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Crystallization and preliminary X-ray crystallographic analysis of DNA polymerase from *Thermus aquaticus*.

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Abstract

Two crystal forms of DNA polymerase from *Thermus aquaticus* have been grown at room temperature. Rhombohedral crystals (form I) grown from ammonium sulfate solution diffracted poorly to 10 Å only and thus are not suitable for X-ray structure determination. Trigonal crystals (form II) grown from polyethylene glycol solution are more suitable for structure determination since their diffraction pattern extends to 2.5 Å at cryogenic temperature upon exposure to synchrotron X-rays. They belong to space group $P3_121$ (or its enantiomorph $P3_221$) and their unit-cell dimensions are $a = 106.7$ and $c = 169.7$ Å for flash-frozen crystals. The presence of one molecule per asymmetric unit gives a crystal volume per protein mass (V_M) of 3.0 Å^3 Da^{-1} and a solvent content of 58% by volume. X-ray data have been collected to 2.7 Å Bragg spacing from native crystals.

Introduction

Taq DNA polymerase, a monomeric protein of 832 amino-acid residues, is a highly thermostable enzyme isolated from *Thermus aquaticus* (Chien, Edgar & Trela, 1976; Kaledin Slyusarenko & Gorodetskii, 1980). It is widely used in amplifying DNA fragments by the polymerase-chain reaction method (Saiki *et al.*, 1985; Mullis & Faloona, 1987) and also in DNA sequencing. The gene encoding *Taq* DNA polymerase has been cloned and its amino-acid sequence has been reported (Lawyer *et al.*, 1989) This revealed that *Taq* DNA polymerase belongs to the family of *Escherichia coli* PolI-like DNA polymerase (Blanco, Bernad, Blasco & Salas, 1991). The N-terminal domain (about 300 amino-acid residues) of *Taq* DNA polymerase has 5′-3′ exonuclease activity and the C-terminal domain (about 410 amino-acid residues) has DNA polymerase activity. When compared with *E. coli* DNA polymerase I, the 3′-5′ exonuclease domain of *Taq* DNA polymerase (about 120 amino-acid residues) is much shorter and, thus, *Taq* DNA polymerase lacks the proof-reading activity. As a consequence, the fidelity of *Taq* DNA polymerase is poor. The structure of the Klenow fragment containing the polymerase domain and the 3′-5′ exonuclease domain has been well characterized (Beese, Friedman & Steitz, 1993; Ollis, Brick, Hamlin, Xuong & Steitz, 1985). However, no X-ray crystallographic study of any 5′-3′ exonuclease domains has been reported. In order to provide a detailed structural basis for understanding the activity of 5′-3′ exonuclease domain and the thermostability of the whole enzyme, we have initiated a structural determination of *Taq* DNA polymerase by X-ray crystallography. As the first step, crystallization and preliminary X-ray data are reported in this paper.

Materials and methods

Crystallization

Taq DNA polymerase, overproduced and purified by the procedure as described previously (Kwon, Lim, Park, Koh & Lee, 1991), was dialyzed against 20 m*M* Tris–HCl buffer at pH 8.2 containing 100 m*M* KCl, 10%(*v*/*v*) glycerol, 0.1 m*M* ethylenediaminetetraacetic acid (EDTA) and 1 m*M* dithiothreitol (DTT). The dialyzed protein was concentrated to 10 mg ml^{-1}.

Crystallization was achieved by the hanging-drop vapor-diffusion method at room temperature (295 ± 2K). Needle-shaped microcrystals were produced with the reservoir solution of 1.7 *M* ammonium sulfate, 2 m*M* DTT and 100 m*M* Tris–HCl, pH 7.5–8.5. When β-octylglucoside was added to the hanging drop at 0.3%(*w*/*v*) concentration, the crystal morphology changed from the needle to rhombohedron. Crystals with approximate dimensions of 0.2 × 0.2 × 0.2 mm could be grown when the reservoir solution contained 2.4 *M* ammoninum sulfate, 1%(*w*/*v*) 2-methyl-2,4-pentanediol, and 100 m*M* Tris–HCl at pH 8.5. These rhombohedral crystals (form I, Fig. 1*a*) diffracted poorly to about 10 Å only and they were abondoned.

