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Crystallization and preliminary X-ray diffraction analysis of a cytochrome P450 (CYP119) from *Sulfolobus solfataricus*

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CYP119 is a cytochrome P450 with a molecular weight of 43 kDa which has been isolated from the thermophilic archaeon *Sulfolobus solfataricus*. This enzyme is extremely stable to high temperature and high pressure. The first crystallization and preliminary crystallographic study of CYP119 is reported here. Crystals of CYP119 were obtained by the sitting-drop vapour-diffusion method using a precipitant solution containing 20%(*w*/*v*) PEG 4000 and 0.2 *M* sodium thiocyanate at pH 6.4. Using synchrotron radiation, the CYP119 crystal diffracted to 1.84 Å resolution. It belongs to the tetragonal space group $P4_32_12$, with unit-cell parameters $a = b = 86.17$ (0.07), $c = 221.11$ (0.04) Å, in which the numbers in parentheses describe the standard deviations. Assuming two molecules of the CYP119 per asymmetric unit, the calculated molar volume (V_m) is 2.38 Å^3 Da^{-1}. Bijvoet and dispersive anomalous difference Patterson maps show a clear peak corresponding to the haem irons. The complete crystallographically defined structure is currently in progress using MIR (multiple isomorphous replacement) and MAD (multiwavelength anomalous diffraction) techniques.

1. Introduction

CYP119 is a haem-enzyme cytochrome P450 encoded in a gene of the archaebacterium *S. solfataricus* (Wright *et al.*, 1996). *S. solfataricus* is an acidothermophilic archaeon that has an optimum growth temperature of 358 K. Therefore, as is the case for other proteins characterized from *S. solfataricus* such as β-glucosidase and ribonuclease P2, CYP119 exhibits extreme stability toward elevated hydrostatic pressure and elevated temperature (McLean *et al.*, 1998). For instance, measurement of thermally induced unfolding by differential scanning calorimetry showed that the melting temperature of this enzyme (363 K) is 40 K higher than that of other cytochrome P450s such as *Pseudomonas putida* cytochrome P450cam. The optical absorption spectrum is also completely resistant to the formation of inactive P450 (the so-called P420) by hydrostatic pressure up to 2×10^8 Pa. The thermostability and barostability of CYP119 suggest numerous commercial applications arising from the ability of the P450 system to functionalize chemically recalcitrant compounds with high degrees of regiospecifity and stereospecificity. A molecular understanding of the origins of the thermostability and barostability demands high-resolution structural information and hence we have pursued the crystal structure of CYP119.

CYP119 belongs to the P450 superfamily and exhibits a particularly high identity (33%) in primary sequence with cytochrome P450eryF. Crystal structures of six P450s have been determined and reported thus far (Peterson & Graham-Lorence, 1995; Poulos *et al.*, 1995; Park *et al.*, 1997). The basic structural characteristics such as the molecular shapes and topologies of the secondary structures bear good resemblance between the six P450 structures. Differences in physiological function between the P450s have been explained in terms of subtle differences in the structure of the active site around the haem. On the other hand, there has been no discussion of the stability of P450s towards temperature or pressure on the basis of their molecular structure. An important goal is to understand the differences between CYP119 and other P450s that convey overall stability to thermal or pressure perturbations. To assess this problem, homology modeling was attempted (McLean *et al.*, 1998); however, a high-resolution crystal structure of CYP119 is required. In the present study, we report the first crystallization of CYP119 and preliminary results of the crystallographic analysis.

2. Material and methods

A recombinant enzyme of CYP119 was expressed from *Escherichia coli* TB-1 using the expression system constructed previously (McLean *et al.*, 1998). The enzyme was purified according to the method reported previously.

Table 1
Crystal parameters and data reduction of CYP119.

Values in parentheses are for the outer resolution shell; this was 1.94–1.84 Å for final resolution 1.84 Å, 2.64–2.50 Å for final resolution 2.50 Å and 2.77–2.63 Å for final resolution 2.63 Å.

Data set	Native1	MAD data		
Unit-cell parameters (Å)	$a = b = 86.17$ (0.07), $c = 221.11$ (0.04)	$a = b = 86.24$ (0.07), $c = 221.37$ (0.04)		
Wavelength (Å)	0.70	1.6500	1.7401	1.7142
Resolution range (Å)	28.0–1.84	31.6–2.50	34.1–2.63	43.1–2.63
No. of reflections measured	526436	211573	181033	180664
No. of unique reflections	72290	29665	25592	25550
Completeness (%)	99.0 (95.7)	99.5 (00.9)	99.1 (96.7)	99.2 (96.6)
$R_{\rm merge}$† (%)	4.4 (28.9)	5.3 (18.0)	4.8 (14.7)	4.7 (14.7)
Redundancy	7.3 (7.1)	7.1 (7.2)	7.1 (6.9)	7.1 (6.9)
Mean $\langle I/\sigma(I)\rangle$	11.2 (2.6)	11.7 (3.9)	12.8 (4.8)	12.8 (4.8)

† $R_{\rm merge} = \sum\sum_i |I(h) - I(h)_i| / \sum\sum_i I(h)$, where $I(h)$ is the mean intensity after rejections.

The purified protein was homogeneous as judged by SDS–PAGE and isoelectric focusing gel electrophoresis. For crystallization, the enzyme solution with an Rz value of 1.55 or greater in 50 m*M* potassium phosphate buffer pH 7 was concentrated to 41 mg ml^{-1} (∼1 m*M*).

