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Crystallization and preliminary X-ray diffraction studies of a DNA excision repair enzyme, UvrB, from *Thermus thermophilus* HB8

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A DNA excision repair enzyme, UvrB, from *Thermus thermophilus* HB8 was crystallized by the vapor-diffusion method using lithium sulfate as the precipitant and β-octylglucoside as an additive. The crystals belong to the trigonal space group $P3_121$ or $P3_221$, with unit-cell dimensions of $a = b = 136.0$ and $c = 108.1$ Å. The crystal is most likely to contain one UvrB protein in an asymmetric unit with the V_m value of 3.8 Å^3 Da^{-1}. The crystals diffracted X-rays beyond 2.9 Å resolution. Although the crystals were sensitive to X-ray irradiation at room temperature, the frozen crystals at 100 K showed no apparent decay during the intensity measurement.

1. Introduction

DNA is easily damaged with UV radiation as well as with various chemical agents. Such damage may cause mutagenesis or cell death. Living organisms have DNA repair systems with which genetic information is preserved (Friedberg *et al.*, 1995). Among various DNA repair systems, nucleotide excision repair is one of the most important repair systems because this is involved in the repair of a wide range of DNA damage (Van Houten, 1990; Grossman & Thiagalingam, 1993; Sancar, 1996). Extensive studies of this repair system for *Escherichia coli* revealed that the UvrA, UvrB, and UvrC proteins are involved in the recognition and excision of the damaged segment of DNA and that UvrD helicase, DNA polymerase I, and DNA ligase are involved in completing the DNA.

It has been shown that the $UvrA_2B$ complex recognizes a lesion in DNA as the first step of this excision process and that after dissociation of the UvrA from the $UvrA_2B$–DNA complex by using ATP hydrolysis the UvrB–DNA complex forms a complex with the UvrC protein to carry out the incision reactions at both 3′ and 5′ sides of the lesion in DNA (Grossman & Thiagalingam, 1993; Orren & Sancar, 1989). Thus, the UvrA, UvrB, and UvrC proteins play a fundamental role in recognition and incision of the lesion in DNA, but little is known about the mechanism.

Recently we cloned and sequenced the *uvrA* (Yamamoto *et al.*, 1996) and *uvrB* (Kato *et al.*, 1996) genes from an extremely thermophilic bacterium, *T. thermophilus* HB8. The deduced amino-acid sequences of the UvrA and UvrB proteins have identities of 59 and 54%, respectively, with the corresponding proteins of *E. coli*. Thus, the functional mechanisms as well as the three-dimensional structures of these proteins are similar in these organisms.

The UvrB protein of *T. thermophilus* HB8 was overexpressed in *E. coli*, and its ATPase and DNA-binding activities have been characterized (Kato *et al.*, 1996). As expected this protein is stable in wide ranges of temperature (278–353 K at pH 7.5) and pH (pH 6–11 at 298 K). The UvrB protein from *T. thermophilus* HB8 is a single polypeptide chain consisting of 665 amino-acid residues. Limited proteolysis of this protein suggested that it consists of four structural domains (N, M, C1, and C2); the N and C1 domains are necessary for ATPase activity, the C1 domain is indispensable for DNA binding, and the N and/or M domains are involved in UvrA binding (Nakagawa *et al.*, 1997). However, the mechanism by which the UvrB protein binds specifically to the lesion in the DNA and participates in excision of the DNA remains unclear. In order to understand the functions of the UvrB protein, we started the analysis of its three-dimensional structure by X-ray crystallography. Here we describe the crystallization and preliminary X-ray diffraction analysis of the UvrB protein from *T. thermophilus* HB8.

2. Experimental and results

2.1. Crystallization

The UvrB protein was purified by the procedure described previously (Kato *et al.*, 1996). The solution of the UvrB protein contained about 14 mg ml^{-1} protein, 10 m*M* β-mercaptoethanol, 1 m*M* EDTA, and 10%(*v*/*v*) glycerol in 50 m*M* Tris–HCl buffer (pH 7.5).

Crystallization experiments of the UvrB protein were performed with the hanging-drop

vapor-diffusion method at 293 K. In a typical experiment, 5 µl of the protein solution was mixed with the same volume of the reservoir solution and equilibrated against 1 ml of the reservoir solution. An initial attempt of crystallization using Crystal Screen I (Hampton Research) was unsuccessful. When lithium sulfate was used as the precipitant at pH 6.0 in various crystallization conditions, small rod-shaped crystals appeared. However they did not grow to sufficient size for X-ray diffraction experiment. Significant improvement in the size of the crystal and reproducibility was achieved when β-octylglucoside was added; bipyramidal-shaped crystals appeared after a few days and reached a maximum size (*ca* $0.5 \times 0.5 \times 0.4$ mm) in a few weeks (Fig. 1). The reservoir solution that gave the best crystallization result contained 1.78 *M* lithium sulfate, 0.5%(*w*/*v*) β-octylglucoside, and 100 m*M* MES (pH 6.0).

