Crystal Structure of a Metal Ion-bound IS200 Transposase

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IS200 transposases, present in many bacteria and Archaea, appear to be distinct from other groups of transposases. To provide a structural basis for understanding the action of IS200 transposases, we have determined the crystal structure of the SSO1474 protein from Sulfolobus solfataricus, a member of the IS200 family, in both Mn2+-bound and Mn2+-free forms. Its monomer fold is distinct from other classes of structurally characterized transposases. Two monomers form a tight dimer by exchanging the C-terminal α-helix and by merging the two central β-sheets into a large β-sheet. Glu55, His62, and four water molecules provide the direct coordination sphere of the catalytically essential metal ion in the Mn2+-bound structure. His16, Asp59, and His60 also play important roles in maintaining the metal binding site. The catalytic site is formed at the interface between monomers. The candidate nucleophile in the transposition mechanism, strictly conserved Tyr121 from the other monomer, is turned away from the active site, suggesting that a conformational change is likely to occur during the catalytic cycle.

Transposable genetic elements or transposons are mobile DNA sequences that can insert themselves into nonhomologous target sites in the genome. They promote genome rearrangements such as duplication, deletion, and inversion (1). Insertion sequences, the smallest transposons, the transposase-encoding gene is flanked by terminal inverted repeats, but some insertion sequences, such as IS200 and IS608, are capable of independent transposition. In most of the insertion sequences, the transposase-encoding gene is flanked by terminal inverted repeats, but some insertion sequences, such as IS200 and IS605, do not carry terminal inverted repeats (2). So far, five protein families have been described that mediate transposition: DDE transposases, Y2 (or rolling circle) transposases, tyrosine transposases, serine transposases, and a fifth family that encodes a combination of reverse transcriptase and endonuclease (RT/En) activities (3). The Mu, Tn5, and DDE transposases, along with retroviral integrases, have been structurally characterized (4–6).

The 707-bp-long insertion sequence IS200, originally identified in Salmonella typhimurium LT2 (7), contains a single open reading frame tnpA, which encodes a unique transposase. Subsequently, IS200-like transposases have been found to be present in a wide variety of Gram-positive and Gram-negative eubacteria and Archaea (8, 9). IS605, IS606, and IS608 from Helicobacter pylori carry two open reading frames, tnpA and tnpB (10). The tnpA open reading frames encode IS200-like transposases. Members of the IS200 transposase family, including that encoded by the H. pylori IS608 tnpA, show high sequence conservation among them, but they do not carry the complete sequence signatures of any of the other known transposases (10). IS608 transposase leads to loss of the transposase activity, indicating that they are essential for catalysis (11). The HuH motif is suggested to be involved in binding a Mg2+ ion (12), which is essential for the catalytic activity of H. pylori IS608 transposase, but its structure does not contain a bound metal ion in the active site (11).

To provide the framework for a better understanding of the unique transposition mechanism of the IS200 transposase family, we have determined the crystal structure of an IS200 transposase encoded by the SSO1474 gene of Sulfolobus solfataricus in both Mn2+-bound and Mn2+-free forms. S. solfataricus transposase shows ~36% sequence identity to the H. pylori IS608 transposase. The structure reveals that the monomer fold of the IS200 transposase family is different from those of other structurally characterized transposase families and that dimerization is necessary for forming the active site at the interface between the monomers. The Mn2+-bound structure also reveals a unique coordination sphere for the metal ion. The present structural data shed light on the action of a new class of IS200 transposase family.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The SSO1474 gene encoding the 133-residue IS200-like transposase was PCR-amplified and cloned into the expression vector pET-21a(+) (Novagen). This construct was overexpressed in Escherichia coli Rosetta2(DE3)pLysS cells using Terrific-Broth culture medium. Protein expression was induced by 1 mM isopropyl β-D-thiogalactopyranoside, and the cells were incubated for an additional 48 h at 18 °C following growth to mid-log phase at 37 °C. The cells were lysed by sonication in 50 mM Tris-HCl (pH 7.0) and 50 mM NaCl. Following heat treatment at 70 °C for 10 min, the sample was centrifuged at 18,000 revolutions/min for 60 min. The supernatant was applied to a HiTrap SP (5 ml) column (Amersham Biosciences), which was previously equilibrated with 50 mM Tris-HCl (pH 7.0). Upon eluting with a gradient of NaCl in the same buffer, SSO1474 transposase was eluted at 650–700 mM NaCl concentration. The protein was further purified by gel filtration on a HiLoad XK-16 Superdex 200 prep-grade
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E. coli Rosetta2(DE3) pLysS cells, we used the M9 culture medium that contained extra amino acids, including SeMet.

