Minor Groove Interactions between Polymerase and DNA: More Essential to Replication than Watson−Crick Hydrogen Bonds?

Juan C. Morales and Eric T. Kool*

Department of Chemistry, University of Rochester Rochester, New York 14627

Received October 5, 1998

Studies of the enzymatic mechanisms responsible for DNA synthesis and mutations have often focused on the structural and energetic issues of base pair geometry and Watson−Crick hydrogen (H) bonding.[1] Until relatively recently, it was thought that a polymerase was not likely to make specific H-bonded interactions with individual DNA bases since the four base structures differ significantly, although earlier work pointed out that H-bond acceptors appear at similar positions for A−T and G−C in the minor groove.[2] Recent X-ray crystal structures of DNA polymerases bound to duplexes,[3] however, have implicated amino acid side chains in H bonding to minor groove acceptor atoms, and thus as potentially important to insertion and extension of base pairs during DNA replication. For example, in the *Bacillus stearothermophilus* DNA polymerase I large fragment,[3c] which is highly homologous to the Klenow fragment of DNA polymerase I from *Escherichia coli*, conserved protein side chains or oriented water molecules anchored to them, form H bonds to the first four base pairs extending from the 3′-primer terminus at N3 of purines and O2 of pyrimidines. Mutagenesis studies of the Klenow fragment (Kf) of *E. coli* DNA polymerase I, human DNA polymerase β (Pol β) and HIV-1 reverse transcriptase have examined the importance to replication of some of the analogous residues.[4] Alanine substitutions of Arg 668 and Gln 849 in Kf and alanine and leucine substitutions of Asn 279 and Arg 238 in Pol β, all thought to be minor groove H-bond donors, markedly decrease DNA-binding affinity and the k_{cat} for DNA synthesis,[4a,b,c] although which groups on the DNA are affected is not clear.

To test these effects, we synthesized a new 9-methyl-1-H-imidazo[(4,5)-*b*]pyridine nucleoside analogue, **1** (denoted Q), which is isoelectronic and isosteric with deoxyadenosine (Figure 1A). It lacks all Watson−Crick H bonding groups, but does have a minor groove acceptor nitrogen, analogous to N3 of adenine. For comparison, we also made use of a 4-methylbenzimidazole deoxynucleoside **2** (denoted Z),[5] which differs from Q only by the absence of N3; it lacks both Watson−Crick pairing and minor groove acceptor ability. For geometric complementarity, we used nucleoside F (Figure 1A), which is a thymidine isostere,[6,7] as a pairing partner, and pairs with natural bases were examined as well. We evaluated the effects with the Klenow polymerase (lacking 3′-5′ exonuclease activity), which is among the best characterized DNA polymerases. Although previous studies have evaluated the importance of Watson−Crick H bonding to nucleotide insertion, the present studies are the first to evaluate minor groove effects for both sides of the minor groove using the same analogues, and both for insertion and extension of base pairs.

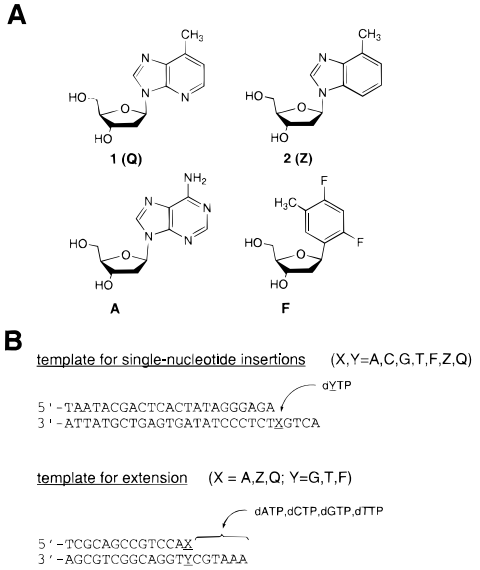

Figure 1. Structures and sequences in this study. (A) Structures of natural DNA bases and analogues in this study. (B) Sequences of primer and template DNAs used in the polymerase experiments.

