Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

A new crystal form of XT6 enables a significant improvement of its diffraction quality and resolution

Maya Bar,[a] Gali Golan,[a] Magda Nechama,[a] Gennady Zolotnitsky,[b] Yuval Shoham[b,c] and Gil Shoham[a]*

[a]Department of Inorganic Chemistry and the Laboratory for Structural Chemistry and Biology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, [b]Department of Biotechnology and Food Engineering, Technion-ITT, Haifa 32000, Israel, and [c]Institute of Catalysis Science and Technology, Technion-ITT, Haifa 32000, Israel

Correspondence e-mail: gil2@vms.huji.ac.il

Received 10 November 2003
Accepted 17 December 2003

Xylanases (1,4-β-D-xylan xylanhydrolases; EC 3.2.1.8) hydrolyze the 1,4-β-D-xylopyranosyl linkage of xylans. The detailed structural characterization of these enzymes is of interest for the elucidation of their catalytic mechanism and for their rational modification toward improved stability and specificity. An extracellular xylanase from *Geobacillus stearothermophilus* T-6 (XT6) has recently been cloned, overexpressed, purified and biochemically characterized. Previous crystallographic efforts resulted in a hexagonal crystal form, which subsequently proved to be of limited use for structural analysis, mainly because of its relatively poor diffraction quality and resolution. A systematic search for more suitable crystals of XT6 recently resulted in a new crystal form of this enzyme with significantly improved diffraction characteristics. The new crystals belong to a *C*-centred monoclinic crystal system (space group *C*2), with unit-cell parameters a = 121.5, b = 61.7, c = 89.1 Å, β = 119.7°. These crystals diffract X-rays to better than 1.5 Å resolution, showing a very clear diffraction pattern of relatively high quality. The crystals are mechanically strong and exhibit excellent radiation-stability when frozen under cold nitrogen gas. A full diffraction data set to 1.45 Å resolution (94.1% completeness, $R_{\rm merge}$ = 7.0%) has been collected from flash-frozen crystals of the native enzyme at 95 K using synchrotron radiation. Crystals of the E159A/E265A catalytic double mutant of XT6 were found to be isomorphous to those of native XT6. They were used for a full measurement of 1.8 Å resolution diffraction data at 100 K (90.9% completeness; $R_{\rm merge}$ = 5.0%). These data are currently being used for the high-resolution structure determination of XT6 and its mutant for mechanistic interpretations and rational introduction of thermostability.

1. Introduction

One of the main factors that limit the widespread use of enzymes in industry is their inherent instability. Like many other proteins, they are readily subject to denaturation by heat, chemicals and extreme pH conditions. Significant improvements of these characteristics are required in order to obtain robust enzymes with long half-lives that can operate under industrial conditions. One approach to overcoming these limitations is to alter the stability of a particular functionally suitable enzyme and make it more stable under extreme conditions by rational chemical and/or genetic modification. For example, one can enhance the stability of a given enzyme *via* a series of specific amino-acid substitutions using its detailed three-dimensional structure as a guide. A xylanase (1–4-β-D-xylanxylanohydrolase; EC 3.2.1.8) from the thermophilic bacterium *Geobacillus stearothermophilus* T-6 (XT6) has been selected in our laboratory in order to examine the practical aspects of such strategy. The study of this enzyme has been particularly focused on rational enhancement of its thermostability on the basis of its three-dimensional structure and its detailed catalytic mechanism.

In addition to serving as a readily available prototype enzyme for such study, XT6 is a very attractive target for various biotechnological applications. Xylanases, like other hemicellulases, have been studied extensively in recent years because of their wide potential range of industrial applications (Shallom & Shoham, 2003). Such applications include their use in biobleaching of paper pulp, bioconversion of lignocellulose material to fermentative products and improvement of animal feedstock digestibility and their potential use in novel organic synthesis (Mackenzie *et al.*, 1998; Saha, 2000; Suurnakki *et al.*, 1997; Wong & Sanddler, 1993). Obviously, for these enzymes to be effectively integrated into industrial production lines they have to be efficient, specific and stable.