2.2. X-ray diffraction studies

The crystal was sealed in a glass capillary tube with a small amount of the mother liquor. Diffraction data were collected on the R-AXIS IV image-plate (Rigaku) system at room temperature using Ni-filtered Cu Kα radiation. X-rays, generated with a Rigaku rotating anode at 40 kV and 100 mA, were focused with Ni-coated double bent mirrors. Diffraction data recorded on the image-plate were processed using the R-AXIS software package (Higashi, 1990). The crystals belong to trigonal system, with unit-cell dimensions $a = b = 136.0$ and $c = 108.1$ Å. The symmetry and the observed systematic absence of the diffraction data are compatible with space group $P3_121$ or $P3_221$. Assuming that one UvrB protein molecule is present in an asymmetric unit, the V_m value is 3.8 Å^3 Da^{-1} (Matthews, 1968). The possibility that the asymmetric unit contains more than one UvrB molecule is less likely based on the fact that the DNA binding proteins with molecular size similar to that of the UvrB protein, such as *Taq* DNA polymerase (Kim *et al.*, 1995) and PcrA helicase (Subramanya *et al.*, 1996), have relatively high V_m values compared with those for standard proteins. The present UvrB protein crystals diffracted X-rays to about 3.2 Å resolution with a conventional X-ray source and to beyond 2.9 Å resolution with synchrotron radiation. An initial intensity data collection with the conventional equipment at room temperature, however, yielded a data set up to about 3.8 Å resolution due to radiation sensitivity of the crystal (crystal 1 in Table 1).

In order to stabilize the crystal against X-ray irradiation the crystal was mounted in the loop and flash-cooled to 100 K using an Oxford Cryosystems Cryostream. As the present crystallization solution contains 10%(*v*/*v*) glycerol, the crystal was successfully frozen without soaking in any other cryoprotectant solution. Upon freezing the cell dimensions changed slightly to $a = b = 133.9$ and $c = 106.6$ Å. The frozen crystals showed no apparent decay against X-ray irradiation; complete data were collected to 3.3 Å resolution with the conventional X-ray source (crystal 2 in Table 1).

Data collection with higher resolution and signal-to-noise ratio [$I/\sigma(I)$] for the frozen crystal was achieved using synchrotron radiation with a screenless Weissenberg camera for macromolecular crystals (Sakabe, 1991) at the BL6B of the Photon Factory at the National Laboratory for High Energy Physics. The radius of the cassette, which contained a Fuji imaging plate (IP) with a detection area of 80×40 cm, was 573 mm. The diffraction intensities recorded on each IP were read out with Rigaku drum scanner. They were processed and scaled with *DENZO* and *SCALEPACK* (Otwinowski, 1993). Results of the intensity measurement are shown in Table 1 (see crystal 3).

Table 1 shows that the present crystals yield diffraction data up to 2.9 Å resolution and are suitable for X-ray analysis at atomic resolution. The search for heavy-atom derivatives and the preparation of the selenomethionyl UvrB protein are under way.

Table 1
Intensity measurements of the UvrB protein crystals.

	Crystal 1	Crystal 2	Crystal 3
Temperature (K)	296	100	100
Wavelength (Å)	1.5418	1.5418	1.00
Oscillation angle (°)	1.5	1.5	3.0
Resolution limit (Å)	3.8	3.3	2.9
Measured reflections	18947	57702	73897
Independent reflections	8360	15635	22881
Completeness (%)†	68.6 (53.7)	88.2 (78.5)	90.9 (82.4)
Mean $I/\sigma(I)$†	5.0 (3.1)	8.5 (4.2)	14.5 (4.8)
$R_{\rm merge}$ (%)†‡	7.7 (14.9)	7.1 (12.9)	7.5 (21.0)

† Reflections with $F < \sigma(F)$ were rejected. Numerals in parentheses are for the data in the highest resolution shell. ‡ $R_{\rm merge} = \sum_h \sum_i |I_{hi} - \langle I_h \rangle| / \sum_h \sum_i I_{hi}$.

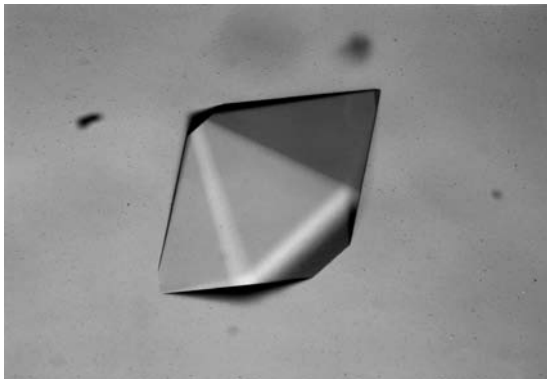

Figure 1
Trigonal crystal of UvrB protein from *T. thermophilus* HB8. The crystal size is approximately $0.5 \times 0.5 \times 0.4$ mm.

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