Coordination of Divalent Metal Ions in the Active Site of Poly(A)-specific Ribonuclease*

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Poly(A)-specific ribonuclease (PARN) is a highly poly(A)-specific 3’-exoribonuclease that efficiently degrades mRNA poly(A) tails. PARN belongs to the DEDD family of nucleases, and four conserved residues are essential for PARN activity, i.e. Asp-28, Glu-30, Asp-292, and Asp-382. Here we have investigated how catalytically important divalent metal ions are coordinated in the active site of PARN. Each of the conserved amino acid residues was substituted with cysteines, and it was found that all four mutants were inactive in the presence of Mg\(^2+\). However, in the presence of Mn\(^2+\), Zn\(^2+\), Co\(^2+\), or Cd\(^2+\), PARN activity was rescued from the PARN(D28C), PARN(D292C), and PARN(D382C) variants, suggesting that these three amino acids interact with catalytically essential metal ions. It was found that the shortest sufficient substrate for PARN activity was adenosine triphosphate (A\(_3\)) in the presence of Mg\(^2+\) or Cd\(^2+\). Interestingly, adenosine dinucleotide (A\(_2\)) was efficiently hydrolyzed in the presence of Mn\(^2+\), Zn\(^2+\), or Co\(^2+\), suggesting that the substrate length requirement for PARN can be modulated by the identity of the divalent metal ion. Finally, introduction of phosphorothioate modifications into the A\(_3\) substrate demonstrated that the scissile bond non-bridging phosphate oxygen in the pro-R position plays an important role during cleavage, most likely by coordinating a catalytically important divalent metal ion. Based on our data we discuss binding and coordination of divalent metal ions in the active site of PARN.

Poly(A)-specific ribonuclease (PARN)\(^1\) is a highly poly(A)-specific 3’-exonuclease that efficiently degrades mRNA poly(A) tails (1–6). PARN is oligomeric, most likely consisting of three subunits (i.e. homotrimer) (6), and interacts with both the 3’-end-located poly(A) tail and the 5’-end cap structure during degradation (6–9). The interaction with the 5’-end cap structure enhances the rate of degradation (6, 7, 9) and amplifies the processivity of PARN activity (9). The functional significance of PARN is still unknown, although its high specificity for poly(A) tail degradation and therefore could play important roles in mRNA decay and initiation of protein synthesis (6–9).

PARN belongs to the DEDD family of nucleases, and four conserved acidic amino acids characteristic of this family are present in PARN (5, 10–12). These amino acids are Asp-28, Glu-30, Asp-292, and Asp-382 in human PARN. Site-directed mutagenesis has shown that these conserved residues are essential for catalytic activity and that they are required for the binding of divalent metal ions to PARN (13). Based on these observations it was proposed (13) that PARN utilizes the two-metal ion mechanism for its catalysis, as suggested for the Escherichia coli 3’-exonuclease domain of DNA polymerase I (pol I) (14, 15), although the stoichiometry of divalent metal ion binding in the active site of PARN is not yet known. To fully understand the catalytic mechanism of PARN it is of fundamental importance to determine metal ion binding coordination and stoichiometry in its active site.

Structural analyses by x-ray or NMR are powerful strategies to reveal the presence of divalent metal ions in the active sites of enzymes. However, despite the high resolution provided by these techniques, these methods frequently fail to unambiguously locate catalytically important divalent metal ions. Furthermore, even if the divalent metal ions are correctly located in the active site by structural analysis, it is still necessary to functionally confirm the interaction between the divalent metal ions and their ligands (16). Thus, other techniques are required to locate functionally important metal ions. This is of particular importance when structural data, as in the case of PARN, is not available. Sulfur substitutions of oxygen believed to be in direct contact with Mg\(^2+\) ions have successfully been used to locate ligands in direct contact with catalytically important Mg\(^2+\) ions. This is commonly referred to as “metal ion switch” experiments, and it has successfully been used to locate metal ions in both enzymes and ribozymes (see for example Refs. 16–22). Chemically, Mn\(^2+\), Zn\(^2+\), Co\(^2+\), and Cd\(^2+\) are soft metal ions and coordinate soft atoms such as sulfur, whereas Mg\(^2+\), which is a hard metal ion, avoids sulfur as a ligand (23) (reviewed in Ref. 24). Thus, the effect of shifting oxygen to sulfur can be compensated to some extent by changing Mg\(^2+\) to a soft metal ion; e.g. Mn\(^2+\), Zn\(^2+\), Co\(^2+\), or Cd\(^2+\).

