Template-dependent variation in the relative fidelity of DNA polymerase I of *Escherichia coli* in the presence of Mg^{2+} versus Mn^{2+}

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Received 3 January 1984; Revised and Accepted 12 March 1984

ABSTRACT

The fidelity of *E. coli* DNA polymerase I in the presence of Mg^{2+} vs Mn^{2+} was examined at many positions along natural DNA templates, by use of an electrophoretic assay of misincorporation. Although there was an overall greater tendency for misincorporation to occur in Mn^{2+} -activated chain elongation, some specific sites on the template were more prone to misincorporation with Mg^{2+} and others with Mn^{2+} . This sequence-dependent effect was seen in spite of the finding that the relative rate of incorporation of the correct nucleotide at different positions on the template was essentially the same with Mg^{2+} and Mn^{2+} . In agreement with previous studies, the fidelity of *E. coli* pol I was higher at activating, than at inhibiting, concentrations of Mg^{2+} . The results reveal new complexities regarding the role of divalent cation in the control of fidelity in DNA synthesis and attest to the dynamic nature of interactions between DNA polymerase, its substrates and divalent metal activator during the course of polymerization on natural templates.

INTRODUCTION

An important property of DNA polymerases is their fidelity, which largely determines the accuracy with which genetic information is transmitted during DNA replication. The control of fidelity in DNA synthesis is intimately built into the catalytic mechanism of DNA replication, as dictated by the specific base pairing that exists within the double helix. In the preceding paper (Hillebrand *et al.*) we described the use of gel electrophoresis to detect the insertion of noncomplementary nucleotides into the growing polymucleotide chain. We measured the elongation of a [5'-³²P]primer annealed to a naturally occurring template strand, in reactions containing DNA polymerase and only three of the four dNTPs (highly purified by HPLC). Under these conditions the rate of chain elongation is a measure of the propensity of a DNA polymerase to incorporate a noncomplementary nucleotide in place of the missing dNTP. Although we originally envisioned this assay of misincorporation as a rapid means for identifying conditions under which the fidelity of DNA synthesis is altered, it quickly became evident that the data obtained in these experiments conveyed information regarding the relative rates of misincorporation at different positions on the template. We showed that the propensity for misincorporation varied a great deal along the template, as evidenced by autoradiographic banding patterns observed after chain elongation proceeded in the presence of only three dNTPs.

Since the most thoroughly documented condition that affects misincorporation is the identity of the divalent metal activator [reviewed by Mildvan and Loeb (1)], we decided to use the electrophoretic assay of misincorporation to verify that replacement of Mg^{2+} by Mn^{2+} results in an increased rate of misincorporation. Surprisingly, experiments with DNA

polymerase I of E. coli revealed that the effect on fidelity of replacement of Mg^{2+} by Mn^{2+} was not as simple as expected from earlier work. We report here that at certain positions on a natural DNA template, DNA synthesis by E. coli pol I is more accurate with Mg^{2+} than with Mn^{2+} , whereas at other positions Mg^{2+} promotes misincorporation, relative to Mn^{2+} .

MATERIALS AND METHODS

Only dNTPs purified twice by HPLC were used in this work. Preparation of the [5'- ^{32}P]G4 template-primers, HPLC purification of dNTPs and the electrophoretic assay of misincorporation were described in detail in the preceding paper (Hillebrand et al.). All other materials were obtained from sources indicated in the preceding paper. Divalent cation concentrations were determined with a Yellow Springs Instruments Model 31 conductivity bridge. The restriction fragment primers used in this study are identified in Table 1 of the preceding paper (Hillebrand et al.).

RESULTS

Activation of DNA Polymerase I of E. coli by Divalent Cations

When the electrophoretic assay of misincorporation is used to compare the fidelity of DNA synthesis under various conditions, one must insure that any differences seen in primer extension in the presence of only three dNTPs are due to differences in fidelity rather than polymerizing activity. For this reason, the polymerase reactions must be controlled, such that an equal amount of correct incorporation occurs in the presence of all four dNTPs under the different reaction conditions.

