Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Flexibility revealed by the 1.85 Å crystal structure of the β sliding-clamp subunit of *Escherichia coli* DNA polymerase III

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Received 18 February 2003
Accepted 6 May 2003

PDB Reference: β subunit of DNA polymerase III, 1mmi, r1mmisf.

The β subunit of the *Escherichia coli* replicative DNA polymerase III holoenzyme is the sliding clamp that interacts with the α (polymerase) subunit to maintain the high processivity of the enzyme. The β protein is a ring-shaped dimer of 40.6 kDa subunits whose structure has previously been determined at a resolution of 2.5 Å [Kong *et al.* (1992), *Cell*, **69**, 425–437]. Here, the construction of a new plasmid that directs overproduction of β to very high levels and a simple procedure for large-scale purification of the protein are described. Crystals grown under slightly modified conditions diffracted to beyond 1.9 Å at 100 K at a synchrotron source. The structure of the β dimer solved at 1.85 Å resolution shows some differences from that reported previously. In particular, it was possible at this resolution to identify residues that differed in position between the two subunits in the unit cell; side chains of these and some other residues were found to occupy alternate conformations. This suggests that these residues are likely to be relatively mobile in solution. Some implications of this flexibility for the function of β are discussed.

1. Introduction

The *Escherichia coli* DNA polymerase III (Pol III) holoenzyme is the complex molecular machine primarily responsible for chromosomal DNA synthesis. Interactions among the ten different protein subunits in the holoenzyme and their associations with other replicative proteins account for the high efficiency, processivity and fidelity of DNA synthesis by the replisome (Kelman & O'Donnell, 1995; Baker & Bell, 1998; O'Donnell *et al.*, 2001).

The β subunit is a ring-shaped dimer of identical 40.6 kDa subunits that encircles the nascent double-stranded DNA at the replication fork, where it travels behind and interacts with the polymerase (α) subunit (O'Donnell *et al.*, 1992; Hingorani & O'Donnell, 2000). The action of β as a sliding clamp confers essentially infinite processivity on the actions of the holoenzyme: DNA synthesis on the leading strand at replication forks may well occur without dissociation of the polymerase during synthesis of half of the chromosome. β is loaded onto primer templates through its interactions with the clamp-loading (γ) complex, a multisubunit component of the holoenzyme that comprises three γ and/or τ subunits, as well as single molecules of the δ, δ', χ and ψ subunits (Jeruzalmi, O'Donnell *et al.*, 2001; Jeruzalmi *et al.*, 2002; O'Donnell *et al.*, 2001).

The structure of the β dimer determined by X-ray crystallography at 2.5 Å resolution was reported by Kong *et al.* (1992), who used data collected at room temperature with crystals of space group $P2_1$. More recently, the structure of a

monomeric mutant form of β in complex with the δ subunit of the γ complex has been determined at 2.5 Å resolution (Jeruzalmi, Yurieva *et al.*, 2001). In addition, we recently reported biochemical experiments that indicate that peptides corresponding in sequence to the β-interacting element of δ identified by crystallography, as well as a putative β-interacting region of α, do interact with β and prevent its binding to both δ and α (Dalrymple *et al.*, 2001). This suggests that the sites of interaction of δ and α on β overlap. With the intention of confirming this directly by solving the crystal structures of complexes of β with various peptides, we first purified β in large quantities from a new overproducing strain of *E. coli* and crystallized it under conditions similar to those used previously (Kong *et al.*, 1992). Collection of data at 100 K at a synchrotron source enabled refinement of the structure of the β dimer at 1.85 Å and this has enabled a detailed examination of the differences between the structures of the two separate subunits in the crystal lattice. These comparisons give new insights into the mobility of regions of the β dimeric ring in solution.

2. Materials and methods

2.1. Overproduction of the β subunit of DNA polymerase III

The plasmid pSJS9 (Blanar *et al.*, 1984), used as source of the *dnaN* gene that encodes β, was a gift from Dr Steven Sandler. The bacteriophage λ promoter vectors pCE30 and pND201 were as described by Elvin *et al.* (1990). Vector pND230 is a derivative of pCE30 that contains sites for cleavage by *Hpa*I and *Nco*I, plus an additional *Bam*HI site, between the *Bam*HI and *Sma*I sites (Elvin *et al.*, 1990). The chromosome-derived DNA in pSJS9 contains *dnaN* within a *Dra*I–*Nco*I fragment that contains two sites for cleavage by *Eco*RV. One of these occurs 49 bp before the *dnaN* start codon; the other is within the gene. The 1985 bp *Dra*I–*Nco*I fragment was first isolated and then cleaved (partially) by *Eco*RV. The 1292 bp *dnaN*$^+$ fragment was isolated and inserted between the *Hpa*I and *Nco*I sites of pND230 to give plasmid pND220 (5328 bp), thus placing *dnaN* between the two *Bam*HI sites. pND220 directed overproduction of β to a modest level (data not shown).