XT6 is an extracellular endo-xylanase belonging to glycoside hydrolase family 10 (GH10). It is secreted from the bacterium into the environment, where it degrades polymeric xylan into smaller units (Khasin *et al.*, 1993). These processed small sugar units are then imported into the bacterium for various purposes. The corresponding gene of this enzyme has been characterized, cloned, sequenced and overexpressed in *Escherichia coli* (Gat *et al.*, 1994; Fishman *et al.*, 1995; Lapidot *et al.*, 1996; Shulami *et al.*, 1999). When overexpressed in *E. coli*, the recombinant XT6 is highly soluble and can make up over 70% of the total host protein. The mature enzyme consists of a single polypeptide chain of 379 amino acids, with a calculated molecular weight of 43 808 Da. The pI values of the purified protein are 9 and 7 under native and denaturating conditions, respectively. The optimal activity of XT6 was shown to occur in the pH range 6.5–7; however, about 60% of the activity is retained at pH 10. At 338 K and pH 7 the enzyme is stable for over 10 h, while at 338 K and pH 9 the half-life of the enzyme was shown to be approximately 6 h. Based on sequence comparison with other GH10 xylanases and biochemical characterization of catalytic mutants, it was shown that Glu159 of XT6 is the acid–base catalytic residue and Glu265 is the catalytic nucleophile (Mechaly, 1998). These findings led to the preparation of several mechanism-oriented mutated enzyme variants in which one or both of these residues have been replaced by site-directed mutagenesis. The E159A/E265A double mutant is hence very important for various structural studies, especially its complexes with bound effectors such as substrates and transition-state analogues.

As a first step in the rational thermostabilization of XT6, we initiated a systematic study of its detailed three-dimensional structure. Our assumption was that such structural information, especially at high resolution, should serve as a good basis for the understanding of its stability characteristics and its mechanism of catalytic action. Moreover, it was expected that such information should be very useful for the rational design of modifications that will make this enzyme more specific, more efficient and more thermostable. The results of this comprehensive study were expected to be subsequently applied for further stabilization of this and related enzymes, particularly for industrial processes that require extreme temperature conditions.

XT6 has been crystallized previously in a hexagonal crystal form (H1 crystals, space group $P3_221$; Teplitsky *et al.*, 1997). These crystals diffracted X-rays to about 2.4 Å resolution, but their usefulness in complete structural analysis was realised to be very limited because of their extraordinary sensitivity to X-ray radiation and their rapid damage on X-ray exposure. Moreover, an extensive search for crystal-freezing and cross-linking techniques that could be used for the reduction of potential crystal radiation damage only resulted in relatively ineffective procedures (Teplitsky *et al.*, 1997; Mechaly *et al.*, 2000).

In an attempt to overcome the practical limitations of the hexagonal crystal form of XT6, we conducted a systematic search for an alternative crystal form with improved diffraction properties. Such a search resulted recently in a new and improved monoclinic crystal form of the enzyme. The new crystals diffract X-rays to significantly higher resolution (better than 1.5 Å) and are relatively less affected by X-ray radiation under flash-cooling conditions. In the following, we describe this new crystal form of XT6 (M2 crystals) and report the measurement of a full X-ray diffraction data set which was obtained from native XT6 using synchrotron radiation to 1.45 Å resolution. A similar X-ray diffraction data set is reported here for the E159A/E265A double mutant of XT6 in the new crystal form, which was obtained at 1.80 Å resolution using a rotating-anode X-ray laboratory source. These data are currently being used for a detailed three-dimensional structure analysis of the native enzyme and its catalytic mutant at high resolution.

2. Enzyme expression and purification from *E. coli*

The expression and purification of XT6 were performed using procedures similar to those described previously (Teplitsky *et al.*, 1997; Mechaly *et al.*, 2000). The mature xylanase gene, without its leader sequence, was cloned into the T7 polymerase expression vector pET9d (Novagen) (Studier *et al.*, 1990) and overexpressed in *E. coli* strain BL21DE3 (Novagen) (Lapidot *et al.*, 1996). The recombinant xylanase contained an additional methionine residue at its N-terminus and as a result the molecular weight of the final recombinant xylanase (consisting of 380 amino acids) was