Here, the metal ion switch approach has been used to locate divalent metal ions in the active site of PARN. Oxygens located both in the polypeptide chain of PARN and in the RNA substrate for PARN were replaced with sulfur. The replacement of Mg\(^2+\) by Mn\(^2+\), Zn\(^2+\), Co\(^2+\), and Cd\(^2+\) could rescue PARN activity from PARN mutants where the conserved amino acids Asp-28, Asp-292, or Asp-382 had been substituted to cysteine residues, indicating that these amino acids play important roles for metal ion coordination. The replacement of oxygen to sulfur in the RNA substrate provided evidence that the pro-R oxygen of the scissile phospho-

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The abbreviations used are: PARN, poly(A)-specific ribonuclease; pol, polymerase.
rus group of the substrate interacts with divalent metal ions.

**EXPERIMENTAL PROCEDURES**

*Site-directed Mutagenesis—* The cysteine mutant plasmids pE33-PARN(D28C), pE33PARN(E30C), pE33PARN(D292C), and pE33-PARN(D382C) were obtained from pE33PARN plasmid (13) using a QuikChange site-directed Mutagenesis kit (Stratagene) following the manufacturer's protocol. The pairs of oligonucleotide primers used were as follows: P28C-5’-5’-GCAGTCTCCGACACCAGGAGTTTCACG-GAATC-3’, P28C-3’ (5’-TTCTGGAAAACCTCCCTGGATGGGCAA-GAAGTGCC-3’); P30C-5’-5’-CTCCGGATCCATGGGCGGGCTTCCAGG-AATCACTGATGTC-3’, P30C-3’ (5’-CATCAGTGATCTCCTGAAACGGCCC-CATCGAGGCGCAAAG-3’); P292C-5’-5’-GACACAATATGCTCTTGGCC-TTACTGATTCCTGAAAACGCCC-3’, P292C-3’ (5’-GAACGTGTTGCATGACGCA-CCAGACGATATTGCTGTC-3’); P382C-5’-5’-CCACGAGGCAGCTACGACGCCC-3’, P382C-3’ (5’-GGCCCTGATGTTAG-GTGGTCAGCTCCGTCCTGTC-3’). The mutations were confirmed by DNA sequencing.

*Expression and Purification of Recombinant PARN—* His-tagged recombinant wild type and mutants PARN(D28C), PARN(E30C), PARN(D292C), and PARN(D382C) were purified from E. coli strain BL21(DE3) using a Ni²⁺ matrix (Novagen Inc.) as described previously (6). The amount of protein was measured using a Bio-Rad protein assay kit, and its purity was analyzed on a SDS-polyacrylamide gel following silver staining. High molecular weight silver staining marker was purchased from Sigma.

*Poly(A)-specific Ribonuclease Activity Assay—* Adenosine di-, tri-, and pentanucleotides (A2, A3, and A5) and A2s-A3s isomers I and II, which were modified with phosphorothioate by the replacement of one of the two non-bridging phosphate oxygen atoms by a sulfur atom between the second and third adenosines from the 5′-end, were synthesized by the manufacturer (ThermoHybaid). The isomers I and II had 6.98- and 8.51-min retention times in the reversed phase high performance liquid chromatography purification according to the purification reports provided by the company. To identify the absolute configurations of A2s-A3s isomers I and II, different concentrations of snake venom phosphodiesterase (Sigma) were used to hydrolyze 5′-32P-labeled As-A3s isomer I or II in 50 mM Tris-HCl (pH 7.9) and 5 mM MgCl₂ at 30 °C (as previously described (25)). The resulting products were analyzed by denaturing gel electrophoresis.

Approximately 20 pmol of As, A₃, and A₅ were labeled with [γ-32P]ATP (3000 Ci/mmol, Amersham Biosciences) by T4 polynucleotide kinase in 20-μl reactions at 37 °C for 1 h. The labeled RNA was then fractionated by electrophoresis on 25% polyacrylamide (19:1 acrylamide/bisacrylamide) gel and subsequently purified. Deadenylation reactions were carried out in buffer HEPES, pH 7.25, 100 mM NaCl, 0.1 μM methylated bovine serum albumin, and 2 mM of indicated divalent metal ions at 30 °C. Recombinant wild type PARN or PARN(D28C), PARN(E30C), PARN(D292C), or PARN(D382C) mutants were incubated at 0.3–30 nM (as indicated) with one of the 5′-32P-labeled As, A₃, or A₅, or As, A₃s substrates at indicated concentration for deadenylation assay. After incubation, the reactions were stopped and the resulting products were separated by electrophoresis in 25% polyacrylamide (19:1 acrylamide/bisacrylamide)/7 M urea gels. The gels were visualized and quantified using a 400S PhosphorImager (Amersham Biosciences).

