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An Equivalent Metal Ion In One- and Two-metal Ion Catalysis

Wei Yang

Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health 9000 Rockville Pike, Bldg. 5, Rm B1-03 Bethesda, MD 20892 USA

Abstract

Nucleotidyl-transfer enzymes, which synthesize, degrade and rearrange DNA and RNA, often depend on metal ions for catalysis. All DNA and RNA polymerases, MutH-or RNase H-like nucleases and recombinases, and group I introns appear to require two divalent cations to form a complete active site. The two-metal ion mechanism has been proposed to orient substrate, facilitate acid-base catalysis, and allow catalytic specificity to exceed substrate-binding specificity owing to the stringent metal-ion (Mg^{2+} in particular) coordination. Not all nucleotidyl-transfer enzymes use two metal ions for catalysis, however. The $\beta\beta\alpha$ -Me and HUH nucleases depend on a single metal ion in the active site for the catalysis. All of these one- and two- metal ion dependent enzymes generate 5'-phosphate and 3'-OH products. We compare and contrast their structures and mechanisms and show that they share a functionally equivalent metal ion.

INTRODUCTION

Polymerases^{1–3} and nucleases^{4–7} using two-metal ion catalysis share a common active site structure. In the enzyme-substrate complexes, the two metal ions are jointly coordinated by the scissile phosphate and an active site Asp (Fig. 1)⁸. They are bisected by the scissile phosphate with metal ion A on the nucleophile side and metal ion B on the leaving group side. After the observation of two metal ions in the active site of alkaline phosphatase and 3'-5' exonuclease^{9, 10}, Steitz and Steitz proposed that metal ions can substitute protein side chains in catalytic RNA and function as general base and acid¹¹. It was proposed that metal ion A deprotonates the nucleophilic water and metal ion B stabilizes the pentacovalent phosphate intermediate^{4, 11}. Recent studies of polymerases and nucleases indicate that metal ion B may destabilize the substrate and promote nucleophilic attack^{6, 8}. In addition the two metal ions are intimately linked, and the distance between the metal ions is likely shortened from ~4 Å in a substrate complex to ~3.5 Å in the transition state^{6, 12}. The closely positioned metal ions can effectively neutralize the highly negatively charged pentacovalent phosphate. Both the number and location of metal ions in the active site are sensitive to the environment and can deviate from the norm in the presence of non-cognate substrates, altered active site residues, or metal ion substitution^{5, 12–16}. This sensitivity to the coordination environment may be the basis for the exceedingly high catalytic specificity of polymerases, restriction endonucleases, and other nucleic acid enzymes that utilize the two-metal ion mechanism⁸.

Many nucleases and recombinases, however, don't use the two-metal ion mechanism. A large fraction of those that generate 5'-phosphate and 3'-OH products use one metal ion for catalysis according to structural and biochemical analyses. To understand the different metal ion requirements, enzymes known to use one-metal ion catalysis are categorized and compared with the two-metal ion dependent enzymes. Surprisingly, these two seemingly different

mechanisms share a conserved metal-ion binding site. The comparative studies and functional implications are summarized here.

RESULTS AND DISCUSSION

Nucleases containing one-metal ion in the active site

A single metal ion has been observed in the active site of endonucleases with the $\beta\beta\alpha$ -Me motif^{17, 18}, which is characterized by the consecutive β , β , α secondary structure elements arranged in a V shape (Fig. 2A–E). The β strands form one arm of the “V”, and the helix the other. A single metal ion located at the opening of the “V” is coordinated by active site residues and the scissile phosphate including the leaving group (3'-O) (Fig. 2A–E)^{19–21}. Many of the $\beta\beta\alpha$ -Me endonucleases like Caspase-Activated DNase (CAD), Serratia, Vvn and E-group colicins hydrolyze nucleic acids with no sequence or ribose (DNA versus RNA) specificity^{19, 22, 23}. Other superfamily members like HNH²⁴ and His-Cys homing endonucleases²⁰ are highly sequence specific, but in these cases protein domains besides the $\beta\beta\alpha$ -Me motif mediate base-specific interactions.

