Different Divalent Cations Alter the Kinetics and Fidelity of DNA Polymerases*

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Divalent metal ions are essential components of DNA polymerases both for catalysis of the nucleotidyl transfer reaction and for base excision. They occupy two sites, A and B, for DNA synthesis. Recently, a third metal ion was shown to be essential for phosphoryl transfer reaction. The metal ion in the A site is coordinated by the carboxylate of two highly conserved acidic residues, water molecules, and the 3′-hydroxyl group of the primer so that the A metal is in an octahedral complex. Its catalytic function is to lower the pKₐ of the hydroxyl group, making it a highly effective nucleophile that can attack the α-phosphorous atom of the incoming dNTP. The metal ion in the B site is coordinated by the same two carboxylates that are affixed to the A metal ion as well as the non-bridging oxygen atoms of the incoming dNTP. The carboxyl oxygen of an adjacent peptide bond serves as the sixth ligand that completes the octahedral coordination geometry of the B metal ion. Similarly, two metal ions are required for proofreading; one helps to lower the pKₐ of the attacking water molecule, and the other helps to stabilize the transition state for nucleotide excision. The role of different divalent cations are discussed in relation to these two activities as well as their influence on base selectivity and misincorporation by DNA polymerases. Some, but not all, of the effects of these different metal ions can be rationalized based on their intrinsic properties, which are tabulated in this review.

All DNA polymerases (DNA pols)² require Mg²⁺ or Mn²⁺ for primer extension and for excision of incorrectly incorporated dNTPs via intrinsic 3′→5′ exonuclease activity (1–5). A two-metal-ion mechanism is used by all DNA pols to catalyze nucleotide addition to a growing primer strand (6). Although DNA polymerases employ the physiologically relevant Mg²⁺, other divalent metal ions can substitute for Mg²⁺, although they tend to reduce the fidelity of DNA replication (7–10). The effect of metal ion cofactors on the fidelity of DNA replication has been studied for various DNA pols including E. coli DNA pol I (11), AMV DNA pol (12), Klenow fragment of E. coli DNA pol I (13), T4 pol (7), T7 pol (7), human pol α (7), pol β (7), and Dpo4 (8). Some metal ions have been shown to be mutagens and carcinogens probably because they reduce the base selectivity of DNA pols (7, 8, 11–15). Different divalent cations influence fidelity check points in the minimal kinetic scheme for the nucleotidyl transfer reaction (Scheme 1). Cations that can substitute for Mg²⁺ affect DNA pols by: 1) altering the ground-state binding affinity of incoming dNTPs to pol-DNA binary complexes (16); 2) decreasing base selectivity by promoting misincorporation during primer extension (8); 3) decreasing the rate of base excision (17); 4) altering primer extension past a mismatch at the primer-template (P/T) terminus (17). This review will address the way various metal ions increase misincorporation based on their physical properties. Our emphasis will be on the effect of different cations on the behavior of RB69 pol, which we have studied extensively in our lab (18). There are other reviews that deal with pol β, another DNA pol that has been thoroughly studied with respect to the influence of different metal ions on its structure and function (19–21).

