J|A|C|S
COMMUNICATIONS

Published on Web 12/28/2002

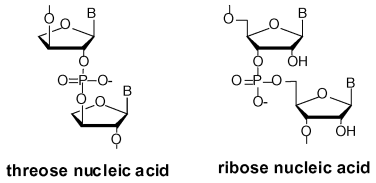
DNA Polymerase-Mediated DNA Synthesis on a TNA Template

John C. Chaput, Justin K. Ichida, and Jack W. Szostak*

Howard Hughes Medical Institute, and Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114

Received September 18, 2002; E-mail: szostak@molbio.mgh.harvard.edu

Systematic evaluation of nucleic acid structure with regard to the chemical etiology of RNA,[1] has led to the recent discovery of (3′,2′)-α-L-threose nucleic acid (TNA) as a candidate evolutionary progenitor of RNA.[2] TNA is capable of antiparallel, Watson−Crick base-pairing with complementary DNA, RNA, and TNA oligonucleotides.[2] This property is remarkable, given that the TNA repeat unit is one atom shorter than that of DNA or RNA. This intersystem



base-pairing allows for the possibility of information transfer between successive genetic systems; in addition, the relative chemical simplicity of threose suggests that the prebiotic synthesis of TNA may have been more facile than that of RNA. These considerations make further investigation of the functional properties of TNA of considerable interest.[3] However, efforts to evolve TNA aptamers or enzymes using in vitro selection would require enzymes capable of replicating TNA.[4,5] As an initial step in this direction, we have examined DNA synthesis by a variety of DNA polymerases on a synthetic TNA template.

To compare the accuracy and efficiency of TNA- versus DNA-directed DNA polymerization we used a primer extension assay (Figure 1).[6] A chimeric DNA/TNA template was constructed containing a DNA primer binding site, followed by six natural nucleotides and nine TNA residues. Thus, each polymerase was given a normal DNA substrate for initial binding and a "running start" of six nucleotides before being challenged to continue DNA synthesis on TNA. A broad range of DNA polymerases was surveyed for activity in this assay.[7]

The results of our screen for DNA polymerase activity on a TNA template are shown in Figure 2A. All enzymes except AMV-RT and Dbh revealed rapid full-length primer extension on an all-DNA template. The most striking result with the TNA template is that most enzymes catalyze the extension of 1−3 nucleotides into the TNA portion of the template, with a few enzymes even showing traces of full-length product. The enzymes with the greatest activity on the TNA template were *Bst Pol*I, the bacteriophage T7 DNA polymerase (exo-) and its mutated commercial version Sequenase, and the viral reverse transcriptase MMLV-RT and its mutated commercial version SuperScript II. We suggest that the generally small amount of primer that is extended by more than three nucleotides on the TNA template reflects the progressive loss of contacts between the polymerase and the template strand as the polymerase translocates into the TNA region of the template.

In an effort to improve the efficiency of TNA-dependent DNA synthesis we explored a variety of conditions. Varying incubation time and temperature, salt conditions, and pH proved ineffective.

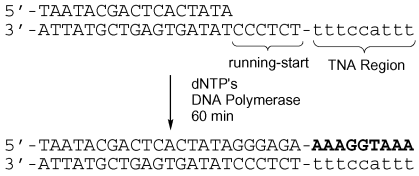


Figure 1. Structure and sequence of primer−template complex. TNA is shown in lower case, and the DNA product of the TNA template is in bold.

