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Crystallization and Preliminary X-Ray Crystallographic Study of *Streptomyces olivaceoviridis* E-86 β-Xylanase

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β-Xylanase from *Streptomyces olivaceoviridis* E-86 has been crystallized by the hanging drop vapor diffusion method from 25% saturated ammonium sulfate and 2% McIlvaine buffer, pH 5.7. The crystals diffract to at least 1.9 Å resolution, and belong to space group $P2_12_12_1$, with unit-cell dimensions of $a=79.6$ Å, $b=95.2$ Å, and $c=140.3$ Å. There are probably two xylanase molecules (MW = 45 K) per asymmetric unit.

Key words: crystallization, *Streptomyces olivaceoviridis*, X-ray crystallography, β-xylanase.

β-Xylanase [EC 3.2.1.8] or endo-1,4-β-D-xylanase hydrolyzes the β-1,4-glycosidic bonds within the xylan backbone. Xylanases are utilized to prepare D-xylose or xylooligosaccharides from xylan, and their commercial significance as bio- or prebleaching agents is increasing in the pulp and paper industries. Bacterial and fungal β-1,4-glucanases can be classified into distinct families (A-F), xylanases belonging to either family F or G, on the basis of the amino acid sequence similarities of their catalytic domains (*1, 2*).

The family F enzymes typically consist of a cellulose-binding domain, a flexible linker peptide and a single catalytic domain. Attempts to crystallize family F enzymes have so far been unsuccessful, probably because of the flexibility of the linker region between the separated domains. Only catalytic domains have been crystallized, and their structures have been determined by X-ray crystallography (*3-6*). The structures are folded into a commonly occurring enzyme motif, *i.e.* the eight-folded α/β-barrel first observed in triose phosphate isomerase (*7*).

Streptomyces is a very active bacterium as to the biochemical decomposition of the lignocellulosic biomass in soil. *Streptomyces olivaceoviridis* E-86 β-xylanase (MW = 45,000) has been used for the production of xylobiose and xylose from commercial hardwood (*8*), and in previous papers, we reported the specificity of this enzyme toward xylan. Analysis of the specificity of this enzyme showed that its mode of action differed among the several side chains of xylan (*9-13*). A genomic DNA sequence study is also in progress, and the deduced amino acid sequence is very homologous to that of *S. lividans* xylanase A, indicating that this enzyme belongs to family F.

We report here the crystallization of *S. olivaceoviridis* E-86 β-xylanase and the preliminary results of X-ray crystallographic studies. This is the first report of the crystallization of an intact family F xylanase. Structural determination of the entire molecule will reveal the spatial relationship between the xylan-binding domain and the catalytic domain, which are separated by the linker peptide, which might be indispensable for the enzyme activity. In addition, the structure might provide new insights into the function of xylan binding as well as a structural basis for the comparison of other family F enzymes in the active site for further understanding of the catalytic mechanism.

The xylanase was purified by the procedure reported by Kusakabe *et al.* (*14*) with modification. The culture supernatant was concentrated and dialyzed against distilled water at 2°C. The concentrated supernatant was charged on a DEAE-cellulose DE-52 (Whatman) column equilibrated with a 20 mM acetate buffer solution (pH 5.6). The non-adsorbed fraction was charged on a QAE-Sephadex A-25 (Pharmacia) column (20×300 mm) equilibrated with a 10 mM ethanolamine-HCl buffer solution (pH 9.4), and the column was washed thoroughly with the same buffer. Elution was carried out with a linear gradient of 0-0.5 M NaCl. The active fractions were then chromatographed on an Ultrogel ACA44 (LKB) column (25×900 mm) equilibrated with a 50 mM phosphate buffer solution (pH 6.4) containing 0.1 M NaCl, and elution was carried out with the same buffer solution. Active fractions were pooled to yield the pure enzyme, which was to be homogeneous judged on SDS-PAGE analysis.

