3.5 (3.62–3.5)
Mosaicity (°)	0.25	0.32
Observed reflections	25092	14166
Unique reflections	3220 (312)	3288 (403)
Redundancy	8.8	4.3
$I/\sigma(I)$	8.7 (7.4)	9.8 (4.7)
Completeness (%)	100.0 (100.0)	97.9 (97.8)
$R_{\rm merge}$ (%)	15.1 (33.5)	8.7 (24.5)
V_m† (Å^3 Da^{-1})	2.66	2.84

† Calculated with one subunit in the asymmetric unit.

involve communication from one domain to the other in the form of a conformational change.

Here, we describe the crystallization and preliminary X-ray diffraction analysis of FadR complexed to CoA or octanoyl-CoA. Evidence is presented for a previously undetected homology to the family of PAS signal-transduction proteins and the initial results of molecular-replacement trials with models constructed on the basis of this homology are described.

2. Materials and methods

2.1. Purification and crystallization

FadR was overexpressed in *E. coli* and purified as described previously (DiRusso *et al.*, 1998). In short, a vector was constructed containing an N-terminal hexahistidine tag. The protein was purified in a single step from crude *E. coli* extract using Ni-agarose beads. The final solution contained 10% glycerol and 50 m*M* phosphate buffer pH 8.0, with the protein at a concentration of 5 mg ml^{-1}.

Crystallization was performed using the hanging-drop vapour-diffusion method with equal volumes of protein and precipitant solution (2–4 µl). EMBL factorial screens (Zeelen *et al.*, 1994) yielded crystals under several conditions. Further optimization gave crystals of FadR with CoA and FadR with octanoyl-CoA under similar conditions: 11% PEG 8000, 100 m*M* citric acid pH 5.0, 2.5% *t*-butanol at 293 K. Both crystal forms appeared as hexagonal plates or rods (Fig. 1*a*), and also as diamond-shaped plates for the FadR–CoA complex. Crystals grew to their maximum size of 0.3 × 0.3 × 0.1 mm within 2–3 weeks.

2.2. Data collection

Data were collected on a Nonius FR591 rotating-anode generator with XRM-216 focusing mirrors (Prophysics) and a MAR345 image-plate detector. Crystals were mounted in quartz capillaries and exposed at room temperature. Crystals of the FadR–octanoyl-CoA complex were aligned such that the long *c* axis of the unit cell was approximately parallel to the φ-axis in order to minimize spot overlaps and optimize data collection. Images were processed using programs from the *HKL* suite (Otwinowski & Minor, 1997).

3. Results and discussion

The crystals that were obtained of the FadR–CoA and FadR–octanoyl-CoA binary complexes diffracted to better than 3.5 Å (Fig. 1) and it was possible to collect complete data sets from single crystals on a home source (Table 1). The mosaicity was low and initial experiments with synchotron radiation have indicated that data to 2.5 Å can be collected provided suitable cryo-conditions can be found. Both crystal forms are reasonably insensitive to radiation, although evidence for some radiation damage became apparent at the end of the data collection (after 12 h), which might account for the high $R_{\rm merge}$ for the data of the FadR–CoA crystal. The space group was determined from the systematic absences, which indicated the presence of a 2_1 screw axis in the FadR–CoA crystal and a 6_5 (or 6_1) screw axis in the FadR–octanoyl-CoA crystals. The calculated Matthews coefficients (Table 1) indicated one subunit per asymmetric unit for both crystal forms.

Although some weak sequence homologies have been reported between FadR and other proteins (see §1), these do not warrant the construction of a reliable model suitable for molecular-replacement experiments. Searching the PDB (Bernstein *et al.*, 1977) with the FadR sequence, however, revealed a significant homology of the C-terminal (acyl-CoA-binding) domain of FadR to the photoactive yellow protein (PYP). The sequence alignment (Fig. 2) shows that 27 out of 120 residues are identical (23%). PYP is a water-soluble photoreceptor responsible for negative phototaxis in *Ectorhodospira halophila* and is thus involved in a signal-transduction pathway (Meyer, 1985; Sprenger *et al.*, 1993). Recently, it has become apparent that PYP can be considered the first structural scaffold for the family of so-called PAS proteins (Pellequer *et al.*, 1998). PAS domains, first identified in *Drosophila* clock proteins (Nambu *et al.*, 1991), have been found in a wide range of organisms (Zhulin & Taylor, 1997). They have been shown to be involved in transcriptional activation and sensing of signals as part of signal-transduction systems (Zhulin & Taylor, 1997). Given the homology to PYP, the C-terminal domain of FadR may well fall into this family of PAS domains, making it the first crystallized example of a multidomain PAS protein.

Efforts to solve the structure by molecular replacement using the PYP structure as search model have so far proven unsuccessful. A search for suitable heavy-atom derivatives has been initiated aimed at resolving the crystal structure at 2.5 Å resolution.

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                130        140        150        160        170
                 |          |          |          |          |
FADR       VLATANEVADHADAFAELDYNIFRGLAFASGNP-IYG--LILNGMKGLYTR------IGR
PYP        MEHVAFGSEDIENTLAKMDDGQLDGLAFGAIQLDGDGNILQYNAAEGDITGRDPKQVIGK
           .   *    *   . * .* .   **** . .    *  *  *   *  *       **.

                180        190        200        210        220        230
                 |          |          |          |          |          |
FADR       HYFANPEARSLALGFYHKLSALCSEGAHDQVYETVRRYGHESGEIWHRMQKNLPGD--LA
PYP        NFFKDVAPCTDSPEFYGKFKEGVASGNLNTMFEYTFDYQMTPTKVKVHMKKALSGDSYWV
           ..*       . .  ** *     . *    ..*    *       .  .*.* * **
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Figure 2
Sequence alignment of the C-terminal domain of FadR (FADR) with the sequence of the photoactive yellow protein (PYP). The residue numbering corresponds to that of FadR.

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