To improve overproduction of β, the natural *dnaN* ribosome-binding site was removed and replaced as follows. Plasmid pND220 was digested with *Bam*HI. The 1326 bp *dnaN*$^+$ fragment was isolated and treated with sufficient exonuclease BAL31 to remove about 60 bp of DNA from each end. The resulting library of blunt-ended fragments was ligated with plasmid pND201 that had been linearized with *Hpa*I (immediately following the strong ribosome-binding site in the vector). We reasoned that high-level expression of *dnaN* might adversely affect growth of the host strain, as had been observed previously with the *dnaG* gene in a similar vector (Stamford *et al.*, 1992). Therefore, 192 ampicillin-resistant transformants of strain AN1459 (Vasudevan *et al.*, 1991) were replica plated on LB agar plates at 303 and 314 K. The 24 strains that were inviable at the higher temperature were grown in LB broth at 303 K to A_{595} = 0.5, then treated for 2 h at 315 K. Analysis by SDS–PAGE showed that four of them directed high-level overproduction of β. The plasmid in one of these was retained as pND261 (Fig. 1). DNA sequence determination using vector primers as described in Elvin *et al.* (1990) showed that the ends of the BAL31 deletion that gave rise to pND261 were at 8 bp before the ATG start codon (Fig. 1) and at 59 bp beyond the TAA stop codon of the *dnaN* gene.

2.2. Purification of the β subunit of DNA polymerase III

E. coli strain AN1459/pND261 was grown in 1 l of LB medium supplemented with thymine and ampicillin (50 mg each) at 303 K until A_{595} = 0.5 and was then heated rapidly to and incubated at 315 K for 3 h. The culture was cooled in ice and cells (3.5 g) were harvested by centrifugation (10 800*g*, 10 min, 275 K), frozen in liquid nitrogen and stored at 203 K. All subsequent steps were carried out at 273–277 K. Thawed cells were resuspended in 30 ml lysis buffer (50 m*M* Tris–HCl pH 7.6, 1 m*M* EDTA, 1 m*M* dithiothreitol, 20 m*M* spermidine·3HCl) and passed twice through a French press (82.7 MPa). The homogenate was clarified by centrifugation (39 000*g*, 30 min). To the supernatant (fraction I; 27.5 ml) was added solid ammonium sulfate (8.25 g) over a period of 30 min, with stirring. Stirring was continued for a further 30 min before the precipitate was harvested by centrifugation

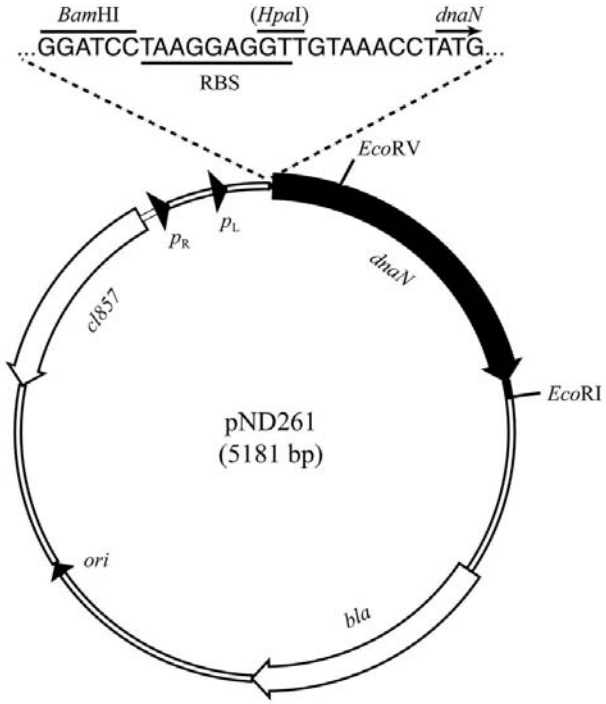

Figure 1
Plasmid pND261, which directs overproduction of the β sliding-clamp subunit of *E. coli* DNA polymerase III, is a bacteriophage λ-promoter plasmid constructed by insertion of a 1168 bp *dnaN*$^+$ fragment (generated by treatment of a larger fragment with exonuclease BAL31) at the *Hpa*I site of vector pND201 (Elvin *et al.*, 1990). This brought the start codon of *dnaN* into close proximity to the strong ribosome-binding site (RBS) in the vector (sequence shown above).

(39 000*g*, 30 min) and redissolved in a small volume of buffer *A* [50 m*M* Tris–HCl pH 7.6, 1 m*M* EDTA, 1 m*M* dithiothreitol, 20%(*v*/*v*) glycerol]. The solution was dialyzed against two changes of 2 l of buffer *A* plus 0.15 *M* NaCl to yield fraction II (13.5 ml). Fraction II was diluted with 27 ml of buffer *A* plus 0.15 *M* NaCl and passed at 1 ml min^{-1} through a column (2.5 × 15 cm) of Toyopearl TSK DEAE-650M anion-exchange resin that had been equilibrated with the same buffer. Under these conditions, the β subunit (fraction III; 64.5 ml) passed unretarded through the column. Fraction III was dialyzed against two changes of 2 l of buffer *A* and then applied to a similar column of the same resin. The column was washed with 100 ml buffer *A*, then β was eluted (in a sharp peak at ∼0.1 *M* NaCl) in a linear gradient (500 ml) of 0–0.5 *M* NaCl in the same buffer. Fractions containing β were pooled (fraction IV; 68 ml), dialyzed against three changes of 2 l buffer *B* (20 m*M* sodium phosphate pH 7.6, 1 m*M* dithiothreitol) and then passed at 1 ml min^{-1} through a column (2.5 × 11 cm) of hydroxyapatite (BioRad) that had been equilibrated in buffer *B* to yield highly purified β (fraction V; 110 ml).

