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Structure of a filament of stacked octamers of human DMC1 recombinase

Eukaryal DMC1 proteins play a central role in homologous recombination in meiosis by assembling at the sites of programmed DNA double-strand breaks and carrying out a search for allelic DNA sequences located on homologous chromatids. They are close homologs of eukaryal Rad51 and archaeal RadA proteins and are remote homologs of bacterial RecA proteins. These recombinases (also called DNA strand-exchange proteins) promote a pivotal strand-exchange reaction between homologous single-stranded and double-stranded DNA substrates. An octameric form of a truncated human DMC1 devoid of its small N-terminal domain (residues 1–83) has been crystallized. The structure of the truncated DMC1 octamer is similar to that of the previously reported full-length DMC1 octamer, which has disordered N-terminal domains. In each protomer, only the ATP cap regions (Asp317–Glu323) show a noticeable conformational difference. The truncated DMC1 octamers further stack with alternate polarity into a filament. Similar filamentous assemblies of DMC1 have been observed to form on DNA by electron microscopy.

1. Introduction

Archaeal RadA (Sandler *et al.*, 1996) and eukaryal Rad51 (Shinohara *et al.*, 1992) and DMC1 (Bishop *et al.*, 1992) proteins, along with the remotely related bacterial RecA proteins (Clark & Margulies, 1965), form a superfamily of recombinases (also called DNA strand-exchange proteins; Seitz & Kowalczykowski, 2000). The RadA, Rad51 and RecA proteins facilitate a pivotal DNA strand-exchange process between a single-stranded DNA (ssDNA) and a homologous double-stranded DNA (dsDNA) in homologous recombination, which appears to be essential in the repair of double-stranded DNA breaks and the restarting of stalled replication forks (Cox, 1998; Cox *et al.*, 2000; Courcelle *et al.*, 2001; Lusetti & Cox, 2002; Kowalczykowski, 2000). DMC1 is a meiosis-specific recombinase that is required for the formation of the synaptonemal complex (Bishop *et al.*, 1992). These proteins are known to be fascinatingly versatile in forming supramolecular assemblies. Electron-microscopic and crystallographic results have revealed strikingly similar 'active' recombinase assemblies in the form of right-handed helical filaments with approximately six monomers per turn (VanLoock *et al.*, 2003; Conway *et al.*, 2004; Wu *et al.*, 2004; Chen *et al.*, 2008; Sheridan *et al.*, 2008; Li *et al.*, 2009; Sehorn *et al.*, 2004). The milestone structures of *Escherichia coli* RecA (EcRecA) in complex with a series of DNA molecules have shed light on the exact mechanism of homologous DNA-strand exchange (Chen *et al.*, 2008). Crystallized 'inactive' EcRecA filaments with shorter helical pitches were also observed two decades ago (Story *et al.*, 1992). Ring-shaped 'inactive' forms of such recombinases with 6–8 protomers have also commonly been observed by electron microscopy (Heuser & Griffith, 1989; Yu & Egelman, 1997; Passy *et al.*, 1999; Yang *et al.*, 2001; Galkin *et al.*, 2006; McIlwraith *et al.*, 2001; Masson *et al.*, 1999) and by crystallography (Shin *et al.*, 2003; Kinebuchi *et al.*, 2004; Du & Luo, 2012). In addition to the three commonly found forms, crystal structures of overwound three-monomer-per-turn filaments (Ariza *et al.*, 2005) and left-handed filaments of *Sulfolobus solfataricus* RadA (Chen *et al.*, 2007;

