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Crystallization and structure determination of the core-binding domain of bacteriophage lambda integrase

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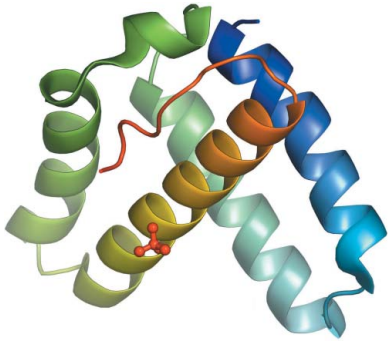

Bacteriophage lambda integrase catalyzes site-specific DNA recombination. A helical bundle domain in the enzyme, called the core-binding domain (Int^{CB}), promotes the catalysis of an intermediate DNA-cleavage reaction that is critical for recombination and is not well folded in solution in the absence of DNA. To gain structural insights into the mechanism behind the accessory role of this domain in catalysis, an attempt was made to crystallize an Int^{CB}–DNA complex, but crystals of free Int^{CB} were fortuitously obtained. The three-dimensional structure of DNA-free Int^{CB} was solved at 2.0 Å resolution by molecular replacement using as the search model the previously available DNA-bound 2.8 Å structure of the Int^{CB} domain in a larger construct of lambda integrase. The crystal structure of DNA-free Int^{CB} resembles the DNA-bound structure of Int^{CB}, but exhibits subtle differences in the DNA-binding face and lacks electron density for ten residues in the C-terminus that form a portion of a linker connecting Int^{CB} to the C-terminal catalytic domain of the enzyme. Thus, this work reveals the domain in the absence of DNA and allows comparison with the DNA-bound form of this catalytically activating domain.

1. Introduction

Bacteriophage lambda integrase (λ-Int), a site-specific DNA recombinase encoded by bacteriophage lambda, catalyzes genetic recombination between the virus and the host bacterium to enable the lytic and the lysogenic phases of the viral life cycle (Campbell, 1962). Recombination is carried out by four molecules of λ-Int acting on conserved genomic sequences, called core sites, and proceeds *via* a Holliday-junction intermediate formed by a series of DNA-cleavage, strand-exchange and ligation reactions (for reviews, see Sadowski, 1993; Radman-Livaja *et al.*, 2006; Landy, 1989). Enzyme activity at the core site is mediated by two of the three domains of λ-Int: the central DNA-binding domain, commonly known as the core-binding domain (Int^{CB}), and the C-terminal catalytic domain (Int^{Cat}), which enables the DNA-cleavage reaction through a conserved tyrosine nucleophile (Tirumalai *et al.*, 1997, 1998). The crystal structures of DNA-bound partial and full-length constructs of λ-Int provide critical knowledge about the organization of the active site and reveal details of interactions of the enzyme with DNA, as well as with the other enzyme molecules in the dimeric or tetrameric assemblies (Aihara *et al.*, 2003; Biswas *et al.*, 2005). These crystal structures show that Int^{CB} and Int^{Cat} bind to opposite faces of the recognition sequence by adopting a clamp-like architecture with an extended linker connecting the two domains. Experiments in our laboratory with isolated protein constructs of Int^{CB} and Int^{Cat} showed that Int^{CB} contributes to an increase in DNA cleavage by Int^{Cat} even when they are not covalently linked together (Subramaniam *et al.*, 2007). We proposed that Int^{CB} might render the substrate DNA more suitable for cleavage by Int^{Cat} by inducing structural changes in the DNA.

Characterization of Int^{CB}–DNA interactions in solution using biophysical techniques revealed that Int^{CB} indeed induces structural changes in the DNA and concomitantly undergoes a transition from a molten globule-like structure to a well folded structure upon binding DNA (Kamadurai & Foster, 2007). In order to characterize the Int^{CB}-mediated DNA structural changes in more detail, we attempted to

crystallize an Int^{CB}–cognate DNA complex; fortuitously, we observed diffraction-quality crystals of Int^{CB} that were free of DNA under certain crystallization conditions, although the presence of DNA in the mother liquor strongly promoted crystallization. The crystal structure of DNA-free Int^{CB} was solved at 2.0 Å resolution by molecular replacement and was found to resemble the DNA-bound conformation observed in the other lower resolution (3.0, 2.8 Å) crystal structures of complete and partial constructs of lambda integrase (Aihara *et al.*, 2003; Biswas *et al.*, 2005), but with subtle changes in the DNA-binding face and in the C-terminus.

