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Crystallization and preliminary X-ray analysis of formate oxidase, an enzyme of the glucose–methanol–choline oxidoreductase family

Formate oxidase (FOD), which catalyzes the oxidation of formate to yield carbon dioxide and hydrogen peroxide, belongs to the glucose–methanol–choline oxidoreductase (GMCO) family. FOD from *Aspergillus oryzae* RIB40, which has a modified FAD as a cofactor, was crystallized at 293 K by the hanging-drop vapour-diffusion method. The crystal was orthorhombic and belonged to space group $C222_1$. Diffraction data were collected from a single crystal to 2.4 Å resolution.

1. Introduction

The enzymes of the glucose–methanol–choline oxidoreductase (GMCO) family exhibit little sequence similarity in their catalytic sites. However, either a His–Asn or a His–His pair is conserved in the catalytic site of all enzymes belonging to the GMCO family. The crystal structures of choline oxidase from *Arthrobacter globiformis* (Quaye *et al.*, 2008), glucose oxidase from *Aspergillus niger* (Hecht *et al.*, 1993), cholesterol oxidase from *Brevibacterium sterolicum* (Vrielink *et al.*, 1991; Lario *et al.*, 2003), the flavin domain of cellobiose dehydrogenase from *Phanerochaete chrysosporium* (Hallberg *et al.*, 2002) and pyranose 2-oxidase from *Peniophora* sp. (Bannwarth *et al.*, 2004) show that they all share a highly conserved catalytic site.

Enzymes that are capable of oxidizing formate to carbon dioxide are classified as formate dehydrogenases and formate oxidases (FODs). Formate dehydrogenases from various microorganisms and higher plants have been well characterized (Boyington *et al.*, 1997; Moura *et al.*, 2004; Tishkov & Popov, 2006; Schirwitz *et al.*, 2007; Shabalin *et al.*, 2009). In contrast, there are only a few reports on FODs. FOD from *Aspergillus nomius* IRI013 was the first FOD to be purified and characterized (Kondo *et al.*, 2002). Recently, we purified and characterized FOD from *Debaryomyces vanrijae* MH201 and subsequently cloned and expressed three FOD genes from *D. vanrijae* MH201 in *Escherichia coli* (Uchida *et al.*, 2007; Maeda *et al.*, 2008). An FOD gene from *A. oryzae* RIB40 was also expressed in *E. coli* (Maeda *et al.*, 2009). The native and recombinant enzymes are all dimeric proteins with subunit molecular masses of about 64 kDa. In the amino-acid sequences deduced from their nucleotide sequences, three FAD-binding site motifs and a His residue are conserved throughout the GMCO family (Dym & Eisenberg, 2001; Fan *et al.*, 2004; Ghanem & Gadda, 2005).

The amino-acid sequence deduced from the nucleotide sequence of the FOD gene from *A. oryzae* RIB40 shows 28.9 and 25.7% similarity to those of choline oxidase from *Arthrobacter globiformis* and glucose oxidase from *Aspergillus niger*, respectively (Fig. 1). A His511–Arg554 pair might exist in the catalytic site of the recombinant *A. oryzae* FOD (FOD_{AO}), instead of the His–Asn and His–His pairs that exist in the catalytic sites of the choline and glucose oxidases, respectively. Also, His508 and Arg551, which correspond to His511 and Arg554, respectively, are found in FOD from *D. vanrijae* MH201. The choline and glucose oxidases contain FAD that is covalently bound to His97 via the 8α-position of the isoalloxazine

Figure 3
X-ray diffraction pattern from a crystal of FOD. The ring indicates a resolution of 2.4 Å.

Table 1
Data-collection and processing statistics.

Beamline	SPRING-8 BL-26B1
Wavelength (Å)	1.00
Detector	R-Axis V
Crystal-to-detector distance (mm)	450
Rotation range per image (°)	0.80
Total rotation range (°)	106
Exposure time per image (s)	15.0
Size of crystal used for data collection (mm)	0.3 × 0.3 × 0.1
Resolution range (Å)	50–2.40 (2.49–2.40)
Space group	C222 ₁
Unit-cell parameters (Å)	<i>a</i> = 155.36, <i>b</i> = 159.97, <i>c</i> = 184.66
Mosaicity (°)	0.578
Total No. of measured intensities	357244 (34120)
No. of independent reflections	86521 (8530)
Mean <i>I</i> σ(<i>I</i>)	13.5 (3.7)
Multiplicity	4.1 (4.0)
Completeness (%)	97.5 (97.5)
<i>R</i> _{merge} † (%)	6.7 (50.3)
Overall <i>B</i> factor from Wilson plot (Å ²)	46.9
No. of molecules per asymmetric unit	3
Matthews coefficient (Å ³ Da ^{−1})	2.92
Solvent content (%)	57.2

† $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ is the intensity of the *i*th measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the mean value of *I(hkl)* for all *i* measurements.

synchrotron radiation of wavelength 1.00 Å at the BL-26B1 station of SPRING-8 (Hyogo, Japan; Ueno *et al.*, 2006). The collected data were processed with *HKL-2000* (Otwinowski & Minor, 1997).

3. Results and discussion

A diamond-shaped crystal of FOD_{AO} appeared in two weeks (Fig. 2). The crystal yielded good diffraction images as shown in Fig. 3. The

crystal parameters and diffraction data statistics are summarized in Table 1. The space group of the crystal was determined to be C222₁, with unit-cell parameters *a* = 155.36, *b* = 156.97, *c* = 184.66 Å. 86 521 independent reflections were obtained from the total of 357 244 measured reflections, with an *R*_{merge} value of 6.7%. The data set was 97.5% complete at the resolution limit of 2.4 Å. The crystal contained three subunits of the enzyme in the asymmetric unit. The *V*_M value (the crystal volume per unit protein molecular weight; Matthews, 1968) was determined to be 2.92 Å³ Da^{−1} and the solvent content was 57.2% assuming the presence of three molecules of the subunit in the asymmetric unit. The *V*_M value and solvent content lie within the range usually found for protein crystals.

All FODs isolated to date are dimeric proteins. The crystal of FOD_{AO} used in this report contains three subunits in the asymmetric unit. In order to obtain information about its catalytic site and modified FAD, structure determination by molecular replacement using choline oxidase (PDB code 2jby; Quaye *et al.*, 2008) and glucose oxidase (PDB code 1cf3; Wohlfahrt *et al.*, 1999) is now in progress.

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