Single-nucleotide insertions were carried out to qualitatively evaluate minor groove effects on the ability of the polymerase to form a base pair. A 5′-^{32}P-labeled 23mer (Figure 1B) was extended one base by inserting nucleotides opposite A, Z, and Q nucleosides in the template by the polymerase. Results evaluated by gel electrophoresis show that dFTP is efficiently inserted opposite Z and Q (Figure 2A, lanes 10 and 15) and that there is a small difference between the two. Among the natural nucleoside triphosphates, dTTP shows some insertion opposite Z and also opposite Q, although to a lesser extent. The other nucleotides are very poorly inserted opposite these nonpairing bases. Overall, the data suggest that the presence or absence of a minor groove acceptor atom in the template strand makes only a small difference in the ability to insert a nucleotide opposite that base.

To test minor groove effects for the incoming nucleotide itself, we also synthesized the nucleoside triphosphate analogue of Q (dQTP) and compared it to dZTP and dATP in its ability to be inserted opposite each of the four natural bases as well as opposite F, Z, and Q in separate template−primer duplexes (Figure 2B).

* To whom correspondence should be addressed. E-mail: etk@etk.chem.rochester.edu.

(1) (a) Loeb, L. A.; Kunkel, T. A. *Annu. Rev. Biochem.* **1982**, *52*, 429−457. (b) Kuchta, R. D.; Mizrahi, V.; Benkovic, P. A.; Johnson, K. A.; Benkovic, S. J. *Biochemistry* **1987**, *26*, 8410−8417. (c) Kuchta, R. D.; Benkovic, P. A.; Benkovic, S. J. *Biochemistry* **1988**, *27*, 6716−6725. (d) Wong, I.; Patel, S. S.; Johnson, K. A. *Biochemistry* **1991**, *30*, 526−537. (e) Kornberg, A.; Baker, T. A. *DNA Replication*, 2nd ed.; W. H. Freeman: New York, 1992. (f) Carroll, S. S.; Benkovic, S. J. *Chem. Rev.* **1990**, *90*, 1291−1307. (g) Echols, H.; Goodman, M. F. *Annu. Rev. Biochem.* **1991**, *60*, 477−511. (h) Petruska, J.; Goodman, M. F. *J. Biol. Chem.* **1995**, *270*, 746−750. (i) Law, S. M.; Eritja, R.; Goodman, M. F.; Breslauer, K. J. *Biochemistry* **1996**, *35*, 12329−12337.

(2) Seeman, N. C.; Rosenberg, J. M.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 804−808.

(3) (a) Pelletier, H.; Sawaya, M. R.; Kumar, A.; Wilson, S. H. *Science* **1994**, *264*, 1891−1903. (b) Doublié, S.; Tabor, S.; Long A. M.; Richardson, C. C.; Ellenberger, T. *Nature* **1998**, *391*, 251−258. (c) Kiefer, J. R.; Mao, C.; Braman, J. C.; Beese, L. S. *Nature* **1998**, *391*, 304−307.

(4) (a) Polesky, A. H.; Steitz, T. A.; Grindley, N. D. F.; Joyce, C. M. *J. Biol. Chem.* **1990**, *265*, 14579−14591. (b) Polesky, A. H.; Dahlberg, M. E.; Benkovic, S. J.; Grindley, N. D. F.; Joyce, C. M. *J. Biol. Chem.* **1992**, *267*, 8417−8428. (c) Beard, W. A.; Osheroff, W. P.; Prasad, R.; Sawaya, M. R.; Jaju, M.; Wood, T. G.; Kraut, J.; Kunkel, T. A.; Wilson, S. H. *J. Biol. Chem.* **1996**, *271*, 12141−12144. (d) Bebenek, K.; Beard, W. A.; Darden, T. A.; Prasad, R.; Luxon, B. A.; Gorenstein, D. G.; Wilson, S. H.; Kunkel, T. A. *Nat. Struct. Biol.* **1997**, *4*, 194−197.

(5) (a) Guckian K. M.; Morales, J. C.; Kool, E. T. *J. Org. Chem.* **1998**, *63*, 9652−9656. (b) Morales, J. C.; Kool, E. T. *Nat. Struct. Biol.* **1998**, *5*, 950−954.