To fulfill this requirement in a comparison of the fidelity of Mg^{2+} - vs Mn^{2+} -activated synthesis by E. coli DNA polymerase I, we electrophoretically assayed the activity of this enzyme in the presence of several concentrations of Mg^{2+} or Mn^{2+} . In this way we could compare the rates of chain elongation obtained with Mg^{2+} vs Mn^{2+} in the presence of all four dNTPs, in the same region of the template that would subsequently be examined in the assay of misincorporation. The A22 template-primer was incubated with E. coli pol I in the presence of all four dNTPs at various Mg^{2+} or Mn^{2+} concentrations. After a short incubation the products were analyzed by gel electrophoresis/autoradiography. As seen in Figure 1A, maximal chain elongation in this region of the G4 genome occurred at about 4mM Mg^{2+} (lane 4) and 0.12mM Mn^{2+} (lane 12). Similar rates of chain elongation occurred at 4mM Mg^{2+} and 0.012mM Mn^{2+} (lanes 4 and 10, respectively). Electrophoretic analysis of the products produced at various times with these two concentrations of divalent metal ions (Figure 1B) revealed that over a 30-minute period the rates of chain elongation in the two reactions were very similar. Moreover, because the autoradiographic banding patterns produced from each reaction were the same, it appears that the rate of incorporation of the correct nucleotide at different positions on the template is not influenced by the identity of the activating divalent metal ion. Therefore, in subsequent comparisons of the accuracy of E. coli pol I in the presence of the two divalent metal ions, using primer A22, we used 4mM Mg^{2+} and .012mM Mn^{2+} . Similar control experiments were conducted with other G4 template-primers prior to their use in the fidelity assays.

Fidelity of E. coli DNA Polymerase I at Different Positions of a Natural DNA Template, in the Presence of Different Divalent Metal Activators

By use of the electrophoretic assay of misincorporation, we compared the fidelity

Figure 1. Electrophoretic Analysis of Mg^{2+} -activated vs Mn^{2+} -activated Chain Elongation in the Presence of all 4 dNTPs. Reaction mixtures contained 38mM Tris-HCl (pH 7.5), 200mM KCl, 7.6mM DTT, 0.16 mg/ml bovine serum albumin, 15% glycerol, 50 μ M dNTPs, 0.4nM (3'-OH termini) A22 template-primer, 8 units/ml DNA polymerase I ('large fragment') of *E. coli* and various concentrations of $MgCl_2$ or $MnCl_2$.

Panel A, Chain Elongation as a Function of Divalent Metal Ion Concentration. After 2 min at 30°C, polymerization was stopped by addition of 20mM EDTA, and the extent of primer elongation was analyzed by gel electrophoresis/autoradiography, as described in the preceding paper (Hillebrand et al.). Lanes 1-6, Mg^{2+} -activated synthesis at 0.12mM, 0.4mM, 1.2mM, 4mM, 12mM and 40mM Mg^{2+} , respectively. Lane 7, ddA. Lanes 8-13, Mn^{2+} -activated synthesis at 0.0012mM, 0.004mM, 0.012mM, 0.04mM, 0.12mM and 0.4mM Mn^{2+} , respectively.

Panel B, Timecourse of Chain Elongation at 4mM Mg^{2+} and 0.012mM Mn^{2+} . Odd-numbered lanes represent products of Mg^{2+} -activated synthesis, and even-numbered lanes represent products of Mn^{2+} -activated synthesis. Reactions were carried out at 30°C for 0 min (Lanes 1 and 2), 1 min (Lanes 3 and 4), 2 min (Lanes 5 and 6), 5 min (Lanes 7 and 8), 15 min (Lanes 9 and 10) and 30 min (Lanes 11 and 12).

of *E. coli* pol I with either Mg^{2+} or Mn^{2+} as the divalent cation activator. Because this assay can measure the sequence dependence of misincorporation by DNA polymerases, we were able to test whether these divalent cations have differential effects on the propensity of the enzyme to misincorporate at several specific points on the G4 template.