A conserved single metal-ion binding site is also found in HUH endonucleases required for Y1-type DNA transposition^{25, 26} and for initiation of rolling-circle DNA replication²⁷, a mechanism used in bacterial conjugation^{28–30} and propagation of ssDNA viruses (Adeno-associated virus (AAV), parvovirus, etc)^{31–33}. The name of HUH comes from the sequence motif of two conserved His's (H) separated by a hydrophobic residue (U)²⁷. These nucleases catalyze a two-step reaction. In the first step a conserved tyrosine serves as the nucleophile to cleave DNA and generate a tyrosyl-5'-phosphate and 3'-OH. After DNA rearrangement or replication, in the second step the phospho-tyrosyl bond is cleaved by a 3'-OH, so the DNA is re-ligated and the active site Tyr regenerated^{26, 27, 34}. The first step has been structurally well studied and shown to be very similar to nucleic acid hydrolysis, while the configuration of the second step of the reaction is less known. The active site of these enzymes consists of β -strands and an α -helix, but they are not contiguous in the amino-acid sequence^{26, 31, 35, 36} (Fig. 2F). The two conserved His's in the HUH motif are involved in metal ion binding as proposed²⁷. Interestingly, the metal ions, scissile phosphates and nucleophiles of the HUH and $\beta\beta\alpha$ -Me enzymes can be superimposed, although the locations of β -strands and α -helix are approximately switched (Fig. 2).

An equivalent metal ion in RNase H and TraI

RNase H, for which several crystal structures along the catalytic pathway are available^{6, 37}, provides an example of two-metal ion catalysis (Fig. 1). The HUH nuclease TraI, crystallized with a ssDNA substrate, Mg^{2+} ion and the nucleophile Tyr mutated to Phe³⁵, reveals the most complete active site that utilizes the one-metal ion mechanism (Fig. 2F). If the Tyr were present, its hydroxyl group would be 2.6 Å from the scissile phosphorus and perfectly inline for nucleophilic attack. Similar arrangement of the metal ion and catalytic residues are observed for other HUH members^{26, 33, 34}. When the active sites of TraI and RNase H are superimposed, metal ion A in the RNase H-substrate complex coincides with the amine group of a highly conserved Lys in TraI (Fig. 3). This Lys, however, is not strictly required for DNA cleavage by TraI³⁸, nor is it conserved among enzymes utilizing one-metal ion catalysis. Nevertheless and perhaps most astonishingly, metal ion B of RNase H coincides with the single Mg^{2+} of TraI (Fig. 3).

Not only are the Mg^{2+} ions of TraI and RNase H (B) coincident relative to the scissile phosphate, they also share similar coordination geometry. Both are devoid of inner-sphere water ligands, and both are coordinated by three active site residues plus two oxygen atoms from the scissile phosphate (Fig. 3). The deviation from the normal octahedral Mg^{2+}

coordination geometry and the absence of water ligands³⁹ indicate that the metal ion and its ligands are likely strained. This conserved metal-ion binding site in RNase H and TraI, which share no structural or functional similarity and use different amino acids (Asp and Glu vs His) for metal ion coordination, suggests an essential and convergent role of the metal ion in degradation of nucleic acid. It is worth noting that metal ion B in polymerases, which catalyze nucleic acid synthesis instead of degradation, has six ligands but is often devoid of water ligand³. As proposed for metal ion B in the two-metal ion mechanism⁸, the single metal ion in TraI may also destabilize the substrate and weaken the scissile bond for breakage.

Similarity between the $\beta\beta\alpha$ -Me and HUH superfamilies

Coordination of the metal ion in the $\beta\beta\alpha$ -Me nucleases colicin E7 and E9 resembles that of TraI (Fig. 2). To crystallize colicin E7 and E9 with DNA substrate, the general base His, which activates the nucleophilic water molecule, was mutated to Ala, Glu or Gln (Fig. 2A–C)^{40–42}. In the presence of Zn^{2+} , the single metal ion in the active site of colicin E7 and E9 is coordinated by three His's and the scissile phosphate as the Mg^{2+} in TraI (Fig. 2B–C). The distance between the Zn^{2+} and the 3'-O leaving group varies from 2.8 to 3.2 Å, which leaves the Zn^{2+} with only 4 ligands in a more or less tetrahedral geometry. When Zn^{2+} in colicin E9 is substituted by Mg^{2+} , which supports the catalysis, one His coordinating the metal ion moves away, and so does the 3'-O leaving group (Fig. 2A)⁴⁰. The active site mutation and consequent changes of the scissile-phosphate orientation and coordination environment may prevent Zn^{2+} and Mg^{2+} from adopting the strained five-ligand coordination and engaging the 3' leaving group.

