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Crystallization and preliminary X-ray crystallographic analysis of malonamidase E2, an amidase signature family member

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Malonamidase E2 from *Bradyrhizobium japonicum* catalyzes the hydrolysis of malonamate. The enzyme belongs to an amidase signature family which has a highly conserved serine- and glycine-rich sequence over a stretch of ~45 amino acids. More than 100 known or predicted members belonging to this family, whose biological functions vary widely, can be identified in sequence databases. Although urgently needed, no three-dimensional structure of any protein of this family is yet available. The crystallization of malonamidase E2 was undertaken as a first step toward the goal of providing information on the canonical structure of the amidase signature family. The enzyme was crystallized using the hanging-drop vapour-diffusion method at 277 K under two different conditions. One crystal form, which is easier to work with than the other form, belongs to the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 104.29$, $b = 95.58$, $c = 74.90$ Å. The unit cell is likely to contain two molecules of MAE2, with a crystal volume per protein mass (V_M) of 2.045 Å^3 Da^{-1} and solvent content of about 39.9% by volume. A native data set to 1.8 Å resolution was obtained from a flash-cooled crystal using synchrotron radiation.

1. Introduction

The amidase signature (AS) family is characterized by a conserved signature sequence which spans approximately 130 amino acids in length and is termed the 'amidase signature sequence'. The C-terminal ~45 amino acids of the signature sequence are more conserved than those in the N-terminal part and this C-terminal part is rich in glycine and serine residues (Patricelli & Cravatt, 2000). Proteins containing the AS sequence are ubiquitously found in nature, ranging from archaea to mammals (Boshoff & Mizrahi, 1998; Cravatt *et al.*, 1996; Ettinger & DeLuca, 1995; Kobayashi *et al.*, 1993; Sako *et al.*, 1996). Although the common biochemical nature of these proteins is the hydrolysis of the amide bond ($CO-NH_2$), the substrate specificities and biological functions of these enzymes vary widely; for example, the hydrolysis of acetamide in fungi (Hynes, 1975) and the degradation of fatty-acid amides in mammals (Cravatt *et al.*, 1996). Site-directed mutagenesis studies provided evidence that AS enzymes are Ser-Lys catalytic dyad hydrolases (Patricelli & Cravatt, 2000), in contrast to most serine proteases, lipases and esterases which contain the characteristic Ser-His-Asp catalytic triad. Although rare, other Ser-Lys dyad hydrolases which do not belong to the AS family have been found, including β-lactamase (Strynadka *et al.*, 1992), a DNA polymerase V accessory protein UmuD (Peat *et al.*, 1996) and LexA repressor (Luo *et al.*, 2001), all of which are from *Escherichia coli* and cleave the peptide bond.

Three malonamidases (MAs), MAE1a, MAE1b and MAE2 from *B. japonicum*, have previously been purified and characterized (Kim & Kang, 1994). These enzymes, first found in *B. japonicum* bacteroids and the plant cytosol of soybean nodules, have been proposed to be involved in the transport of fixed nitrogen from bacteroids to the plant cell in symbiotic nitrogen metabolism (Kim & Chae, 1990). Among the three enzymes, MAE2 is highly specific for the hydrosylaminolysis and hydrolysis of malonamate. MAE2 belongs to the AS family, exhibiting a well conserved AS sequence (Koo *et al.*, 2000). A series of site-directed mutagenesis studies and kinetic experiments showed that Ser155 is essential for catalytic activity and that Gln151 and Lys169 are also important for catalysis (Koo *et al.*, 2000). Ser155 is an invariant residue and Lys169 is strictly conserved as lysine or arginine in the AS family. Whether the catalytic importance of Gln151, which is conserved as aspartic acid in most of the AS family, is specific for MAE2 is not clear at this point.

The functional diversity of the AS family underscores the importance of structural information on this family. The lack of a three-dimensional structure of any member of this