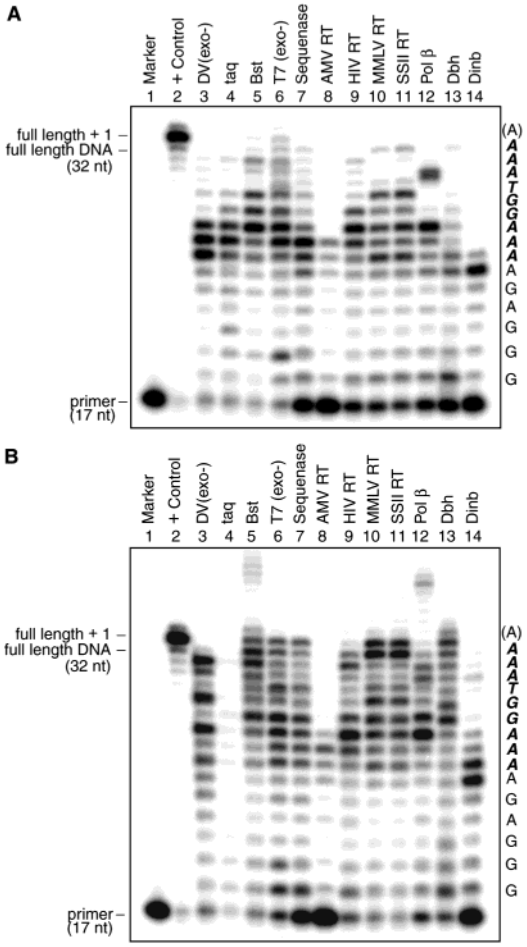


Figure 2. Primer-extension experiments. Primer was 5′-labeled with ^{32}P and annealed to a DNA template (control) or to a DNA/TNA chimeric template. Polymerization reactions were performed with 125 nM primer/template and equimolar ratios of all four dNTPs (250 μM) by incubating for 60 min with 0.5 μL of enzyme under conditions optimal for each enzyme. Reactions were analyzed by denaturing polyacrylamide gel electrophoresis. (A) Standard conditions. (B) Reactions supplemented with 1.5 mM Mn^{2+}.

The presence of Mn^{2+} ions is known to relax the specificity of many DNA polymerases.[8,9] We found that supplementing standard polymerase reaction mixtures with 1.5 mM $MnCl_2$ had a dramatic

10.1021/ja028589k CCC: $25.00

Table 1. Fidelity of DNA Synthesis on DNA and TNA Templates[a]

	Sequenase		Superscript II	
	DNA	TNA	DNA	TNA
nucleotides	1937	1207	2049	1049
transitions	3	5	6	7
transversions	10	8	3	5
deletions	1	0	0	1
insertions	0	0	0	0
error rate	0.007	0.011	0.004	0.011

[a] The error rate equals (transition + transversion mutations)/total nucleotides sequenced.

effect on the activity of several polymerases (Figure 2B). This effect was most pronounced among the reverse transcriptases (Figure 2, lanes 9−11). In the case of MMLV-RT and SS II, the fraction of full-length product increased from 2 to 3% in the absence of Mn^{2+} to as much as 28% in the presence of Mn^{2+}. The presence of Mn^{2+} also had particularly strong effects on the thermophilic polymerase Deep Vent (exo-) and the Sulfolobus repair enzyme Dbh (Figure 2, lanes 3 and 13, respectively).

We were interested in testing the possibility that the loss, distortion, or weakening of enzyme−template contacts might result in a decrease in the fidelity of DNA synthesis on a TNA template. We therefore sequenced full-length DNA products from primer-extension reactions performed in the presence of 1.5 mM Mn^{2+} with either Sequenase or SS II.[10,11] Approximately 150 sequences (1000−2000 nucleotides) were determined for each of four template/polymerase combinations (Table 1). The error-rate of DNA synthesis on a TNA template was not significantly different from that on a DNA template for Sequenase (0.7% vs 1.1%), but the difference (0.4% vs 1.1%) was highly significant ($p < 0.001$) in the case of SS II. The relatively high observed error rates on DNA of 0.4−0.7% probably reflect the combined use of nonediting polymerases in the presence of the mutagenic Mn^{2+} ion.

In the course of the analysis described above, we observed a class of sequences that are best explained by partial primer extension on the TNA template followed by dissociation and continued synthesis using a free primer as a second template. This template switching was observed only for primer extension on the TNA template and explains the presence of longer than full-length products in some of the primer-extension reactions (Figure 2B, lanes 5 and 12). Template switching is an integral part of the retroviral life cycle,[12] and not surprisingly the frequency of template switching was significantly higher for SS II than for Sequenase (0.17 versus 0.02, respectfully). Since no examples of template switching were observed with the DNA control template, it is likely that the slower rate of synthesis on a TNA template allowed time for a slow strand-switching process to occur.

Crystallographic studies of the mechanism of DNA polymerase-mediated DNA synthesis reveal the presence of numerous contacts between the enzyme and its DNA and dNTP substrates,[13] including H-bonding and electrostatic interactions with the template strand.[14] Our observation of DNA synthesis on a TNA template suggests that most of the critical enzyme−substrate contacts are maintained in this context and also suggests that a DNA/TNA heteroduplex adopts a conformation generally similar to that adopted by a DNA/DNA duplex within the enzyme active site. However, the much slower rate of DNA synthesis on a TNA template suggests that at least some enzyme−substrate contacts are missing or suboptimal. The most direct explanation is the loss or weakening of contacts with the TNA template strand, but it is also possible that the altered geometry of a TNA/DNA duplex leads indirectly to loss or weakening of contacts with the primer strand or the incoming dNTP. The presence of Mn^{2+} ions may relax constraints on the geometry of either the enzyme or the substrate, allowing partial restoration of these lost or weakened contacts.