2. Materials and methods

The Int^{CB} construct used in this study contains residues 75–176 of the full-length 356-residue lambda integrase and includes a starting methionine. It was obtained by deletion mutagenesis of an Int^{CB} construct encoding residues 63–176 (Tirumalai *et al.*, 1998). As a ligand, a 15-base-pair cognate DNA duplex, 5′-d(GCT CAA GTT AGT ACG)-3′, and its complement were used. Oligonucleotide preparation, protein expression and purification were performed using methods described previously (Subramaniam *et al.*, 2003, 2007; Kamadurai *et al.*, 2003). The Int^{CB}–DNA complex was assembled in 25 m*M* Tris pH 8.5 and 10 m*M* sodium chloride (assembly buffer) at a final protein concentration of 0.6 m*M* with a 10% stoichiometric excess of DNA or at an equimolar concentration of 1.75 m*M*. In all crystallization trials, 1 µl stock solution was mixed with 1 µl reservoir solution at room temperature and the crystallization trays were incubated at 277 K. Initial crystals were obtained *via* the sitting-drop method with reservoir solution condition No. B5 from the Crystal Screen HT kit (Hampton Research) containing 0.1 *M* Tris pH 8.5, 0.2 *M* lithium sulfate and 30% PEG 4000. Subsequent trials were carried out with and without the DNA ligand with the same reservoir solution composition but prepared in-house. Crystal constituents were identified by dissolving the crystal in the assembly buffer

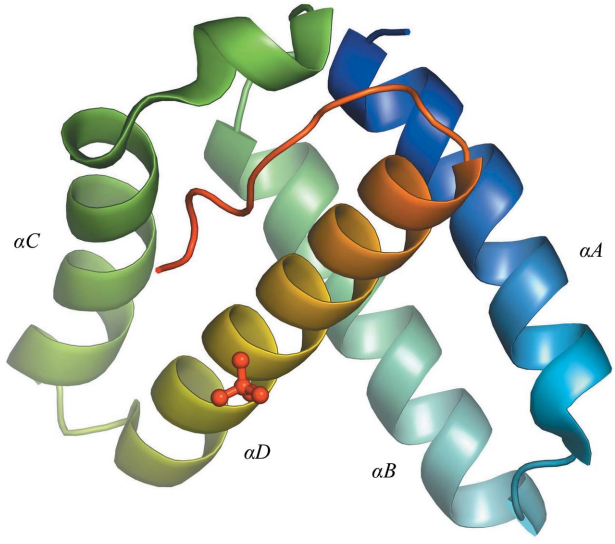

Figure 1
Cartoon representation of the crystal structure of DNA-free Int^{CB}. The structure is shown in a rainbow color gradient from the N-terminus (blue) to the C-terminus (red). The lone sulfate ion is shown as a ball-and-stick model. Int^{CB} folds into a four-helix bundle (helices *A*–*D*) with a flexible linker region at the C-terminus. The Int^{CB} construct used in this study contains residues 75–176 of the full-length lambda integrase and a starting methionine (residue 74), but electron density was only observed between residues 74 and 166. The *B* factors are nearly uniform through out the protein, averaging 24 ± 5 Å^2.

Table 1
Diffraction data and crystallographic refinement statistics.

Values in parentheses are for the highest resolution shell.

Data collection	
Space group	$P4_12_12$
Unit-cell parameters (Å, °)	$a = b = 46.69$, $c = 111.53$, $\alpha = \beta = \gamma = 90$
Resolution range (Å)	43.07–2.00 (2.07–2.00)
Total reflections	57414 (3708)
Unique reflections	8503 (641)
R_{merge} (%)	3.5 (17.4)
Completeness (%)	95.4 (74.5)
$I/\sigma(I)$	34.2 (7.9)
Refinement	
R_{cryst} (%)	22.8
R_{free} (%)	28.4
R.m.s. deviations from ideal geometry	
Bond lengths (Å)	0.02
Bond angles (°)	1.6
Ramachandran plot statistics	
Residues in most favored regions (%)	95.2
Residues in additional allowed regions (%)	4.8
Residues in disallowed regions (%)	0
Average *B* value, all atoms (Å^2)	23.91

followed by analysis using Coomassie Blue-stained SDS–PAGE and ethidium bromide-stained DNA agarose gels. To derivatize with platinum, a saturated aqueous solution of K_2PtCl_4 was diluted with the pre-equilibrated reservoir solution and a crystal was soaked in this K_2PtCl_4-containing reservoir solution for 5 min prior to data collection.

The crystals were mounted on nylon loops (Hampton Research) and were flash-frozen either in a nitrogen–helium stream at 93 K or in liquid nitrogen, with the reservoir solution serving as the cryo-protectant. Data were collected using a Rigaku rotating-anode generator equipped with an R-AXIS IV^{++} detector. The data were scaled and averaged using *CrystalClear* (Rigaku Inc.) and then imported into the *CCP*4 software suite (Collaborative Computational Project, Number 4, 1994) for subsequent analysis.