3. Results and discussion

3.1. Crystallization of CYP119

A single crystal of CYP119 in the ferric state was obtained by the vapour-diffusion method using the sitting-drop technique. Crystals were grown at 293 K in 200 m*M* sodium thiocyanate (NaSCN) solution at pH 6.4 using 20%(*w*/*v*) PEG 4000 as a precipitant. The initial droplets contained 1 µl protein solution (41 mg ml^{-1}) and 1 µl precipitant solution and were equilibrated against a reservoir containing 800 µl precipitant solution. CYP119 crystals were obtained after one week at 293 K. Crystals grown under these conditions reached their maximum size within 5 d; their typical dimensions are approximately 0.7 × 0.4 × 0.3 mm (Fig. 1).

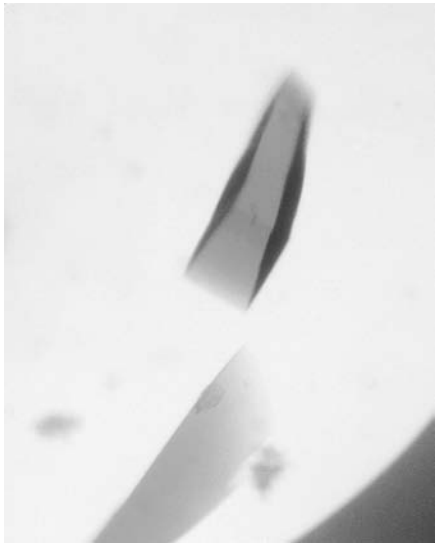

Figure 1
A single crystal of cytochrome P450 (CYP119) grown from 20%(*w*/*v*) PEG 4000, 0.2 *M* sodium thiocyanate pH 5.6. Approximate dimensions of the crystal are 0.5 × 0.3 × 0.1 mm.

3.2. Diffraction data collection and multiwavelength anomalous diffraction (MAD) analysis

High-resolution diffraction data were obtained using a synchrotron-radiation source at the RIKEN beamline 2 (BL44B2) station, SPring-8, Harima, Japan (Adachi *et al.*, 1996). Intensity data were collected with a MAR CCD detector mounted on a Huber alignment table. The cryogenic head of the Rigaku Cryosystems cryostream was mounted close to the goniometer head. The crystal was mounted with the *c* axis as the axis of rotation; its distance from the CCD detector was 200 mm. Measurements were performed at 100 K and the wavelength of the incident X-rays was 0.7 Å. Diffraction data were integrated and scaled with the programs *MOSFLM* (Leslie, 1994) and *SCALA* (Collaborative Computational Project, Number 4, 1994).

The crystal was found to belong to the tetragonal space group $P4_32_12$, with unit-cell parameters $a = b = 86.17$ (0.07), $c = 221.11$ (0.04) Å. Assuming two molecules of CYP119 per asymmetric unit, the crystal volume per protein mass (V_m) is calculated to be 2.36 Å^3 Da^{-1}, which is within the range of values observed in protein crystals (Matthews, 1968). This V_m value corresponds to a solvent content of approximately 48%. The reflection data have an $R_{\rm merge}$ value of 4.4% for 72 290 independent reflections derived from 526 436 total observations. The completeness of the data set is 99.0% at 28.0–1.84 Å. The data-collection statistics are summarized in Table 1.

Since CYP119 contains one haem iron per protein molecule, we collected additional diffraction data using the multiwavelength anomalous diffraction (MAD) method in order to obtain initial phase information for structural determination. Prior to collection of the diffraction data, the X-ray fluorescence spectrum of the CYP119 crystal was measured in order to determine the absorption edge of the haem iron using a Si-Pin photodiode X-ray detector (Amptec Inc. XR-00CR). Three data sets for MAD calculation were collected, with wavelengths of 1.7401 (peak), 1.7142 (edge) and 1.6500 Å (remote), using one crystal. No serious radiation damage to the crystal was detected during the data collection at the four different wavelengths. The distance between the crystal and detector was set to 150 mm. The data were processed as described above and the results are summarized in Table 1.

3.3. Position of the haem Fe atom from anomalous dispersion

Fig. 2 shows the Harker section of the Bijvoet anomalous difference Patterson map using data collected at 1.7401 Å. The maps show clear haem iron–iron self-vectors on the Harker section, with more than six times the root-mean-square deviation of the map. The haem Fe positions were refined to (0.058, 0.119, 0.109) and (0.454, 0.864, 0.046) by vector-space refinement (Collaborative Computational Project, Number 4, 1994). We attempted to solve the structure using MAD data, but the anomalous signals were

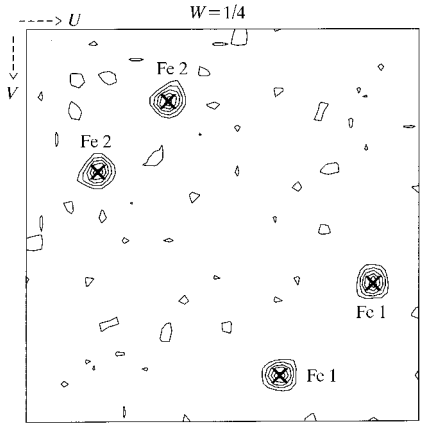

Figure 2
Bijvoet difference Patterson maps using the data collected at 1.74 Å. Diffraction data between 10 and 3 Å resolution were used for calculation. Cross symbols correspond to haem iron–iron self-vectors. The positions of the haem irons refined to (0.058, 0.119, 0.109) and (0.454, 0.864, 0.046) by vector-space refinement.

not large enough for complete phase determination to obtain an interpretable electron-density map. We also attempted structural determination of CYP119 by the molecular-replacement technique using the P450 structures available so far, but this also failed. Preparation of heavy-atom derivatives for phase determinations are in progress and the fine structural analysis is ongoing.

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