Figure 2. Electrophoretic Analysis of Mg^{2+} -activated vs Mn^{2+} -activated Primer Elongation in the Presence of 3 dNTPs. Reactions with primer A22 (lanes 1-13) were carried out at 30°C for 30 min in the presence of 4mM $MgCl_2$ or 0.012mM $MnCl_2$, as described in Figure 1. Lane 1, ddA. Lanes 2 and 3, '-A' reactions with Mg^{2+} and Mn^{2+} , respectively. Lane 4, ddG. Lanes 5 and 6, '-G' reactions with Mg^{2+} and Mn^{2+} , respectively. Lane 7, no polymerase added. Lane 8, ddC. Lanes 9 and 10, '-C' reactions with Mg^{2+} and Mn^{2+} , respectively. Lane 11, ddT. Lanes 12 and 13, '-T' reactions with Mg^{2+} and Mn^{2+} , respectively. Lanes 14-16, ddT, '-T' (Mg^{2+}) and '-T' (Mn^{2+}) reactions, conducted with primer F66 (under similar conditions as with A22).

Figure 2 depicts the results of such an experiment, in which the A22 template-primer was acted upon by *E. coli* DNA polymerase I in the presence of only three dNTPs.

The electrophoretic mobility of the unelongated primer is shown in lane 7, while products of the dideoxy sequencing reactions are displayed in lanes 1, 4, 8 and 11 (ddA, ddG, ddC and ddT, respectively). The bands produced in each dideoxy reaction represent points on the template where misincorporation must take place in the corresponding 'mimic'

reaction if the primer is to be elongated further. Lanes 2 and 3 portray the products produced in '-A' reactions activated by Mg^{2+} and Mn^{2+} , respectively. Unlike the parallel 'complete' reactions of Figure 1B, which gave the same relative band intensities for Mg^{2+} - and Mn^{2+} -activated reactions, the two '-A' reactions produced quite different autoradiographic banding patterns. Note that few of the primers in the Mg^{2+} -activated reaction (lane 2) were elongated past the first point where dATP was needed, as evidenced by the heavy intensity of the band marked by arrow a. However, nearly all of the primers in the Mn^{2+} -activated reaction were elongated past this point, as evidenced by the absence of a band at arrow a, lane 3. Thus, at this point on the template Mn^{2+} promotes misincorporation by *E. coli* pol I, relative to Mg^{2+} . Also note that practically all of the primers that had undergone one misincorporation in the Mg^{2+} -activated reaction also underwent a second misincorporation, as evidenced by the low intensity of the band marked by arrow b, compared to the band above it. In contrast to the Mg^{2+} -activated reaction, in the Mn^{2+} -activated reaction, only a very small fraction of primers underwent a second misincorporation in place of dATP, as indicated by the heavy intensity of the band at arrow b, lane 3. Therefore, at the first T residue in the template from the end of the A22 primer, Mn^{2+} promoted misincorporation by *E. coli* pol I, relative to Mg^{2+} , whereas the converse was seen at the second T in the template. In Figure 2 (eg., lanes 2 and 6) are seen several 'minus' bands with the same electrophoretic mobility as the corresponding 'dideoxy' bands. As discussed in the preceding paper (Hillebrand *et al.*), these bands represent postmisincorporation pause sites.