The PEG-grown form II crystals (Fig. 1*b*) were found to be more suitable for X-ray studies. Typical conditions to grow form II crystals are as follows. The reservoir solution is prepared by mixing 100 µl of 1.0 *M* sodium citrate, at pH 5.5, 500 µl of 30%(*w*/*v*) PEG 8000 (Sigma), 20 µl of 3.0 *M* ammonium sulfate, 10 µl of 200 m*M* DTT, 10 µl of 20%(*w*/*v*) sodium azide and 360 µl of distilled water, giving the final concentrations of 15%(*w*/*v*) PEG 8000m 60 m*M* ammonium sulfate, 2 m*M* DTT, and 0.2%(*w*/*v*) sodium azide in the buffer of 100 m*M* sodium critrate (final pH 5.8). The protein solution was prepared by mixing 3 µl of 7%(*w*/*v*) β-octylglucoside and 27 µl of the dialyzed protein solution at 10 mg ml^{-1}, giving a concentration of 9 mg ml^{-1} *Taq* DNA polymerase in 18 m*M* Tris–HCl, 0.09 m*M* EDTA, 0.9 m*M* DTT, 90 m*M* KCl, 9%(*v*/*v*) glycerol, 0.7%(*w*/*v*) β-octylglucoside (final pH 8.2). The hanging drop (typically 6 µl) was prepared by mixing equal volumes of the above reservoir and protein solutions. The pH of the hanging drop immediately after mixing was 6.0. For different batches of the enzyme preparation, the above condition had to be adjusted slightly by lowering the pH of the 1.0 *M* sodium citrate solution to 5.25, by varying the

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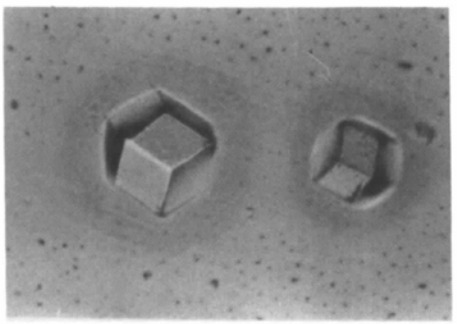
ammonium sulfate concentration in the reservoir from 30 to 90 m*M*, and by increasing the PEG 8000 concentration up to 18%(*w*/*v*). Trigonal rod crystals started to grow from the amorphous precipitate in 3 d and they reached typical dimensions of 0.1 × 0.1 × 0.8 mm in two weeks (Fig. 1*b*).

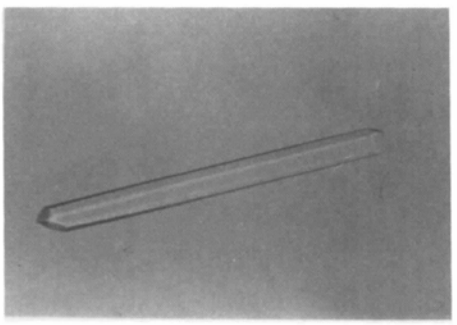
X-ray crystallographic studies

For the initial X-ray experiments at Seoul National University, crystals were mounted in thin-walled quartz capillaries. X-ray diffraction data were collected at 290 K on a FAST diffractometer system (Enraf–Nonius, Delft, The Netherlands) using the *MADNES* software (Messerschmidt & Pflugarth, 1987). Graphite-monochromatized Cu $K\alpha$ X-rays from a rotating-anode generator (Rigaku RU-200) were used.