For crystallization, the protein was concentrated by precipitation with ammonium sulfate (0.5 g ml^{-1}) and dialyzed against buffer *A* for storage at 203 K. It was further concentrated by use of an Ultrafree-4 centrifugal filter unit equipped with a Biomax-5K membrane (Millipore) and then diayzed against 20 m*M* Tris–HCl pH 7.5, 0.5 m*M* EDTA, 20%(*v*/*v*) glycerol. Protein concentration (determined as molarity of subunits) in freshly dialyzed solutions of purified β were determined spectrophotometrically at 280 nm, using a calculated value of ε_{280} (Gill & von Hippel, 1989) of 14 650 M^{-1} cm^{-1}. The subunit molecular weight was determined by ESI–MS using a VG Quattro II mass spectrometer with a sample that had been dialyzed extensively into 0.1% formic acid.

Table 1
Crystallographic data and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data processing	
Resolution range (Å)	50–1.85 (1.92–1.85)
No. of reflections	62043 (6056)
Completeness (%)	93.4 (91.7)
Redundancy	1.92 (1.84)
$R_{\rm merge}$† (%)	5.6 (46.7)
$I/\sigma(I)$	12.09 (1.76)
Final model statistics	
Resolution range (Å)	50–1.85 (1.90–1.85)
R‡	18.84 (25.4)
$R_{\rm free}$‡	24.46 (31.6)
$R_{\rm free}$ completeness (%)	5.0
No. of protein atoms	5865
No. of water molecules	665
Root-mean-square deviations from ideal geometry	
Bond lengths (Å)	0.008
Bond angles (°)	1.370
Chiral volumes (Å^3)	0.109
Planar groups (Å)	0.021
B factor (Å^2)	4.8
Ramachandran plot statistics	
Residues in most favoured regions (%)	92.0
Residues in generously allowed regions (%)	99.2

† $R_{\rm merge} = \sum_{hkl}\sum_i |I(hkl)_i - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i \langle I(hkl)_i\rangle$. ‡ $R = \sum_{hkl} |F_o(hkl) - F_c(hkl)| / \sum_{hkl} |F_o(hkl)|$, where F_o and F_c are the observed and calculated structure factors, respectively.

2.3. DNA-replication assays

The activity of β in DNA replication was determined by monitoring its ability to promote DnaA protein-dependent conversion of the single-stranded DNA of a recombinant bacteriophage bearing the γ2 replication origin of plasmid R6K (Masai *et al.*, 1990) to its duplex replicative form (Allen *et al.*, 1993). Highly purified replication proteins were prepared as described in Stamford *et al.* (1992) and San Martin *et al.* (1995), except for DnaA protein (Love, 1996) and DNA polymerase III* (Wold *et al.*, 1996), which were gifts from Drs Chris Love and Elliott Crooke, respectively. Assay mixtures (25 µl each) contained Tris–HCl pH 7.5 (20 m*M*), potassium glutamate (125 m*M*), dithiothreitol (8 m*M*), bovine serum albumin (2.5 µg), magnesium acetate (8 m*M*), Brij-58 [0.01%(*w*/*v*)], ATP (1 m*M*), CTP, GTP and UTP (0.25 m*M* each), dATP, dCTP and dGTP (50 µ*M* each), (^{3}H)-dTTP (50 µ*M*; 5 Bq pmol^{-1}), M13-A site single-stranded DNA (230 pmol as nucleotide), single-stranded DNA-binding protein (600 ng), DNA polymerase III* (20 units), DnaA protein (51 ng), DnaG primase (87.5 ng) and the DnaB–DnaC complex (415 ng). The reaction mixtures were assembled at 273 K and then, after addition of the β subunit (0–8 ng), were treated for 10 min at 303 K. DNA synthesis was terminated by cooling to 273 K and addition of 1 ml of 10%(*w*/*v*) trichloroacetic acid containing 0.1 *M* sodium pyrophosphate. Acid precipitates were collected on Whatman GF/C filter discs, which were washed with 1 *M* HCl, 0.1 *M* sodium pyrophosphate and ethanol, dried and counted using a liquid-scintillation counter.

2.4. Crystallization and data collection

Crystals of the β sliding clamp were grown at 294 K by vapour diffusion and were isomorphous with the type III (space group $P2_1$) crystals obtained by Kong *et al.* (1992). Equal volumes (2–7 µl) of solutions of β (45–60 mg ml^{-1}) and reservoir buffer [100 m*M* Na MES pH 6.0, 50–60 m*M* $CaCl_2$, 30%(*v*/*v*) PEG 400] were suspended in hanging drops over 1 ml of reservoir buffer. Crystals appeared after 6–8 h and grew as rods with dimensions of 0.5 × 0.2 × 0.2 mm within 1–2 d. Crystals were transferred to a cryobuffer [100 m*M* Na MES pH 6.0, 100 m*M* $CaCl_2$, 40%(*v*/*v*) PEG 400] and flash-frozen in liquid N_2 for data collection.