Crystallization—Crystals were grown by the hanging drop vapor diffusion method at 24°C by mixing equal volumes (2 μl each) of the protein solution (19 mg ml−1 concentration in 50 mM Tris-HCl (pH 7.0) and 200 mM NaCl) and the reservoir solution. To grow crystals of the native protein, we used a reservoir solution consisting of 1.4 M trisodium citrate and 100 mM sodium HEPES (pH 7.5). The crystals grew to the approximate dimensions of 0.2 × 0.2 × 0.1 mm within a few days. The SeMet-substituted protein was crystallized under crystallization conditions identical to those for the native crystals, except for the presence of 10 mM dithiothreitol in the protein solution. Mn2⁺-bound crystals of the native protein were prepared by replacing the hanging drop solution of the Mn2⁺-free crystals with a solution consisting of 100 mM sodium HEPES (pH 7.5), 25% (w/v) polyethylene glycol 3350, and 100 mM manganese chloride over a period of ~30 min in about ten steps before cryoprotection.

X-ray Data Collection and Structure Determination—A crystal of the SeMet-substituted protein was frozen using a cryoprotectant solution containing 10% (v/v) glycerol in the crystallization mother liquor. X-ray diffraction data were collected at 100 K on an Area Detector Systems Corporation (ADSC) Quantum 210 charge-coupled device area detector system at the BL-4A experimental station of the Pohang Light Source, Pohang, Korea. For each image, the crystal was rotated by 1°, and the crystal-to-detector distance was set to 270 mm. The raw data were processed and scaled using the program suite HKL2000 (13). The crystal belongs to the space group C2, with unit cell parameters of a = 93.1 Å, b = 68.2 Å, c = 65.5 Å, α = γ = 90°, β = 129.1°. Two monomers are in the asymmetric unit, giving the crystal volume/protein mass (V/c) of 2.52 Å³ Da⁻¹ and a solvent content of 51.2%. Table 1 summarizes statistics of multiwavelength anomalous diffraction data collection.

Two of the four expected selenium atoms of two monomers in each crystallographic asymmetric unit were located with the program SOLVE (14), and the selenium sites were used to calculate the phases with RESOLVE (15). Phasing statistics are summarized in Table 1. Model building was done using the program O (16). The model was refined with the program CNS (17), and several rounds of model building, simulated annealing, positional refinement, and individual B-factor refinement were performed. The 2-fold non-crystallographic symmetry restraints were relaxed in successive rounds of refinement. Water molecules were added using the program CNS, followed by visual inspection and B-factor refinement.

X-ray diffraction data from a native crystal in the Mn2⁺-free form were collected at 100 K on an ADSC Quantum 4R charge-coupled device area detector system at the BL-38B1 experimental station of SPring-8, Harima, Japan. X-ray diffraction data of the Mn2⁺-bound

| Data collection, phasing, and refinement statistics |
|-----------------------------------------------|
| Data set                                      | SeMet A2 (edge) | SeMet A3 (remote) |
| Wavelength (Å)                                | 0.97920         | 0.97912           |
| Resolution range (Å)                         | 50–1.65         | 50–1.65           |
| Total/unique reflections                     | 463,069/38,315  | 475,183/38,406    |
| Rmerge (%)                                    | 7.6 (30.5)*     | 8.1 (38.8)*       |
| Rwork (%)                                     | 3.0 (9.2)*      | 4.1 (12.4)*       |
| Figure of merit for multiwavelength anomalous diffraction phasing (50–1.70 Å) | 0.31/0.48 (before/after density modification) |

