Structure of the type II restriction endonuclease XveII from *Xanthomonas campestris*

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Although structural studies have been performed on various type II restriction endonucleases, the molecular mechanism of SmaI endonuclease family is still poorly understood due to the lack of common sequence motifs. To gain insights toward DNA recognition, cleavage mechanism, active-site arrangement, and overall structure of this enzyme family, we have cloned, purified and crystallized a SmaI isoschizermer XveII-I from *Xanthomonas campestris* pv. vesicatoria strain 7-1. Similar to SmaI, XveII-I recognizes the palindromic sequence 5'-CCCGGG-3' and cleaves at the center of this site to generate blunt-ended fragments. Therefore the functionally relevant structural features of XveII-1 should be applicable to other members of this enzyme family.

The XveII crystals belong to tetragonal space group $P4_12_12$ with cell parameters of a=b=118.5, c=156.8 Å. And the crystal structure of XveII has been determined by Se-MAD approach by collecting diffraction data at three different wavelengths at Taiwan beamline SP12B2 (SPring8, Japan). Preliminary analyses on the experimental electron density map and a partial main-chain atomic model indicate that XveII-I folds as a curved 10-stranded antiparallel β-sheet with one side exposed to solvent and the other backed by helices. As there are four XveII-I molecules per asymmetric units and there are several crystallographic two-fold axes present in this space group. It is highly likely that a functionally relevant XveII-I dimer will be identified. In addition, because Mg^{2+} is required for crystallization, it is expected that the active site will be recognized after the Mg^{2+} ions are located.

Anomalous scattering data collection of Ni bounded ColE7 Dnase domain combines with Imm protein or DNA

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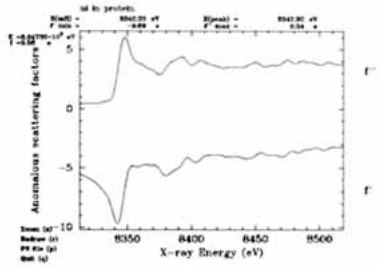
HNH motif, and metal binding nuclease motif which is involved in digestion, homing or repairing of DNA. The folding of ββα-metal active site processes the DNA binding and cleavage mechanism. Binding of metal ion in activity site with key residues and nucleotide is required for DNase activity. A HNH family Dnase domain of ColE7 complex with Imm protein and Ni2+ was crystallized for clarity of geometry relationship between metal ion and activity site.

For confirming the position of Ni ion in Dnase active site, we collected anomalous x-ray diffraction data by a chosen energy closed to absorption peak of Ni.

Energy scanning of metal bounded crystal around absorption edge of Ni^{2+} monitoring by fluorescence



Ni in protein

Total points integrated : 139718

Integration limits low/high: 832.19 33332.08

First/last data points at : 8313.01 8518.54

Energy scale increment : 0.233

	E (eV)	f'	f"
Inflection point	8342.34	-9.7	3.2
Peak	8347.92	-6.9	6.0

The details of mechanism of hydrolysis DNA of ColE7 is still unclear. Metal ion was removed by EDTA treatment to prevent cleavage of DNA during crystallization in our pervious ColE7 Dnase domain/ DNA complex structure. However, inactivated ColE7 Dnase domain mutants combined with different length of DNA were crystallized and diffraction data was collected during this beam line schedule.

Preliminary statistic table is following

	ColE7 T2A/ 18mer DNA
Cell constant	Tetragonal 60.26 Å, 106.6 Å,106.6 Å 90°, 90°, 90°
Reflection observed	114550
Unique reflections	30091
Resolution	30.0 ~2.9 Å (35% I/σ < 2 in last shell)
Rsym linear 30.0 ~2.9 Å	6.9% (37% for last shell)
Completeness	99% (100% for last shell)

	ColE7 T2A 493Q / 8mer DNA
Cell constant	Monoclinic 47.1 Å, 105.5Å, 180.1Å 90°, 90.144°, 90°
Reflection observed	51023
Unique reflections	29017
Resolution	30.0 ~3.03. Å (56% I/σ < 2 in last shell)
Rsym linear 30.0 ~3.03 Å	5.5% (66 % for last shell)
Completeness	83.7% (79 % for last shell)