To kinetically compare the reactions generating A₂, using 5′-32P-end-labeled As, As, and A₃s substrates, deadenylation reactions were performed under single turnover conditions (0.2 nM RNA substrate, 55 nM PARN, 25 mM HEPES, pH 7.25, 100 mM NaCl, 0.1 μM methylated bovine serum albumin, and 1 mM of indicated divalent metal ion). The reactions were followed over time and terminated at eight different time points. Subsequently, the products were separated by gel electrophoresis, and the total amount of As and A₃ products was quantified at each time point using a 400S PhosphorImager (Amersham Biosciences). The total amount of A₃ and A₅ products is identical to the amount turned over from A₂ to A₃ because product A₃ turns over to A₃ (see “Results” and Fig. 2). The calculated total amount of A₃ generated in the presence of each divalent metal ion, was plotted versus time. Observed rate constants of cleavage (kₐobs) were determined by fitting the data to the equation for a single exponential:

\[ k_{\text{obs}} \text{cleaved} = k_{\text{end point}} \left(1 - e^{-k_{\text{obs}}t} \right), \]

where \( k_{\text{obs}} \text{cleaved} \) is fraction of A₂ cleaved to A₃, \( t \) is time, \( k_{\text{end point}} \) is maximum fraction of A₂ cleaved to A₃. The calculated \( k_{\text{obs}} \text{cleaved} \) values were based on at least three independent experiments.

**RESULTS**

*Minimal Substrate Length for PARN—* To determine the minimal RNA substrate requirement for PARN degradation, it was investigated if PARN could degrade three short RNA substrates, i.e. adenosine penta-, tri-, and dinucleotides (A₅, A₃, and A₂). The substrates A₅ and A₃ were efficiently degraded to adenosine A₂, in the presence of Mg²⁺, whereas the A₂ substrate was much less efficiently degraded under these conditions (Fig. 1). A similar result was obtained when Mg²⁺ was replaced by Cd²⁺, although hydrolysis was less efficient in the presence of Cd²⁺ (Fig. 2). In the presence of Mn²⁺, Zn²⁺, or Cu²⁺, all three substrates were efficiently degraded to mononucleotides (Fig. 2). These observations suggest that A₂ is the shortest efficient RNA substrate for PARN in the presence of Mg²⁺ and that the substrate length requirement for PARN can be modulated by the identity of the divalent metal ion.

**PARN Mutants with Cysteine Substitution in the Active Site Are Inactive in the Presence of Mg²⁺ but Can Be Rescued in the Presence of Soft Divalent Metal Ion—** It has previously been shown that four conserved acidic amino acid residues Asp-28, Glu-30, Asp-292, and Asp-382 of PARN are essential for PARN activity, in keeping with our previous results (13). Furthermore, Fe²⁺-mediated hydroxyl radical cleavage provided evidence that Asp-28, Glu-30, and Asp-382, were required for coordination of catalytically important metal ions (13). Fig. 3 shows that single cysteine substitutions of the conserved acidic amino acid residues severely deteriorated PARN activity in the presence of Mg²⁺. Some activity could be recovered from the PARN(D382C) mutant after several hours of incubation, although we never managed to detect any hydrolytic activity when using the other cysteine-substituted PARN mutants (data not shown). This suggests that all these conserved acidic amino acid residues are essential for PARN activity, in keeping with our previous results (13).

