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Crystallization and preliminary X-ray analysis of carboxypeptidase 1 from *Thermus thermophilus*

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Carboxypeptidase 1 from the thermophilic eubacterium *Thermus thermophilus* (*Tth*CP1, 58 kDa), a member of the M32 family of metallocarboxypeptidases, was crystallized by the sitting-drop vapour-diffusion method using PEG 8000 as the precipitant. The crystals diffracted X-rays to beyond 2.6 Å resolution using a synchrotron-radiation source. The crystals belonged to the orthorhombic space group $C222_1$, with unit-cell parameters a = 171.0, b = 231.6, c = 124.9 Å. The crystal contains three molecules in an asymmetric unit (V_M = 2.11 Å^3 Da^{-1}) and has a solvent content of 61.5%.

1. Introduction

Carboxypeptidases hydrolyse peptide bonds from the C-terminus of peptides and proteins. Metallocarboxypeptidases (EC 3.4.17), which comprise the largest class of carboxypeptidases, are ubiquitous in nature and typically have a single Zn^{2+} bound to the active site. Thermostable carboxypeptidases (EC 3.4.17.19), which are most active around 353–373 K, are a subclass of metallocarboxypeptidases which are distributed in bacteria and archaea and release a C-terminal amino acid with broad substrate specificity, with the exception of Pro (Lee *et al.*, 1992; Cheng *et al.*, 1999). Their thermostability and broad substrate specificity are suitable for use in C-terminal ladder sequencing of peptides and proteins (Cheng *et al.*, 1999). Instead of the His-Xaa-Xaa-Glu-His motif that is characteristic of classical metallocarboxypeptidases (carboxypeptidases A, B and T), thermostable carboxypeptidases contain a His-Glu-Xaa-Xaa-His motif that coordinates a divalent cation such as Zn^{2+} or Co^{2+} (Lee *et al.*, 1992, 1994, 1996; Cheng *et al.*, 1999) as in other families of metalloproteases (thermolysin and deformylase) and thus are classified as the new peptidase family M32 (Rawlings *et al.*, 2004). Biochemical studies showed that bacterial and archaeal thermostable carboxypeptidases vary in aggregation state and metal-ion dependency. For instance, the carboxypeptidase from the thermophilic bacterium *Thermus aquaticus* YT-1 (CPase *Taq* or *Taq*CP) exists as a monomer (56 kDa) in solution and is activated by both Co^{2+} and Zn^{2+} (Lee *et al.*, 1992, 1994, 1996); in contrast, the carboxypeptidase from the hyperthermophilic archaeon *Pyrococcus furiosus* (*Pfu*CP) exists as a homodimer (2 × 59 kDa) in solution and is activated by Co^{2+} but not by Zn^{2+} (Cheng *et al.*, 1999). Although the crystal structure of an archaeal enzyme, *Pfu*CP, has been solved (Arndt *et al.*, 2002), no crystallization or crystal structure determination has been reported for bacterial enzymes belonging to this family. Here, we describe the crystallization of the carboxypeptidase 1 from the thermophilic bacterium *T. thermophilus* HB8 (*Tth*CP1, 58 kDa), which is 86% identical to *Taq*CP and 35% identical to *Pfu*CP in amino-acid sequence.

2. Methods and results

2.1. Protein purification and crystallization

Recombinant *Tth*CP1 was overexpressed in *Escherichia coli* using the pET system (Novagen). *Tth*CP1 was purified from the *E. coli* lysate by the following five steps of column chromatography: Resource ISO 6 ml, Resource Q 6 ml, Hydroxyapatite CHT10, Resource ISO 6 ml and HiLoad 16/60 Superdex 200 pg (Amersham Biosciences and Bio-Rad). Crystallization trials were performed by the sitting-drop vapour-diffusion method using the crystallization screening kits Crystal Screens 1 and 2 (Hampton Research) and Wizard I and II (Emerald Biostructures).