For molecular replacement, the coordinates corresponding to Int^{CB} in PDB entry 1p7d (chain *A*, residues 74–176; Aihara *et al.*, 2003) were used as the search model and the search was implemented using the automated search feature in *Phaser* (McCoy, 2007). The initial structure from molecular replacement was then refined using *REFMAC* (Murshudov *et al.*, 1997) followed by visual inspection and structure manipulation using *Coot* (Emsley & Cowtan, 2004). The stereochemical quality of the final structure, listed in Table 1, was assessed using *PROCHECK* (Laskowski *et al.*, 1993).

3. Results and discussion

Large single crystals were obtained in crystallization wells containing an Int^{CB}–DNA mixture after one week of incubation at 277 K. Even though the starting material contained both Int^{CB} and the DNA ligand, analysis of these crystals on SDS–PAGE and DNA agarose gels revealed only the presence of Int^{CB}, indicating that Int^{CB} crystallized without the DNA. Crystal structures of DNA-bound Int^{CB} in larger constructs of lambda integrase (Aihara *et al.*, 2003; Biswas *et al.*, 2005) show that Int^{CB}–DNA interactions are mediated predominantly by electrostatic interactions involving the phosphate backbone of the DNA ligand. Consistent with this observation, binding studies showed that Int^{CB}–DNA interactions are weakened upon an increase in ionic strength (Kamadurai, 2007). The absence of DNA in these Int^{CB} crystals could be attributed to weakened protein–DNA interactions arising from the moderately high ionic strength of the

crystallization condition. Nevertheless, the presence of the DNA oligonucleotide strongly promoted protein crystallization, as omission of the DNA ligand significantly reduced the yield of diffraction-quality crystals.

Int^{CB} crystals belonged to space group $P4_12_12$ and in general had good diffraction qualities, with the highest resolution in the region of 2.0 Å. A K_2PtCl_4-soaked crystal showed exceptionally good diffraction data [R_{merge}, $I/\sigma(I)$] and completeness and hence the data set collected on this crystal was used for structure determination, although no density was observed for the heavy metal. Matthews coefficient analysis with the mass of Int^{CB} and the observed unit-cell parameters ($a = b = 46.69$, $c = 111.53$ Å) showed that the asymmetric unit consists of only one copy of Int^{CB}. We were able to find a unique molecular-replacement solution using the Int^{CB} portion of the crystal structure of a DNA-bound partial λ-Int construct that contains both Int^{CB} and Int^{Cat} (PDB code 1p7d; Aihara *et al.*, 2003) as the search model. The Int^{CB} structure was refined to a final R_{cryst} of 22.8% and an R_{free} of 28.4%. The difference in these values is moderately higher than that expected at this resolution; however, comparison of the model against a composite OMIT map confirmed the accuracy of the model and additional refinement to improve statistics did not result in significant changes in the model. Refinement and stereochemical statistics of the final structure are included in Table 1.

The refined structure of DNA-free Int^{CB} reveals a four-helix bundle architecture defined by the helices *A*–*D* (Fig. 1). The crystal-packing interactions involved the fringes of the helices, including the helix–turn–helix DNA-binding motifs constituted by helices *A* and *B* and by helices *C* and *D*, and occlude the space for DNA, which may also explain why DNA failed to cocrystallize. The arrangement of Int^{CB} in the crystal is unlike the arrangement in the functionally important tetramer assembly (Biswas *et al.*, 2005), suggesting that the interprotomer interactions observed in the tetrameric assembly were not critical for stabilizing the Int^{CB} structure in the crystal.

The final structure of DNA-free Int^{CB} resembles the DNA-bound structure (Aihara *et al.*, 2003) with an average backbone r.m.s.d. of 0.6 Å (Fig. 2*a*). Modest but noticeable structural differences between the two structures are observed in regions that are proximal to the DNA (Fig. 2*b*). A sulfate ion bound to Arg152 that mimics the interactions of the residue with the phosphate backbone in the DNA-bound structure (Aihara *et al.*, 2003) was observed in the structure, as well as 98 ordered water molecules. No electron density was observed for ten C-terminal residues (167–176) that form a part of the flexible linker region connecting Int^{CB} to the C-terminal catalytic domain of lambda integrase (Aihara *et al.*, 2003). Electron densities were also lacking for some of the surface-exposed side chains, particularly those of Lys93, Lys95 and Arg109 that interact with the phosphate backbone, suggesting disorder in the absence of DNA. However, the side chain of Asn99, which makes a base-specific contact with DNA (Aihara *et al.*, 2003), is ordered and is observed to interact with the side chain of Lys103. Similarly, interaction with the sulfate ion and crystal-packing interactions appeared to have contributed to the ordering of the side chains of Arg152 and residues 116–120, respectively.