Products of the other 'mimic' reactions are shown in lanes 5 and 6 ('-G', Mg^{2+} vs Mn^{2+}), lanes 9 and 10 ('-C', Mg^{2+} vs Mn^{2+}) and lanes 12 and 13 ('-T', Mg^{2+} vs Mn^{2+}), each pair preceded by the corresponding 'dideoxy' lane. The greater extent of elongation in the Mn^{2+} -activated '-G' and '-C' reactions, compared with the Mg^{2+} -activated reactions, would suggest that overall, *E. coli* DNA polymerase I misincorporates in place of dGTP and dCTP more readily with Mn^{2+} than with Mg^{2+} . This conclusion is supported by results obtained with other primers, although we have identified template positions at which the converse is true (data not shown), as with misincorporation in place of dATP. Although the '-T' reactions with primer A22 (lanes 12 and 13, Figure 2) showed little difference in misincorporation with Mg^{2+} vs Mn^{2+} , with most primers examined *E. coli* pol I misincorporated in place of dTTP more readily with Mn^{2+} than with Mg^{2+} . A typical '-T' result is depicted in lanes 14-16 of Figure 2 (data obtained with primer F66).

Figure 3 displays reaction products formed by elongation of primer RA50. The upper part represents a timecourse of elongation of this primer in the presence of all four dNTPs. These data [obtained using the higher resolution thin gel system of Sanger (2)] reinforce the phenomenon shown in Figure 1, that the relative rate of phosphodiester bond formation varies greatly along the template, and confirm that the identity of the divalent metal activator (Mg^{2+} vs Mn^{2+}) does not affect the relative polymerization rate at different sites on the template. A timecourse of elongation in the corresponding 'mimic' reactions with the RA50 primer is shown in the lower part of Figure 3. Misincorporation in place of dATP is shown in lanes 3-5 for Mg^{2+} and in lanes 6-8 for Mn^{2+} . Again, the data indicate a pronounced sequence dependence of misincorporation rates in the presence of Mg^{2+} vs Mn^{2+} . Comparison of '-A' bands in lane 3 with the 'ddA' bands of lane 2 reveals that misincorporation at the first and third T residues in the template occurred more readily with Mn^{2+} than with Mg^{2+} , whereas misincorporation at the second T

occurred more readily with Mg^{2+} than with Mn^{2+} . Misincorporation in place of dGTP (lanes 10-15) and dCTP (lanes 17-22) occurred more readily with Mn^{2+} than with Mg^{2+} at the few sites examined with the RA50 primer. Misincorporation in '-T' reactions was not dramatically different for Mg^{2+} and Mn^{2+} (lanes 24-29), although differences in band intensities suggest some sequence dependence.

The results shown in Figures 1-3 are representative of experiments conducted with many different template-primers. The data indicate that the relative effect of Mg^{2+} and Mn^{2+} on the fidelity of *E. coli* pol I is strongly sequence-dependent. Although there is an overall tendency for greater misincorporation in Mn^{2+} -activated reactions than in Mg^{2+} -activated reactions, the data indicate the reverse effect at many sites along the template. Similar results with rat liver DNA polymerase alpha and AMV reverse transcriptase (unpublished data) suggest that this sequence-dependent phenomenon may be exhibited by many DNA polymerases.

DISCUSSION

In the preceding paper (Hillebrand *et al.*) we described an electrophoretic method for assessment of the fidelity of DNA synthesis at many positions on a natural template. With this assay of misincorporation we observed that the propensity for incorporation of noncomplementary nucleotides varies a great deal at different positions on a naturally occurring DNA template. The data of Figures 1 and 3 (this paper) show that the rate of incorporation of complementary nucleotides also varies at different points along the template. Furthermore, the relative rates of phosphodiester bond formation (in the presence of 4 dNTPs) at different points on the template are not influenced by a change (from Mg^{2+} to Mn^{2+}) in the divalent metal ion used to activate polymerization by *E. coli* DNA polymerase I. In contrast, the nature of the variation in noncomplementary incorporation along the template (reflected by the pattern of band intensities for 'mimic' reactions) is markedly dependent on the identity of the divalent metal activator. Although most template positions did not exhibit clear differences with regard to fidelity in the presence of Mg^{2+} vs Mn^{2+} , among those that did, greater misincorporation generally occurred with Mn^{2+} than with Mg^{2+} . However, the reverse was true at many positions. Thus, the relative fidelity of DNA synthesis with *E. coli* DNA polymerase I in the presence of