Crystals appeared in the presence of polyethylene glycol (PEG) as the precipitant. After refinement of the crystallization conditions, crystals suitable for X-ray analysis were obtained in two weeks by mixing 1.0 µl protein solution (10 mg ml^{-1} in 20 m*M* Tris–HCl pH 8.0, 0.15 *M* NaCl, 1 m*M* DTT) and 1.0 µl reservoir solution consisting of 10%(*w*/*v*) PEG 8000, 100 m*M* Tris–HCl buffer pH 7.0 and 0.2 *M* $MgCl_2$. Drops were equilibrated against 300 µl reservoir solution at 278 K. Fig. 1 shows typical crystals (0.1 × 0.1 × 0.35 mm).

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Figure 1
Single crystals of *Tth*CP1 grown at 278 K using PEG 8000 as the precipitant.

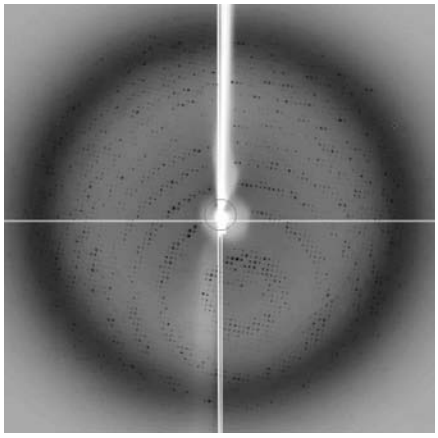

Figure 2
A diffraction image (1° oscillation) from a *Tth*CP1 crystal. The edge of the detector corresponds to a resolution of 2.6 Å. The data were collected at NW-12, PF-AR.

2.2. X-ray data collection and processing

The crystal of *Tth*CP1 was picked up in a nylon loop (Hampton Research), transferred to a cryoprotectant solution containing 30%(*v*/*v*) glycerol, 7%(*w*/*v*) PEG 8000 and 70 m*M* Tris–HCl buffer pH 7.0, 0.14 *M* $MgCl_2$ and then mounted for flash-cooling at 100 K using a Rigaku cryostat. Diffraction data were collected using an ADSC Quantum 210 detector system at beamline PF-AR NW12 at the Photon Factory (Tsukuba, Japan). The wavelength was set to 1.000 Å and the distance between the crystal and the detector was 200 mm. The crystals diffracted to 2.6 Å resolution (Fig. 2). The diffraction data were indexed and scaled with *HKL*2000 (Otwinowski & Minor, 1997). The crystals belonged to space group $C222_1$, with unit-cell parameters $a = 171.0$, $b = 231.6$, $c = 124.9$ Å. The crystals contain three molecules per asymmetric unit, according to the Matthews coefficient ($V_M = 2.11$ Å^3 Da^{-1}; Matthews, 1968). The data statistics are given in Table 1.

Table 1
Crystal parameters of *Tth*CP1.

Values in parentheses are for the highest resolution shell.

X-ray source	PF-AR NW12
Wavelength (Å)	1.000
Unit-cell parameters (Å)	$a = 171.0$, $b = 231.6$, $c = 124.9$
Resolution range (Å)	41.2–2.60 (2.74–2.60)
Observed reflections	276748
Unique reflections	75787
Data completeness (%)	99.4 (98.1)
Redundancy	3.7 (3.2)
$R_{\rm merge}$†	0.087 (0.357)
$\langle I\rangle/\langle\sigma(I)\rangle$	6.9 (2.1)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

X-ray absorption fluorescence spectroscopy (XAFS) experiments at beamline BL38B1 at SPring-8 (Harima, Japan) revealed that the crystal contained Zn, but not Mn, Fe, Ni or Cu (Fig. 3). The XAFS data showed that the recombinant *Tth*CP1 used in this study existed as the Zn^{2+}-bound form.

Structure determination by molecular replacement using the coordinates of *Pfu*CP (PDB code 1ka2; Arndt *et al.*, 2002) as a search model is currently under way.

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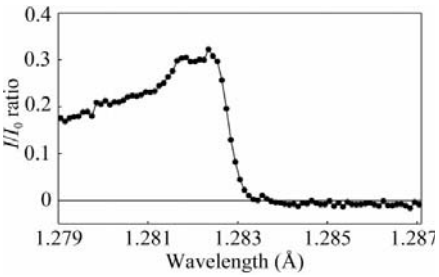

Figure 3
X-ray absorption fluorescence spectrum (XAFS) of a *Tth*CP1 crystal showing that the crystal contains Zn. The data were collected at BL38B1, SPring-8.

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