Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic analysis of chitinase F1 (ChiF1) from the alkaliphilic *Nocardiopsis* sp. strain F96

Tsutomu Matsui,[a] Takashi Kumasaka,[a] Kimiko Endo,[b] Takao Sato,[a] Satoshi Nakamura[b] and Nobuo Tanaka[a]*

[a]Department of Life Science, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan, and [b]Department of Bioengineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

Correspondence e-mail: ntanaka@bio.titech.ac.jp

Received 2 July 2004
Accepted 18 August 2004

Chitinase F1 (ChiF1) isolated from the alkaliphilic *Nocardiopsis* sp. strain F96 is a family 18 chitinase that hydrolyzes chitin, an insoluble β-1,4-linked polymer of *N*-acetylglucosamine. Crystals of recombinant ChiF1 with molecular weight of 33 000 Da were grown to a suitable size for X-ray structure analysis using 18%(*w*/*v*) polyethylene glycol 8000, 200 m*M* zinc acetate dehydrate and 100 m*M* sodium cacodylate buffer pH 6.5. Diffraction data were collected at SPring-8 and show that the crystals belong to the trigonal space group $P3_112$ or $P3_212$, with unit-cell parameters a = 56.0, c = 179.5 Å, and diffract X-rays beyond 1.2 Å resolution. Crystallographic analysis was carried out using the multiwavelength anomalous diffraction (MAD) method using zinc as the anomalous scatter. The binding of Zn atoms was clarified from the Bijvoet and dispersive Patterson functions, which gave prominent zinc–zinc self-vectors on the Harker section.

1. Introduction

Chitinases (EC 3.2.1.14) hydrolyze chitin, a homopolymer of β-1,4-linked *N*-acetylglucosamine and the second most abundant biopolymer on Earth, after cellulose. Degradation of a polymer to a monomeric sugar is an important process that enables humans to utilize such abundant resources. Because of the fact that chitin is insoluble in neutral and alkaline water and is slightly soluble in acidic solutions, heterogeneous phase reactions between soluble chitinases and insoluble chitin take place during this process.

The gene encoding chitinase F1 (ChiF1) was cloned from the alkaliphilic *Nocardiopsis* sp. strain F96 and DNA-sequence analysis revealed that ChiF1 belongs to family 18 of the glycosyl hydrolases (Endo *et al.*, 2004). No chitinase from an alkaliphile has been studied previously except for the chitinase from an alkaliphilic actinomycete (Tsujibo *et al.*, 1992). The gene encoding ChiF1 (the *chiF1* gene) was successfully expressed in *Escherichia coli* and ChiF1 was secreted into the culture supernatant (Endo *et al.*, 2003). ChiF1 exhibited chitin hydrolytic activity, even under alkaline conditions. This implies that ChiF1 is a good candidate for the reutilization of chitin because its activity is not dependent on pH.

According to classification by amino-acid sequence similarity (Henrissat & Davies, 1997), chitinases are classified into families 18 and 19 of the glycosyl hydrolases; most non-plant chitinases belong to family 18. Chitinase B from *Serratia marcescens*, the crystal structure of which has been reported (van Aalten *et al.*, 2000), shares the highest sequence identity (31%) to ChiF1 of the reported structures. No crystal structure of a family 18 chitinase from alkaliphilic microbes has yet been reported, although an initial catalytic mechanism for family 18 chitinases has been proposed (Brameld & Goddard, 1998; Knapp *et al.*, 1996; Papanikolau *et al.*, 2001; Tews *et al.*, 1997; Uchiyama *et al.*, 2001; van Aalten *et al.*, 2001) based on the crystal structures of several other chitinases (van Aalten *et al.*, 2000, 2001; Kolstad *et al.*, 2002). This paper reports the preliminary results of a crystallographic study in order to elucidate the mechanism of insoluble chitin hydrolysis under alkaline conditions.

2. Experimental procedures and results

2.1. Purification of ChiF1

Expression of the *chiF1* gene has been described previously (Endo *et al.*, 2003). Recombinant ChiF1 was purified from the culture supernatant of *E. coli* carrying the *chiF1* gene by ammonium sulfate fractionation (50–70% saturation) and DEAE-Toyopearl 650M (Tosoh Corporation) column chromatography. Active fractions were collected and subjected to SDS–PAGE (Laemmli, 1970). The purified ChiF1 showed a single band at 35 kDa. Chitinase activity was measured as described elsewhere (Endo *et al.*, 2004).

2.2. Crystallization of ChiF1

ChiF1 was crystallized at 298 K using the sitting-drop vapour-diffusion method. Initial crystallization conditions were determined by

© 2004 International Union of Crystallography
Printed in Denmark – all rights reserved

Table 1
X-ray diffraction data statistics for a ChiF1 crystal.

Values in parentheses refer to the outer shell.