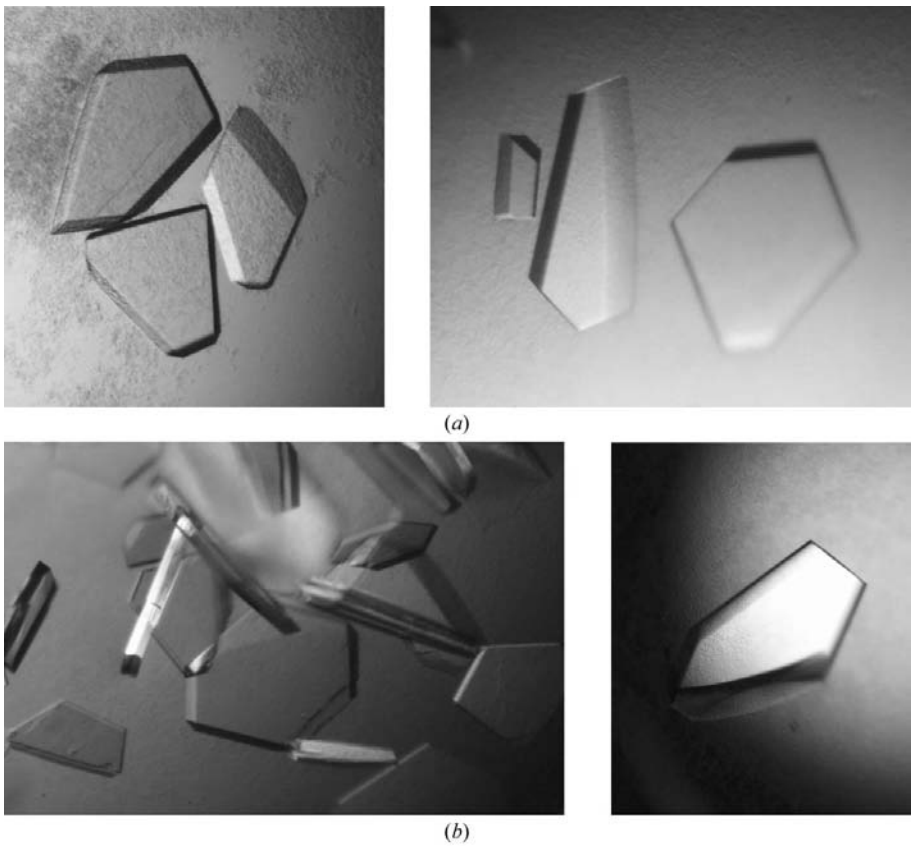

Figure 1
Typical single crystals of XT6 in the new monoclinic (M2) crystal form (space group *C*2; magnification of about ×100). These crystals were used for a diffraction data measurement of the E159A/E265A double mutant of XT6 to 1.80 Å resolution (*a*) and for the diffraction measurement of native XT6 to 1.45 Å resolution (*b*). Note the variety of shapes and the sharp extinction of these crystals under polarized light.

43 939 Da. Under this expression system, XT6 comprised over 70% of the soluble protein content of the growing *E. coli* and no inclusion bodies were detected. Using this system, we routinely obtained over 3 g of purified XT6 enzyme per litre of *E. coli* cultures grown overnight on Terrific Broth (Sambrook *et al.*, 1989).

3. Crystallization and crystal forms

3.1. The old H1 crystal form

As mentioned above, preliminary crystallographic analysis has already been performed in our laboratory on another crystal form (H1) of XT6. The H1 crystals of XT6 were obtained using a reservoir solution consisting of 1.6–1.8 *M* ammonium sulfate and 8% MPD and drops containing 8–12 mg ml^{-1} protein and 50% reservoir solution (Teplitsky *et al.*, 1997). The resulting H1 crystals appeared usually as transparent 'diamonds' with very distinct faces and edges. The dimensions of these crystals were usually around 0.3 × 0.3 × 0.2 mm and they displayed X-ray diffraction to approximately 2.4 Å resolution (using strong synchrotron radiation). From the diffraction pattern, it was determined that these crystals belong to the hexagonal space group $P3_221$, with unit-cell parameters $a = b = 112.2$, $c = 122.9$ Å. Subsequently, these H1 crystals of XT6 proved to be of limited use, mainly because of their rapid rate of crystal deterioration during exposure to X-ray radiation. There were also various difficulties in the effectiveness of flash-freezing procedures for crystallographic data measurement of these crystals at low temperatures (Teplitsky *et al.*, 1997; Mechaly *et al.*, 2000), as described below.