Subsequently, it was investigated if PARN activity could be rescued from the cysteine-substituted PARN mutants in the presence of the soft divalent metal ions Mn²⁺, Zn²⁺, or Cd²⁺. The rational behind this experiment was that introduction of cysteines at specific positions could potentially bring a sulfur group into the active site of PARN and replace an oxygen coordination partner for a catalytically important Mg²⁺ ion.
Such a rescue effect has previously been observed for the V(D)J recombinase (26). If this is also the case for PARN, it should be possible to recover PARN activity from the cysteine-substituted mutants by the addition of a soft divalent metal ion, such as Mn$^{2+}$. Fig. 3 shows that PARN activity was indeed recovered in the presence of Mn$^{2+}$ for the mutants PARN(D28C), PARN(D292C), and PARN(D382C). The restoration of PARN activity for PARN(D28C), PARN(D292C), and PARN(D382C). The restoration of PARN activity for PARN(D28C), PARN(D292C), and PARN(D382C). The restoration of PARN activity was recovered in the presence of Mg$^{2+}$ or Mn$^{2+}$ (1 mM). Reacted RNA was recovered and fractionated by electrophoresis using 25% polyacrylamide:bisacrylamide (19:1, v/v). The resulting fluorogram is shown. A$_r$, A$_p$, and A$_i$ denote the locations of adenosine tri-, di-, and mono-nucleotides, respectively.

Fig. 2. Deadenylation activities of PARN and PARN(D382C) in the presence of different divalent metal ions. The deadenylation reactions were performed using 5'-32P-labeled A$_r$ (0.1 nM) and PARN (0.3 nM) or PARN(D382C) (30 nM) (as indicated) at 30 °C for 30 min in the presence of 2 mM of indicated divalent metal ions of Mg$^{2+}$, Cd$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, and Co$^{2+}$. Reacted RNA was recovered and fractionated by electrophoresis using 25% polyacrylamide:bisacrylamide (19:1, v/v). The resulting fluorogram is shown. In lane NC the RNA substrate A$_r$ was incubated in the absence of PARN. A$_r$, A$_p$, and A$_i$ denote the locations of adenosine tri-, di-, and mono-nucleotides, respectively.

Fig. 3. Deadenylation activities of wild type PARN and PARN mutants in the presence of Mg$^{2+}$ and Mn$^{2+}$. The deadenylation reactions were performed with 5'-32P-labeled A$_r$ (0.1 nM) RNA substrate and wild type PARN (0.3 nM) or indicated PARN mutants (30 nM) at 30 °C for 5 min in the presence of Mg$^{2+}$ or Mn$^{2+}$ (1 mM). Reacted RNA was recovered and fractionated by electrophoresis using 25% polyacrylamide:bisacrylamide (19:1, v/v). The resulting fluorogram is shown. A$_r$, A$_p$, and A$_i$ denote the locations of adenosine tri-, di-, and mono-nucleotides, respectively.

Phosphorothioate Modification of RNA Substrate Interferes with PARN Activity—To investigate how the one or more catalytically important divalent metal ions are coordinated by the RNA substrate, one of the non-bridging oxygen atoms of the phosphodiester bond between the second and third adenosine residues of the A$_r$ substrate was replaced by sulfur (as shown in Fig. 4). This substrate was referred to as A$_r$S (5'-AAAAs-3') and existed in two diastereomeric isomers, R$_p$- and S$_p$-configurations, respectively. Each isomer was purified, and subsequently the configuration of the purified isomers was investigated by snake venom phosphodiesterase digestion. Fig. 5 shows that the A$_r$S isomer I was hydrolyzed much faster by snake venom phosphodiesterase than the isomer II. Thus, the A$_r$S isomer I was assigned the R$_p$-configuration and the A$_r$S isomer II the S$_p$-configuration because R$_p$-diastereomeric RNA is the preferred substrate for snake venom phosphodiesterase (25). This conclusion was further supported by the reversed phase high performance liquid chromatography purification profile in which the isomer I had a shorter retention time than the isomer II (see “Experimental Procedures”). The separation of R$_p$- and S$_p$-isomers of a singly thio-substituted oligoribonucleotide by high performance liquid chromatography has been well documented in the literature (25, 27).