Figure 3. Electrophoretic Analysis of Mg^{2+} - vs Mn^{2+} -activated Elongation of Primer RA50. Reaction mixtures contained 32mM Tris-HCl (pH 7.5), 200mM KCl, 5mM $MgCl_2$ or 0.12mM $MnCl_2$, 50μM dNTPs, 0.9nM (3'-OH termini) RA50 template-primer and 40 units/ml DNA polymerase I ('large fragment') of *E. coli*.

Upper Panel, Timecourse of Primer Elongation in the Presence of all 4 dNTPs. Lanes 1, 2, 27 and 28, ddA, ddG, ddC and ddT, respectively. Lanes 3-14, Mg^{2+} -activated elongation monitored at 15-sec intervals over a 3-min period. Lanes 15-26, Mn^{2+} -activated elongation monitored at 15-sec intervals over a 3-min period.

Lower Panel, Primer Elongation in the Presence of 3 dNTPs. Lane 1, no polymerase added. Lanes 2, 9, 16 and 23, ddA, ddG, ddC and ddT, respectively. Lanes 3-5, '-A' reaction (Mg^{2+}) carried out for 1, 3 and 6 hr, respectively. Lanes 6-8, '-A' reaction (Mn^{2+}) carried out for 1, 3 and 6 hr, respectively. Lanes 10-12, '-G' reaction (Mg^{2+}) carried out for 1, 3 and 6 hr, respectively. Lanes 13-15, '-G' reaction (Mn^{2+}) carried out for 1, 3 and 6 hr, respectively. Lanes 17-19, '-C' reaction (Mg^{2+}) carried out for 1, 3 and 6 hr, respectively. Lanes 20-22, '-C' reaction (Mn^{2+}) carried out for 1, 3 and 6 hr, respectively. Lanes 24-26, '-T' reaction (Mg^{2+}) carried out for 1, 3 and 6 hr, respectively. Lanes 27-29, '-T' reaction (Mn^{2+}) carried out for 1, 3 and 6 hr, respectively.

CGCATACGATCGGCAAAATTAAT A GCTTGCACAAACACCTGCGCC
 CCGTAAGCTGGTACGCAATCAAT CGGCAAAATAAATGACCTGGCA
 AATGTCCTCAATTAATCTCTCAT CAGCTTGCCTGCTCCATGCTG
 GCTCTGTCCTCCCTCTTTCGTC T G8GCTGCTGCTGTGTGACTCACG
 GCGTGGTATAAATTAATGCGCCCT CAAAACATCTCTTGTG8GACCG
 TTCCCGGGGATTCG8TGCACTC CAGCGCAAGCTTGGCACTGG
 GCTCTCAAAACATCTCTTGG8 A CCGCTTGG8GGTGTGCTG8TAC
 GACGCGCTGGTGGTGGTGGTGA C TCTTTTGG8ATGGT8ATATGG
 CATCAATCGCGCAAAATAATA C GTTGCACAAACACGTGG8GCTTT
 GCTCCAATTAACCTCTACGA C TTGCTGCTCATTAACAAACCCC
 TCTGAGGATTATAAATGGGG8 G 8TACGCTTGCCTAACTCTTAA
 TCTGCTGTGTCCTCCCTCTTCGT C 8TGGCGCTGTGGTGTGACTCA
 CCTCAAAACATCTCTTGG8 A CCGCTTGG8GGTGTG8TGACTT
 GCTGCTGCTGCTGCTGCTG8 A CTCACGCGCTG8ATATGCTTTC
 TGGTGTGCTGGTGACTCTTTCG A GTGTGATG8TGTGGCGCTC
 TATGGTACGCTGGACCTTTGT A G8ATACCTTCGCTTCTGCTGAC

