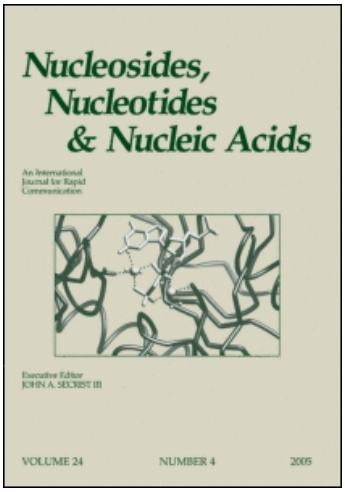


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

DNA Polymerase Recognition of 2'-Deoxy-2'-Fluoroarabinonucleoside 5'-Triphosphates (2'F-Arantps)

Chang G. Peng[a]; Masad J. Damha[a]
[a] McGill University, Montreal, Quebec, Canada

To cite this Article Peng, Chang G. and Damha, Masad J.(2007) 'DNA Polymerase Recognition of 2'-Deoxy-2'-Fluoroarabinonucleoside 5'-Triphosphates (2'F-Arantps)', Nucleosides, Nucleotides and Nucleic Acids, 26: 8, 1189 — 1193
To link to this Article: DOI: 10.1080/15257770701527786
URL: http://dx.doi.org/10.1080/15257770701527786

Nucleosides, Nucleotides, and Nucleic Acids, 26:1189–1193, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701527786

Taylor & Francis
Taylor & Francis Group

DNA POLYMERASE RECOGNITION OF 2′-DEOXY-2′-FLUOROARABINONUCLEOSIDE 5′-TRIPHOSPHATES (2′F-araNTPs)

Chang G. Peng and Masad J. Damha □ *McGill University, Montreal, Quebec, Canada*

□ *We examined the ability of 2′-deoxy-2′-fluroarabinonucleoside 5′-triphosphates (2′F-araNTPs) to serve as substrates of various DNA polymerases. In addition, we also examined the ability of these polymerases to accept DNA-FANA (2′-deoxy-2′-fluoroarabinonucleic acids) chimeras as template strands while synthesizing a DNA or FANA-DNA complementary strand. We provide preliminary data demonstrating that 2′F-araNTPs are indeed substrates of several DNA polymerases, and that FANA-DNA chimeric templates are generally well recognized by these polymerase enzymes.*

Keywords 2′F-araNTPs; DNA polymerases

INTRODUCTION

Chemical modifications of nucleic acids offer flexibility in the design and utility of these molecules, particularly in development of antisense, siRNA and aptamer therapeutics. For example, most chemically modified RNA aptamers evolved through SELEX[1,2] make use of the 2′F or 2′NH_2 ribose modifications. Post-SELEX modifications, especially 2′-*O*-methylation of RNA, are commonly introduced to further improve the nuclease stability and/or pharmacokinetics of the aptamer nucleic acid.[3] Even fewer examples exist with modified deoxyribonucleoside 5′-triphosphates (dNTPs).[4,5] This is mainly due to the limitations of the polymerases, which can efficiently accept only a handful of chemically modified nucleoside 5′-triphosphates. In fact, SELEX processes using only modified dNTPs or ribonucleoside 5′-triphosphate (rNTPs) and modified templates are very difficult to carry out. In this study, we examined various polymerases for their activity to incorporate pyrimidine 2′F-araNTPs. In addition, we also examined the ability of

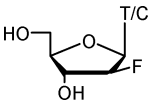
This work was supported by grants from the Canadian Institute of Health Research (CIHR) and Topigen Pharmaceuticals, Inc. C.G. Peng is recipient of FQRNT fellowship and a Clifford Wong McGill Major Fellowship. M.J.D. is recipient of the Bernard Belleau Award (CSC).

Address correspondence to Masad J. Damha, Department of Chemistry, McGill University, Montreal, QC H3A 2K6, Canada. E-mail: masad.damha@mcgill.ca

TABLE 1 Base sequence of oligonucleotide primer and templates

Code	Sequence[a]
PF20	5′-*TAATACGACTCACTATA*-3′
PF31	5′-**TTACCTTT-**<u>**CTCTTCTCCC**</u>**-***TATAGTGAGTCGTATTA***-3′**
PF33	5′-TCGGTGGATCATAGACAGTA-<u>CTCTTCTCCC</u>-*TATAGTGAGTCGTATTA*-3′
PF34	5′-CTCTATGTGCACGCA-<u>CTCTTCTCCC</u>-*TATAGTGAGTCGTATTA*-3′

[a] *Note*: Capital letters: DNA; *Italized sequence: primer binding sequence and primer (17nt)*; Underlined sequence: a running start (10nt); **Bold and capital letter:** FANA units.



these polymerases to accept FANA-DNA chimeras as template strands while synthesizing a DNA or FANA-DNA complementary strand.

