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Purification, crystallization and preliminary X-ray analysis of the *Enterococcus faecalis* protein EF0377

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The *EF0377* gene of *Enterococcus faecalis* was cloned and overexpressed in *Escherichia coli*. The protein has been purified and crystallized in three forms. Type III crystals belong to space group $P2_1$, with unit-cell parameters a = 72.11, b = 94.97, c = 80.77 Å, β = 111.93°. There are four molecules per asymmetric unit and diffraction is observed to beyond 1.65 Å under cryoconditions (100 K) using synchrotron radiation. An almost complete set of X-ray diffraction data was collected to 1.9 Å from the native crystal.

1. Introduction

Recent advances in genome sequencing, as well as breakthroughs in computer software technology and third-generation synchrotron beamlines for macromolecular crystallography, have allowed structural genomics to expand in the past few years. One ultimate goal of structural genomics projects is to map the entire protein-structure space. It is postulated that this can be accomplished by determining the structures of a large number of unique proteins that show no significant sequence homology to known protein structures and as such are likely to include the majority of new unique protein folds (Vitkup *et al.*, 2001). For many proteins where function cannot be assigned by amino-acid sequence comparisons alone, it is expected that structural genomics will provide important new data. The selection of proteins for structure determination is key to the structural genomics approach (Linial & Yona, 2000). In this work, we applied three criteria in choosing proteins: (i) little or no structural homology (more likely to be a novel fold), (ii) unknown protein function (structure may aid functional assignment) and (iii) origin from a pathogenic bacterium (may lead to a novel drug target). These criteria were met by gene *EF0377*, which encodes a protein of 231 amino acids from *Enterococcus faecalis*. EF0377 shows no significant sequence similarity to any other protein as determined by analysis of the ProtoMap database (Portugaly & Linial, 2000; http://www.protomap.cs.huji.ac.il). EF0377 has been shown by sequence alignment to contain three ankyrin repeats known to function as protein–protein interaction mediators. Many pathogenic bacteria, *e.g. Pseudomonas aeriginosa* and *Escherichia coli* 0157, express similarly conserved hypothetical proteins with ankyrin repeats, suggesting a probable role in virulence.

2. Materials and methods

2.1. Expression and purification

The *EF0377* gene was amplified by PCR and cloned between the *Bam*HI and *Hin*dIII sites of the pQE-10 vector (Qiagen) in frame with an N-terminal His_6 tag. This vector was then transformed into *E. coli* strain BL21 (DE3). Eight 1 l cultures of LB media were each inoculated with 10 ml of an overnight culture and shaken at 200 rev min^{-1} at 310 K until $OD_{600} \simeq 0.5$ was reached. Expression of the His_6-tagged fusion protein was induced with 1 m*M* isopropyl-β-D-thiogalactoside (IPTG) for 3 h before cells were harvested by centrifugation at 5000*g* for 15 min at 277 K, resuspended in 25 m*M* Tris pH 7.5, 150 m*M* NaCl and 4 m*M* imidazole (~10 ml per litre pellet) and lysed by sonication. The fusion protein was purified by affinity chromatography using Ni–NTA Superflow resin (Qiagen) under native conditions according to the manufacturer's guidelines.

2.2. Crystallization

All crystallization trials were carried out by the hanging-drop vapour-diffusion method in 24-well Linbro plates using the sparse-matrix screens Crystal Screen (Jancarik & Kim, 1991) and Crystal Screen II as well as commercially available grid screens from the same company (Hampton Research, Laguna, CA, USA).

The concentration of the freshly prepared protein was determined by UV absorbance using an extinction coefficient of 0.551 M^{-1} cm^{-1} (based on the amino-acid

sequence) and adjusted to 8 mg ml^{-1}. The protein and reservoir solutions were carefully mixed on a siliconized cover slip in equal volumes (4 µl in total). The drop was equilibrated over a reservoir containing 1 ml of the screening solution and was incubated at 293 K. Crystals grew in 2–3 weeks and reached maximum size (0.2 × 0.1 × 0.1 mm) in one month. EF0377 crystals were mounted in a fibre loop (Hampton Research, Laguna, CA, USA; Teng, 1990).

2.3. X-ray data collection

Data were collected on type III crystals at beamline ID13 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. Crystals were removed from the drop, passed through a drop of dried paraffin oil which acted as a cryoprotectant (Riboldi-Tunnicliffe & Hilgenfeld, 1999) and were flash-cooled to 100 K in a stream of nitrogen gas supplied by an Oxford Cryosystems Cryostream. The diffraction patterns were collected on a MAR CCD detector using exposure times of 3–5 s; the crystal was rotated by 1° per frame. Owing to the intensity of the beam, the crystal was translated several times during data collection. Indexing and integration of the diffraction images were performed using the program *MOSFLM* (Leslie, 1992) and merging and scaling of the reflections was performed using the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