The only procedure that seemed to provide a significant reduction in the radiation damage to the H1 crystals involved their soaking for 20–30 min in a solution containing 25% glucose, followed by the usual flash-freezing techniques. This freezing procedure turned out to be relatively complicated to perform and difficult to reproduce. This procedure also seemed to significantly reduce the practical resolution limit of the diffraction of the crystals. Moreover, the use of high concentration of glucose in the freezing solution made it impossible to diffuse substrates and analogues into the XT6 crystals. Preliminary soaking experiments of this type indicated that several glucose molecules (originating from the glucose freezing solution) bind specifically to the enzyme and thus prevent the binding of substrates and analogues in the corresponding binding sites. These limitations present obvious disadvantages for the structural study of substrate binding to XT6 and its implications for the detailed catalytic pathway of this enzyme.

3.2. A systematic search for an alternative crystal form

Considering the limitations of the H1 crystal form of XT6 for meaningful study of its structure and catalytic mechanism, it was clear that an alternative crystal type should be obtained and/or an alternative crystal-freezing procedure should be developed.

Unfortunately, refinement of the original crystallization conditions and the glucose-based freezing procedures did not result in significant improvement in either the diffraction quality or the crystal-freezing effectiveness. Systematic variations around the reported procedures indicated that the crystals are extremely sensitive to even small changes in the original crystallization conditions and especially to the introduction of organic solvents and organic additives, as well as the addition of cryosolvents such as glycerol and polyethylene glycol (PEG). It was therefore concluded that efforts to improve the old crystals should be discontinued and efforts should be concentrated on a systematic search for new crystallization conditions, which should be completely different. In the new search, we tried to avoid high salt concentrations and focused particularly on solutions with flash-freezing potential (*i.e.* glycerol content of >10% and/or PEG content of >20%). With these crystallization guidelines, it was expected that a new crystal form of XT6, if obtained, would be less sensitive to X-ray radiation and easier to freeze for more efficient diffraction data collection.

In order to find new starting conditions for XT6 crystallization, we re-screened the crystallization conditions for native XT6 with a series of solutions of different buffers, precipitants, salts (<1 *M*), cryoprotectants (PEG *etc.*) and other additives, following the guidelines described above. Such systematic crystallization screens have been used recently in our laboratory and proved to be successful for a number of related hemicellulases from *G. stearothermophilus* T-6 (Teplitsky *et al.*, 1997, 1999, 2000). Applying these rationally designed screens for native XT6 indicated that a new type of crystal (M2) is obtained for this enzyme with polyethylene glycol (PEG; 20–30%) as a precipitant (and cryoprotectant) in the pH range 6.0–6.5. Further refinement of these initial conditions led to the following hanging-drop procedure, which produced the best crystals for crystallographic analysis.

3.3. The new M2 crystal form

The crystallization procedure that led to the new M2 crystal form used a reservoir solution containing 25% PEG 550 monomethyl ether (MME), 0.1 *M* MES buffer pH 6.5 and 10 m*M* $ZnSO_4$. The M2 crystals were obtained by mixing 5 µl protein solution (containing about 5 mg ml^{-1} protein) with 4 µl reservoir solution and with 1 µl of 50% methyl pentanediol (MPD) as an organic additive in the drop (the final MPD content in the drop was ∼5%). Under these conditions at room temperature (294–295 K), initial crystals could be observed within 3–4 weeks and grew to their full size in about 2–3 months. Most of the M2 crystals appeared as flat polygons, each with three to six sharp edges and typical dimensions of around 0.5 × 0.3 × 0.02 mm. Two crystals grown according to this procedure were used for full diffraction data collection of native XT6 at 1.45 Å resolution, as described below.