-GGTACGCATCAATCGGCAAA T AATATGCTTCAGAAAACACCT-
 -CACAATGCTCCAATTAACTC T ACTCAGCTTGCTGCTACTAA-
 -AATTGGCGGCTCAAAACTA T CTCTTGGACGCTGGTGGTG-
 -TAACTTCGGTAAGCTGGTAC G CATCAATCGGCAAAATAATG-
 -TAAAAATGGCGGCTCAAAA C TATCTTGGAGGAGGCTGGTG-

A genetic assay for detection of misincorporation on a natural template has also been used to study the fidelity of DNA synthesis (3,8). In this system a restriction fragment is annealed to a template consisting of the viral strand of bacteriophage ϕ X174, bearing the am3 mutation, 85 nucleotides downstream from the 3'-OH terminus of the primer. Chain elongation is allowed to proceed past the mutant site, then the product DNA is used in a transfection experiment, which detects revertant nucleotide sequences that arose during the *in vitro* DNA synthesis. Measurements of the fidelity of *E. coli* pol I in the presence of Mg^{2+} vs Mn^{2+} using this genetic system also indicated a greater propensity for misincorporation in the presence of Mn^{2+} , compared with Mg^{2+} (3). In the ϕ X174 am3 system most of the revertants arose from misincorporation opposite an adenine residue in the template (9), this type of misincorporation is equivalent to that observed in a '-T' reaction in the electrophoretic assay. We have found that with most primers the results of the '-T' reaction with *E. coli* pol I are like that shown in lanes 14-16 of Figure 2, i.e., misincorporation opposite A residues is usually enhanced by Mn^{2+} , relative to Mg^{2+} . Therefore, our data are in agreement with the results observed with the ϕ X174 am3 assay of fidelity. In light of our observation that at certain points on the template, misincorporation occurs more readily with Mg^{2+} than with Mn^{2+} , we would predict that at certain mutant sites on the ϕ X174 genome, the appearance of revertant nucleotide sequences during *in vitro* DNA synthesis should occur more frequently with Mg^{2+} than with Mn^{2+} . A strong influence of neighboring nucleotide sequence on the production of revertants in the ϕ X174 genome with Mn^{2+} vs Mg^{2+} has in fact been reported. Comparison of the reversion frequency at two different amber mutant sites on the ϕ X174 genome following *in vitro* replication catalyzed by the multienzyme complex of bacteriophage T4 in the presence of Mg^{2+} vs Mn^{2+} revealed that the enhancement of replicational errors by Mn^{2+} was much greater at one mutant site than at the other (10).

The sequence dependence of the relative fidelity of DNA synthesis with Mg^{2+} vs Mn^{2+} must reflect variations in the interactions that occur between the DNA polymerase, template-primer, dNTPs and divalent metal ions during DNA synthesis. Although the mechanism of DNA synthesis is not understood in detail, it is known that with *E. coli* DNA polymerase I, divalent metal activator (Mg^{2+} , Mn^{2+} , or Co^{2+}) is required for binding of a dNTP to the enzyme (1,11). The divalent metal activator apparently binds to the enzyme and also chelates the terminal phosphoryl group on the dNTP (12,13). Although binding of dNTP to the polymerase can occur in the absence of DNA, experiments conducted in the presence of template suggest that multiple dNTP-binding sites exist, and that binding is strongest when the dNTP is complementary to the template (14). Goodman et al. (15) recently obtained evidence with DNA polymerase of bacteriophage T4 that Mn^{2+} increases the error frequency (compared with Mg^{2+}) primarily by differentially increasing the residence time of a noncomplementary (vs complementary) dNTP bound to the polymerase-template-primer complex. A kinetic enhancement of noncomplementary dNTP binding by Mn^{2+} could contribute to the overall enhancement of misincorporation by Mn^{2+} (compared with Mg^{2+}) observed in this study. However, the strong sequence-dependent variation that we observed for fidelity in the presence of Mn^{2+} vs Mg^{2+} suggests a more complex explanation, involving additional interactions, which vary according to the position on the template.