A similar alignment of the metal ion, scissile phosphate and nucleophile is also observed in the substrate complexes of other $\beta\beta\alpha$ -Me enzymes, I-PpoI, I-HmuI, Vvn and T4 endo VII^{19–21} (Fig. 2D–E), although a mixture of His, Asn, Asp, Glu, Ser, etc instead of three His's are used as the metal ion ligands. One of the three protein residues participating in the metal ion coordination is difficult to identify due to the lack of conservation in amino acid type and location in the tertiary structure^{19, 20, 24} (Fig. 2A–E). This elusive metal-ion ligand is also mobile like the flexible His in the colicin E's and may be located > 4 Å from the metal ion in crystal structures due to non-functional metal ion substitution (Fig. 2E)⁴³ or active site mutations (Fig. 2A, D)^{21, 40, 44, 45}. However, there is a notably conserved feature among $\beta\beta\alpha$ -Me nucleases. Two catalytically essential residues are adjacent in the sequence and structure (at the end of the first β strand): the invariable His, which serves as the general base, and its N-terminal neighbor, which coordinates the metal ion (circled in Fig. 2A–E). The metal ion ligand varies from His in colicins, Glu in Vvn, Ser in I-PpoI, to Asp in T4 endo VII and I-HmuI (Fig. 2A–E), but the peptide bond between the general base activating the nucleophile and the metal-ion ligand that orients the scissile phosphate is conserved and likely essential for the catalysis. This is reminiscent of the coupling of nucleophile and scissile phosphate by the A and B metal ions jointly coordinated by the conserved Asp in the two-metal ion mechanism (Fig. 1, 2A–E). The coupling may be such that the nucleophile doesn't form unless the scissile bond is aligned and strained.

Other known HUH enzymes share a similar active site configuration with TraI, although the third metal ion ligand from protein varies in residue type (His, Gln or Glu) and location^{26, 28, 31, 33, 34}. Coupling between the attacking nucleophile and leaving group is not evident in the HUH family. This may be due to the two-step reaction that HUH enzymes catalyze, during which the nucleophile Tyr in the first step would become the leaving group in the second step^{26, 27}. In addition, DNA exchange or replication primed by the cleavage product 3'-OH takes place in between the two steps^{26, 27, 34}. It is not clear whether the substrate is reoriented so that the phospho-tyrosyl bond replaces the phosphodiester bond of the first cleavage step or the phospho-tyrosine remains more or less in the same place, but the leaving group 3'-OH

in the first step becomes the attacking nucleophile. Either way, some rearrangement in the active site is needed³⁷.

Differences between one- and two-metal ion catalysis

The hallmark of two-metal ion dependent nucleic acid enzymes is an absolutely conserved Asp (Fig. 1), which coordinates both metal ions and is substituted by a backbone phosphate in ribozymes⁸. Among the one-metal ion dependent enzymes, there is no conserved Asp. The $\beta\beta\alpha$ -Me superfamily is marked by a conserved general base His, and the HUH enzymes by two histidines required for metal ion coordination (Fig. 2). Another major difference is the degree of difficulty in capturing catalytically essential metal ions in crystal structures. The two metal ions are elusive even in enzyme-substrate complexes⁸, but one-metal ion dependent enzymes can bind the metal ion in the absence of substrate^{19, 20, 29, 31, 46}.

In general, enzymes using one-metal ion catalysis are less stringent in metal ion selection and substrate specificity than two-metal ion-dependent enzymes. Mg^{2+} , Ca^{2+} , Mn^{2+} , Zn^{2+} , Cu^{2+} , Cd^{2+} , Co^{2+} and Ni^{2+} can support one-metal ion catalysis to varying degrees in many enzymes^{19, 20, 38}, while Mg^{2+} is typically required for two-metal ion catalysis⁸. The preference for Mg^{2+} may result from the chemical environment and the stringency imposed by coordination of two metal ions within 3–4 Å. The stringent requirement for two- Mg^{2+} coordination is likely the basis for catalytic specificity by the two-metal ion mechanism⁸. In contrast, catalytic specificity of one-metal ion dependent homing and HUH nucleases appears to derive from substrate binding^{20, 21, 26, 28, 35}.

