Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and initial X-ray analysis of alkaline xylanase

Ning Lu, Hideaki Moriyama, Satoshi Nakamura,† Takao Sato and Nobuo Tanaka*

Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

† Present address: Japan Synchrotron Radiation Research Institute, 323 Mihara, Mikazuki-cho, Sayo-gun, Hyougo 679-5891, Japan.

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

Received 7 September 1999
Accepted 18 January 2000

Crystals of alkaline xylanase (xylanase J) from alkaliphilic *Bacillus* sp. 41M-1 were grown by decreasing the temperature of the protein solution. Initial X-ray analysis of the crystal showed a tetragonal system with space group $P4_1$ or $P4_3$ and unit-cell parameters $a = b = 115.7$, $c = 46.0$ Å. Assuming two molecules per asymmetric unit, $V_m = 2.31$ Å^3 Da^{-1}. The crystals diffract X-rays to 2.6 Å resolution at 100 K.

As a hemicellulase which hydrolyzes β-1,4-xylan into monomeric sugars, β-1,4-xylanase (1,4-β-D-xylan xylohydrolase; E.C. 3.2.1.8) is expected to play a important role in biotechnological methods directed towards increasing the global food supply. A variety of xylanases are widely distributed in heterotrophic bacteria, having characteristics dependent on their particular living conditions (Wong *et al.*, 1988). Alkaliphilic *Bacillus* sp. 41M-1 isolated from forest soil in Japan (Nakamura *et al.*, 1993*a*) produces an alkaline xylanase (xylanase J) consisting of 327 amino-acid residues, with a molecular weight of 35 922 Da calculated from its amino-acid composition. As previously described (Nakai *et al.*, 1994), the gene encoding xylanase J has been cloned, sequenced and expressed in *Escherichia coli* (Nakamura *et al.*, 1993*b*). Xylanase J has an isoelectric point at pH 5.3 and exhibits optimum activity at pH 9.0, 323 K. In order to determine its structure, we have subjected it to crystallization and performed X-ray analysis on the resultant crystals.

Based on preliminary experiments showing that the enzyme's solubility decreased markedly with decreasing temperature, a protein solution (~20 mg ml^{-1}) was prepared at 293 K in 10 m*M* sodium phosphate buffer pH 7.0. On storage at 275–277 K for 24 h, many small needle-shaped crystals appeared even though no precipitants were added; these disappeared quickly when the solution was warmed to about 293 K. Centrifugation was used to collect the crystals, which were placed into fresh protein solution as seeds; after several seeding cycles, plate-shaped crystals (Fig. 1) grew to dimensions of 0.8 × 0.2 × 0.05 mm.

Initial X-ray analysis of a crystal was undertaken with an R-AXIS IIc image-plate detector on a Rigaku rotating-anode fine focus X-ray generator operated at 40 kV and 100 mA and providing mirror-focused Cu $K\alpha$ radiation. The crystal equilibrated in the cryoprotectant solution [reservoir solution plus 20%(*w*/*v*) glycerol] diffracts X-rays beyond 2.6 Å resolution at 100 K without any serious decay in intensity, although the addition of an antifreeze reagent widened the mosaic spread to 0.7°. The crystal has a tetragonal 4/*m* intensity distribution symmetry, with unit-cell parameters $a = b = 115.7$, $c = 46.0$ Å. The absence of (00*l*) reflections for $l \neq 4n$ indicated the space group to be either $P4_1$ or $P4_3$. Two molecules exist per asymmetric structure and $V_m = 2.31$ Å^3 Da^{-1}. The data to 2.6 Å resolution were indexed, integrated and reduced on a Silicon Graphics Indigo workstation using the Unix version of the *R-AXIS* software provided with the instrument. A total of 35 326 measured reflections were merged and scaled into 14 707

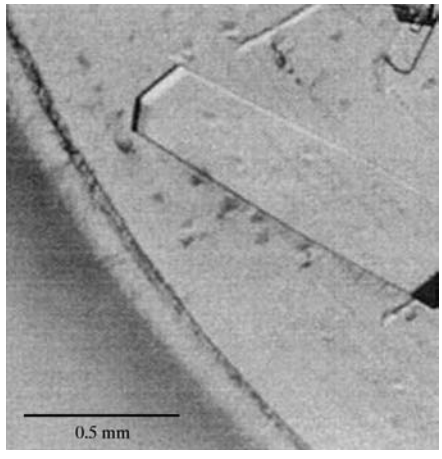

Figure 1
Crystal of xylanase J. The crystal was grown by decreasing the temperature of the protein solution. Its dimensions are approximately 0.3 × 0.2 × 0.05 mm.

© 2000 International Union of Crystallography
Printed in Denmark – all rights reserved

unique reflections with an $R_{\rm merge}$ (on intensity) of 9.5%.

To elucidate the crystal structure, the molecular-replacement method was applied using the *X-PLOR* (Brünger, 1993) and *AMoRe* (Navaza, 1994) programs and *B. pumilus* xylanase as a model (Moriyama *et al.*, 1987). Since the actual molecule is 1.5 times bigger than the model, we unfortunately could not determine the correct orientation or position of the two molecules in the asymmetric unit, nor could an alternative structure be acquired owing to the unusual crystallization conditions. A search for heavy-atom derivatives is now in progress so that the multiple isomorphous method can subsequently be applied.

The authors would like to express their thanks to Dr M. Nakasako of Tokyo University for his help with the cryo-experiments.

References

Brünger, A. T. (1993). *X-PLOR Version* 3.1. Yale University, New Haven, CT, USA.

Moriyama, H., Hata, Y., Yamaguchi, H., Sato, M., Shinmyo, A., Tanaka, N., Okada, H. & Katsube, Y. (1987). *J. Mol. Biol.* **193**, 237–238.

Nakamura, S., Wakabayashi, K., Nakai, R., Aono, R. & Horikoshi, K. (1993*a*). *World J. Microbiol. Biotechnol.* **9**, 221–224.

Nakamura, S., Wakabayashi, K., Nakai, R., Aono, R. & Horikoshi, K. (1993*b*). *Appl. Environ. Microbiol.* **59**, 2311–2316.

Nakai, R., Wakabayashi, K., Asano, T., Aono, R., Horikoshi, K. & Nakamura, S. (1994). *Nucleic Acids Symp. Ser.* **31**, 235–236.

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.

Wong, K. K. Y, Tan, L. U. L. & Saddler, J. N. (1988). *Microbiol. Rev.* **52**, 305–317.