(6) (a) Schweitzer, B. A.; Kool, E. T. *J. Org. Chem.* **1994**, *59*, 7238. (b) Moran, S.; Ren, R. X.-F.; Sheils, C. J.; Kool, E. T. *Nucleic Acids Res.* **1996**, *24*, 2044. (c) Chaudhuri, N. C.; Ren, R. X.-F.; Kool, E. T. *Synlett* **1997**, 341. (d) Guckian, K. M.; Kool, E. T. *Angew. Chem., Int. Ed.* **1998**, *36*, 2825−2828.

(7) (a) Moran, S.; Ren, R. X.-F.; Rumney, S.; Kool, E. T. *J. Am. Chem. Soc.* **1997**, *119*, 2056−2057. (b) Moran, S.; Ren, R. X.-F.; Kool, E. T. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10506−10511. (c) Liu, D.; Moran, S.; Kool, E. T. *Chem. Biol.* **1997**, *4*, 919−926.

10.1021/ja983502+ CCC: $18.00
Published on Web 02/24/1999

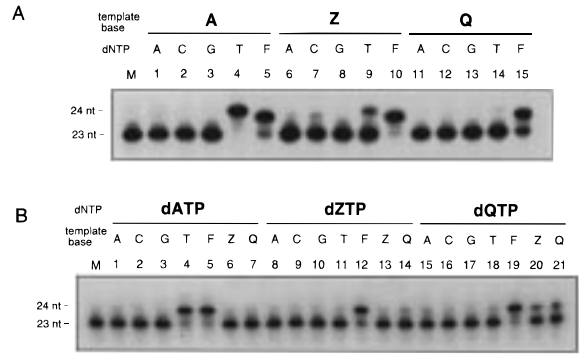

Figure 2. Autoradiograms of denaturing PAGE gels showing minor groove effects on nucleotide insertion. (A) Minor groove interaction effects on single-nucleotide insertions opposite A, Z, and Q in the template. (B) Minor groove interaction effects on single-nucleotide insertions of dATP, dZTP, and dQTP. The primer (23nt) is elongated by one nucleotide to give the product (24nt) when insertion is successful. The data were taken at 37 °C using KF (exo-) 0.2 units/μL, 5 μM primer/template duplex, 20 μM dNTP, and the reactions were stopped after 1 min (B) and 2 min (A).

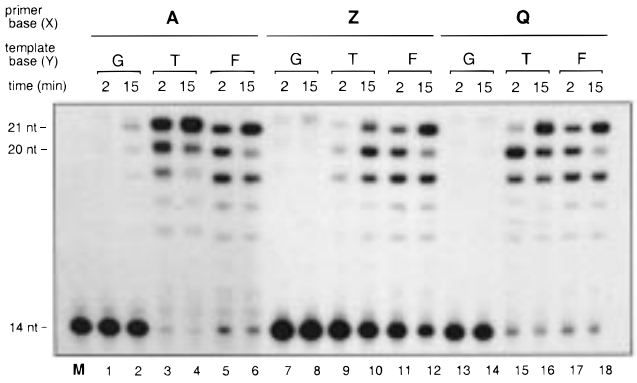

Figure 3. Autoradiogram of denaturing PAGE gel showing minor groove interaction effects on extension of normal and modified base pairs. The data were taken at 37 °C using KF (exo-) 0.2 units/μL, 200 nM primer/template duplex, and 20 μM dNTP. 14nt band is unextended primer.

The results showed selective and relatively efficient nucleotide insertion for dZTP or dQTP opposite F in the template, and almost no insertion opposite the natural bases. The results suggest, therefore, that minor groove interactions have a qualitatively small effect overall for the base pair being formed, in contrast to previous findings (see below). The nonpolar Q−F pair behaves similarly to a Z−F pair, despite the latter having little possibility of H bonding between the polymerase and the minor groove of the DNA in either template or primer strand.

