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Initial crystallographic study of human PCNA in complex with a peptide containing the noncanonical PIP-box sequence of human DNA polymerase ι

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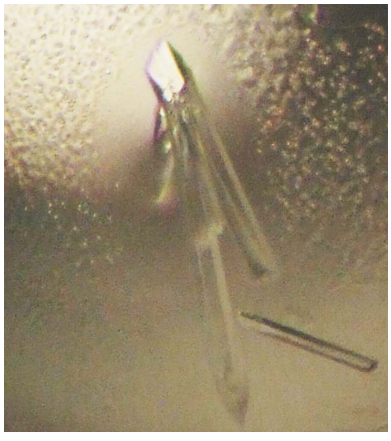
Human DNA polymerase ι (Polι) is one of the Y-family DNA polymerases involved in translesion synthesis (TLS), which allows continued replication at damaged DNA templates. Polι has a noncanonical PCNA-interacting protein box (PIP-box) within an internal region of the protein. Polι activity is stimulated by PCNA binding through the noncanonical PIP-box. To clarify the interaction of PCNA with the noncanonical PIP-box of Polι, PCNA and a Polι peptide carrying the noncanonical PIP-box complex have been cocrystallized. The crystal belongs to space group *C*2, with unit-cell parameters $a = 167.1$, $b = 68.7$, $c = 90.0$ Å, $\beta = 95.1^\circ$. Structural analysis by molecular replacement is in progress.

1. Introduction

Genomic DNA is constantly damaged by endogenous and environmental factors. DNA damage in the S-phase often stalls DNA replication by the replicative polymerases Polδ or Polε because they possess compact active sites. Translesion synthesis (TLS) is one of the DNA damage-tolerance mechanisms which allows the continuation of DNA synthesis at damaged templates. During TLS, Polδ or Polε are replaced by specialized DNA polymerases which can perform DNA synthesis using damaged templates. The specialized polymerases are termed 'TLS polymerases' and include Y-family DNA polymerases such as Polη, Polκ and Polι (Ohmori *et al.*, 2001). Because of their wide active sites, the three TLS polymerases mentioned above can accommodate DNA templates containing bulky damaged bases. Thus, TLS polymerases can perform bypass synthesis using damaged DNA templates in a lesion-specific manner. For instance, Polη is able to efficiently incorporate two adenines opposite a thymine–thymine (T–T) cyclobutane pyrimidine dimer (Masutani *et al.*, 2000), which is one of the major photoproducts resulting from UV irradiation. Polι efficiently incorporates an adenine opposite the 3′-T of the 6–4 T–T pyrimidine–pyrimidone photoproduct (Tissier *et al.*, 2000; Vaisman *et al.*, 2003). Polκ bypasses dG-N2-BPDE (benzo[*a*]pyrene diol epoxide) by inserting the correct cytosine opposite the bulky lesion (Suzuki *et al.*, 2002; Zhang *et al.*, 2000, 2002).

Proliferating cell nuclear antigen (PCNA) forms a ring-shaped homotrimer with a molecular weight of ∼90 kDa and functions as a sliding clamp which tethers DNA polymerases onto primer termini. Many proteins involved in replication, repair, the cell cycle, chromatin assembly and sister-chromatid cohesion interact with PCNA (Moldovan *et al.*, 2007). Most such proteins have a PCNA-interacting protein box (termed a 'PIP-box'; Warbrick, 1998). The canonical PIP-box is composed of eight amino-acid residues Q*xxhxxaa*, where the first residue Q is conserved, *h* is a hydrophobic residue such as Met, Leu or Ile and *a* is an aromatic residue such as Phe or Tyr. To date, the structures of human PCNA bound to peptides derived from human p21, the p66 subunit of human Polδ, an artificial PL peptide and the full-length human flap endonuclease I protein have been determined (Gulbis *et al.*, 1996; Bruning & Shamoo, 2004; Kontopidis *et al.*, 2005;



Sakurai *et al.*, 2005). The peptides and protein all include a canonical PIP-box located at the extreme C-terminus and the structures revealed that the interactions of the canonical PIP-boxes with PCNA are very similar to each other. In contrast, the PIP-boxes of the abovementioned three TLS polymerases do not contain the canonical sequence: they lack the conserved Gln residue. The canonical Gln residue is replaced by Met, Lys and Lys in Polη, Polκ and Polι, respectively, and it has been reported that DNA synthesis by these TLS polymerases is stimulated by PCNA binding *via* their non-canonical PIP-boxes (Haracska *et al.*, 2001*a*,*b*; Haracska *et al.*, 2002; Vidal *et al.*, 2004).

Human Polι is composed of 715 amino acids and has a molecular weight of 80 kDa (McDonald *et al.*, 1999). The noncanonical PIP-box of Polι has been identified as 420-KKGLIDYY-427 (Vidal *et al.*, 2004) and is located in an internal region of the protein, whereas Polη and Polκ have noncanonical PIP-boxes at their extreme C-termini. To reveal the interaction of human PCNA with the internal noncanonical PIP-box of Polι, we performed a structural study of the PCNA–Polι peptide complex. Here, we report a crystallization and X-ray diffraction study of human PCNA in complex with a Polι peptide carrying the noncanonical PIP-box.