One type of interaction that would vary at different sites on a natural template is base stacking interactions, which are stronger between certain nearest neighbor base pairs

than others (16-19). Differential stacking interactions along the template could impart some sequence specificity to the binding of noncomplementary dNTPs at the site of phosphodiester bond formation. The identity of the divalent metal activator may influence the sequence specificity of base stacking interactions in such a way that a change from Mg^{2+} to Mn^{2+} might result in stabilization of noncomplementary dNTP binding (adjacent to primer termini) at certain template positions, and destabilization at other positions. In this regard, the available information on divalent metal ion binding to nucleotide residues is consistent with the promotion of misincorporation by Mn^{2+} , compared with Mg^{2+} at template positions 5' to G•C base pairs. Evidence exists for strengthening of base stacking upon binding of divalent metal ions to nucleotides (20). Although both Mg^{2+} and Mn^{2+} bind to the phosphate groups in nucleotide residues, Mn^{2+} has the additional capability to form macrochelates with GMP residues, involving both phosphoryl group and N7 of the purine (20,21). If the preferential binding of Mn^{2+} to G•C base pairs imparts additional stacking potential at these sites, then one would predict a correspondingly increased stabilization of misincorporated nucleotides next to these sites, consistent with the results depicted in Figure 4. Interestingly, the am3 site on the ϕ X174 genome (lowermost sequence listed in Figure 4), at which misincorporation was also promoted by Mn^{2+} , is flanked on the 3' side by a G•C base pair, like the sites identified in this study as more prone to misincorporation in the presence of Mn^{2+} .

Sequence-dependent stacking interactions could also influence the probability of removal of misincorporated nucleotides (by the 3'-exonuclease activity present on *E. coli* DNA polymerase I and many other DNA polymerases). Evidence exists for a greater participation of this editing activity in Mn^{2+} -activated synthesis than in Mg^{2+} -activated synthesis by *E. coli* pol I (22), which may account for the greater fidelity of incorporation that we observed at some template positions in the presence of Mn^{2+} , compared with Mg^{2+} . The formation of macrochelates between Mn^{2+} and GMP residues might act to stabilize mispairs both prior to phosphodiester bond formation (as discussed above) and after misincorporation. The latter effect would be expected to protect misincorporated nucleotide residues from 3'-exonuclease action in a sequence-dependent fashion, and therefore might contribute to the observed sequence dependence of the relative fidelity of DNA synthesis in the presence of Mg^{2+} vs Mn^{2+} . A factor that might impart considerable sequence dependence to the efficiency of 3'-exonucleolytic error-correction is the rate at which a misincorporated residue is stabilized by subsequent chain elongation. It has recently been reported (23) that with DNA polymerase alpha from calf thymus (which lacks 3'-exonuclease activity) elongation of synthetic template-primers containing 3'-terminal mismatches is promoted by Mn^{2+} , relative to Mg^{2+} . A greater efficiency of postmisincorporation elongation by pol I could account for some of the increased rate of chain elongation observed in 'mimic' reactions carried out with Mn^{2+} , compared with Mg^{2+} .

The results reported in this paper testify to the dynamic nature of the interactions that take place between DNA polymerase and its substrates and metal ion cofactor during the course of polymerization on natural templates. Detailed physicochemical studies of these interactions, combined with more extensive characterization of the sequence dependence of fidelity in the presence of different divalent metal ions, may eventually yield a molecular explanation for this phenomenon.

ACKNOWLEDGEMENTS

Work supported by Grant GM25530 from the National Institutes of Health and Grant Q819 from the Robert A. Welch Foundation. G.G.H. was recipient of a Robert A. Welch Predoctoral Fellowship. K.L.B. is recipient of Research Career Development Award CA00891 from the National Cancer Institute. The authors thank Mr. Alan McCluskey and Ms. Katherine Abbott for technical assistance.

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