| Refinement Statistics                        | Mn2⁺-free       | Mn2⁺-bound       |
|----------------------------------------------|-----------------|-----------------|
| Data set                                     | SPring-8 BL-38B1| PLS BL-4A       |
| X-ray source                                 | 93.5, 68.2, 65.5| 91.9, 67.9, 65.3|
| Number of protein/atom/pressor/Å²            | 21.0/22.2       | 22.3/23.8       |
| No. of water molecules/pressor/Å²            | 2,164/260 (19.9)| 2,164/260 (24.6)|
| No. of Mn²⁺/pressor/Å²                       | 380 (37.2)      | 155 (35.8)      |
| r.m.s.d. deviation from ideal geometry       | 0               | 2 (23.5)        |
| Bond lengths/angles/Å                        | 0.005/1.45      | 0.0075/1.40     |
| Ramachandran plot (Most favorable (%))       | 90.0            | 87.6            |
| Allowed (%)                                  | 9.1             | 10.7            |
| Generously allowed (%)                       | 0.9             | 1.7             |
RESULTS AND DISCUSSION

Structure of IS200 Transposase

Monomer Structure—We have determined the crystal structure of an IS200 transposase encoded by the SSO1474 gene using the multiwavelength anomalous diffraction method (Table 1). The refined models of both Mn$^{2+}$-free and Mn$^{2+}$-bound forms contain 260 residues of the IS200 transposase. β-Strands are shown as arrows and α-helices as cylinders. C, ribbon diagram of a dimer. Monomers 1 and 2 are in deep salmon and light blue, respectively. The primed secondary structure elements belong to the second monomer of the dimer. D, stereo C-α trace of S. solfataricus transposase. Every tenth residue is marked by a dot and labeled. Two bound manganese (Mn) ions are also indicated. Five signature sequence motifs are highlighted in colored lines: motif I, (V/V)HUL/V/L(F/Y)LXX/P, at positions 59–69 in red; motif II, KG/LLSR, at positions 81–86 in green; motif IV, KWXXS/GYF/FYKT/TSXG, at positions 100–111 in cyan; and motif V, (I/H)VXXWXX, at positions 118–125 in blue, respectively.

Comparison of Monomer Structures—The monomer structure of S. solfataricus transposase is similar to that of the H. pylori IS608 transposase (11) (Protein Data Bank [PDB] code 2A6M, sequence identity = 36%, r.m.s. deviation = 1.39 Å for 130 C-α atoms of structurally aligned residues of the Mn$^{2+}$-free structure, monomer A). A major difference is the presence of an additional helix at the C terminus of the 155-residue H. pylori transposase. This region is not conserved (Fig. 2), and thus it may be dispensable for the transposition activity. Comparisons with the structural data base in the Protein Data Bank using the program server DALI (18) found only a low level (Z score

Ser$^{114}$). Two monomers of the Mn$^{2+}$-bound S. solfataricus transposase in the asymmetric unit overlap with an r.m.s. difference of 0.30 Å for 130 C-α atom pairs. Large deviations occur at the residues 88–94, with a maximum of 2.25 Å at Gly$^{98}$. Between the Mn$^{2+}$-free and Mn$^{2+}$-bound monomers, the r.m.s. differences range between 0.27 and 0.48 Å for 130 C-α atom pairs. Large deviations occur at the residues 92–98, with a maximum of 2.25 Å at Gly$^{98}$. Unless otherwise stated, we take monomer A for describing the structural features.

The central core of the S. solfataricus transposase monomer is an antiparallel, four-stranded β-sheet made of the strands β1 (residues 15–25), β2 (50–57), β3 (60–67), and β5 (105–110) (Fig. 1, A and B). A short β1 strand (residues 6–9) at the N terminus makes an antiparallel, two-stranded minisheet with the N-terminal part of the longest strand β2 (Fig. 1, A and B). Two α-helices (α1, α2) cover one side of the central β-sheet. The other side of the central β-sheet is open in a monomer, but in a dimer, it is partly covered by the C-terminal helix α3 and a preceding loop from the second monomer (described under "Dimer Structure").