Data were collected at 100 K on beamline 14-C at the Advanced Photon Source (X-ray wavelength 1.000 Å), Argonne, IL, USA using an Area Detector Corporation of America Quantum 4 detector. Reflections corresponding to diffraction spacings to 1.7 Å were observed. Data were collected and processed with the *HKL* package (Otwinowski & Minor, 1997). Statistics for the data are shown in Table 1.

2.5. Structure determination and refinement

The coordinates of the published structure of β (Kong *et al.*, 1992; PDB code 2pol) with all non-protein atoms removed were used to generate the starting model for refinement. Since the space group and unit-cell parameters of the crystals used in this study were identical to those used by Kong and coworkers, molecular replacement was unnecessary.

CNS (Brünger *et al.*, 1998) was used for the initial part of the refinement. The starting model (R = 36.1%; $R_{\rm free}$ = 35.8%) was subjected to rigid-body refinement (with the two monomers treated as separate bodies), giving model R1 (R = 32.4%; $R_{\rm free}$ = 31.8%). Electron-density maps calculated with σ_A-weighted $2F_o - F_c$ and $F_o - F_c$ coefficients were generated and inspected. The electron density clearly indicated that the use of coordinates from Kong *et al.* (1992) was justified. This model was subjected to simulated annealing and restrained temperature-factor refinement, reducing the R factor to 28.0% and $R_{\rm free}$ to 31.5%. At this stage, many water molecules could be observed in electron-density maps and thus 20 cycles of *ARP*/*wARP* (Perrakis *et al.*, 2001) refinement were carried out to add water molecules. The resulting model, containing 679 solvent atoms, was subjected to another cycle of energy minimization and temperature-factor refinement, giving model B2 (R = 23.85; $R_{\rm free}$ = 27.8%). The model was used to produce further σ_A-weighted $2F_o - F_c$ and $F_o - F_c$ maps and the model was manually rebuilt using *O* (Jones *et al.*, 1991), resulting in removal of some of the water molecules. At this stage, the maximum-likelihood method as implemented in *REFMAC* (Murshudov *et al.*, 1997) was used and the resulting model was manually rebuilt again. Two further cycles of rebuilding and refinement were performed, during which alternate conformations were built for some residues and additional solvent molecules were included in the model. The statistics for the final model were derived using *REFMAC* (Murshudov *et al.*, 1997) and *PROCHECK* (*CCP*4 suite; Collaborative Computational Project, Number 4, 1994) and are given in Table 1. Although the X-ray data in the highest resolution bin were weak [$I/\sigma(I)$ = 1.76], their inclusion in the refinement improved the electron-density maps.

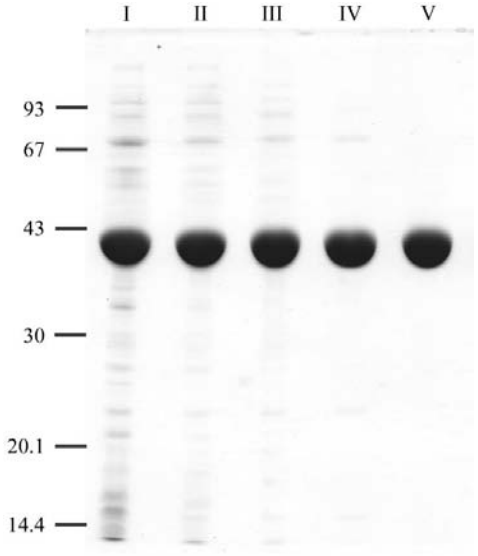

Figure 2
Overproduction and purification of the β sliding clamp. SDS–PAGE (12%) analysis of samples of fractions in successive steps of purification of β. Lanes are marked with fraction numbers I–V (as in Table 2). Equal amounts of β activity (3.0×10^5 units) were loaded in each lane. The gel was stained with Coomassie Blue. The mobilities of molecular-weight markers (sizes in kDa) were as indicated.

Table 2
Purification of the β subunit of *E. coli* DNA polymerase III holoenzyme.

	Fraction	Protein† (mg)	Activity‡ (10^{-9} units)	Specific activity (10^{-6} units mg^{-1})
I	Cell-free extract	206	1.38	6.7
II	Ammonium sulfate	181	1.32	7.3
III	DEAE flowthrough	143	1.07	7.5
IV	DEAE gradient	120	0.90	7.5
V	Hydroxyapatite	103	0.78	7.6

† Protein concentrations were measured by the method of Bradford (1976), with bovine serum albumin as standard. ‡ Activity of β in DnaA protein-dependent replication of M13-A site single-stranded DNA. One unit of activity represents the amount of the β subunit that promotes incorporation of one picomole of deoxynucleotide into *de novo* synthesized DNA in 1 min.

2.6. Structure analysis

Coordinates of representative structures of β-clamp-type proteins were obtained from the Protein Data Bank. These were the bacteriophage RB69 sliding clamp (PDB code 1b77; Shamoo & Steitz, 1999), herpes simplex virus (HSV) UL42 protein (1dml; Zuccola *et al.*, 2000) and the *Saccharomyces cerevisiae* proliferating cell nuclear antigen (PCNA; 1plq; Krishna *et al.*, 1994). These files were divided up such that each new file contained one repeat of the β-clamp motif. The protomer of *E. coli* β has three repeats and all the others have two. All domains were aligned structurally using *LSQMAN* (Kleywegt, 1996). *STAMP* (Russell & Barton, 1992) was used to create a structure-based multiple-sequence alignment. Secondary structures were analyzed using *DSSP* (Kabsch & Sander, 1983). The packing of hydrophobic residues in the cores of these domains was analyzed with *ClusterOne* (A. J. Oakley, unpublished program). *ClusterOne* determines all non-bonded interactions shorter than 4.0 Å in a protein and searches for contiguous clusters of hydrophobic groups. An atom needs to be an aromatic or aliphatic C atom with at least two non-bonded contacts to other such atoms to be considered as part of a cluster. A quorum of at least four atoms is needed to form a 'cluster'.