Catalytic role of the metal ion

We propose that the $\beta\beta\alpha$ -Me and HUH nucleotidyl-transfer enzymes use a common one-metal ion mechanism for catalysis. The single metal ion appears to be spatially and functionally equivalent to metal ion B of two-metal ion catalysis (Fig. 3). The metal ion probably plays a catalytic role in phosphoryl transfer reactions. Firstly, the high charge density of divalent cations obviously can stabilize the electron-rich pentacoordinate phosphate intermediate more efficiently than protein sidechains. Secondly, the conserved metal ion is likely coordinated by three protein ligands and two oxygen atoms of the scissile phosphate as observed for TraI and RNase H (Fig. 3). Such coordination deviates from the usual octahedral or tetrahedral geometry preferred by divalent cations⁴⁷ and leads to a strained metal ion. By engaging two oxygen atoms from the scissile phosphate as ligands, which necessarily results in an unfavorably coordination angle (O-Me-O) smaller than 90° (Fig. 3), the metal ion can directly destabilize the scissile bond and facilitate the nucleophilic attack. Finally, the conserved metal ion in RNase H³⁷ and TraI³⁵ as well as metal ion B in many DNA and RNA polymerases^{3, 8} is also devoid of water ligand and is thus at a high-energy state^{39, 48}. The metal ion may accelerate product release and turnover rate by regaining water ligands.

The catalytic role of the conserved metal ion in nucleotidyl transfer reactions rationalizes why ligands of metal ion B in two-metal ion catalysis are less mutable than those of metal ion A^{37, 49}. The absence of a second metal ion in the one-metal ion mechanism may be compensated by the presence of a good general base in the $\beta\beta\alpha$ -Me family (His) or a better nucleophile in the HUH family (Tyr), which is easier to deprotonate than a water molecule.

METHODS

Structural superimposition of the active site between $\beta\beta\alpha$ -Me and HUH enzymes and between TraI and RNase H were carried out manually using ONO⁵⁰. These enzymes share no sequence or structural conservation. But they all generate 5' phosphate and 3'-OH. In theory the orientation of nucleophile and scissile phosphate relative to the active site should be conserved,

and indeed they were superimposable. Moreover, the metal ions in $\beta\beta\alpha$ -Me and HUH were found to have similar locations and thus were included for superposition. Superposition of RNase H and TraI was also achieved by optimizing the alignment of scissile phosphates, nucleophiles and metal ions (B in RNase H).

Structure rendering and distance measurement were made using PyMol (www.pymol.com).

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Figure 1. A diagram of two-metal ion catalysis by RNase H

Coordination of metal ions A and B is indicated by the dashed lines. The Asp conserved in all nucleotidyl-transfer enzymes using two-metal ions is highlighted in pink. This Asp is replaced by a backbone phosphate in group I introns. The oxygen atoms from the protein and scissile phosphate that chelate metal ion B are highlighted in pink, and the attacking nucleophile is highlighted in blue.

Figure 2. Examples of one-metal ion-dependent nucleases

(A–E) The $\beta\beta\alpha$ -Me family. (F) The flipped $\beta\beta\alpha$ -Me family. The active site residues are shown in green(C)/blue(N)/red(O) and the DNA substrate centered on the scissile phosphate in yellow (C)/blue/red/orange(P) stick models. The Na^+ , Mg^{2+} and Zn^{2+} in the active site are shown as color-coded spheres. Coordination of metal ion is represented by pink dashed lines. The mobile metal-ion ligand is indicated by a yellow arrow. When determined, the nucleophile water is shown as a red sphere. The adjoining two active site residues in the $\beta\beta\alpha$ -Me nucleases are highlighted by the orange ovals. Mutations of the active site residues, which enable crystallization of enzyme-substrate complexes are labeled.

RNase H (PDB: 1ZBL)

TraI (PDB: 2A0I)

Figure 3. Comparison of the active site of RNase H and TraI in stereoview

The nucleic acid is shown in light grey, and the protein in yellow with the N (blue), O (red), P (magenta) highlighted. The Mg^{2+} ions are shown as purple spheres, and their coordination by macromolecules is indicated by dashed pink lines. The active site mutations (D192N and Y16F) that prevent the chemistry are labeled. Alignments of the metal ions (or Lys) between the two enzymes are shown by the semi-transparent grey lines. The orange arrows represent the nucleophilic attack.