2. Methods and results

Recombinant PCNA was expressed in *Escherichia coli* BL21 (DE3) harbouring a pT7-PCNA expression vector (Fukuda *et al.*, 1995). To date, various procedures for the preparation of recombinant hPCNA have been reported (Gulbis *et al.*, 1996; Zheleva *et al.*, 2000; Sakurai *et al.*, 2005; Bruning & Shamoo, 2004; Kontopidis *et al.*, 2005). However, the protein was purified in a similar manner to that described previously for the PCNA G178S mutant (Hishiki *et al.*, 2008). In brief, bacterial cells were lysed by sonication and the supernatant of the cell lysate was purified using HiTrap Q HP and HiTrap Phenyl HP columns (GE Healthcare). The fractions containing PCNA were finally purified using a HiLoad 26/60 Superdex 200 column (GE Healthcare) equilibrated with 10 m*M* HEPES–NaOH pH 7.4 and 100 m*M* NaCl. The purified protein was concentrated to 10 mg ml^{-1} using Amicon Ultra (Millipore) and frozen using liquid N_2.

A peptide composed of residues 415–437 of human Polι was commercially synthesized (GenScript Co., USA). A tenfold molar excess of the Polι peptide (ALNTAKKGLIDYYLMPSLSTTSR) was incubated with PCNA (7.0 mg ml^{-1}). Cocrystals of PCNA–Polι peptide were grown by hanging-drop vapour diffusion at 293 K. Typical hanging drops were prepared by mixing 0.9 µl protein solution and 0.9 µl reservoir solution containing 25% PEG 2K MME, 100 m*M* sodium cacodylate pH 6.4 and 200 m*M* potassium thiocyanate. Rod-shaped crystals were obtained in a few weeks (Fig. 1). Crystals were transferred to a cryo-buffer (22.5% PEG 3350, 100 m*M* sodium cacodylate pH 6.4, 200 m*M* potassium thiocyanate and 20% ethylene glycol) for cryoprotection. Crystals were then flash-frozen in an N_2-gas stream at 100 K. X-ray diffraction data were collected from PCNA–Polι peptide crystals using an ADSC Quantum 210 CCD detector on beamline NW12A at Photon Factory Advanced Ring (PF-AR; Fig. 2). The diffraction data were indexed, integrated and scaled using *HKL*-2000 (Otwinowski & Minor, 1997). Diffraction data statistics are given in Table 1. The crystal belongs to space group *C*2, with unit-cell parameters $a = 167.1$, $b = 68.7$, $c = 90.0$ Å, $\beta = 95.1^\circ$. The asymmetric unit is estimated to contain one trimer of PCNA–Polι peptide, with a corresponding crystal volume per protein weight (V_M)

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell (2.59–2.50 Å).

Space group	*C*2
Unit-cell parameters	
a (Å)	167.1
b (Å)	68.7
c (Å)	90.0
β (°)	95.1
V_M (Å^3 Da^{-1})	3.0
Solvent content (%)	59
Wavelength (Å)	1.0000
Resolution range (Å)	50.0–2.50
Measured reflections	122188
Unique reflections	35169
Completeness (%)	99.1 (97.4)
Mean $I/\sigma(I)$	9.9 (4.2)
R_{merge}† (%)	8.9 (27.6)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

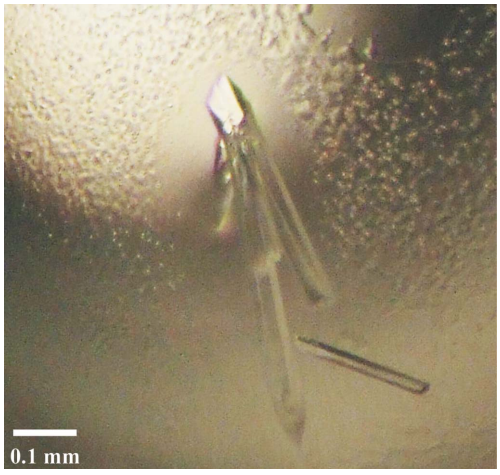


Figure 1
Crystals of the PCNA–Polι peptide complex.

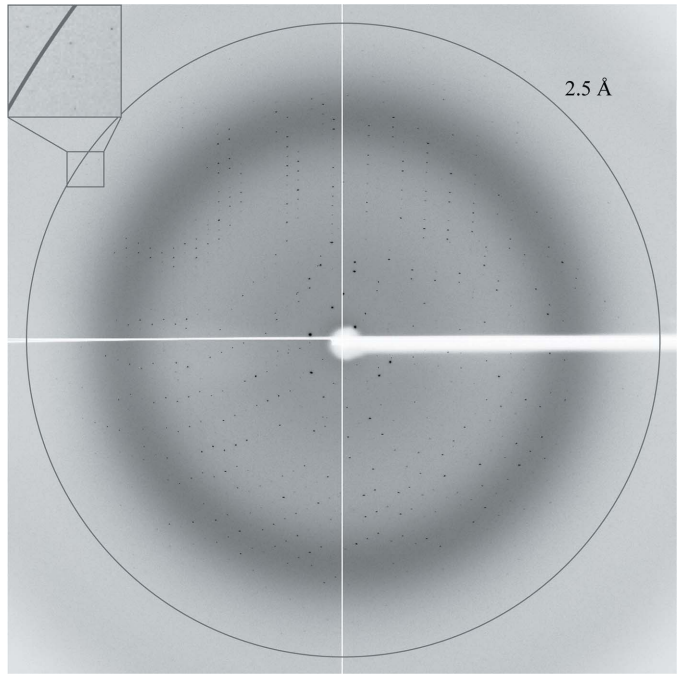


Figure 2
A diffraction pattern from a crystal of the PCNA–Polι peptide complex.

of 3.0 Å^3 Da^{-1} and a solvent content of 59%. Structural determination by molecular replacement is now in progress.

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