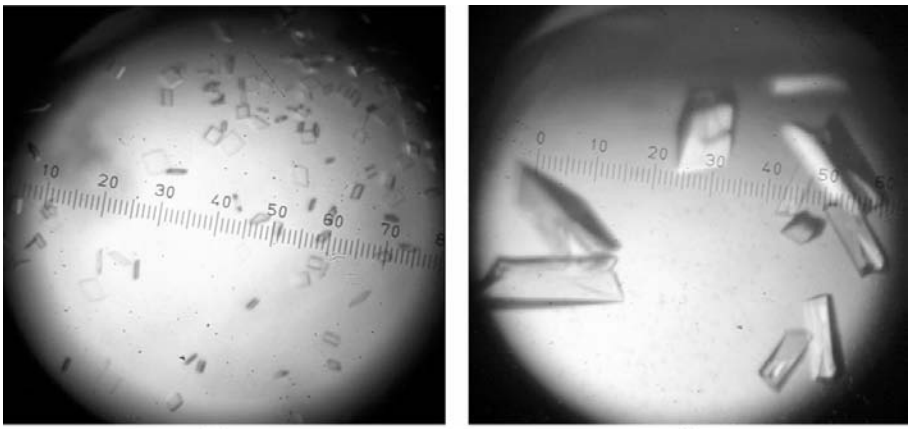

Figure 1
Crystals of MAE2. (*a*) Slow-growing crystals; (*b*) fast-growing crystals. Eight divisions on the ruler indicate 0.1 mm.

Table 1
Crystal information and data-collection statistics of slow-growing (native I) and fast-growing crystals (native II) of MAE2.

Values in parentheses are for the highest resolution shell.

Source	Native I 6B, PAL†	Native II 6B, PAL
Wavelength (Å)	1.12713	1.12714
Space group	$P2_1$	$P2_12_12$
Unit-cell parameters (Å, °)	a = 62.09, b = 79.67, c = 82.88, β = 103.37	a = 104.29, b = 95.58, c = 74.90
Resolution range (Å)	30–2.2	30–1.8
Completeness (>1σ) (%)	90.3 (78.0)	97.4 (93.4)
R_{sym}‡ (%)	9.5 (24.5)	3.7 (11.3)
$I/\sigma(I)$	12.17 (3.44)	20.76 (6.81)

† Beamline 6B at Pohang Accelerator Laboratory, Pohang, Korea. ‡ $R_{sym} = \sum|I_{obs} - I_{avg}|/I_{obs}$, where I_{obs} is the observed intensity of an individual reflection and I_{avg} is the average over symmetry equivalents.

family prompted us to initiate the structural study of MAE2. In this report, we describe the preliminary X-ray crystallographic analyses of MAE2.

2. Protein purification

In the early stage of this study, we used MAE2 with a His_6 tag at the N-terminus for crystallization trials without success, mainly owing to a strong tendency towards precipitation in the crystallization of the protein sample. Subsequently, we resorted to MAE2 without a tag, which was expressed in *E. coli* AD494 (DE3) (Novagen) and purified by employing a series of column purifications as described in Koo *et al.* (2000). Typically, ~16 mg of the enzyme was obtained from 4 l of cell culture. This wild-type enzyme was concentrated to 10 mg ml^{-1} in 20 m*M* Tris–HCl buffer pH 7.4.

3. Crystallization and X-ray crystallographic study

Crystals of MAE2 were obtained by the hanging-drop vapour-diffusion method using 24-well plates at 277 K. Two crystallization conditions were found in the screening process using Crystal Screens I and II (Hampton Research) and Wizard I and II (Emerald BioStructures). 2 µl droplets of the final purified protein solution were mixed with an equal volume of precipitant solution and the mixture was equilibrated on a cover slip against 1 ml of the precipitant solution. In one condition (18% polyethylene glycol 8000, 0.1 *M* sodium cacodylate pH 6.5, 0.2 *M* zinc acetate) crystals of MAE2 (Fig. 1*a*) appeared in three months and grew very slowly over six months. In the other condition (20% polyethylene glycol 1000, 100 m*M* Tris–HCl pH 7.0), crystals of MAE2 (Fig. 1*b*) appeared in 2 d and grew over 10 d to full size. The final size of the fast-growing crystals is much larger than that of the slow-growing crystals. Diffraction data for each crystal form were collected from a flash-cooled crystal using synchrotron radiation. Before data collection, the crystals were briefly immersed in each of the precipitant solutions containing 10–15% glycerol and cooled to 100 K using a cryocooling system (Oxford Cryosystems). Diffraction data (Table 1) were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The slow-growing crystals belonged to space group $P2_1$, while the fast-growing crystals belonged to space group $P2_12_12$. We calculated that the slow-growing crystals contain two molecules of MAE2 (calculated molecular mass 45 637.19) in the asymmetric unit, corresponding to a crystal volume per unit molecular weight (V_M) of 2.19 Å^3 Da^{-1} and a solvent content of 43.7% (Matthews, 1968) when one unit cell was assumed to contain four protomers. The fast-growing crystals are likely to contain two molecules of MAE2 in the asymmetric unit, corresponding to a V_M of 2.04 Å^3 Da^{-1} and a solvent content of 39.9% when one unit cell was assumed to contain eight protomers. A search for suitable heavy-atom derivative crystals is in progress using the fast-growing MAE2 crystals.

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