Comparison of Monomer Structures—The monomer structure of S. solfataricus transposase is similar to that of the H. pylori IS608 transposase (11) (Protein Data Bank [PDB] code 2A6M, sequence identity = 36%, r.m.s. deviation = 1.39 Å for 130 C-α atoms of structurally aligned residues of the Mn$^{2+}$-free structure, monomer A). A major difference is the presence of an additional helix at the C terminus of the 155-residue H. pylori transposase. This region is not conserved (Fig. 2), and thus it may be dispensable for the transposition activity. Comparisons with the structural data base in the Protein Data Bank using the program server DALI (18) found only a low level (Z score
A close-up view of a metal ion binding by the HUH motif. A, $2F_{o} - F_{c}$ electron density map around the Mn$^{2+}$ binding site. Residues near the HUH motif (His$^{60}$ and His$^{62}$) are shown in sticks. Octahedral coordination of Mn$^{2+}$ is represented by black dotted lines, whereas blue dotted lines denote hydrogen bonds. Red balls represent water molecules. B, superposition of the Mn$^{2+}$-bound (orange sticks) and Mn$^{2+}$-free (cyan sticks) S. solfataricus transposase structures. Black dotted lines indicate Mn$^{2+}$ coordination, whereas green dotted lines denote hydrogen bonds. C, superposition of the Mn$^{2+}$-bound S. solfataricus transposase (orange sticks) and Mn$^{2+}$-free structure of the H. pylori IS608 transposase (green sticks). Black dotted lines denote Mn$^{2+}$ coordination and hydrogen bonds. D, binding of a divalent metal ion by the HUH motif in other proteins. Black dotted lines denote Mn$^{2+}$ coordination and hydrogen bonds.
(Wat9, Wat18, Wat7, and Wat68), respectively. Essentiality of His62 and His65 in metal ion coordination explains the finding that mutations of the corresponding residues (His64 and His66 of H. pylori IS608 transposase causes severe reductions in transposition activity (11).

The orientation of the His62 imidazole ring is maintained by hydrogen bonds with His16 and Glu55 (2.9 Å between the His62 N-ε2 atom and the His16 N-ε2 atom; 3.0 Å between the His62 N-ε2 atom and the O-ε1 atom of Glu55). His16, His65, and His62 are strictly conserved, whereas Glu55 is more variable among IS200 transposases (Fig. 2). However, the residues corresponding to Glu55 have side chains that are capable of hydrogen bonding. Thus, the observed coordination of the metal ion is likely to be retained in other members of the IS200 transposase family. The imidazole ring of His62 is likely to be in the rare NH2 tautomeration state due to an interaction with Asp55 (Fig. 3, A–C). The essential roles played by His16 and Asp55 in maintaining the metal ion binding site explain the finding that mutation of the corresponding residues (His20 and Asp59) of H. pylori IS608 transposase cause severe reductions in the in vitro DNA nicking activity (11).

It is hypothesized that the two histidine residues of the HUH motif may be involved in metal ion coordination (12). Our structure of the Mn2+–bound S. solfataricus transposase confirmed the proposed role of the HUH motif in the IS200 transposase family. The detailed view of the metal ion binding site provided in this study also has significant implications for a better understanding of the catalytic mechanism of IS200 transposases, because Mg2+ ions are essential for DNA nicking and the formation of a covalent intermediate between H. pylori IS608 transposase and DNA (11).

The observed manganese ion binding in the S. solfataricus transposase active site is distinct from the modes of metal ion binding to the HUH motifs of the adeno-associated virus type 5 Rep (AAV Rep), the F plasmid Tral relaxase domain, and the conjugal relaxase TrwC (22–24). In Rep, Tral, and TrwC, two histidines of the HUH motif, along with a third residue located on the preceding β-strand (Asp in Rep; His in Tral and TrwC), directly coordinate a zinc, a manganese, and a zinc ion, respectively (Fig. 3D). In comparison, only the second histidine of the HUH motif directly coordinates the metal ion in S. solfataricus transposase, and the first histidine makes an indirect, water-mediated coordination. Our observed structure is also different from the proposed model of the magnesium ion binding to the H. pylori IS608 transposase (11). Our structure indicates that the third Mg2+–coordinating ligand is the H. pylori IS608 transposase is most likely Glu55, instead of Asp61 (11) (Fig. 3C).