The Two-metal-ion Mechanism for Nucleotidyl Transfer Reaction

All DNA pols require two divalent metal ions for primer extension (6). One metal ion occupies the “A site” and helps to lower the pKₐ of the terminal 3′-OH group on the primer and coordinates both the α-phosphate of the incoming dNTP and the 3′-OH of the primer strand, which facilitates its nucleophilic attack on the α-phosphorous atom of the incoming dNTP (6). The other metal ion, occupying the “B site,” coordinates the α-, β-, and γ- non-bridging phosphate oxygens of the incoming dNTP, helping to neutralize the developing negative charge as the ternary complex approaches the transition state in the nucleotidyl transfer reaction, and assists in the departure of the PPᵢ product. Yang et al. (22) have shown that for pol β, the dNTP-metal ion complex in the “B site” alone is unable to induce closing of the fingers, a necessary step for the phosphoryl transfer reaction to proceed. Both A and B metal ions are thus required to prepare the active site for nucleotidyl transfer (22). Tsai and co-workers (23) have used Rh³⁺ as an exchange-inert cation complexed with an incoming dNTP to selectively fill the “B site” in pol β so that the effect of binding a metal ion in the “A site” could be studied independently of the cation in the B site. Their results showed that the closing of the fingers could occur before occupancy of the A site but that Mg²⁺ could diffuse into the A site, although pol β was in the closed form (23). In contrast, studies with RB69 pol by Wang and co-workers (16) showed that although the fingers can close in the absence of an “A” metal ion, the fingers have to reopen for...
a divalent cation to bind in the “A site.” Recent studies by Yang and co-workers (24) observed the transient presence of a third metal ion of pol η after the nucleotidylation reaction was initiated, but before release of the products, using time-resolved x-ray crystallography (24). The third transient metal ion was coordinated to four water molecules in addition to an oxygen atom (which acts as a bridge between the α- and β-phospho atoms) and to the non-bridging oxygen of the α-phosphate. Yang and co-workers (24) proposed that in addition to the A and B metal ions, the third metal ion (Mg²⁺) participates in neutralizing the negative charges built up in the transition state and is likely involved in facilitating the protonation of the pyrophosphate leaving group. Recent studies by Gao and Yang (25) have shown that the third metal ion is indeed required for catalysis by pol η.

The Nature of Metal Ion Coordination Complexes with Various DNA pols

Several groups have solved the crystal structures of DNA pols with metal ions and an incoming dNTP bound in the polymerase active site (6, 9, 26–29). RB69 pol has been one of the most extensively studied DNA pols in the B family and is the only DNA pol where all combinations of mispaired bases were captured in ternary complexes (26). For this purpose, four mutations were required adjacent to the nucleotide binding pocket. Among these the triple mutant (tm) was used to capture a ternary complex in the presence of Mn²⁺ and dUpNpp, a non-hydrolyzable dNTP analogue (Fig. 1) (6). In this structure, Mn²⁺, bound in the “B site,” has nearly ideal octahedral geometry and is coordinated by the triphosphate tail of the incoming dUpNpp along with the carboxylate side chains of Asp⁴¹¹ and Asp⁶²⁴, and the backbone carbonyl oxygen of Leu⁴¹² (Fig. 1b). Mn²⁺ bound in the “A site,” however, has highly distorted octahedral geometry because in this structure, it is coordinated with the 3’-OH group of the primer, the two carboxylate side chains of Asp⁴¹¹ and Asp⁶²⁴, and the oxygen of the α-phosphate of the incoming dNTP. However, the distance between the 3’-OH group and the α-phosphate of the incoming dNTP is too great to support phosphodiester bond formation (6). Xia et al. (6) proposed that as the reaction approaches the transition state, metal ion A helps to reduce the 3’-OH-α distance, facilitating covalent bond formation (Fig. 1c). Similar results have been observed when Mg²⁺ occupies both the A sites and B sites, suggesting that Mg²⁺ and Mn²⁺ share similar coordination geometries in RB69 pol ternary complexes (6).

In addition to RB69 pol (6, 30), T7 pol (31, 32), Klkenow fragment (33–35), and Dpo4 (8, 10, 36), pol β has also been extensively studied (9, 19–21, 23). Although the topology of the Palm domain of pol β, which contains the two conserved catalytic carboxylates, differs between RB69 pol and pol β, the metal ion coordination geometries are nearly identical. In fact, crystal structures of all DNA pols have the same coordination geometry of the A and B metal ions (for examples, see Fig. 2).

DNA Polymerases Can Use Different Metal Ions for Catalyzing Phosphoryl Transfer

Early studies by Sirover and Loeb (15, 37) measured perturbations in the fidelity of DNA synthesis using AMV DNA pol. They identified several metal ions as being mutagenic or carcinogenic including Ag⁺, Be²⁺, Cd²⁺, Co²⁺, Cr³⁺, Mn²⁺, Ni²⁺, and Pb²⁺ (15). Subsequent studies with E. coli DNA pol I (11, 38) showed that Co²⁺ and Mn²⁺ could effectively replace Mg²⁺ but caused an increase in misincorporation. Similar results were reported with Mn²⁺ and Co²⁺ for human pol α and pol β (39). Later studies by Snow et al. (7) showed that Ni²⁺, albeit active, was an inefficient activator for a variety of DNA pols including AMV pol, human pol α, T4 pol, Klkenow fragment, and T7 pol.