Table 1

Data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data collection	
Space group	<i>P</i> 4
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 124.00, <i>c</i> = 91.713, $\alpha = \beta = \gamma = 90$
Resolution (Å)	47.5–2.60 (2.69–2.60)
<i>R</i> _{merge}	0.068 (0.419)
$\langle I/\sigma(I) \rangle$	25.7 (2.7)
Completeness (%)	97.0 (82.1)
Unique reflections	42118 (6906)
Multiplicity	7.1 (6.0)
Refinement	
Resolution (Å)	47.5–2.6
No. of reflections	39104
<i>R</i> _{work} / <i>R</i> _{free}	0.179/0.246
No. of atoms	
Total	7632
Protein	7536
Ligand/ion	0
Water	62
<i>B</i> factors (Å ²)	
Overall	44.6
Protein	44.4
Water	60.6
R.m.s. deviations	
Bond lengths (Å)	0.014
Bond angles (°)	1.91

Chang *et al.*, 2009) have also been observed. Here, we report the crystal structure of human DMC1 devoid of the first 83 amino-acid residues of its N-terminal domain (Δ_{83} HsRadA). The truncated protein formed octamers similar to those in the crystal structure of full-length HsDMC1 (Kinebuchi *et al.*, 2004), which has disordered N-terminal domains. Crystal-packing analysis indicates that the truncated octamers further stack into a filament that is strikingly similar to those observed previously in HsDMC1–dsDNA complexes by electron microscopy (Masson *et al.*, 1999; Passy *et al.*, 1999).

2. Experimental procedures

2.1. Cloning, protein preparation and crystallization

The open reading frame of residues 84–340 of DMC1 from *Homo sapiens* was inserted between the *Nco*I and *Xho*I sites of plasmid pET28a (Novagen). The resulting plasmid was verified by DNA

sequencing using T7 promoter and terminator primers. The recombinant Δ_{83} HsDMC1 was overexpressed in *E. coli* BL21 Rosetta2 (DE3) cells (Novagen) at 310 K for 4 h using 0.5 mM isopropyl β -D-1-thiogalactopyranoside as the inducer. The cells were disrupted by sonication. The insoluble particles were removed by centrifugation at 12 000g. Soluble protein was first separated by nickel-affinity chromatography. The expressed protein had eight extra C-terminal amino-acid residues (LEHHHHHH). Gel-filtration chromatography was performed on a Sephacryl S-300 HR column (GE Healthcare) using a buffer composed of 0.5 M NaCl, 30 mM Tris–HCl pH 7.9. The purified protein was concentrated to ~ 20 mg ml^{−1} by ultrafiltration.

2.2. Crystallization of Δ_{83} HsDMC1 and diffraction data collection

The Δ_{83} HsDMC1 crystals (space group *P*4) were grown by the hanging-drop method and grew to maximum dimensions of 0.4 × 0.05 × 0.05 mm. The optimal well solution consisted of 6% polyethylene glycol 3350, 0.5 M NaCl, 0.05 M MgCl₂, 0.05 M HEPES–NaOH buffer pH 7.4 and 0.06% thymol. A crystal was transferred into well solution supplemented with 20% glycerol, looped out of the solution and cooled in a nitrogen cryostream at 100 K. The diffraction data set was collected at a wavelength of 0.98 Å on beamline ID08 at the Canadian Light Source and was processed using *HKL*-2000 (Otwinowski & Minor, 1997). The statistics of the diffraction data are listed in Table 1.

2.3. Structural determination and refinement

The previously reported HsDMC1 model (PDB entry 1v5w; Kinebuchi *et al.*, 2004) was used as the search model for molecular-replacement solution using *Phaser* (McCoy *et al.*, 2007). Four monomers were located in the asymmetric unit. The model was iteratively rebuilt using *Coot* (Emsley & Cowtan, 2004) and refined using *REFMAC5* (Murshudov *et al.*, 2011) from the *CCP4* package (Winn *et al.*, 2011). Statistics of the refinement and model geometry are also given in Table 1. 91.3% of nonglycine residues fell into the most favored region of the Ramachandran map. Two residues (Ser318 in two chains) were found in the disallowed region. These Ser318 residues were found in the ATP cap regions with low electron density. Fig. 1 was generated using *MolScript* (Kraulis, 1991) and rendered using *Raster3D* (Bacon & Anderson, 1988). Fig. 2 was rendered using *CCP4mg* (Potterton *et al.*, 2004). The coordinates and structure

