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Cloning, expression, purification, crystallization and preliminary diffraction analysis of the C-terminal catalytic domain of human poly(ADP-ribose) polymerase

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Two fragments of the C-terminal catalytic domain of human poly(ADP-ribose) polymerase (catPARP), Met-catPARP and Gly-Ser-catPARP, were purified and crystallized. Both catPARP crystals belong to space group *C*2, with almost the same unit-cell parameters. However, the shapes and harvest periods of both crystals were quite different owing to the slight mutation at the N-terminal position. Gly-Ser-catPARP was found to be more suitable for X-ray crystallography and crystals showed diffraction to at least 3.5 Å resolution.

1. Introduction

Poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30), a eukaryotic DNA-binding enzyme that participates in cell recovery from DNA damage (Oei *et al.*, 1997), consists of three functional domains: an N-terminal DNA-binding domain, a C-terminal catalytic domain and a central automodification domain (de Murcia & Menissier de Murcia, 1994). PARP binds to and is activated by DNA-strand breaks and catalyses the synthesis of homopolymers of ADP-ribose from NAD^+ onto nuclear acceptor proteins. PARP itself is the main protein acceptor (automodification), but the enzyme has also been shown to modify histones, topoisomerases, DNA polymerases and ligases (D'Amours *et al.*, 1999). The formation of these negatively charged polymers in the vicinity of the DNA nick is thought to cause electrostatic repulsion of PARP from the DNA and to facilitate recruitment of the base-excision repair complex (Dantzer *et al.*, 1999). The ADP-ribose polymers formed by PARP are cleaved by the cellular hydrolase poly(ADP-ribose) glycohydrolase. PARP activation and the rapid synthesis and degradation of ADP polymers can result in abrupt and profound cellular NAD^+ depletion.

Cytotoxic drugs or radiation can induce activation of PARP and it has been demonstrated that inhibitors of PARP can potentiate the DNA-damaging and cytotoxic effects of chemotherapy and irradiation (Delaney *et al.*, 2000). Because of the essential function of PARP in cellular repair and survival, evaluation of the potential of novel potent PARP inhibitors for the treatment of cancer in combination with selected cytotoxic agents has been described. These inhibitors have been designed to mimic the substrate–protein interactions of NAD^+ and PARP using the crystal structure of chicken catPARP (Ruf *et al.*, 1998; White *et al.*, 2000; Koch *et al.*, 2002). However, it should be advantageous to use the human structure in order to design more selective and effective drugs for humans. Therefore, we searched for suitable conditions for X-ray analysis of the catalytic domain of human PARP.

2. Methods

2.1. Cloning and expression

The PARP gene for Gly-Ser-catPARP was PCR amplified using an EST clone (Incyte Genomics) and cloned into the pGEX4T-2 vector (Amersham Pharmacia) by the *Eco*RI enzyme with a fused protein, glutathione *S*-transferase (GST), and a thrombin-cleavage site. Sequencing of the recombinant DNA performed using a PRISM310 genetic analyser (Applied Biosystems) confirmed the integrity of the cloned DNA. Luria broth medium containing 50 mg ml^{-1} ampicillin was inoculated with a preculture of the DH5α strain (Toyobo) containing the Gly-Ser-catPARP construct. Bacterial growth was performed at 310 K to an OD_{650nm} of 0.5. Expression was induced with 1 m*M* isopropyl-β-D-1-thio-galactopyranoside for 4 h at 310 K. The cells were harvested by centrifugation and washed with buffer *A* (50 m*M* Tris–HCl, 50 m*M* NaCl, 1 m*M* EDTA, 1 m*M* dithiothreitol pH 8.0). The cell pellet was stored frozen at 243 K. At the time of purification, the cell pellet was thawed and the cells were resuspended in buffer *A*. After addition of up to 1% Triton X-100, the suspension was sonicated and cell debris was removed by centrifugation. The supernatant containing the GST-fused protein was checked by SDS–PAGE and Western-blotting experiments with anti-GST antibody (Amersham Pharmacia) and anti-PARP antibody (Santa Cruz).

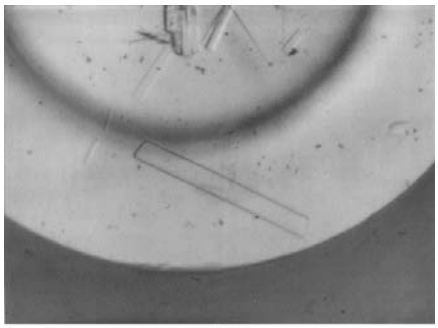
2.2. Purification

The supernatant was mixed with glutathione Sepharose 4B (Amersham Pharmacia) and incubated for 3 h at 277 K. The resin was washed five times with 10 ml buffer *B* [0.5% Triton X-100 (Sigma) in PBS (Shinyo Chemical)] and incubated after the addition of 50 U ml^{-1} thrombin (Amersham Pharmacia) overnight. Harvest supernatant contained the desired PARP, as checked by SDS–PAGE and Western-blotting experiments with anti-PARP antibody. The Gly-Ser-catPARP protein was concentrated and loaded onto a Superdex 200 HR (Amersham Pharmacia) size-exclusion column. Homogeneous protein was purified from this column by isocratic elution with buffer *C* (50 m*M* Tris–HCl, 150 m*M* NaCl, 1.5 m*M* dithiothreitol pH 7.5).

