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Solution structure of an "open" *E. coli* Pol III clamp loader sliding clamp complex

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ABSTRACT

Sliding clamps are opened and loaded onto primer template junctions by clamp loaders, and once loaded on DNA, confer processivity to replicative polymerases. Previously determined crystal structures of eukaryotic and T4 clamp loader-clamp complexes have captured the sliding clamps in either closed or only partially open interface conformations. In these solution structure studies, we have captured for the first time the clamp loader-sliding clamp complex from *Escherichia coli* using size exclusion chromatography coupled to small angle X-ray scattering (SEC-SAXS). The data suggests the sliding clamp is in an open conformation which is wide enough to permit duplex DNA binding. The data also provides information about spatial arrangement of the sliding clamp with respect to the clamp loader subunits and is compared to complex crystal structures determined from other organisms.

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1. Introduction

During DNA synthesis and replication the daughter strands are copied at a fast rate and in an accurate manner by DNA polymerases (Benkovic et al., 2001; Kong et al., 1992; Onrust et al., 1991; Pomerantz and O'Donnell, 2007; Stukenberg et al., 1991). The accuracy is an inherent property of DNA polymerases but the processivity (up to 1000 nts in a single binding event) is conferred through binding with sliding clamps (Shamoo and Steitz, 1999; Kong et al., 1992; Onrust et al., 1991; Stukenberg et al., 1991). The sliding clamps anchor and topologically link the polymerases to the DNA and prevent premature dissociation. A sliding clamp is loaded onto the primer template junction on the DNA lagging strand every 2–3 s in *Escherichia coli* (Wu et al., 1992; Moolman et al., 2014).

Because of the inherent closed ring nature of the majority of uncomplexed sliding clamps, a multi-subunit macromolecule referred to as clamp loader is required for loading of the clamp. Clamp loaders are members of family of ATPase associated with various cellular activities (AAA+) which use ATP binding and hydrolysis to bind, open and load sliding clamps in the correct orientation onto primer template junctions (Erzberger and Berger, 2006; Neuwald et al., 1999). In contrast to other members of AAA+ family of ATPases, clamp loaders are heteropentameric. The absence of a sixth subunit provides a gap for binding of DNA/RNA primer template substrates (Bowman et al., 2004; Simonetta et al., 2009). ATP binding increases the affinity of the clamp loader for binding the sliding clamp through induction of conformational changes. These conformational changes are necessary for interaction of the loader with the clamp as well as with the primer template DNA (Bowman et al., 2004; Jeruzalmi et al., 2001b; Simonetta et al., 2009; Hingorani and O'Donnell, 1998). Once the sliding clamp is loaded onto the DNA, the clamp loader releases and closes the sliding clamp around the DNA. These steps are coupled to ATP hydrolysis and dissociation of ADP from the clamp loader (Jarvis et al., 1989; Turner et al., 1999). Once the sliding clamp is released the polymerase binds the clamp and carries out DNA replication.

Previously, the X-ray crystal structure of the eukaryotic pentameric clamp loader, replication factor C (RFC), bound to a closed homotrimeric sliding clamp referred to as proliferating cellular nuclear antigen (PCNA) from *Saccharomyces cerevisiae* has been determined (Bowman et al., 2004). Because ATP hydrolysis weakens the interaction of sliding clamp and the clamp loader, a variant of the clamp loader complex (where the arginine finger of the SRC motifs in RFC-B, RFC-C, RFC-D and RFC-E subunits were mutated to glutamine) was used which resulted in the capture of the clamp loader-clamp structure, but the PCNA clamp was in a closed conformation. The crystal structure revealed a spiral arrangement of ATPase domains of clamp loader with the N-terminal domains of the subunits interacting with the PCNA ring and DNA modeled within the central hole of the PCNA and primer template binding

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channel of RFC also revealed that the RFC spiral complemented the grooves of the modeled duplex DNA. This crystal structure presented the "screw cap" interaction of RFC with primer template DNA.

Further understanding of the mechanism of clamp loading reaction has been gained from the crystal structure of an ATP bound state of the bacteriophage T4 clamp loader bound to the gp45 sliding clamp and primer-template DNA (Kelch et al., 2011). Previous studies on the T4 sliding clamp has shown the clamp having an open conformation in solution in the absence of the loader (Alley et al., 1999; Millar et al., 2004). However, the X-ray crystal structure of the T4 clamp loader-clamp-DNA complex revealed a partially opened sliding clamp. The opening at the clamp interface was approximately 10 Å, not wide enough to thread duplex DNA. The spiral conformation of the clamp matched the spiral of the primer-template DNA as well as the clamp loader. This structure also revealed extensive interactions between all the five subunits N-terminal domains of the clamp loader with the bacteriophage gp45 sliding clamp.

In *E. coli*, the sliding clamp is referred to as the β sliding clamp and the clamp loader complex as either the γ or τ complex. The crystal structure of the β sliding clamp revealed the molecule to be a homodimer (with an outer and inner diameter of 80 and 40 Å, respectively). Each of the monomers (comprised of 366 residues) with head to tail interaction arrangement, that results in the β sliding clamp having two distinct faces (Kong et al., 1992). The β sliding clamp interacts with several protein partners during DNA replication and repair using one of these faces (which has several surface protruding loops). Although the X-ray crystal structure of the clamp was of a closed ring, solution studies of the clamp using hydrogen exchange mass spectroscopy have revealed that β clamp is not a static closed ring in solution but highly flexible (Fang et al., 2011). The same studies also suggested that intermediate conformations of the clamp might facilitate interactions with different protein partners.