Pelletier et al. (9) have reported structures of pol β ternary complexes in the presence of several different metal ions and showed that apart from Mg²⁺ and Mn²⁺, only Cd²⁺ and Zn²⁺ catalyzed primer extension with a blunt-end DNA substrate.
Egli and co-workers (36) carried out kinetic studies using Dpo4 pol with a variety of divalent cations and found that only Mg\(^{2+}\), Mn\(^{2+}\), and Ca\(^{2+}\) could support Dpo4-catalyzed polymerization but that Sr\(^{2+}\), Ba\(^{2+}\), Zn\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\), and Co\(^{2+}\) were inactive. Recent work by Vashishtha and Konigsberg (30) on RB69 pol showed that, apart from Mg\(^{2+}\) and Mn\(^{2+}\), Co\(^{2+}\), and to a lesser extent Ni\(^{2+}\), were the only divalent cations that could support both pol and exo activities. The metal ion preferences for different DNA pols are summarized in Table 1. Thus, DNA pols from different families can utilize different metal ions as cofactors, but they do not follow a pattern that can be predicted from their physical properties.

**Metal Ions Affect Various Fidelity Checkpoints during DNA Replication**

Divalent metal ions can alter the fidelity of DNA replication at various points along the reaction pathway by: 1) affecting the ground-state binding affinity of correct and incorrect dNTPs to DNA pol P/T binary complexes (16); 2) promoting misincorporation during primer extension (8); and 3) influencing exonuclease activity (17). Scheme 1 shows the various fidelity checkpoints employed by DNA pols that minimize dNMP misincorporation. The effect of divalent cations on each of these checkpoints will be addressed in the following sections.

**The Effect of Metal Ions on Ground-state Binding Affinity of Incoming dNTPs for pol P/T Binary Complexes**

Different divalent cations can affect the ground-state equilibrium dissociation constant \((K_{d,p})\) for incoming dNTPs (Scheme 2). Zhang et al. (40) used a dideoxy P/T containing 2AP opposite an incoming dNTP (the templating position) and measured the \(K_{d,p}\) for dTTP binding, which was 9 \(\mu M\) in the presence of Mg\(^{2+}\). This equilibrium binding assay was based on the observation that, upon formation of the pol-P/T binary complex, 2AP becomes unstacked and exists in a high fluorescence state (41, 42). The addition of dTTP results in 2AP stacking (2AP is translocated from \(n\) position to \(n + 1\) position) and quenching of the 2AP fluorescence (41). In the same study (40), there was no change in 2AP fluorescence when it was present at a location one residue downstream from the templating position when dTTP was added opposite dG as the templating base; therefore the \(K_{d,p}\) for binding of the incoming dNTPs could not be determined. In a similar study carried out by Wang and co-workers (16) in the presence of Ca\(^{2+}\), the \(K_{d,p}\) values were determined to be 53 nM and 53 \(\mu M\) for dTTP (correct) and dCTP (incorrect) binding, respectively, opposite 2AP (when 2AP was present in the templating position), suggesting that the identity of the divalent cation cofactor has a profound effect on the ground-state equilibrium dissociation constant. Hariran et al. (41) also reported a \(K_{d,p}\) value of 31 \(\mu M\) for dTTP binding opposite 2AP with T4 pol in the presence of Mg\(^{2+}\), similar to the value reported for RB69 pol (16). A more comprehensive study on the effect of different divalent cations on ground-state binding affinity for incoming dNTPs was carried out by Vashishtha and Konigsberg (30), where the \(K_{d,p}\) values were measured in the presence of Mg\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\), and Ca\(^{2+}\) with RB69 pol. Their results showed that the dissociation constants for dTTP binding opposite 2AP in RB69 pol ternary complexes