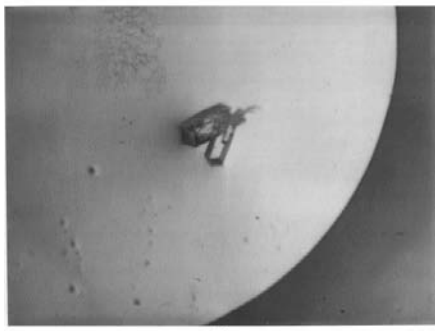
Met-catPARP protein was purchased from Trevigen and purified in a single step using the same type of size-exclusion column as used for the Gly-Ser-catPARP protein.

2.3. Crystallization

Initial crystals were obtained by the hanging-drop vapour-diffusion method using a Crystal Screen kit (Hampton Research). Crystals were obtained under condition No. 39 (0.1 *M* HEPES, 2.0 *M* ammonium sulfate, 2% polyethyleneglycol pH 7.5) and conditions were refined using the sitting-drop vapour-diffusion method. The best crystals were obtained using 4 µl protein solution at 14 mg ml^{-1} in buffer *D* [25 m*M* Tris–HCl, 3.4 m*M* β-mercaptoethanol, 0.1%(*w*/*v*) β-octyl-glucopyranoside pH 8.5] mixed with 4 µl mother liquor.



(*a*)



(*b*)

Figure 1
CatPARP crystals obtained using the conditions described in Table 1. (*a*) Met-catPARP. (*b*) Gly-Ser-PARP.

Table 1
Crystallization conditions and X-ray data-collection characteristics of catPARPs.

	Met-catPARP	Gly-Ser-catPARP
Crystallization conditions	1.9–2.0 *M* $(NH_4)_2SO_4$, 2% PEG 400, 0.1 *M* Tris–HCl pH 8.0	2.1–2.2 *M* $(NH_4)_2SO_4$, 2% PEG 400, 0.1 *M* Tris–HCl pH 8.0
Crystallization period	6 months	3 d
Crystal size (mm)	0.6 × 0.05 × <0.01	0.2 × 0.04 × 0.04
Space group	*C*2 (?)	*C*2
Unit-cell parameters (Å, °)	*a* = 181, *b* = 54, *c* = 91, β = 114	*a* = 179.82, *b* = 53.77, *c* = 92.01, β = 114.4
Upper diffraction limit (Å)	5.0	3.5

2.4. X-ray diffraction data collection

Crystals of each protein were mounted in a nylon loop (Hampton Research) and flash-cooled to 100 K in an N_2 gas stream. Diffraction data sets were collected at beamline 6B of the Photon Factory (PF-KEK) using an imaging-plate detector (Sakabe *et al.*, 1997). A wavelength of 1.00 Å and a crystal-to-detector distance of 573 mm were used. Data integration was performed with *DENZO* and scaling and merging were performed using *SCALEPACK*; both programs are from the *HKL* package (Otwinowski & Minor, 1997).

3. Results and discussion

Initially, thin plate-shaped crystals of Met-catPARP protein were obtained (Fig. 1*a*). However, these crystals were unsuitable for crystallographic experiments because of weak diffraction arising from their plate-like shape. Furthermore, six months were necessary for crystal growth. Therefore, we gave attempts to modify of the Met-catPARP crystals and designed the Gly-Ser-catPARP protein for the next crystallization for the following reasons: (i) the N-terminal portion of each protein has similar spatial dimensions for crystal packing and similar crystallization conditions could be expected; (ii) the solubility change may provide a new molecular interaction. As a result, prism-shaped Gly-Ser-catPARP crystals (Fig. 1*b*) were obtained under similar conditions to those used to crystallize Met-catPARP. However, the shape of the Gly-Ser-catPARP crystals was much better than that of the Met-catPARP crystals, even though the crystals have almost the same crystallographic parameters (Table 1). Furthermore, the harvest period of the Gly-Ser-catPARP crystals was shortened to 3 d compared with the six months required for the Met-catPARP crystals. These favourable features of the Gly-Ser-catPARP crystals may be because of fast growth along the *a* axis, which is the slowest-growing and therefore the thinnest direction in the Met-catPARP crystals. Full data-processing statistics can be found in Table 2.

Table 2
Data-collection and processing statistics for Gly-Ser-catPARP.

Values in parentheses refer to data in highest resolution shell.

Wavelength (Å)	1.00
Resolution (Å)	50.0–3.5
Total No. of reflections	38475
No. of unique reflections	9423
Completeness (%)	96.4 (95.8)
R_{sym} (%)	7.5 (24.9)
$I/\sigma(I)$	9.0 (2.1)

Molecular replacement with a starting model of chicken catPARP (PDB code 1a26), which has 87% identical residues to the human protein, led to two solutions. With two monomers in the asymmetric unit, the determined Matthews coefficient is 2.6 $Å^3\ Da^{-1}$ (Matthews, 1968) and the solvent content is 53%. Some portions of the obtained electron-density map were ambiguous.

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