	MAD			
	Edge	Peak	Remote	Single
Wavelength (Å)	1.282900	1.282323	1.200000	1.000000
Space group	$P3_112$ or $P3_212$			$P3_112$ or $P3_212$
Unit-cell parameters (Å)	a = 56.0, c = 179.5			a = 55.8, c = 179.4
Resolution (Å)	1.94 (2.01–1.94)	1.94 (2.01–1.94)	1.94 (2.01–1.94)	1.24 (1.28–1.24)
No. measured reflections	237929	237960	255071	933200
No. unique reflections†	44027	44042	46076	87187
Average redundancy	5.40 (4.33)	5.40 (4.36)	5.54 (5.29)	10.70 (8.81)
Completeness (%)	95.0 (67.8)	95.0 (68.1)	98.7 (97.5)	95.6 (76.1)
$R_{\rm merge}$‡ (%)	0.087 (0.249)	0.090 (0.277)	0.086 (0.239)	0.082 (0.299)
$\langle I/\sigma(I)\rangle$	13.6 (4.9)	12.9 (4.9)	13.2 (5.7)	14.1 (6.5)
Mosaicity (°)	0.33	0.33	0.34	0.34

† In MAD data, anomalous reflections, I^+ and I^-, were distinguished and then counted individually. ‡ $R_{\rm merge} = \sum\sum[|I(h) - I(h)_i|/I(h)]$, where $I(h)$ is the mean intensity after rejections.

the sparse-matrix method as implemented in Crystal Screens I and II (Hampton Research), Structure Screens I and II (Molecular Dimensions Ltd) and Wizard Screens I and II (deCode Genetics). Crystals could be obtained in several drops using 18–20%(*w*/*v*) polyethylene glycol (PEG) as the precipitant. The molecular weight, which was in the range 3000–8000, and the concentration of PEG were not critical for crystallization. However, either Ca^{2+} or Zn^{2+} had to be added in order to obtain crystals suitable for X-ray analysis.

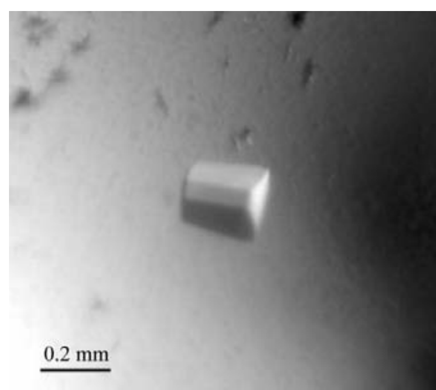

Figure 1
ChiF1 crystal. The crystal has approximate dimensions of 0.2 × 0.2 × 0.2 mm.

The best crystals for data collection were grown under the following conditions. A sitting drop was made up by mixing 3 µl protein solution (5 mg ml^{-1} ChiF1 in 10 m*M* Tris–HCl buffer pH 7.5) with 2 µl reservoir solution [18%(*w*/*v*) PEG 8000, 0.2 *M* zinc acetate dehydrate, 0.1 *M* sodium cacodylate pH 6.5]. Crystals grew to a size of approximately 0.2 × 0.2 × 0.2 mm at 298 K in 5 d (Fig. 1).

2.3. X-ray diffraction analysis of ChiF1

Before the X-ray experiment, crystals were soaked in a cryoprotectant solution [20%(*v*/*v*) glycerol in the reservoir solution] and were flash-cooled in liquid nitrogen. An X-ray fluorescence spectrum was recorded and was used to select the optimal wavelength for MAD data collection (Hendrickson & Ogata, 1996). It was thought that the protein specifically bound to Zn^{2+} ions because the reservoir solution contained 0.2 *M* zinc acetate dehydrate. MAD data were collected from frozen crystals at 100 K using the Rigaku/MSC Jupiter 210 CCD detector installed on beamline BL26B2 of SPring-8, Japan. MAD data collection was carried out by applying 0.5° oscillation per image at 1.282900 Å (the inflection point of the fluorescence spectrum, f' minimum), 1.282323 Å (the maximum of the fluorescence spectrum, f'' maximum) and 1.200000 Å (a remote high-energy wavelength). After MAD data collection, high-resolution data were collected at 1.0000 Å using a Rigaku R-AXIS V imaging-plate system at the same beamline.

Diffraction intensities were integrated, merged and scaled using *d*TREK* (Pflugrath, 1999) from the *CrystalClear* suite (Rigaku/MSC). Data-collection statistics are given in Table 1. Crystals were found to belong to the trigonal space group $P3_112$ or $P3_212$, with unit-cell parameters $a = b = 56.0$, c = 179.5 Å, and diffracted X-rays beyond 1.24 Å resolution with 95.6% completeness (high-resolution data). Assuming the presence of one molecule in the asymmetric unit, with a molecular weight of 33 000 Da, the Matthews coefficient ($V_{\rm M}$) and solvent content were calculated to be 2.46 Å^3 Da^{-1} and 50%(*v*/*v*), respectively (Matthews, 1968).