In summary, we have found that certain DNA polymerases are able, despite the significant differences in the sugar−phosphate backbone, to copy limited stretches of a TNA template. Given the high activity of wild-type polymerases, we suggest that it may be possible to evolve a TNA-directed DNA polymerase with improved activity. In conjunction with an enzyme evolved to synthesize TNA on a DNA template, this would enable directed evolution experiments with TNA sequences.

Acknowledgment. We gratefully acknowledge A. Eschenmoser and G. F. Joyce for helpful discussions, X. Wu for synthesis of the DNA/TNA chimeric oligonucleotide, and R. Krishnamurthy, X. Wu, S. Kuhns, and G. F. Joyce for sharing unpublished exploratory experiments using this template. We also thank L. Silvian and T. Ellenberger for samples of the T7 (exo-), Pol β, Dbh, and DinB polymerases, and D. Das and M. Georgiadis for the plasmid used to express MMLV-RT. This work was supported by grants from the NIH and the NASA Astrobiology Institute. J.W.S. is an Investigator, and J.C.C. is a Research Associate of the Howard Hughes Medical Institute. J.K.I. was supported by a Ford Foundation Predoctoral Fellowship.

References

(1) Eschenmoser, A. *Science* **1999**, *284*, 2118−2124.
(2) Schoning, K.-U.; Scholz, P.; Guntha, S.; Wu, X.; Krishnamurthy, R.; Eschenmoser, A. *Science* **2000**, *290*, 1347−1351.
(3) (a) Orgel, L. E. *Science* **2000**, *290*, 1306−1307. (b) Joyce, G. F. *Nature* **2002**, *418*, 214−221.
(4) (a) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505−510. (b) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818−822.
(5) For recent review see: (a) Famulok, M. *Curr. Opin. Struct. Biol.* **1999**, *9*, 324−329. (b) Wilson, D. S.; Szostak, J. W. *Annu. Rev. Biochem.* **1999**, *68*, 611−647. (c) Breaker, R. R. *Chem. Rev.* **1997**, *97*, 371−390.
(6) The assay and oligonucleotides used in this study were designed by Gerald F. Joyce.
(7) Recombinant DNA polymerases activities and sources. Deep Vent (exo-) (2 units/μL), Thermus Aquaticus (2 units/μL), and *Bst* DNA polymerase (8 units/μL), New England Biolabs; Superscript II RNase H (200 units/μL), Invitrogen; DNA Sequenase (13 units/μL) and AMV reverse transcriptase (20 units/μL), Sigma; and HIV reverse transcriptase (27.3 units/μL), Worthington Biochemical Corp.
(8) Cadwell, R. C.; Joyce, G. F. *PCR Methods Appl.* **1992**, *2*, 28−33.
(9) Tabor, S.; Richardson, C. C. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 4076−4080.
(10) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1989.
(11) Gel-purified DNA products were extended by 4−6 C residues by incubating with dCTP and terminal deoxynucleotidyl transferase (Promega). PCR amplification and subsequent digestion with *Eco*RI (New England Biolabs) allowed individual sequences to be concatenated into multimers with T4 DNA ligase (New England Biolabs). Oligonucleotides were cloned into a TOPO vector (Invitrogen) and sequenced (MGH Molecular Biology Core Facility).
(12) Kulpa, D.; Topping, R.; Telesnitsky, A. *EMBO J.* **1997**, *16*, 856−865.
(13) (a) Ollis, D. L.; Brick, P.; Hamlin, R.; Xuong, N. G.; Steitz, T. A. *Nature* **1985**, *313*, 762−766. (b) Doublie, 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. (d) Li, Y.; Korolev, S.; Waksman, G. *EMBO J.* **1998**, *17*, 7514−7525. (e) Franklin, M. C.; Wang, J.; Steitz, T. A. *Cell* **2001**, *105*, 657−667. (f) Silvian, L. F.; Toth, E. A.; Pham, P.; Goodman, M. F.; Ellenberger, T. *Nat. Struct. Biol.* **2001**, *8*, 984−989.
(14) Kunkel, T. A.; Bebenek, K. *Annu. Rev. Biochem.* **2000**, *69*, 497−529.

JA028589K