Refinement of the crystallization conditions of the initial M2 crystal form resulted in larger crystals (Fig. 1), faster crystal growth and improved stability of the crystals in the X-ray beam. In the best conditions, the initial drop contained 5–9 mg ml^{-1} XT6 protein (native or mutant), 7% PEG 4000–6000, 5–10 m*M* zinc salt ($ZnSO_4$ or $ZnCl_2$) and 0.1 *M* MES buffer pH 6.45. They were equilibrated against a reservoir solution of

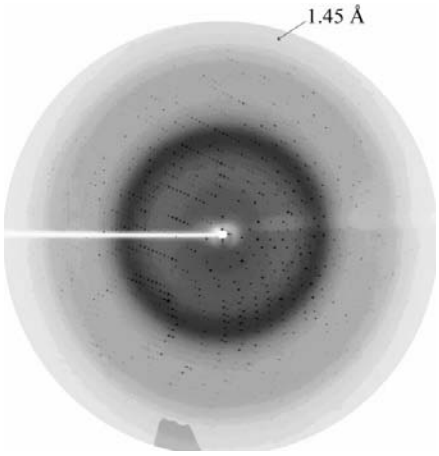

Figure 2
Representative two-dimensional section of the three-dimensional diffraction pattern of native XT6 in its new M2 crystal form. This diffraction pattern was obtained at the X8C beamline of the NSLS synchrotron facility (Brookhaven National Laboratory, NY, USA) using X-ray radiation of λ = 1.0093 Å. This image demonstrates the exceptional quality of the diffraction, especially in the outer part of the diffraction pattern. The 1.45 Å resolution limit is indicated by an arrow.

14% PEG 4000–6000, 10 m*M* $ZnSO_4$ and 0.1 *M* MES pH 6.45 at room temperature. Addition of MPD appeared to reduce the quality of the XT6 crystals under these conditions and was therefore avoided in the final crystallization procedures. In these experiments, the first XT6 crystals could be observed in the drop after about 2–3 d and grow to their full size in about 3–4 weeks. The resulting crystals appeared as transparent flat polygons, similar to the M2 crystals observed previously. However, these crystals appear to be significantly thicker than the original M2 crystals and often grew to dimensions of around 0.5 × 0.5 × 0.1 mm. Most of these crystals had distinct faces and edges and showed very clear and homogenous polarization effects under polarized light (Fig. 1). These crystals were recently used for a full diffraction data collection for the E159A/E265A double mutant of XT6 at 1.80 Å resolution, as described below.

4. Diffraction data measurement and processing

4.1. Crystallographic data set for native XT6

Several crystals of the new M2 crystal form of native XT6 have been used for crystallographic characterization and measurement of X-ray diffraction data under cryogenic conditions. These experiments were performed using synchrotron radiation (λ = 0.92–1.12 Å) and a CCD area detector (Quantum 4, ADSC Inc., USA) at the X8C and X26C beamlines, National Synchrotron Light Source facility (NSLS), Brookhaven National Laboratory (NY, USA). The crystal-freezing procedure used for these experiments included a short soaking of the target crystal (about 2–3 min) in a cryoprotectant solution consisting of the original crystallization reservoir solution of these crystals (25% PEG 550 MME, 0.1 *M* MES pH 6.5, 10 m*M* $ZnSO_4$) and 10%(*v*/*v*) glycerol. The pre-soaked crystal was then submitted to immediate flash-freezing directly in a cold nitrogen-gas stream (95 K; Oxford Cryosystems). The observed diffraction pattern of some of these crystals exceeded the 1.5 Å resolution limit and only a relatively small amount of radiation damage could be detected even at long exposure times (about 8% of the adjusted decay in the diffraction intensity after about 6 h). The observed diffraction pattern of these crystals was sharp and clear and indicated that the crystals belong to a *C*-centred monoclinic crystal system (space group *C*2), with average crystallographic unit-cell parameters a = 121.5, b = 61.7, c = 89.1 Å, β = 119.7°. Different crystals of this batch gave similar unit-cell parameters, with an overall deviation from these average values of less than 0.3%.

Two such crystals were used for a full X-ray diffraction data measurement. The first oscillation data set ($\Delta\varphi$ = 0.5°), was measured on a single crystal at the NSLS/X8C beamline (λ = 1.0093 Å, 95 K). A representative diffraction pattern of this data set is shown in Fig. 2. The second oscillation data set ($\Delta\varphi$ = 0.5°) was measured on another single crystal at the NSLS/X26C beamline (λ = 1.100 Å, 95 K). These two data sets were merged together in order to increase the redundancy and the completeness of the diffraction data. The raw CCD diffraction images were processed with the *DENZO* and *SCALEPACK* software packages (Otwinowski, 1993). A total of 383 803 accepted reflections [$F > 1.0\sigma(F)$] were measured in the 40.0–1.45 Å resolution range and resulted in 95 911 independent reflections with 94.1% completeness to 1.45 Å resolution (49% completeness for the highest resolution shell 1.48–1.45 Å). The overall redundancy of the combined data set was 2, the overall mosaicity was 0.48, the average $\langle I/\sigma(I)\rangle$ was 10.3 and the total $R_{\rm merge}$ was 7.0%. These parameters demonstrate the relatively good quality of the M2 diffraction data and its suitability for a full crystallographic structural analysis.