The *E. coli* clamp loader complex is a multi-subunit macromolecule comprised of the seven subunits δ, δ', ψ and χ and three protomers of the *dnaX* gene products (either γ or τ). It was demonstrated that the clamp loader with any combination of γ or τ subunits ($\gamma_2\tau$, $\gamma\tau_2$, γ_3 or τ_3) were equally active in loading the clamp onto DNA (McInerney et al., 2007). Several X-ray crystal structures of the γ clamp loader have been determined but the $\chi\psi$ subunits, which are required for bridging the interaction of single stranded DNA binding protein (SSB) with the clamp loader, were absent (Jeruzalmi et al., 2001b; Kazmirski et al., 2004; Simonetta et al., 2009). The omission of $\chi\psi$ subunits was because of the inherent flexibility of the ψ subunit 27 N-terminal residues that anchor the $\chi\psi$ heterodimer to the core five subunit (Glover and McHenry, 1998). The ψ subunit interacts with the C-terminal region of γ subunit (Gao and McHenry, 2001). These two subunits together are essential for bridging the interaction between the clamp loader and SSB in *E. coli* (Glover and McHenry, 1998). The ψ subunit plays a role in stabilizing the conformational changes induced by ATP binding and the χ subunit directly interacts with the C terminus of SSB (8 amino acid residues). The $\chi\psi$ complex also plays a role in increasing the affinity of τ and γ for $\delta.\delta'$ to a physiologically relevant range and stabilization of the complex (Olson et al., 1995). In a recent study on the solution structure of the γ clamp loader, the location of $\chi\psi$ was revealed (Tondnevis et al., 2015). The $\chi\psi$ heterodimer interacts with the C-terminal collar region of the loader and was positioned opposite of the ssDNA exit channel on δ subunit and closer to δ'.

To date there is no X-ray crystal or cryo-electron microscopy (cryo-EM) structure of any clamp loader-clamp complex in which the sliding clamp has been shown to be open wide enough at the interface for the binding of duplex DNA. In these studies, we have captured a solution structure of the *E. coli* β sliding clamp loader-clamp complex using small angle X-ray scattering coupled to size exclusion chromatography (SEC-SAXS). Rigid body modeling of the clamp loader-clamp to fit the 1D-scattering data shows the topological arrangement of the β sliding clamp with respect to the γ clamp loader. The clamp makes interactions with the N-terminal domains of all the core subunits. This arrangement is similar to that seen in the RFC-PCNA crystal structure, bacteriophage T4 clamp loader-clamp-DNA complex structure and the archaeal *Pyrococcus furiosus* (*Pfu*) clamp loader-clamp-DNA ternary structure previously determined using X-ray crystallography and cryo-EM (Bowman et al., 2004; Kelch et al., 2011; Miyata et al., 2005). Our model also suggests that the clamp exists in an open conformation in complex with the clamp loader and has an out of plane opening wide enough (~25 Å) for the binding of duplex DNA.

2. Materials and methods

2.1. Protein preparation and purification

E. coli β sliding clamp was purified as described previously with minor modifications (Anderson et al., 2009; Johanson et al., 1986). The stock concentration for β sliding clamp was 90 μM (7.4 mg/ml) for the dimer. The protein was >95% pure as measured by SDS–PAGE gel. The storage buffer was 20 mM Tris–HCl at pH 7.5, 0.5 mM EDTA, 5 mM DTT, 10% glycerol. For expression of the γ clamp loader, *E. coli* BL21 (DE3) cells were transformed with pCOLADuet-1 and pETDuet-1 vectors. The plasmids were generous gifts from Linda Bloom & Lauren Douma (University of Florida. Department of Biochemistry and Molecular Biology). The pCOLADuet-1 vector contained the coding sequence for δ subunit as well as γ subunit and the pETDuet-1 vector contained the coding sequence for the δ' as well as $\psi\chi$ subunits (ψ and χ were part of a single operon). Transformation colonies were used to grow 20 ml of starter culture containing ampicillin and kanamycin as selective markers. The starter culture was grown to optimum density and centrifuged and the pellet was re-suspended in fresh media. For large-scale expression of the γ clamp loader, 2L of LB media supplemented with ampicillin and kanamycin (100 μg/ml and 50 μg/ml) was inoculated with *E. coli* starter culture. The culture was incubated at 37 °C with shaking until $O.D._{600}$ reached 0.6. Protein expression was induced using IPTG to a final concentration of 1 mM followed by an additional 3-h incubation of the culture at 37 °C with shaking. The culture was then centrifuged at 5000*g* at 4 °C for 30 min. Pellets were then frozen at −80 °C.

For purification of γ clamp loader, thawed frozen pellets were re-suspended in re-suspension buffer containing 20 mM Tris–HCl at pH 7.5, 0.5 mM EDTA, 2 mM DTT, 50 mM NaCl and 10% glycerol by stirring. Cells were lysed using French pressure cell. Cells were passed through the French press 3 times on high pressure (1000 psi). Lysed cells were then centrifuges at 18,000 RFC using a JA 20 rotor (Beckman) at 4 °C for 1 h. The cleared lysate was collected and loaded onto a regenerated 2 × 5 ml HiTrap Heparin column (GE Healthcare). The unbound proteins were washed away using the loading buffer (same as re-suspension) until the UV absorbance was stabilized. The protein was eluted using a linear gradient (50–1000 mM NaCl). The fractions containing clamp loader were pooled and dialyzed against 2L of dialysis buffer, which contained 20 mM Tris–HCl at pH 7.5, 0.5 mM EDTA, 2 mM DTT, 100 mM NaCl and 10% glycerol. For purifying the protein further, the dialyzed protein was loaded onto a regenerated 8 ml Mono Q column (Pharmacia). The unbound proteins were washed away using 40 ml of loading buffer (same as dialysis buffer). The clamp loader was eluted using a linear gradient (100–1000 mM NaCl). Clamp loader eluted around 450 mM NaCl. The peak fractions were