**TABLE 1**

**Summary of metal ion preferences of different DNA polymerases**

| DNA polymerase | Mn\(^{2+}\) | Co\(^{2+}\) | Fe\(^{2+}\) | Ni\(^{2+}\) | Zn\(^{2+}\) | Cd\(^{2+}\) | Sr\(^{2+}\) | Ba\(^{2+}\) | Cu\(^{2+}\) | Ca\(^{2+}\) |
|---------------|------------|------------|------------|------------|--------|--------|--------|--------|--------|--------|
| DNA pol I     | +          | +          | -          | -          | -      | -      | -      | -      | -      | -      |
| Human pol α   | +          | +          | -          | -          | -      | -      | -      | -      | -      | -      |
| pol β         | +          | +\(^a\)    | -          | -          | +      | +      | +      | -      | -      | -      |
| AMV DNA pol   | +          | +          | -          | -          | +      | +      | +      | -      | -      | -      |
| T4 pol        | +          | +          | -          | -          | +      | +      | +      | -      | -      | -      |
| T7 pol        | +          | +          | -          | -          | +      | +      | +      | -      | -      | -      |
| RB69pol       | +          | +          | -          | -          | +      | +      | +      | -      | -      | -      |
| Bst pol       | -a         | +          | -          | -          | -      | -      | -      | -      | -      | -      |
| Dpo4 pol      | +          | +          | -          | -          | -      | -      | -      | -      | -      | -      |

\(^a\) Studies by Pelletier et al. (9) and Egli and co-workers (36) previously claimed that human pol β and Dpo4 were not able to utilize Co\(^{2+}\) as cofactor. However, recent studies by Vashishtha and Konigsberg (30) showed that both pol β and Dpo4 can catalyze primer extension in the presence of Co\(^{2+}\) as explained in the text.

\(^b\) See Footnote 3.

FIGURE 2. Comparison of metal ion bound structures of the tm RB69 pol with other DNA polymerases. (a) superposition of the tm RB69 pol with T7 DNA pol (PDB accession number: 1T7P, green); HIV reverse transcriptase (HIV RT) (PDB accession number: 1RTD, cyan), and Dpo4 (PDB accession number: 2AGQ, magenta); (b) superposition of the tm RB69 pol with pol β (PDB accession number: 2FMS, golden).

SCHEME 2. Minimal kinetic scheme for DNA polymerases depicting the ground-state binding affinity \((K_{d,p})\) and apparent binding affinity \((K_{d,app})\) for an incoming dNTP. EDN represents the open conformation of the ternary collision complex, whereas FDN represents the closed conformation.
complexes decreased from Mg\(^{2+}\), Co\(^{2+}\), Mn\(^{2+}\), and Ca\(^{2+}\) in that order. A similar pattern of dissociation constants was observed for dCTP binding opposite 2AP. In general, the \(K_{d,E}\) values were substantially lower with Mn\(^{2+}\) as compared with Mg\(^{2+}\) (30). This may be due to the ability of Mn\(^{2+}\), in contrast to Mg\(^{2+}\), to accommodate base pairs other than the regular Watson-Crick base pairs in the nucleotide binding pocket. The possible reasons for this are discussed in the following sections.

**Effect of Metal Ions on Base Selectivity**

Numerous studies have shown that various divalent cations affect the base selectivity of pols to different extents (13, 14, 43–46). Substitution of Mg\(^{2+}\) by Mn\(^{2+}\) generally results in a decrease in the fidelity of DNA pols including T4 pol (14, 47), T7 pol (31), *E. coli* DNA pol I (13, 48), AMV DNA pol (45), and pol β (9). Beckman *et al.* (48) showed that at very low [Mn\(^{2+}\)] (<1 \(\mu\)M), the fidelity of DNA replication is similar to that observed with Mg\(^{2+}\) and that the decrease in fidelity is only observed at elevated [Mn\(^{2+}\)] (<100 \(\mu\)M). The possible reasons for this behavior include the binding of Mn\(^{2+}\) to the DNA template at elevated [Mn\(^{2+}\)]. Other divalent cations including Co\(^{2+}\) and Ni\(^{2+}\) have been reported to have similar effects on base selectivity as a function of the metal ion concentration (12). Pre-steady-state kinetic studies by Vashishtha and Konigsberg (30) showed that Mn\(^{2+}\) and Co\(^{2+}\) are cofactors for RB69 pol-catalyzed reactions. Surprisingly, the incorporation efficiency for correct incoming dNTPs was higher with Co\(^{2+}\) than with Mg\(^{2+}\) or Mn\(^{2+}\). In contrast, base selectivity was decreased with Co\(^{2+}\) versus Mg\(^{2+}\) but not nearly as much as when Mn\(^{2+}\) replaced Mg\(^{2+}\) (30). Of the all the divalent cations tested, Mn\(^{2+}\) is the most highly mutagenic as Mn\(^{2+}\) promotes misincorporation by increasing the rate of incorporation \(k_\text{pol}\) or \(V_{\text{max}}\) as well as by decreasing the \(K_{d,\text{app}}\) or \(K_m\) values for incorrect incoming dNTPs (14, 30) (where \(k_\text{pol}\) is the maximum rate of dNMP incorporation, and \(K_{d,\text{app}}\) is the apparent equilibrium dissociation constant for [dNTP] that supports the half-maximal rate of dNMP incorporation).