Figure 1

Comparison of the full-length and truncated HsDMC1 structures. (a) One Δ_{83} HsDMC1 octamer is shown in ribbons colored by peptide chain. The L1, L2 and ATP cap regions of one monomer (green) are highlighted in cyan, blue and salmon, respectively. One full-length HsDMC1 structure is shown in gray. (b) Enlarged view of the boxed area in stereo.

factors have been deposited in the Protein Data Bank (Bernstein *et al.*, 1977; Berman *et al.*, 2000, 2003) as entry 4hyy.

3. Results

3.1. The overall structure of a storage form of Δ_{83} HsDMC1

The structure of Δ_{83} HsDMC1 was solved using the molecular-replacement method. The peptide chain was largely ordered except for residues 272–285 in the DNA-interacting L2 region (Asn268–Arg300) as well as residues 339–340 and the eight-residue tag at the C-terminus. The root-mean-square deviation (r.m.s.d.) between the 241 ordered C^α atoms ranged between 0.31 Å (monomer *B* versus monomer *D*) and 0.45 Å (monomer *C* versus monomer *D*). The four monomers of Δ_{83} HsDMC1 in the asymmetric unit formed two stacked octameric rings around the crystallographic fourfold axis.

Each octameric ring showed approximate eightfold rotational symmetry (Fig. 1*a*), with a central pore of 23 Å in width.

3.2. Conformational variation of HsDMC1

The DNA-interacting L1 regions (Arg230–Arg242) were observed in similar conformations in both crystal structures (shown in cyan in Fig. 1). In the L2 regions (shown in blue in Fig. 1), the full-length HsDMC1 structure has a slightly larger section of disordered amino-acid residues (residues 271–289). A two-turn helix (Gly290–Ala296) was clearly observed in the L2 region of the truncated protein, but was not as clearly seen in the full-length protein, and corresponds to helix *G* in EcRecA (Story *et al.*, 1992; Kelley De Zutter *et al.*, 2001). However, the ATP cap regions (shown in salmon in Fig. 1) were observed to be in distinctively different conformations. Since the two monomers in the full-length DMC1 structure (Kinebuchi *et al.*, 2004)

Figure 2

Filament of stacked HsDMC1 octamers. Top, two central octamers are shown as molecular surfaces and four flanking octamers are shown as ribbons. The presentations are colored by peptide chain. Bottom, electron-microscopic micrograph of DMC1 rings formed on dsDNA with an ssDNA tail (reproduced from Fig. 1*c* of Passy *et al.*, 1999, copyright, 1999, National Academy of Sciences, USA).

are essentially identical, with an r.m.s.d. value of 0.02 Å, we only compared the amino-acid residues in the segments 84–269, 291–316 and 324–338 in each monomer of the Δ_{83} HsDMC1 structure with monomer A of the full-length structure. The r.m.s.d. values for 227 C α atoms were between 0.73 Å (monomer A of Δ_{83} HsDMC1) and 0.80 Å (monomer C of Δ_{83} HsDMC1). When all of these segments in the two Δ_{83} HsDMC1 octamers and the full-length octamer were compared, similar r.m.s.d. values of 0.75 and 0.90 Å were obtained for 1816 superimposed C α atoms. The two truncated octamers are more similar to each other, with an r.m.s.d. value of 0.55 Å. It appears that the arrangement of monomers in the three octameric assemblies is essentially identical.

