X-Ray Crystallographic Studies on DNA Repair Enzymes

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Various chemical agents and physical agents such as UV light as well as replication error of DNA may lead to damage or errors in DNA, which induce mutagenesis and carcinogenesis. Living organisms have DNA repair systems with which genetic information is preserved. UvrB protein acts as a central role in nucleotide excision, one of the most important repair systems [1]. The UvrB protein of *Thermus thermophilus* HB8 used in this study is a single polypeptide chain consisting of 665 amino acid residues [2].

The UvrB protein was expressed, purified, and characterized as described [2]. The crystals of this protein obtained by the vapor diffusion method belong to the space group $P3_121$ or $P3_221$, with unit-cell dimensions of a=b=136.0 Å and c=108.1 Å [3]. The crystal was flash cooled to 100K. Its diffraction data were recorded on the imaging plates (IPs) with oscillation mode ($\Delta\omega$=2°) using the camera at BL41XU. Data were processed and scaled in a standard way. Summary of the data collection is shown in Table 1.

Table 1. Summary of the data collection

	Native-1	Native-2	Pt-Derivative	Hg-Derivative
Wavelength (Å)	0.708	0.864	0.864	0.864
Crystal to IP distance (mm)	400	400	400	400
No. of IPs	40	30	40	33
Resolution (Å)	1.8	2.8	2.8	2.2
No. unique reflections	97,801	24,721	26,741	55,815
Completeness (%)	95.0	90.1	96.6	98.9
R_{merge} (%)	6.7	4.2	4.7	5.2

Remarkably, the data to 1.8 Å resolution were collected for the native crystal (<I/σ>= 2.2 and R_{merge}=26.4% for 1.83-1.80 Å shell). The anomalous (Fig. 1) and isomorphous difference Patterson maps for the Pt-deriv. have shown peaks at the same sites.

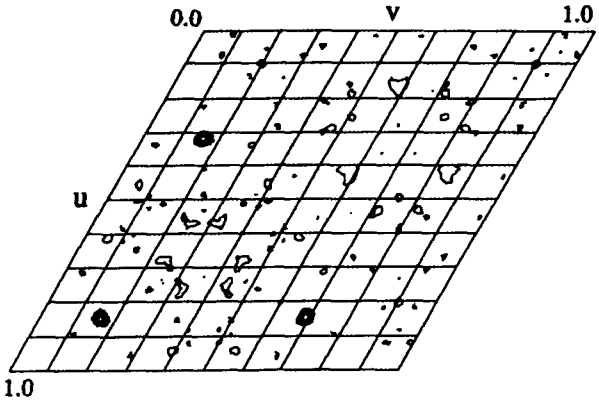

Fig. 1. Anomalous diff. Patterson map at 4.5 Å

References

1) E.C. Friedberg, G.C. Walker & W. Siede. DNA Repair and Mutagenesis. Amer. Soc. Microb. Press, Washington D.C. (1995).
2) R. Kato *et al. J. Biol. Chem.* **271**, 9612-9618 (1996).
3) A. Shibata *et al. Acta Crystallogr. Sec. D,* in press.