A significant change in the side chain conformation of Glu55 occurs upon Mn2+ binding to S. solfataricus transposase (Fig. 3B). The side chain of Glu55 changes its orientation so that the terminal oxygen atoms move toward the Mn2+ ion. The side chain of His62 also undergoes a slight reorientation to coordinate the Mn2+ ion. Binding of the metal ion also pulls the side chains of His62 and Asp55 toward the metal site (Fig. 3B). The extended side chain of Arg25 is located at the dimer interface between the side chains of Asp55 and Glu123 in the Mn2+–free state (supplemental Fig. S1). The primed residue comes from the second monomer of the dimer. However, the side chain of Glu123 changes its direction, and a salt bridge between Arg25 and Glu123 is broken in the Mn2+–bound state (supplemental Fig. S1). Glu123 resides on the C-terminal helix α3, and the breakage of a strong salt bridge upon Mn2+ binding might have a functional implication. This is because a large conformational change involving the rearrangement of the C-terminal helix α3 appears to be necessary to bring the strictly conserved Tyr121 into the proximity of the metal ion and its ligands for catalysis (described under “Location of the Strictly Conserved Tyrosine and Implications for Catalysis”).

Sequence Motifs and Roles of Conserved Residues—The residues that are highly conserved among the members of the IS200 transposase family can be grouped into five sequence motifs (Fig. 2). Motif I (Y/V)HUU(W/F)XX(K/R)YRR encompasses Tyr15–Arg25 of S. solfataricus transposase (Fig. 2, boxed in purple). Motif II, DHU(L/I)(L/I)FUXXXYP, corresponds to the HUH motif (12). It covers Asp59–Pro69 (Fig. 2, boxed in red). Motif III, KGSXSR, covers Lys81–Arg86 (Fig. 2, boxed in green), whereas Motif IV, LWXX(S/G)Y(F/Y)UXXXYG, covers Leu100–Gly111 (Fig. 2, boxed in cyan). Motif V, (I/V)XXLYXXQ, covers Ile118–Gln126 (Fig. 2, boxed in blue). It contains the strictly conserved Tyr121. U is a hydrophobic residue, X stands for any amino acid, and the residues that are strictly conserved in Fig. 2 are in bold face.

Conserved positively charged residues (Lys85/Arg84 from motif I and Lys81/Arg86 from motif III), along with semiconserved Arg67, are clustered on one side of the S. solfataricus dimer (marked with blue circles in Fig. 2 and labeled in Fig. 4A). On the other hand, the opposite face of the dimer is rich in neutral and negatively charged residues (Fig. 4A). The corresponding region of strong positive electrostatic potential has been shown to be the binding site for a stem-loop DNA in the H. pylori IS608 transposase (11). The strictly conserved Lys85 and Gly86 of H. pylori IS608 transposase (corresponding to Lys81 and Gly82 of S. solfataricus transposase) have been shown to be crucial for binding the stem-loop DNA (11). We confirmed that S. solfataricus transposase could bind to...
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double-stranded DNA by electrophoretic mobility shift assay (supplemental data and Fig. S2).

When we superimposed the S. solfataricus transposase structure into the stem-loop-bound model of the H. pylori IS608 transposase (11) (PDB code 2A6O), reasonable shape and charge complementarities existed between S. solfataricus transposase and the stem-loop DNA (Fig. 4B). The protruding side chain of Arg 2 (corresponding to His 11 of IS608) penetrated deeply into the major groove, possibly contacting a base pair of the target DNA. In the H. pylori IS608 transposase, His 11 interacts with G18 in the major groove (11). Lys 22 (corresponding to Lys 26 of IS608) neutralizes the negative charge of the phosphate backbone of the stem-loop DNA. The conserved Arg 16 (corresponding to Arg 28 of IS608) is located near the entrance of the active site and may be essential for interactions with DNA upon possible conformational changes of S. solfataricus transposase (Fig. 4A and supplemental Fig. S3). Arg 16 (corresponding to Arg 86 of IS608) is positioned near the apex of the stem-loop, where the DNA backbone makes a turn.