Crystallization trials were performed by the hanging drop vapor equilibration method. Each droplet consisted of a 20 mg/ml protein solution and a reservoir solution composed of 25% ammonium sulfate and 2% McIlvaine buffer (a mixture of 0.1 M citric acid and 0.2 M Na_2HPO_4, pH 5.7), in the ratio of 1:1, and was equilibrated against 1.5 ml of the same reservoir solution. Prismatic crystals of xylanase were obtained in a 4°C room approximately after 2 weeks. Figure 1 shows a typical crystal (0.3×0.3×1.0 mm) suitable for X-ray measurement.

Diffraction experiments on the native crystals were carried out at the Photon Factory synchrotron facility of the

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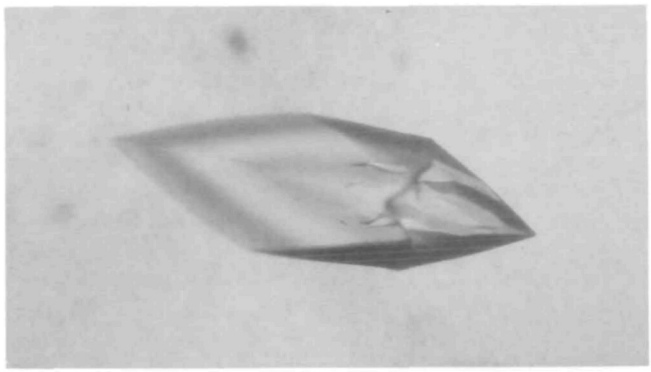

Fig. 1. **A typical crystal of *Streptomyces olivaceoviridis* E-86 β-xylanase grown by the hanging drop vapor diffusion method** (0.3×0.3×1.0 mm in size).

TABLE I. **Summary of X-ray data collection statistics for the native xylanase.**

Resolution (Å)	Average intensity (I)	Average $I/\sigma(I)$	No. of		
			R-factor	Reflections	Complete
100.00-4.09	3,479	30.6	0.035	8,916	0.998
4.09-3.25	2,755	31.0	0.041	8,603	0.998
3.25-2.84	1,308	27.7	0.055	8,368	0.981
2.84-2.58	699	23.0	0.073	8,200	0.968
2.58-2.39	518	18.6	0.091	8,099	0.954
2.39-2.25	392	14.1	0.117	8,031	0.950
2.25-2.14	324	10.7	0.143	7,887	0.935
2.14-2.05	264	8.1	0.175	7,830	0.933
2.05-1.97	208	6.1	0.225	7,750	0.919
1.97-1.90	167	4.9	0.254	6,512	0.777
All reflections	1,146	22.4	0.055	80,196	0.942

R-factor $= \Sigma_h \Sigma_i |I(h)_i - \langle I(h) \rangle| / \Sigma_h \Sigma_i I(h)_i$, where $\langle I(h) \rangle$ is the average intensity of i observation of reflection h.

National Laboratory for High Energy Physics in Tsukuba. Diffraction patterns were measured with a Weissenberg camera for macromolecules (*15*). The wavelength used was 1.00 Å, with a 0.1 mm collimator. Intensity data covering a total rotation of 178° were collected using one crystal. These diffraction data were digitized with a Fuji BA100 image reader. The data set to 1.9 Å resolution was processed using the program, WEIS (*16*) or Denzo (*17*), and scaled using the program, SCALEPACK (*17*).

The collected native intensity data set involved a total of 441,579 observations, which were reduced to 80,196 unique reflections with a completeness of 94.2% and a merging R-factor of 0.055 (Table I). The crystals are orthorhombic and belong to space group $P2_12_12_1$, with cell dimensions of $a=79.6$ Å, $b=95.2$ Å, and $c=140.3$ Å. Assuming two molecules per asymmetric unit, the volume per unit mass, V_m, value for the crystals is 2.95 Å^3/Da, indicating a solvent content of 58% (*18*).

Initial heavy-atom screening was conducted by the soaking method with K_2HgI_4 and K_2PtCl_4 solutions. The Hg-derivative difference Patterson synthesis calculated using the PHASES package (*19*) indicated a single peak corresponding to a Hg-binding site in the protein. More heavy-atom screenings to obtain other derivatives are in progress.

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