The purified A$_r$S-R$_p$ and A$_r$S-S$_p$ isomeric substrates were incubated with PARN, and the R$_p$-phosphorothioate-modified A$_r$S-R$_p$ substrate could be deadenylated both in the presence of Mg$^{2+}$ or Mn$^{2+}$ (Fig. 6). In contrast to this, no PARN activity was recovered when the A$_r$S-S$_p$ substrate was used in the presence of Mg$^{2+}$ or Mn$^{2+}$ (Fig. 6). Notably, no PARN activity was recovered when using the A$_r$S-S$_p$ substrate even after 7 h of incubation or in the presence of Cd$^{2+}$, Mn$^{2+}$, or Zn$^{2+}$ (data not shown). The PARN activities under single turnover conditions when using the A$_r$ or the A$_r$S-R$_p$ substrates in the presence of different divalent metal ions were subsequently quantified. The results from this analysis are summarized in Table I. From this analysis it is concluded that the A$_r$S-R$_p$ substrate is ~60-fold less efficiently cleaved than the A$_r$ substrate in the presence of Mg$^{2+}$. However, when Mg$^{2+}$ was replaced by either...
Mn$^{2+}$ or Zn$^{2+}$ only 2.3- or 1.3-fold differences in relative catalytic efficiency, respectively, were observed between the two substrates. Thus, replacement of Mg$^{2+}$ with any of the soft divalent metal ions Mn$^{2+}$ or Zn$^{2+}$ rescued the PARN activity significantly and improved the deadenylation reaction for the A$_{3s}$-Rp substrate relative to the A$_3$ substrate. In summary, these data suggest that the pro-R oxygen atom of the scissile phosphorus plays a key role during the cleavage of A$_3$ to A$_2$.

**DISCUSSION**

We (13) have recently suggested that the PARN active site resembles the 3'-5' exonuclease active site of DNA pol I (14, 28–30) where three aspartic and one glutamic acid residues coordinate two metal ions either directly or indirectly via bridging water molecules (Fig. 7). In this site of DNA pol I the nuclease (water or hydroxide ion, which is oriented by metal ion A, Glu-357 and Tyr-497 of DNA pol I) attacks the scissile phosphodiester bond, the negative charge on the leaving group is stabilized by metal ion B (17, 31). Herein, we tested this model for PARN experimentally by probing the importance of oxygen atoms coordinating divalent metal ions according to the model. Potentially important oxygens located both in PARN and the RNA substrate were replaced by sulfur atoms, either by replacing acidic amino acid residues in the active site of PARN with cysteines or by replacing oxygen atoms to sulfur in the scissile bond of the RNA substrate. Subsequently, it was investigated how these changes affected PARN activity in the presence of Mg$^{2+}$ and a variety of soft divalent metal ions.

**TABLE I**

Cleavage of A$_3$ and A$_{3s}$-Rp RNA substrates

| Me(II) | Substrate | Rate $k_{obs} \times 10^3$ | Catalytic efficiency $k_{cat/K_m} \times 10^{-5}$ | Relative |
|--------|-----------|---------------------------|---------------------------------|---------|
| Mg(II) | A$_3$     | 4.2 $\pm$ 0.7             | 7.7                             | 1.0     |
| Mg(II) | A$_{3s}$-Rp | 0.07 $\pm$ 0.03          | 0.13                            | 0.017   |
| Mn(II) | A$_3$     | 13 $\pm$ 4               | 23                              | 3.0     |
| Mn(II) | A$_{3s}$-Rp | 5.6 $\pm$ 1.3           | 10                              | 1.3     |
| Zn(II) | A$_3$     | 3.0 $\pm$ 1.0            | 5.5                             | 0.71    |
| Zn(II) | A$_{3s}$-Rp | 2.3 $\pm$ 1.1           | 4.2                             | 0.55    |

* The observed rate constants ($k_{obs}$) of A$_3$ or A$_{3s}$-Rp under single turnover conditions were determined as described under “Experimental Procedures.” The given values are average ± experimental error of at least three independent experiments.

* The catalytic efficiency was calculated by dividing the $k_{obs}$ value with the concentration of PARN (55 nM). The obtained value, determined under single turnover conditions, reflects the $k_{cat/K_m}$ value, because the reaction was not saturated with respect to the PARN concentration.