Determination of the ChiF1 structure by the MAD method is currently in progress. Anomalous and dispersive Patterson maps were calculated using the program *FFT* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Zn-atom sites were confirmed by Patterson

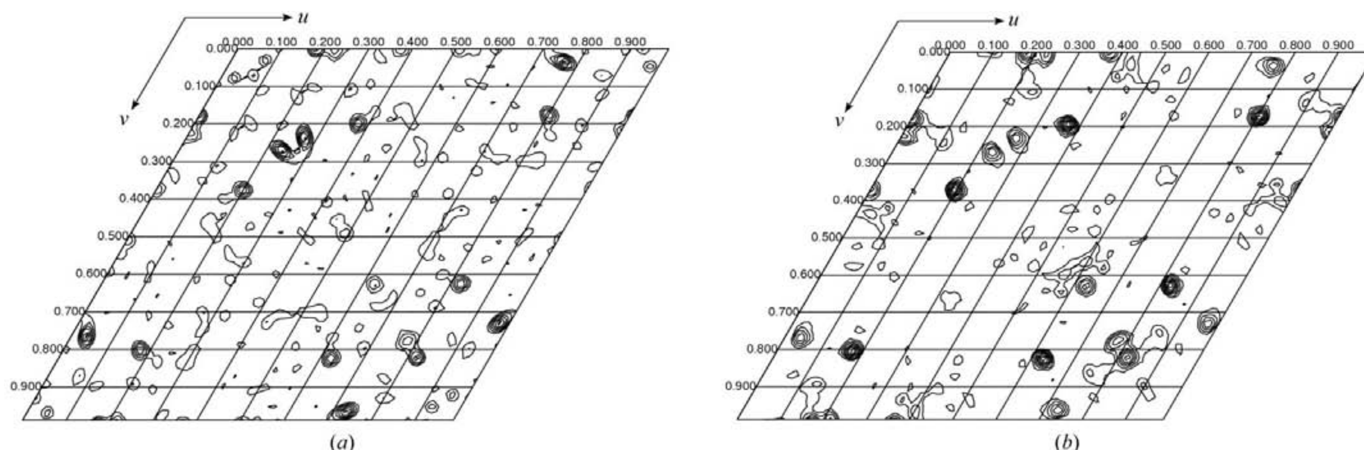

Figure 2
(*a*) Bijvoet and (*b*) dispersive Patterson maps. The two maps show prominent zinc–zinc self-vectors at the same positions on the Harker section (w = 1/3).

maps, which gave prominent zinc–zinc self-vectors on the Harker section (Fig. 2).

3. Discussion

In the initial survey of crystallization conditions, crystal growth occurred with or without additives. Further refinement of the crystallization conditions resulted in suitable crystal growth by adding either Ca^{2+} or Zn^{2+} to the crystallization solvent. Such crystals diffracted X-rays beyond 1.24 Å resolution, but without additives crystals diffracted X-rays to less than 5 Å resolution. The MAD data are promising for elucidation of the detailed structure and should provide further knowledge regarding the effective hydrolysis of chitin.

Use of the BL26B2 beamline of SPring-8 is acknowledged for diffraction data collection. This study was supported in part by a grant from the National Project on Protein Structural and Functional Analyses of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Aalten, D. M. F. van, Komander, D., Synstad, B., Gåseidnes, S., Peter, M. G. & Eijsink, V. G. H. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 8979–8984.
Aalten, D. M. F. van, Synstad, B., Brurberg, M. B., Hough, E., Riize, B. W., Eijsink, V. G. H. & Wierenga, R. K. (2000). *Proc. Natl Acad. Sci. USA*, **97**, 5842–5847.
Brameld, K. A. & Goddard, W. A. (1998). *J. Am. Chem. Soc.* **120**, 3571–3580.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Endo, K., Fukazawa, T., Yatsunami, R. & Nakamura, S. (2003). *Chitin Chitosan Res.* **9**, 150–151.
Endo, K., Fukazawa, T., Yatsunami, R. & Nakamura, S. (2004). In the press.
Hendrickson, W. A. & Ogata, C. M. (1996). *Methods Enzymol.* **276**, 494–522.
Henrissat, B. & Davies, G. (1997). *Curr. Opin. Struct. Biol.* **7**, 637–644.
Knapp, S., Vocadlo, D., Gao, Z. N., Kirk, B., Lou, J. P. & Withers, G. G. (1996). *J. Am. Chem. Soc.* **118**, 6804–6805.
Kolstad, G., Synstad, B., Eijsink, V. G. H. & van Aalten, D. M. F. (2002). *Acta Cryst.* D**58**, 377–379.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Papanikolau, Y., Prag, G., Tavlas, G., Vorgias, C. E., Oppenheim, A. B. & Petratos, K. (2001). *Biochemistry*, **40**, 11338–11343.
Pflugrath, J. W. (1999). *Acta Cryst.* D**55**, 1718–1725.
Tews, I., van Scheltinga, A. C. T., Perrakis, A., Wilson, K. S. & Dijkstra, B. W. (1997). *J. Am. Chem. Soc.* **119**, 7954–7959.
Tsujibo, H., Yoshida, Y., Miyamoto, K., Hasegawa, T. & Inamori, Y. (1992). *Biosci. Biotechnol. Biochem.* **56**, 1304–1305.
Uchiyama, T., Katouno, F., Nikaidou, N., Nonaka, T., Sugiyama, J. & Watanabe, T. (2001). *J. Biol. Chem.* **276**, 41343–41349.