In summary, we report the crystal structure of the core-binding domain of the prototypical site-specific DNA recombinase in the absence of DNA. Although the protein is not well folded in solution, by including a DNA ligand in the crystallizing solution high-quality protein-only crystals were observed. Overall, the structure of the free

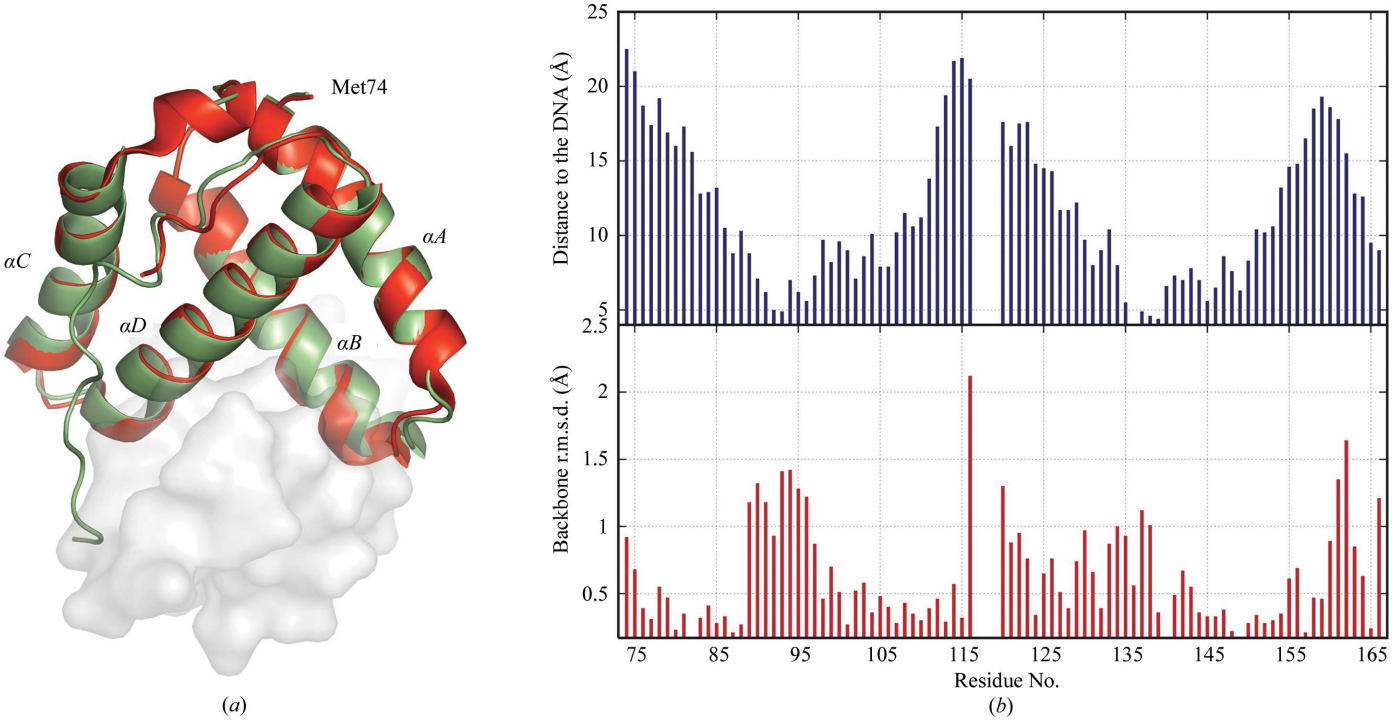

Figure 2
Structural differences between free and DNA-bound Int^{CB}. (*a*) Superimposition of free and DNA-bound crystal structures of Int^{CB}. The structure without DNA is shown in red and with DNA (PDB code 1p7d; Aihara *et al.*, 2003) in green; the DNA is shown as a semi-transparent surface. Overall, the structures overlay well, but subtle changes are observed in the DNA-binding region; also, a portion of a loop (residues 167–176) is missing in the DNA-free Int^{CB} structure. Unlike the DNA-bound structure, the free structure contains a 3_{10}-helix between helices *B* and *C*. (*b*) Correlation between structural differences between free and DNA-bound Int^{CB} (PDB code 1p7d; Aihara *et al.*, 2003) and proximity to DNA. Top, the closest approach of any backbone atoms in each residue in Int^{CB} to the DNA; r.m.s.d. values and the distances to the DNA were calculated for the amide N, C^{α} and carbonyl C atoms using *MOLMOL* (Koradi *et al.*, 1996). Bottom, the backbone r.m.s.d. between the structures in (*a*). These values could not be computed for residues 117–119 as the DNA-bound structure lacks electron density in this segment.

protein resembles the DNA-bound structure, but with subtle differences in the backbone and side chains of residues involved in DNA binding.

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