For the cryogenic experiments, trigonal crystals were soaked for at least 2 d in the cryosolvent, which contained 100 m*M* sodium citrate, 60 m*M* ammonium sulfate, 28%(*w*/*v*) PEG 8000, 0.5%(*w*/*v*) β-octyl glucoside, and 10%(*v*/*v*) glycerol (final pH 5.8). The crystals were suspended in the film of cryosolvent in a nylon loop and were flash-frozen by plunging into a melting mixture of solid/liquid propane (Teng, 1990; D. Jeruzalmi & T. A. Steitz, personal communication). X-ray diffraction data were collected in the nitrogen-gas stream at 100 K. The diffraction pattern from flash-frozen crystals were recorded either on the R-AXIS IIc image-plate detector system using Cu $K\alpha$ X-rays or on the Weissenberg camera using 1.00 Å synchrotron X-rays at the beamline BL-6A2 of the Photon Factory, Japan (Sakabe, 1991). R-AXIS data were processed with *DENZO* (Z. Otwinowski, unpublished work) and scaled with *SCALEPACK* (Z. Otwinowski, unpublished work). Photon Factory data were processed with both *WEIS* (Higashi, 1989) and *DENZO* (Z. Otwinowski, unpublished work) and scaled with *SCALEPACK*.



(*a*)



(*b*)

Fig. 1. Two crystal forms of DNA polymerase from *Thermus aquaticus*. (*a*) Rhombohedral crystals (form I) grown from ammonium sulfate solution. Their dimensions are approximately 0.2 × 0.2 × 0.2 mm. (*b*) A trigonal crystal (form II) grown from polyethylene glycol solution. Its dimensions are approximately 0.1 × 0.1 × 0.8 mm.

Results and discussion

Two crystal forms of DNA polymerase from *T. aquaticus* have been grown at room temperature. When exposed to Cu $K\alpha$ X-rays at 290 K rhombohedral crystals (form I, Fig. 1*a*) grown from ammonium sulfate solution diffracted poorly to 10 Å only and, thus, are not suitable for X-ray structure determination. Trigonal crystals (form II, Fig. 1*b*) grown from polyethylene glycol solution are more suitable for structure detemination, since the diffraction pattern extends to 2.5 Å at cryogenic temperatures upon exposure to synchrotron X-rays.

When rod-shaped trigonal crystals (Fig. 1*b*) were exposed to Cu $K\alpha$ X-rays at 290 K significant diffraction intensities were observable on the FAST area detector to about 3.5 Å Bragg spacing. However, these crystals experienced severe radiation damage at this temperature and lost diffraction intensities beyond about 5 Å within 2 h. The unit-cell parameters determined from a set of low-resolution data were $a = 110$ and $c = 172$ Å.

The radiation damage of trigonal crystals was overcome by collecting diffraction data at cryogenic temperature (about 100 K). With Cu $K\alpha$ X-rays, the diffraction pattern from flash-frozen crystals could be observed to about 3.2 Å on the image-plate detector of the R-AXIS IIc system. With 1.00 Å synchrotron X-rays, diffraction spots to 2.5 Å could be observed on the image plate. Unit-cell parameters were determined as $a = 106.7$ and $c = 169.7$ Å by the program *SCALEPACK*. The flash-freezing process gave reproducible unit-cell dimensions. The space group was determined to be $P3_121$ (or its enantiomorph $P3_221$) by inspecting the intensity distribution of the data. The asymmetric unit contains a single molecule, giving a crystal volume per protein mass (V_m) of 3.0 Å^3 Da^{-1} and a solvent content of 58% by volume, using a molecular mass of 94 000 Da (Lawyer *et al.*, 1989). A nearly complete set of native data has been collected to 2.7 Å Bragg spacing from a flash-frozen crystal at Photon Factory. The final data set consists of 160 820 measurements of 32 463 unique reflections with an $R_{\rm merge}$ (on intensity) of 8.3%. This represents 92% of theoretically observable reflections up to 2.70 Å. The outermost shell of data between 2.70 and 2.82 Å is 55.1% complete and has an average $I/\sigma(I)$ of 2.2 The next shell between 2.82 and 2.97 Å is 92.4% complete and has an average $I/\sigma(I)$ of 3.7. Structure determination by a combination of multiple isomorphous replacement and molecular replacement is underway.

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