We then used the same nucleoside analogues to evaluate the influence of minor groove interactions with an already formed pair when it is being extended by a polymerase. These studies were carried out with primer−template duplexes containing natural or modified bases in either the primer or template strand. Relative extension efficiencies were monitored qualitatively by extending radiolabeled primers in the presence of all four natural nucleoside triphosphates. The results show that when Z or Q are in the template (see Supporting Information), there is no substantial difference in extension efficiency. The pairs modeled after a T−A pair—T−Z and T−Q (denoting primer-template bases)—are elongated with similar efficiency, and to a lesser extent than the T−A control, establishing that a minor groove interaction is not important with the template strand. Nevertheless, geometry is very important for extension, since mismatches G−Z and G−Q and G−A are not extended at all. However, when Z and Q are placed in the primer (Figure 3), base pairs Q−T and Q−F are elongated completely after 2 min, whereas Z−T and Z−F are not fully elongated even after 15 min. These results imply that a minor groove interaction between the polymerase and the base at the 3′-end of the primer is essential to incorporation of the next base. Consistent with these results, we also found poor extension for F−A, F−Z, and F−Q base pairs when compared to T−A, T−Z, and T−Q (see Supporting Information).

To quantitate this strong effect, steady-state kinetics for extension were evaluated for the three most relevant cases: extension of A−T, Q−F, and Z−F pairs.[8] The steady-state efficiencies (V_{max}/K_m) for extension of these three pairs were found to be 2.7×10^7, 1.7×10^5, and 5.6×10^2 % min^{-1} M^{-1}, respectively. Thus, the results show that Q−F is extended more efficiently than Z−F by a factor of 300-fold, and is less efficient than an A−T base pair by a smaller factor of 160-fold. This implies that a H bond between the enzyme and the N3 of the base at the primer strand is essential for extension, and that it is as important as H bonds between the bases themselves. The fact that Q−F is not extended as well as an A−T base pair may reflect the added size of Q−F, which may cause some misalignment of the 3′-OH primer teminus.

Other recent studies have evaluated polymerase minor groove interactions using modified nucleosides. Spratt used a 3-deazaguanine nucleoside to test the effects of a putative Arg283 H bond to the 3-position in a template strand.[9] The loss of nitrogen donor was observed to cause a 170-fold decrease in insertion efficiency. However, our data suggest a smaller difference between the analogues containing CH versus N at the 3-position, and dFTP seems to insert even better opposite Z than opposite Q. It is possible that different rate-limiting steps may explain these relatively small differences, and pre-steady-state kinetic measurements may help to clarify this. As for the primer strand, minor groove interactions have been tested with dCTP or dTTP analogues lacking minor groove keto groups[10] and with 3-deaza-dATP.[11] A complete lack of insertion, and polymerase inhibition, was observed with the former analogues; this was attributed to loss of a minor groove interaction. The present data show, by contrast, that both dZTP and dQTP insert relatively efficiently and similarly opposite F in the template. It is difficult to rationalize the previous findings, since negative results can have multiple explanations. As for extension (distinct from insertion), neither study addressed this step, which we find to be considerably more sensitive to a minor groove interaction in the primer strand.

In summary, we find that minor groove interactions are of considerable importance to DNA synthesis by the Kf enzyme and that these putative H bonds have the greatest influence on one side of the minor groove during extension of that pair. The present data establish that (i) minor groove interactions are more important for extension than for insertion of base pairs, (ii) the interactions are apparently stronger in the primer strand than the template, (iii) a single minor groove interaction in the primer strand can make a 300-fold difference in extension efficiency, and (iv) the polymerase may be even more highly sensitive to base pairing geometry in extension of base pairs than in inserting them in the first place.

Acknowledgment. We thank the National Institutes of Health (GM52956) for support and the Spanish Ministry of Education and Culture for a postdoctoral fellowship to J.C.M.

Supporting Information Available: Details of synthesis and characterization of nucleoside analogue Q, oligonucleotide synthesis, and methods for enzymatic DNA synthesis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA983502+

(8) (a) Goodman, M. F., Creighton, S., Bloom, L. B.; Petruska, J. *Crit. Rev. Bioch. Mol. Biol.* **1993**, *28*, 83−126. (b) Fygenson, D. K.; Goodman, M. F. *J. Biol. Chem.* **1997**, *272*, 27931−27935.
(9) Spratt, T. E. *Biochemistry* **1997**, *36*, 13292−13297.
(10) Guo, M.-J.; Hidbrand, S.; Leumann, C. J.; McLaughlin, L. W.; Waring, M. *Nucleic Acids Res.* **1998**, *26*, 1863−1869.
(11) Cosstick, R.; Li, X.; Tuli, D. K.; Williams, D. M.; Connoly, B. A.; Newman, P. C. *Nucleic Acids Res.* **1990**, *18*, 4771−4778.