**Rare Tautomer Hypothesis for Mutagenesis When Mn\(^{2+}\) Is Present**

Occasionally, replicative DNA pols incorporate mismatched nucleotides (49) via the formation of high-energy tautomers (50–52). In fact, Beeke and co-workers (53) provided structural evidence for the presence of these rare tautomers using a D598A/F710Y double mutant of a catalytically competent fragment of *Bacillus stearothermophilus* pol. Beeke and co-workers (53) showed that, in the presence of Mn\(^{2+}\), the C/A mismatch adopts a tautomer cognate base pair shape that is virtually indistinguishable from the canonical, Watson-crick base pair in double-stranded DNA at the insertion site. With Mn\(^{2+}\), the triphosphate tail was properly aligned for catalysis, and the polymerase was in a closed conformation, facilitating the misincorporation. In contrast, in the presence of Mg\(^{2+}\), the C/A mismatch forms a non-cognate wobble base pair, and the polymerase adopts an “ajar” or partially closed conformation, which prevented mismatch incorporation. In addition, the triphosphate tail of the incoming incorrect dNTP was not properly aligned for catalysis, which helped to prevent misincorporation. These results provide a structural rationale for the mutagenic behavior of Mn\(^{2+}\) in this situation.

**Effect of Metal Ions on the Exonuclease Activity**

Similar to the polymerase active site, the exonuclease site also requires divalent cations to catalyze the 3'-5' exonuclease activity associated with several DNA pols (5, 30, 32, 35, 54, 55). Divalent cations are required but have a varying effect on the exonuclease activity. Results with RB69 pol (30) showed that, as compared with Mn\(^{2+}\) and Co\(^{2+}\), Mg\(^{2+}\) was most effective in promoting base excision but the exo rates varied only slightly among these three metal ions. Ni\(^{2+}\) on the other hand caused a dramatic decrease in exo activity (33-fold with Ni\(^{2+}\) versus Mg\(^{2+}\)). Similarly, the rates of base excision were reported to be nearly identical for *E. coli* DNA pol I with Mg\(^{2+}\), Mn\(^{2+}\), and Co\(^{2+}\) (11).

**All DNA pols Can Utilize Co\(^{2+}\) as Cofactor**

In addition to Mg\(^{2+}\), most other DNA pols can also use Mn\(^{2+}\) and Co\(^{2+}\), albeit with reduced fidelity (11–13, 15, 30). Pelletier *et al.* (9) and Egli and co-workers (36) have shown that pol β and Dpo4 are the two known exceptions that cannot be activated by Co\(^{2+}\). In contrast, Vashishtha and Konigsberg (30) showed that these DNA pols can actually catalyze primer extension in the presence of Co\(^{2+}\). The apparent conflicting results can be rationalized based on the fact that different assay conditions were used by each of these groups. For example, Pelletier *et al.* (9) used blunt-ended DNA with pol β, whereas Vashishtha and Konigsberg (30) used a P/T with a four-base overhang 5’ to the templating base. In the Egli and co-workers (36) experiments, 2 mM DTT was included in their assay with Dpo4, which reduced Co\(^{2+}\) to Co\(^{3+}\) \(\left( E^0 = -0.33 \text{ V for DTT versus } -0.28 \text{ V for Co}^{3+}\right)\). DTT was omitted by Vashishtha and Konigsberg (30) in their assays of Dpo4, so cobalt remained as Co\(^{2+}\). Based on these results, it appears that Co\(^{2+}\) can support catalysis for all DNA pols that have been studied to date.