3. Results

3.1. Overproduction and purification of the β sliding clamp

To generate the plasmid pND261 (Fig. 1) which directs overproduction of the β subunit of DNA polymerase III holoenzyme, a fragment of DNA bearing the *dnaN* gene was first isolated and trimmed to within a few nucleotides of the ATG start codon by use of the exonuclease BAL31 and then

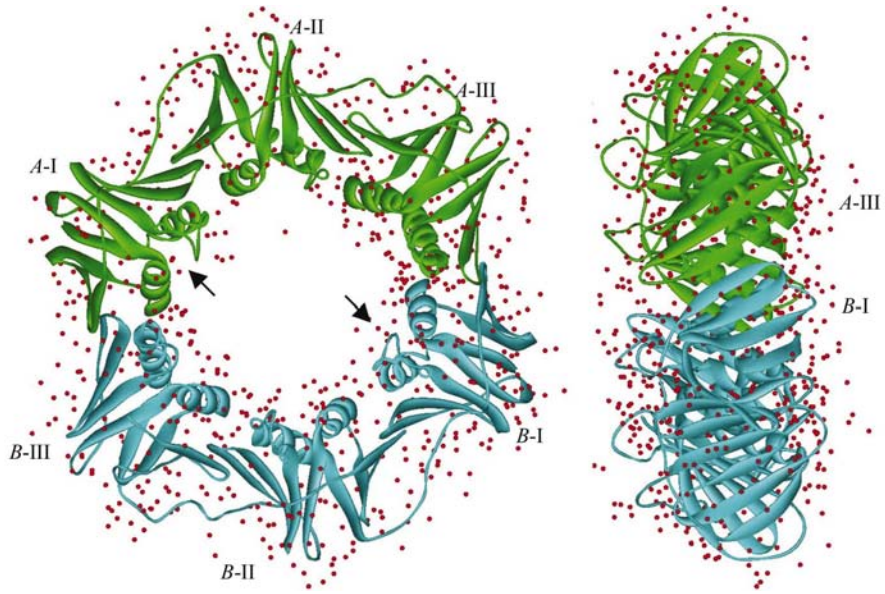

Figure 3
Orthogonal views of the β-clamp dimer as drawn by *WebLab Viewer* (Accelrys, San Diego, CA, USA). The two monomers are shown as green (monomer *A*) and cyan (monomer *B*) ribbons. Water molecules are shown as red spheres. The three β-clamp domains in each monomer are labelled I, II and III. Arrows indicate the residue 21–27 loops.

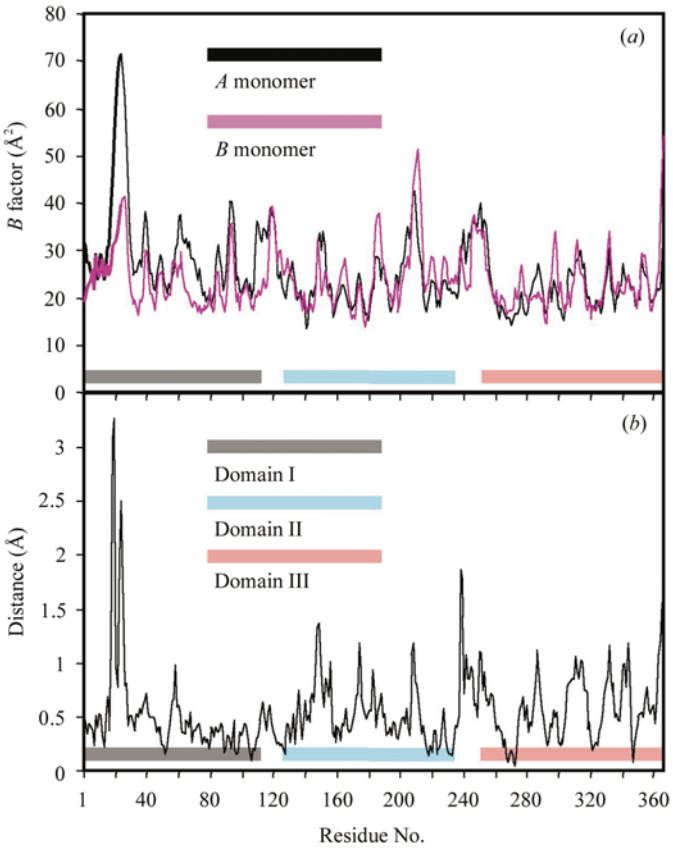

Figure 4
(*a*) Plot of average *B* factor *versus* residue number. (*b*) Differences in C^{α} position of superimposed β-clamp monomers *versus* residue number.