The volume of the M2 crystallographic unit cell as determined from the mean value of the unit-cell parameters at 95 K is 5.84×10^5 Å^3. Assuming that the specific ratio of volume to protein in the crystal ($V_{\rm M}$) is within the normal range of $V_{\rm M}$ values observed for soluble protein crystals (1.68–3.5 Å^3 Da^{-1}; Matthews, 1968), there should be 4–7 XT6 monomers (379 amino acids; MW 43 808 Da) in the unit cell. Since there are four symmetry operations in the *C*2 space group (and hence four copies of the asymmetric unit), this number should be four for the present crystals, indicating that there is a monomer of XT6 in the crystallographic asymmetric unit. With four molecules in the unit cell of the M2 crystals, the calculated $V_{\rm M}$ is 3.3 Å^3 Da^{-1}, a reasonable value according to Matthews (1968). The presence of only one monomer per asymmetric unit in a relatively small unit cell, together with the excellent diffraction qualities demonstrated above, make the M2 crystal form an ideal system for high-resolution structural analysis of XT6 and its potential variants and complexes. This analysis is currently in progress using an initial model obtained from the low-resolution data of the H1 crystal form of native XT6.

4.2. Crystallographic data set for the E159A/E265A double mutant of XT6

A single crystal of the M2 crystal form (0.4 × 0.3 × 0.12 mm) was used for full diffraction data collection on the E159A/E265A double mutant of XT6. This diffraction data was measured at 100 K using a rotating-anode X-ray laboratory source (nickel-filtered Cu $K\alpha$ radiation; λ = 1.54 Å) at the Hebrew University of Jerusalem (Jerusalem, Israel). Crystal freezing included a short soaking of the crystal (1–2 min) in a cryoprotectant solution containing the original crystallization reservoir solution (14% PEG 4000, 10 m*M* $ZnSO_4$, 0.1 *M* MES pH 6.45) and 27%(*v*/*v*) PEG 4000. The pre-soaked crystal was then submitted to immediate flash-freezing directly within a cold nitrogen-gas stream (Oxford Cryosystems). The diffraction pattern of this crystal exceeded the 1.7 Å resolution limit, but owing to technical limitations it was possible to collect only a 1.8 Å resolution data set. As for the native XT6 crystals of the M2 form, the diffraction indicated a *C*-centred monoclinic system (space group *C*2), with unit-cell parameters a = 121.5, b = 61.7, c = 89.1 Å, β = 119.7°, demonstrating that these crystals are closely isomorphous to those of native XT6. These crystal parameters were determined from a combination of still images and oscillation images on an imaging-plate area detector (Rigaku R-AXIS IV^{++} imaging-plate system mounted on a Rigaku RU-300 rotating-anode generator; Shibata, 1990; Sato *et al.*, 1992).

Similarly obtained oscillation images (each with 0.5° rotation angle) were used for a full data measurement. As for the previous data set, the raw imaging-plate diffraction data were processed with the *DENZO* and *SCALEPACK* software packages (Otwinowski, 1993). A total of 113 567 accepted reflections [$F > 1.0\sigma(F)$] were measured in the 40.0–1.80 Å resolution range and resulted in 48 254 independent reflections with 90.9% completeness to 1.80 Å resolution (88% completeness for the highest resolution shell 1.85–1.80 Å), an overall redundancy of 3, an overall mosaicity of 0.9, an average $\langle I/\sigma(I)\rangle$ of 13.3 and a total $R_{\rm merge}$ of 5.0%.

The data set collected and processed for the E159A/E265A double mutant of XT6 represents a diffraction data set of relatively high quality (especially for measurement on a rotating-anode X-ray source) and as such

will be used for detailed crystallographic structural analysis of this important double mutant.