**Properties of Divalent Cations That Can Activate DNA Polymerases**

Magnesium is in the second row of the periodic table and has 2s electrons that are typically lost when it becomes Mg\(^{2+}\) (56). Together with two 3d orbitals, Mg\(^{2+}\) forms unoccupied stable \(sp^3d^2\) hybrid orbitals for six coordination ligands. Because of the involvement of 3d orbitals, the coordination bonds are very strong. The average covalent length is 2.09 Å when all high-resolution Mg\(^{2+}\)-containing protein structures are compared (6, 29, 57). When carboxylate groups are ligands, the coordination bond lengths are typically reduced slightly relative to non-carboxylate ligands. Manganese is in the third row of the periodic table and has two electrons in 3d orbitals, which are more stable than its 3s electrons. When it loses two 3s electrons, it becomes Mn\(^{2+}\), and leaves two 3d electrons in parallel configurations with two separate 3d orbitals in a high-spin state (56). Upon hybridization in \(sp^3d^2\) orbitals, the coordination geometry is also octahedral. Due to the involvement of 3s orbitals, the coordination bond lengths increase to 2.22 Å when all the
Table 2
Ionic radii, coordination geometry, and pKₐ of water molecules coordinated to Mg²⁺, Mn²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, and Ca²⁺

| Metal ion | Ionic radius (Å) | Coordination | Geometry |
|-----------|-----------------|--------------|----------|
| Mg²⁺      | 0.86            | Oct          | Td       |
| Mn²⁺      | 0.81            | Oct          | Td       |
| Co²⁺      | 0.89            | Oct          | Sq       |
| Ni²⁺      | 0.83            | Oct          | TBP      |
| Zn²⁺      | 0.88            | Oct          | HBP      |
| Cd²⁺      | 0.95            | Oct          | Oct      |
| Ca²⁺      | 1.16            | Oct          | Oct      |

pKₐ of the water molecule

|          | 11.4 | 11.5 | 10.0 | 10.6 | 7.0  | 9.0  | 12.8 |

³ Although Zn²⁺ can form octahedral complexes, the majority of Zn²⁺ complexes are tetrahedral.

Mn²⁺-containing high-resolution protein structures are compared (6, 9, 57). The increased distance between adjacent oxygen ligands of Mn²⁺ (2.22 Å relative to 2.09 Å for Mg²⁺) permits the Mn²⁺ coordination octahedron to access ligands that have larger deviations of coordination bond lengths than Mg²⁺. This is also true for ligands from the triphosphate moieties of mismatched dNTPs.

Proceeding from Mg²⁺, to Mn²⁺, to Ca²⁺, the coordination bond lengths continue to increase from 2.09, 2.22, and 2.40 Å for corresponding metal ion-containing high-resolution protein structures (6) However, increased coordination bond lengths also pose problems for reducing the apical ptO3−-Pα distance in the transition state. In fact, RB69 pol is completely ineffective with Ca²⁺, because the shortest estimated ptO3−-Pα distance estimated would be about 3.3 Å, which is too great for nucelophilic attack of ptO3− on the Pα center (6). The reason why Mn²⁺ is catalytically active is that certain Mn²⁺ coordination bond lengths can be reduced to those of Mg²⁺ at the expense of increasing the bond lengths of its adjacent ligands, i.e., distortion of the octahedrons (6). Although Mn²⁺ remains in a high-spin state in most protein structures, it can be converted to low spin in the transition state where two of its 3d electrons occupy the same orbital and the coordination bond lengths are reduced to those of Mg²⁺ (57). This conversion can accelerate incorporation of any dNMP, correct or incorrect, once they are stabilized in a closed ternary complex. When going from Mn²⁺ to Co²⁺, two more electrons are added to the 3d orbital, which results in a low-spin state. As a consequence, the Co²⁺ coordination bond lengths are reduced to 2.09 Å, almost identical to those of Mg²⁺, which could account for activation of various DNA pols by Co²⁺. From Co²⁺ to Zn²⁺, the preferred coordination geometry becomes tetrahedral instead of octahedral, notably in Zn²⁺-binding motifs that involve Cys and His residues. Thus, neither Zn²⁺ nor Cd²⁺ can be used by most DNA pols as catalytic metal ions, pol β (9) and B. stearothermophilus DNA pol I large fragment² are the only two DNA pols that can be activated by Zn²⁺ and Cd²⁺, but the reason for this is not known.