inserted at the *Hpa*I site of vector pND201 (Elvin *et al.*, 1990). This provided *dnaN* with a ribosome-binding site perfectly complementary to the 3′ end of the 16S ribosomal RNA and placed its transcription under the control of tandem heat-inducible bacteriophage λ p_R and p_L promoters. On treatment of cultures of *E. coli* AN1459/pND261 at 315 K, β accumulated over several hours to extraordinarily high levels; it was found to comprise >85% of proteins in the soluble fraction following lysis of the cells using a French press (Fig. 2, lane I; Table 2). The protein was purified from this fraction in yields of over 100 mg per litre of culture (Fig. 2; Table 2) by a simple procedure beginning with ammonium sulfate precipitation and followed by anion-exchange chromatography in buffer containing 0.15 *M* NaCl to remove contaminating nucleic acids that otherwise affected resolution in the next chromatographic step. After dialysis to remove salt, β was applied to the same resin, from which it eluted in a sharp peak following application of an NaCl gradient. Traces of contaminating proteins, including a protease that degraded β during long-term treatment under the conditions of its crystallization, were then removed by passage of this fraction through a column of hydroxyapatite.

The purified protein was fully active in complementing the other subunits of Pol III holoenzyme in DnaA protein-dependent conversion of a single-stranded phage DNA template to its double-stranded replicative form (Table 2). SDS–PAGE analysis of samples containing equal activity from the various purification steps indicated that the protein remained fully active during purification (Fig. 2). The subunit mass of β determined by ESI–MS (40 588 $\pm$ 2 Da) agreed with that calculated from its amino-acid composition (40 586.7 Da).

3.2. Crystal structure of the β sliding clamp

The structure of the β sliding clamp is of high quality as judged by electron-density maps, *R* and R_{free} factors, deviations from ideal geometry, the root-mean-square deviations of *B* factors between bonded atoms and the Ramachandran plot (Table 1).

The high-resolution structure is similar to the previously published model in most regards. The protein is a toroid-shaped dimer of identical subunits, each consisting of three repeats of the conserved β-clamp structural domain (Fig. 3). One loop, spanning residues 21–27 in the *A* monomer, has been built differently in comparison with the previous structure. Examination of the electron density of this loop suggests that it is poorly ordered and our observation of a different conformation in each of the two monomers is probably a reflection of its inherent flexibility.

Features not previously observed for the β subunit are present in the new model. These include ordered solvent

molecules (the model contains 665 water molecules) and alternate conformations for 18 (of 742) residues. These are all hydrophilic residues on the surface of the protein.

4. Discussion

One of the advantages of high-resolution data is that they allow the analysis of subtle differences between non-crystallographic symmetry-related molecules. Some asymmetry between the monomers of the β sliding clamp was observed (Fig. 4). Overall, there is less deviation in the C^{α} position in the first domain compared with domains II and III. The differences are in general subtle and are mostly less than 1 Å in distance. The only exception to this is found with residues 21–27 on the inside surface of the ring, which are poorly ordered in both monomers.

The γ clamp-loading complex binds to and opens the β clamp though interactions of the δ subunit with domains II and III, such that the interactions of domain III of one monomer and domain I of the other are disrupted (Jeruzalmi, Yurieva *et al.*, 2001). Superposition of domain I of the structure presented here on that of the corresponding domain of (monomeric) β in its complex with δ (not shown) confirms the observations of Jeruzalmi, Yurieva *et al.* (2001). Consistent differences in C^{α} positions occur in domain II and greater differences are seen in domain III. These are a consequence of whole-domain movements about inter-domain linkers (for both domains II and III) and of further movements of some loops and β-strands (most marked in domain III). In particular, the regions spanning residues 272–280 and 294–304 are shifted by contact of β with δ. Residues 294–304 do not contact δ directly, but move as a result of shifts in the region spanning residues 272–280. Binding of δ to domains II and IIII results in straightening of the crescent-shaped β monomer, presumably leading to opening of the ring-shaped clamp.

Of the residues known to be involved in binding to δ, Met362 appears to be particularly flexible. Its side chain is located differently in the *A* and *B* monomers and in the *B* monomer it is present in two configurations. Moreover, binding of δ to β results in further movement of this residue. The other side chains involved in binding to δ (Gly174, His175, Leu177, Pro242, Val247, Val360, Pro363 and Arg365) are nevertheless relatively well ordered.

Many structured water molecules, most of which were not observed in the earlier structure of the β clamp, are found in this model (Fig. 3). The central channel features many water molecules engaged in hydrogen-bond interactions with the basic and hydrophilic residues that line the cavity. Since the nascent double-stranded DNA passes through the channel, these water molecules are likely to be important mediators of interactions of the β clamp with DNA. The transient nature of water–protein and water–water interactions is doubtless conducive to the sliding action of the clamp as it passes along DNA. Although many water molecules are also observed to bind to the outer surfaces of β, only five of them need to be displaced upon binding to δ. Nevertheless, many other water–protein interactions would probably be destroyed as the aforementioned loops are shifted as a result of contacts between β and δ.