It is planned to perform the full structural analysis of the double mutant only after the completion of the structure determination of the native enzyme and to use the final structure of native XT6 as the starting model for it. Once determined, the structure of the catalytic mutant of XT6 should address important structural and functional questions related to the active site of the enzyme, such as the possibility of significant structural changes around the mutated catalytic amino-acid residues, if any.

This study was a part of the Amirim project of MB at the Hebrew University, Jerusalem. The study was supported by The Israel Science Foundation (ISf; grant No. 676/00 to GS and YS), the Israeli Ministry of Science and the Arts (IMSA; grant Nos. 5932 and No. 4935 to YS and GS) and the French–Israeli Association for Scientific and Technological Research (AFIRST; to YS) and by the German–Israeli Foundation for scientific research and development (GIF; to YS and GS). GG was supported by a graduate student fellowship of the Wolf Foundation and the Abraham Jaglom Fund. Technical support was provided by the Technion Otto Meyerhof Biotechnology Laboratories, established by the Minerva Foundation, Germany. We also thank the staff at the National Synchrotron Light Source of the Brookhaven National Laboratory (X8C and X26C beamlines) for X-ray beam time, for the use of the crystal-cooling facility and for their helpful support and assistance in synchrotron data measurement and analysis.

References

Fishman, A., Berk, Z. & Shoham, Y. (1995). *Appl. Microbiol. Biotechnol.* **44**, 88–93.
Gat, O., Lapidot, A., Alchanati, I., Regueros, C. & Shoham, Y. (1994). *Appl. Environ. Microbiol.* **60**, 1889–1896.
Khasin, A., Alchanati, I. & Shoham, Y. (1993). *Appl. Environ. Microbiol.* **59**, 1725–1730.
Lapidot, A., Mechaly, A. & Shoham, Y. (1996). *J. Biotechnol.* **51**, 259–264.
Mackenzie, L. F., Wang, Q., Warren, R. A. & Withers, S. G. (1998). *J. Am. Chem. Soc.* **120**, 5583–5584.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mechaly, A. (1998). PhD thesis, Technion, Haifa, Israel.
Mechaly, A., Teplitsky, A., Belakhov, V., Baasov, T., Shoham, G. & Shoham, Y. (2000). *J. Biotechnol.* **78**, 83–86.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
Saha, B. C. (2000). *Biotechnol. Adv.* **18**, 403–423.
Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, New York, USA: Cold Spring Harbor Laboratory Press.
Sato, M., Yamamoto, M., Imada, K., Katsube, Y., Tanaka, N. & Higashi, T. A. (1992). *J. Appl. Cryst.* **25**, 348–357.
Shallom, D. & Shoham, Y. (2003). *Curr. Opin. Microbiol.* **6**, 219–228.
Shibata, A. (1990). *Rigaku J.* **7**, 28–32.
Shulami, S., Gat, O., Sonenshein, A. L. & Shoham, Y. (1999). *J. Bacteriol.* **181**, 3695–3704.
Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990). *Methods Enzymol.* **185**, 60–89.
Suurnakki, A., Tenkanen, M., Buchert, J. & Viikari, L. (1997). *Adv. Biochem. Eng. Biotechnol.* **57**, 261–287.
Teplitsky, A., Feinberg, H., Gilboa, R., Lapidot, A., Mechaly, A., Stojanoff, V., Capel, M., Shoham, Y. & Shoham, G. (1997). *Acta Cryst.* D**53**, 608–611.
Teplitsky, A., Shulami, S., Moryles, S., Shoham, Y. & Shoham, G. (2000). *Acta Cryst.* D**56**, 181–184.
Teplitsky, A., Shulami, S., Moryles, S., Zaide, G., Shoham, Y. & Shoham, G. (1999). *Acta Cryst.* D**55**, 869–872.
White, A., Withers, S. G., Gilkes, N. R. & Rose, D. R. (1994). *Biochemistry*, **33**, 12546–12552.
Wong, K. K. Y. & Sanddler, J. N. (1993). *Hemicellulose and Hemicellulases*, edited by M. P. Coughlan & G. P. Hazlewood, pp. 127–143. London: Portland Press,.