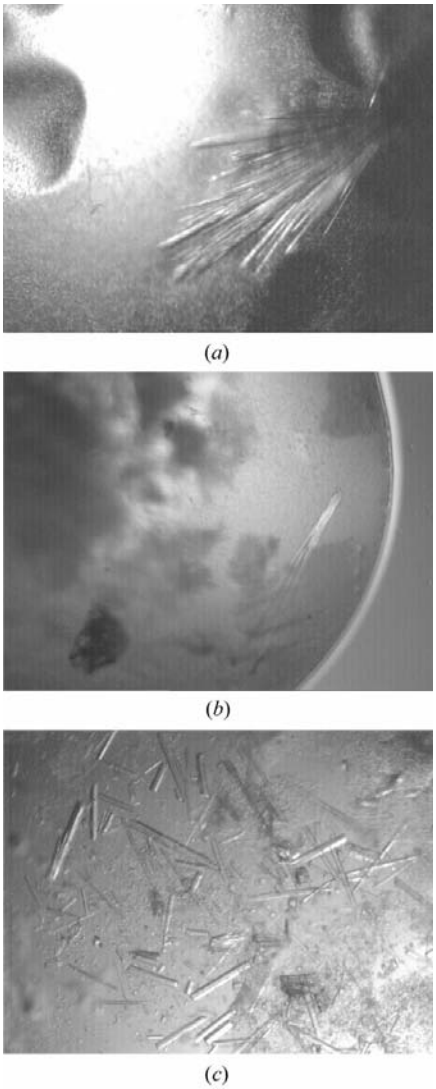

Figure 1
Crystals of EF0377. (*a*) Type I. (*b*) Type II. (*c*) Type III.

3. Results

3.1. Crystallization and preliminary X-ray analysis of EF0377

Crystals were obtained under three different conditions (Fig. 1). Type I crystals grew from 65% MPD and 100 m*M* HEPES pH 7.0 as needles to maximum dimensions of 400 × 70 × 70 µm after 7–10 d. Type II crystals grew from 30%(*w*/*v*) PEG 6K and 100 m*M* Tris pH 8.0 as individual rods in 2–3 weeks to maximum dimensions of 300 × 40 × 40 µm and were suitable for X-ray analysis. Type III crystals were obtained from Crystal Screen (Hampton Research, Laguna, CA, USA) condition No. 22: 30%(*w*/*v*) PEG 4K, 100 m*M* Tris–HCl pH 8.5, 200 m*M* sodium acetate trihydrate. These crystals took one month to reach a maximum size of 200 × 100 × 100 µm.

Initial diffraction experiments (in-house) with type I crystals gave no diffraction. Experiments using type II crystals gave a maximum resolution of 4.0 Å using X-rays from a rotating-anode generator (Nonius FR591 generator operating at 100 kV and 50 mA with 30 min exposures and a MacScience DIP2020 image plate). Type II crystals were not tested at the synchrotron. Using larger type III crystals at a synchrotron source revealed them to be twinned/split, as a number of lattices could be observed on each frame (Fig. 2*a*). However, smaller type III crystals appeared to be single (Fig. 2*b*).

The space group of the EF0377 type III crystals was $P2_1$, with unit-cell parameters $a = 72.10$, $b = 94.97$, $c = 80.77$ Å, $\beta = 111.93°$. The mosaicity was estimated to be 0.4°. Although the crystals were not stable in the strong synchrotron X-ray beam and had to be translated every 20–25 frames, an almost complete data set (220 frames) was collected from a single crystal (Table 1). Data were observed to a maximum resolution of 1.6 Å; however, the data were weak and incomplete and were therefore processed to 1.9 Å.

Table 1
Data-collection and refinement statistics.

Values in brackets refer to the highest resolution shell.

Resolution (Å)	22.7–1.90 (2.02–1.90)
Wavelength (Å)	0.9755
V_M (Å^3 Da^{-1})	2.52
Solvent content (%)	50.70
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 72.11$, $b = 94.97$, $c = 80.77$, $\beta = 111.93$
Total observations	674561
Unique reflections	84147
Average $I/\sigma(I)$	3.9 (2.3)
Redundancy	6.6 (1.9)
Completeness (%)	98.0 (92.6)
R_{sym} (%)	12.7 (31.4)

A single anomalous dispersion (SAD) experiment has since been performed at 1.7 Å to utilize the signal from the S atoms

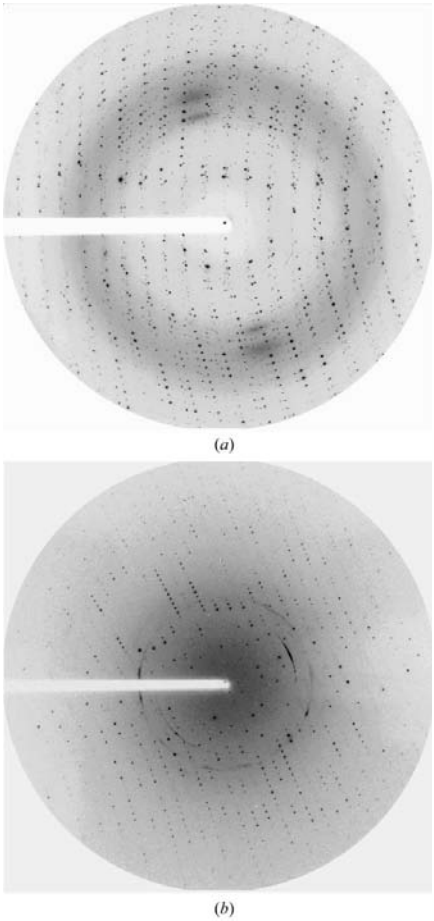

Figure 2
X-ray diffraction images from the EF0377 crystal. (*a*) 1.0° oscillation image taken from a large type III crystal (400 × 50 × 50 µm). Larger crystals showed multiple lattices. This image was taken at the ESRF using a MAR CCD detector. The resolution is 1.6 Å at the edge of the plate. (*b*) Smaller type III crystals (30 × 5 × 5 µm) resulted in a single lattice.

within the protein. Attempts are under way to locate these S atoms and subsequently calculate phases.

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