The ability to reduce the pKₐ of bound water is very similar for Mg²⁺ and Mn²⁺ but is considerably higher for Co²⁺, Ni²⁺, Zn²⁺, and Cd²⁺ (Table 2). Based on this property alone, Co²⁺, Ni²⁺, Zn²⁺, and Cd²⁺ should be more effective as cofactors than Mg²⁺ and Mn²⁺, but this does not agree with the experimental data obtained with nearly all DNA pols (11–13, 15, 30, 36–38, 58), so other issues must be involved. The ionic radii of metal ion A play a crucial role in determining the proximal distance between the 3′-hydroxyl group and α-phosphorous atom of the incoming dNTP as the transition state is approached. The ionic radii of Mn²⁺, Co²⁺, Ni²⁺, and Zn²⁺ are very close to that of Mg²⁺ (Table 2), enabling all these metal ions to potentially bring the 3′-hydroxyl group and α-phosphorous atom of the incoming dNTP close enough for reaction as opposed to Cd²⁺ and Ca²⁺, whose ionic radii are larger than that Mg²⁺ (56). Despite its ability to lower the pKₐ of bound water and despite having similar ionic radii to Mg²⁺, Zn²⁺ is not able to activate most DNA pols. Based on its properties (Table 2), Ni²⁺ would be expected to substitute for Mg²⁺, but it is not clear why Ni²⁺ is such a poor cofactor for DNA pols despite having similar physical properties as Mg²⁺.

Ca²⁺ does not support primer extension with DNA pols with the exception of Dpo4 (6). One possible reason for this is the inability of Ca²⁺ to lower the pKₐ of the 3′-hydroxyl group of the primer as compared with Mg²⁺ (12.8 versus 11.4). The ionic radius of Ca²⁺ is also significantly larger than that of Mg²⁺ (1.1 Å versus 0.86 Å), which renders Ca²⁺ ineffective in polarizing the hydroxyl group for nucleophilic attack. Dpo4 is the only DNA polymerase that can be activated by Ca²⁺, although its ability to act as a cofactor is much reduced as compared with Mg²⁺ (36).

Mn²⁺ has been reported to be highly mutagenic, and this behavior can be rationalized based on the fact that Mn²⁺ is a softer metal ion than Mg²⁺, suggesting that Mn²⁺ is more polarizable than Mg²⁺ (44). In terms of a hexahydrated complex of Mn[OH₂]₆²⁺ and Mg[OH₂]₆²⁺, there is a greater energy penalty with Mg²⁺ as compared with Mn²⁺ when the inner sphere coordination number is changed from 6→5→4, indicating more rigid coordination requirements for Mg²⁺ complexes, which allows less freedom for mismatched dNTPs to be accessible to the nucleotide binding pocket (44). Transition metal ions such as Mn²⁺ bind more tightly to carboxylate groups and the triphosphate moiety of dNTPs as compared with Mg²⁺ (9), which could explain its ability to reduce base selectivity.

To summarize, DNA pols from different families are able to utilize different divalent cations as cofactors to catalyze primer extension. Crystal soaking experiments with pol β have shown that Zn²⁺ and Cd²⁺ were active (9), whereas these metal ions were not able to activate RB69 pol for catalysis (30). Ni²⁺ can support primer extension with all DNA polymerases, albeit with greatly reduced activity except for Dpo4 (36), human pol α (43), and pol β (9). Moreover, Dpo4 is the only polymerase that can utilize Ca²⁺, although Ca²⁺ is much less effective than Mg²⁺ (36). Thus, it seems that the abilities of different metal ions to lower the pKₐ of the primer’s 3′-OH group, the respective coordination geometries, and size are the main but not the only determinants of metal ion activation of DNA pols for catalysis of nucleotidyl transfer and base excision.

³ A. K. Vashishtha, J. Wang, and W. H. Konigsberg, unpublished data.
MINIREVIEW: Effect of Metal Ions on DNA Pol Kinetics and Fidelity

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MINIREVIEW: Effect of Metal Ions on DNA pol Kinetics and Fidelity

SEPTEMBER 30, 2016 • VOLUME 291 • NUMBER 40

JOURNAL OF BIOLOGICAL CHEMISTRY

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