There is evidence that many of the basic residues lining the inside of the channel in the β torus are highly flexible. Many of these have high *B* factors and in some cases (*e.g.* Lys12, Arg73, Lys198) the side chains are completely disor-

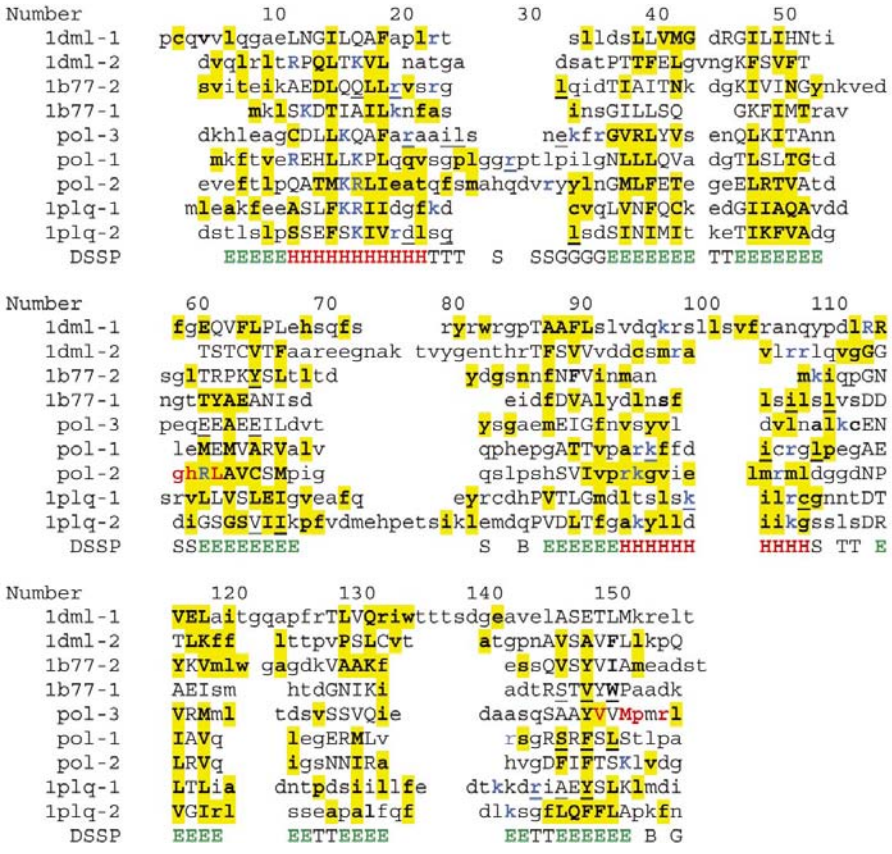

Figure 5
STAMP alignment of representative β-clamp domains from the bacteriophage RB69 sliding clamp (PDB code 1b77), HSV UL42 protein (1dml) and *S. cerevisiae* PCNA (1plq). Each protein was divided up into separate domains, which were numbered consecutively. Each sequence is named according to its PDB code, except for the *E. coli* β-clamp domains, which are designated pol-1 to pol-3. Residues that show structural similarity ($P_{ij} > 0.5$) are capitalized. All other regions are in lower case. Residues involved in hydrophobic clustering (as determined by *ClusterOne*) are highlighted in yellow. Basic residues that could interact with DNA are represented in blue. The *DSSP* designation of the secondary structure of domain I of the *E. coli* β clamp is shown below the sequences. These designations are H, 4-helix (α-helix); B, residue in isolated β-bridge; E, extended strand, participates in β-ladder; G, 3-helix (3_{10}-helix); T, hydrogen-bonded turn; S, bend. Residues in β that interact with δ are shown in red. Residues involved in inter-monomer interactions are underlined.

dered. The temperature factors for such residues typically range from about 24 Å^2 for the C$^\alpha$ atom to over 40 Å^2 for the terminal side-chain atom. Moreover, multiple conformations are observed for some of these residues (*e.g.* Arg176, Arg205, Arg279) and several adopt different conformations in the *A* compared with the *B* monomer (*e.g.* Arg24, Arg80, Lys198, Arg205). The ordering of these residues upon interaction with DNA backbone phosphate groups would be entropically unfavourable. However, since the β clamp must slide along double-stranded DNA, the predominantly electrostatic interactions of these basic residues with DNA would seem to most likely be transitory. This notion of a lack of specificity is reinforced by the observation of poor conservation of basic residues in the various clamp domains (Fig. 5). Although all of the domains feature some basic residues on the inner surface that are presumed to form transient contacts with DNA, the residues vary both in number and in physical location. Dissociation of β from DNA can only take place following a ring-opening event, which requires major structural changes in the protein (Jeruzalmi, Yurieva *et al.*, 2001). Electrostatic attraction of the basic residues to the DNA backbone probably increases the lifetime of the β clamp on the DNA by helping to keep the ring closed.