The data presented are consistent with the proposal in that the three aspartic acid residues Asp-28, Asp-292, and Asp-382 of PARN interact with divalent metal ions during at least one of the reaction steps. However, it remains to be shown that the glutamic acid residue Glu-30 coordinates a divalent metal ion, although available data show that Glu-30 is essential for PARN activity (Fig. 3) and important for the interaction of divalent metal ions (13). Most likely the introduction of a cysteine residue at this position severely affects both the divalent metal ion coordination properties, caused by the replacement of an oxygen atom by sulfur, and the configuration of the PARN active site, because cysteine lacks one methylene group in its side chain compared with glutamate, making it impossible to rescue this particular mutant by replacing Mg$^{2+}$ with a soft divalent metal ion. Another interesting reason for the inability to rescue the PARN(D30C) mutant could be that this glutamate may have two roles as proposed for the corresponding glutamate in the 3'-exonucleolytic active site of DNA pol I (i.e. to coordinate metal ion A as well as the attacking nucleophile, see Fig. 7) and that both these roles can not be fulfilled by cysteine at this position. In light of the latter possibility it is surprising that the two mutants PARN(D28C) and PARN(D292C) could be rescued in the presence of Mn$^{2+}$, albeit the activity of the mutant enzymes were significantly lower than for the wild type enzyme under the same conditions (Fig. 3). The two corresponding aspartates in the 3'-exonucleolytic active site of *E. coli* DNA pol I are each in contact with two ligands; Asp-355 contacts both divalent metal ions while Asp-424 is complexed to metal ion B through two bridging water molecules (Fig. 7). Our rescue data, therefore, suggest that this dual coordination property of Asp-28 and Asp-292 is not obligatory for PARN activity. It remains to be investigated if this is a property shared between the two corresponding active sites of PARN and *E. coli* DNA pol I or if this is a unique property of the PARN active site.

In the crystal structure (14, 17, 32, 33) of the 3'-exonuclease active site of DNA pol I (Fig. 7), it was observed that the pro-S oxygen of the scissile bond coordinated two metal ions while an interaction between the pro-R oxygen and a divalent metal was not seen. In comparison, current PARN data suggest that the pro-R oxygen of the scissile bond interacts with a divalent metal ion during at least one stage of the PARN reaction, whereas conclusive data showing that pro-S oxygen coordinates a metal ion has not yet been obtained. This potential discrepancy is most easily explained by the simple fact that
the two active sites are not identical, PARN belonging to the DEDDh group of the DEDD superfamily of nucleases, whereas the 3′-exonucleolytic active site of DNA pol I belongs to the DEDDy group (12). In the DEDDy group the catalytically important tyrosine residue characteristic of the DEDDy group (e.g. Tyr-497 of DNA pol I, see Fig. 7) is replaced by histidine, which expectedly would have a major effect on catalytic activity (12). However, two other possibilities could very well explain the apparent discrepancy between the two sites. First, our kinetic data do not unambiguously show that the pro-R oxygen coordinates a divalent metal ion during the chemical step of the PARN reaction, because we were only able to compare the catalytic efficiency by which the two substrates A₃ and A₃-S⁻Rₚ were cleaved under single turnover conditions when the reactions were not saturated with respect to the PARN concentrations. Thus, the rescue effect could have occurred during substrate binding. Second, our data do not exclude the possibility that the pro-S oxygen of the scissile bond coordinates a divalent metal ion, because we could not recover PARN activity using the A₃-S⁻ isomeric substrate. Similarly, Brautigam and Steitz (17, 32, 33) could not detect the 3′-exonucleolytic activity of DNA pol I when they used an S⁻ₚ isomeric phosphorothioate oligonucleotide as substrate. Based on their structural analysis they proposed that the bulky sulfur atom on the sulfur-substituted substrate inactivated the enzyme by excluding cations in the 3′-exonucleolytic active site. Finally, we note that the stoichiometry of the catalytically essential divalent metal ions in the active site of PARN is not yet known. Thus, the intriguing possibility that both non-bridging oxygens of the scissile bond coordinate divalent metal ions in the active site of PARN must still be considered. It will be an important task for future kinetic and structural studies to investigate if this is the case or not.

In this study it was found that PARN was active in the presence of either Zn²⁺, which normally is coordinated tetrahedral, or a variety of divalent metal ions that are preferentially coordinated octahedral, i.e. Mg²⁺, Mn²⁺, Cd²⁺, and Co²⁺. This must reflect a considerable amount of divalent metal ion flexibility in the active site of PARN. In the corresponding 3′-exonucleolytic site of DNA pol I it was observed (17), when both Zn²⁺ and Mg²⁺ ions were present, that the binding site for metal ion A preferentially harbored Zn²⁺, coordinated in a distorted tetrahedral fashion, whereas the binding site for metal ion B contained most likely an Mg²⁺ ion, liganded in a roughly octahedral geometry. Furthermore, it was observed that both sites preferentially contained Zn²⁺ when an Rₚ isomeric phosphorothioate oligonucleotide was used as the DNA substrate, even if Mg²⁺ was present in excess over Zn²⁺ in the soaking solution (17). Thus, the capacity to bind a variety of different divalent metal ions in the active sites of PARN or the 3′-exonucleolytic site of DNA pol I seems to be a common property shared between the two active sites.

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