3.3. Filamentous assembly of Δ_{83} HsDMC1

The Δ_{83} HsDMC1 protein is composed solely of the conserved ATPase domain found in RecA orthologs. Such domains start with a polymerization motif (Pellegrini *et al.*, 2002) centered around a hydrophobic residue (Phe85 in HsDMC1) which protrudes into a hydrophobic pocket in an adjacent monomer in the recombinase polymer. Similarly truncated ATPase domains of RadA from *Methanococcus voltae* (MvRadA) have been crystallized as either helical filaments (Galkin *et al.*, 2006) or hexamers (Du & Luo, 2012). As observed in full-length HsDMC1, the Δ_{83} HsDMC1 protein also formed octamers in the crystal. Each unit cell contained 16 protomers of Δ_{83} HsDMC1 arranged in two ring-shaped octamers with alternate polarity (shown as molecular surfaces in Fig. 2) around the crystallographic fourfold axis. Interestingly, this pattern repeats every 91.7 Å along the crystallographic *c* axis to form a filament of stacked rings. The octamer–octamer separation also alternates between 41.4 and 50.3 Å (Fig. 2). The separation between two symmetry-related octamers in the full-length HsDMC1 structure (Kinebuchi *et al.*, 2004) is 45.0 Å. These values are slightly shorter than the average separation of 55 Å derived from electron micrographs of full-length HsDMC1–dsDNA complexes (Passy *et al.*, 1999). Additionally, the full-length and truncated DMC1 octamers both pack rim to rim along the *a* and *b* cell axes to form layers of octamers in the crystals. Both crystals share almost identical *a* and *b* unit-cell parameters of 124 Å, which corresponds to the diameter of the DMC1 octamers.

4. Discussion

The most striking feature of the Δ_{83} HsDMC1 structure is the filament made up of stacked octamers. The bipolar nature of the neighboring octamers in the filament clearly resembles what has been observed previously for the HsDMC1–dsDNA complex using electron microscopy (Fig. 1c of Passy *et al.*, 1999, as reproduced in Fig. 2). The formation of ring-shaped oligomers generally leads to extended and flattened areas on both sides of the rings for stacking. With a central pore of 23 Å in width, dsDNA could find ample space to thread along the filament axis through the octamers. This may explain the previous findings using electron microscopy.

The helix *G* region of Δ_{83} HsDMC1 is ordered in the crystal structure. In the structures of filamentous or hexameric RadA proteins from *M. voltae* and *M. maripaludis*, disorder of helix *G* has been correlated with either the presence of post-hydrolysis ADP or the absence of proper cationic bridging between the C-terminal carbonyl groups of helix *G* and the γ -phosphate of the ATP analog AMPNP. This structural feature of Δ_{83} HsDMC1 is consistent with the notions that helix *G* is intrinsically stable (Du & Luo, 2012) and that ATP hydrolysis in recombinase filaments triggers disordering of

helix *G* and further renders the L2 region incompatible for binding DNA (Qian *et al.*, 2005; Li *et al.*, 2009).

It is also worth noting that the ATP cap region of HsDMC1, which is equivalent to the region between Asp302 and Asp308 that was first identified to interact with the ATP analog AMPNP in the helical filament of MvRadA (Wu *et al.*, 2004), has been observed in different conformations in the truncated and full-length HsDMC1 structures. This ATP cap region has been shown to determine the potassium-ion preference of MvRadA (Qian *et al.*, 2006) and to modulate HsRad51–DNA filament stability (Amunugama *et al.*, 2012). The equivalent region of EcRecA has also been observed to interact with the ATP analog in the active EcRecA–DNA complexes (Chen *et al.*, 2008). Since crystals of HsDMC1 have not been obtained in complex with an ATP analog, it is not surprising that this region shows conformational variability.

The observation of stacked octamer rings of DMC1 by electron microscopy and crystallography is intriguing. Only the right-handed filaments of RecA-like proteins have been correlated with DNA strand exchange. The apparent nucleation of such octameric filaments of HsDMC1 on dsDNA at the dsDNA–ssDNA junction (Passy *et al.*, 1999) may imply a yet undefined function at the very site of DNA strand exchange. It is not difficult to envision that coating adjacent DNA repeats would suppress nonallelic homologous recombination, which is known to cause genome evolution as well as genetic disease (recently reviewed by Liu *et al.*, 2012).

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