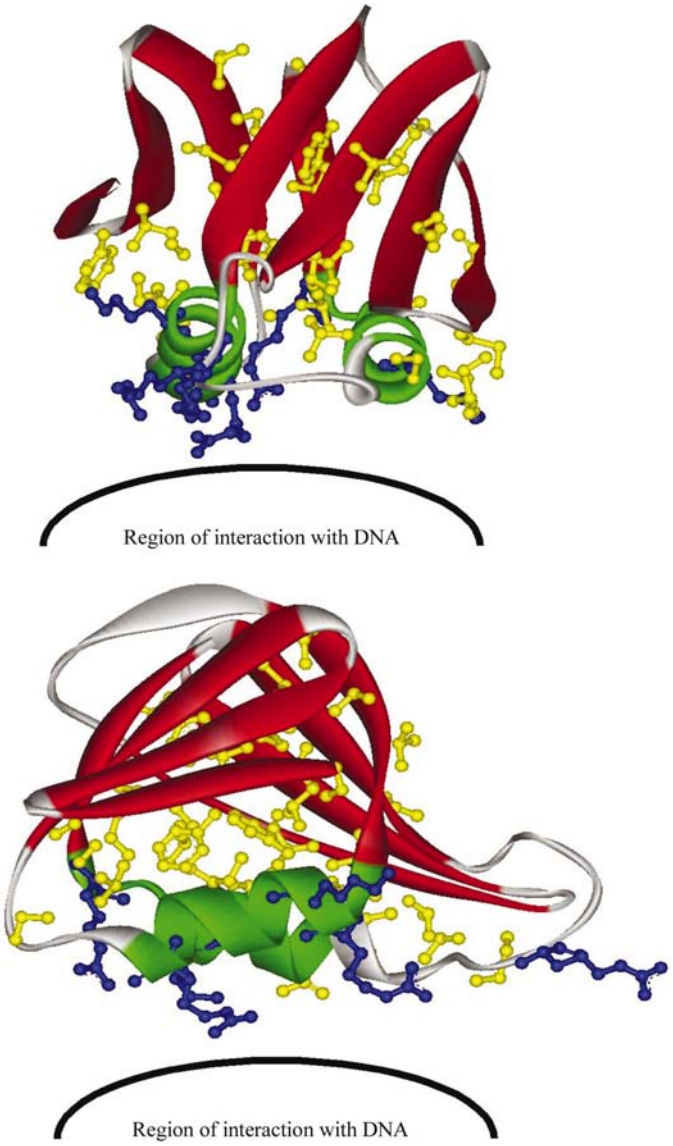

Figure 6
Orthogonal views of the β-clamp domain I from *E. coli* in ribbon format as drawn by *WebLab Viewer*. The ribbon is coloured red (β-strand) or green (α-helix) in accordance with the *DSSP* output. Side chains of residues identified by *ClusterOne* as forming hydrophobic clusters are represented in ball-and-stick representation in yellow, while basic residues lining the inside of the β-clamp are represented similarly in blue. These residues potentially interact with the phosphate backbone of DNA *via* transient salt bridges.

Structures are known of several DNA polymerase-associated sliding-clamp proteins related to β. These include the sliding clamps from bacteriophages RB69 (Shamoo & Steitz, 1999) and T4 (gene 45 protein; Moarefi *et al.*, 2000), PCNAs from yeast (*S. cerevisiae*; Krishna *et al.*, 1994), human (Gulbis *et al.*, 1996) and an archeon (*Pyrococcus furiosus*; Matsumiya *et al.*, 2001), as well as HSV UL42 protein (Zuccola *et al.*, 2000). Subunits of all of these proteins contain either three (in β) or two (in the others) repeats of a structurally related β-clamp domain and all except HSV UL42 form toroidal oligomeric structures containing six such domains. Although there is no significant sequence identity among representative β-clamp domains from the three families (*e.g.* from *E. coli* β, yeast PCNA and HSV UL42), there are several regions that align closely (Fig. 5). These correspond to several of the β-strands and one of the two helices. Although the first helix appears to be structurally conserved in all protomers, the second is not. For example, in HSV UL42 the second helix in both domains does not align well with the helices from the other sliding-clamp domains. The significance of the structural conservation of helix 1 but not helix 2 needs further investigation.

Analysis with *ClusterOne* shows that the clustering of hydrophobic residues in the cores of all of the β-clamp domains are similar (Figs. 5 and 6). This may indicate a common evolutionary origin for the β-clamp domain even though no 'memory' of this (in terms of sequence identity) is retained. In particular, there is a pattern of two hydrophobic residues (separated by three residues) in helix 1 that appears to be conserved in all cases. These residues are located on the same side of the helix and probably represent an essential 'hydrophobic staple' that tethers this helix to the core of the protein. Less well conserved are the patterns of alternating hydrophobic/hydrophilic residues that make up the β-strands, although all possess hydrophobic side chains on alternating residues that contribute bulk to the cores of each domain. While residues corresponding to the second helix tend to be hydrophobic, the patterns of hydrophobic clustering are less well conserved than for helix 1. This observation helps explain the lack of structural conservation in helix 2.

The separate domains of the β clamps are joined in part through a hydrogen-bond ladder linking the β-sheets of each. This applies to inter-monomer interactions as well as inter-domain interactions. Additional hydrophobic contacts are used to maintain the interactions between adjacent domains within the monomers, but this does not extend to the inter-

monomer interactions (Fig. 5). Although hydrophobic contacts do exist between monomers, they are not as extensive as those of intra-monomer domain interactions. This observation can be reconciled by considering the function of the γ clamp-loader complex, which must disrupt the inter-monomer interactions. Too extensive a buried hydrophobic surface would make clamp opening entropically unfavourable.

We thank Drs Steven Sandler, Elliott Crooke and Chris Love for provision of plasmids and proteins, Dr Brian Dalrymple for helpful comments and the Australian Research Council for financial support. AJO holds an Australian Postdoctoral Fellowship.

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