In addition to its role in binding DNA, Lys 81 may also contribute to stabilization of the monomer. In the S. solfataricus transposase structures, the side chain of Lys 81 of motif III points toward the suggested nucleophile Tyr 121 (6.6–7.0 Å between the NZ atom of Lys 81 and the hydroxyl oxygen atom of Tyr 121). The NZ atom of Lys 81 makes hydrogen bonds with the backbone oxygen atoms of Thr 102 and Ser 104 (2.7–2.8 and 2.8 Å, respectively) (supplemental Fig. S3B). The latter two residues are not conserved. The aliphatic part of the Lys 81 side chain also makes nonpolar interactions with two conserved tryptophan residues (Trp 19 and Trp 101) (supplemental Fig. S1).

Strictly conserved Leu 100 and Trp 101 from sequence motif IV, together with neighboring residues (Phe 17, Trp 19, Leu 100, Phe 80, and Lys 81) contribute to the hydrophobic core of a monomer (supplemental Fig. S1). The conserved Thr 100 from sequence motif IV, located on β5 (Figs. 1D and 2), interacts with the C-terminal helix α3’ from the second monomer and appears to contribute to dimerization (Fig. 1D). The side chain OG1 atom of Thr 100 makes a hydrogen bond with the backbone oxygen of Ala 110 (2.7 Å). The conserved Lys 49 makes a salt bridge with the semiconserved Glu 56 at the start of strand β3 (supplemental Fig. S1). This interaction may contribute to stabilizing the N-terminal mini-β-sheet.

Location of the Strictly Conserved Tyrosine and Implications for Catalysis—Tyr 121 residing on the C-terminal helix α3 is the only tyrosine that is strictly conserved among the IS200 transposase family members (Fig. 2). Mutation of the corresponding residue (Tyr 127) in the H. pylori IS608 transposase caused loss of the ability to form a covalent intermediate and abolished transposition activity to the background level, whereas mutation of other tyrosines did not significantly affect transposition (11). This result clearly establishes that Tyr 121 acts as the nucleophile in the catalytic mechanism and forms a covalent phosphotyrosine intermediate.

In both our Mn 2+ -free and Mn 2+ -bound structures of S. solfataricus transposase, however, the side chain of Tyr 121 (coming from the second subunit) is turned away from Glu 56, His 69, and His 65, which contribute to formation of the metal binding site (Fig. 3A and supplemental Fig. S1). The distance from the hydroxyl oxygen atom of Tyr 121 (to Mn 2+ is 13.5 Å. Although the precise role of the metal ion in the catalytic mechanism has yet to be determined, formation of the covalent intermediate requires Mg 2+ (11). This necessitates a conformational change to bring Tyr 121 into the proximity of the metal ion so that the hydroxyl group of Tyr 121 can make a nucleophilic attack on the target DNA. In both DNA-bound and DNA-unbound structures of the H. pylori IS608 transposase, Tyr 127 is similarly turned away from the HUH motif (11). It has been suggested that an ~90° twist of helix αD would be required to juxtapose Tyr 127 of IS608 transposase with the HUH motif (11). The suggested movement may also accompany a change in the side chain orientation of Tyr 121. This is because the hydroxyl group of Tyr 121 can possibly move a distance of up to a few Å from Mn 2+ by simply rotating the torsional angle of its side chain. This kind of motion would be made possible if a conformational change in helix α3’ removes steric hindrance because of nearby residues, such as Val 18 (supplemental Fig. S1). To confirm such a conformational change involving the C-terminal helix α3’, it may be necessary to determine the structure of the metal ion-bound S. solfataricus transposase in complex with the substrate DNA. A possible catalytic mechanism for transposition by S. solfataricus transposase, adapted from Ref. 11, is shown in supplemental Fig. S4, in which the metal ion coordination is based on the present work.

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