MATERIALS AND METHODS

Template and Primer Design

DNA and FANA-DNA templates are shown in Table 1 with the primers used. The primer (PF20) is a 17nt DNA sequence 5′-*TAATACGA CTCACTATA*-3′. Each template strand comprises three sequence segments: a 17nt long primer binding sequence *5′-TATAGTGAGTCGTATTA-3′*, a 10nt long running start sequence 5′-<u>CTCTTCTCCC</u>-3′,[6] and a variable "test sequences" made up of an all-DNA (PF33) or chimeric DNA-FANA (PF34) or FANA segment (PF31). Oligonucleotide FANA-DNA templates were synthesized according to the published procedures.[7]

Polymerases and Primer Extension Assays

We examined seven DNA polymerases, namely: (a) five thermophilic DNA polymerases (source: New England Biolabs (NEB)): Deep Vent (3′→5′ exo-) (DV, 2 units/μL), 9° Nm^{TM} (9N, 2 units/μL), *Bst* large fragment (*Bst*, 8 units/μL), *Taq* (*Taq*, 5 units/μL), Phusion High-Fidelity (Ph, 5 units/μL); (b) one Mesophilic DNA polymerase (source: NEB): Klenow fragment (3′→5′ exo-) (KL, 5 units/μL); (c) one reverse transcriptase (RT): HIV-RT (recombinant) (HIV; source: Worthington Biochemical Corp. 27.3 units/μL). Reaction buffer for DV, 9N, *Bst* and *Taq* is 20 mM Tris-HCl (pH 8.8 at 25°C), 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton X-100; for Ph, 5x Phusion HF Buffer; for KL, 10 mM Tris-HCl (pH 7.5 at 25°C), 5 mM $MgCl_2$, 7.5 mM DTT; for HIV, 50 mM Tris-HCl (pH 7.8 at 25°C), 60 mM KCl, 2.5 mM $MgCl_2$. In primer extension assays, the primer (PF20) was 5′-labeled with ^{32}P and annealed to a template with the final concentration of 85 nM for the primer and 255 nM for the template. The cocktail of reaction buffer, triphosphates (dNTPs or 2′F-araNTPs), water, the

primer and template mixture was prepared according to pre-calculated reaction volume and concentrations. A polymerase was added to initiate the polymerization reactions. 4 μL aliquot from the reaction mixture at different time points was taken, quenched by the same volume of a stopping dye solution (98% deionized formamide, 10 mM EDTA, 1 mg/ml bromophenol blue and 1 mg/ml xylene cyanol), and analyzed by 12% gel electrophoresis.

RESULTS AND DISCUSSIONS

Direct evolution in SELEX using modified 5′-triphosphates requires that modified triphosphates be very good substrates of polymerases, and that the resulting modified oligonucleotide strands can serve as templates for cDNA synthesis. First we conducted primer extension assays to assess the incorporation of six 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) thymine 5′-triphosphates (2′F-araTTPs) and six 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine 5′-triphosphates (2′F-araCTPs) on the DNA template PF33 (Figure 1). Thermophilic enzymes (DV, 9N, Ph) can incorporate 2′F-araNTPs almost as effectively as dNTPs except that Ph shows a "pausing" effect during the last 2′F-araCTP incorporation, as assessed by comparison with dideoxynucleoside 5′-triphosphate (ddNTP) termination assays (lanes

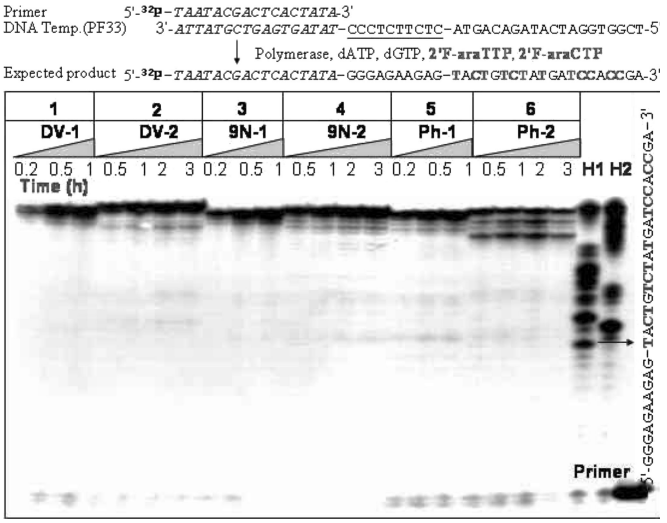


FIGURE 1 Incorporation 6 × 2′F-araT and 6x 2′F-araC units by DNA polymerases on a DNA template. Groups 1, 3, 5: 0.2 mM dNTPs; groups 2, 4, 6: 0.2 mM dATP, dGTP 2′F-araTTP, 2′F-araCTP; 20 μL reaction volume at 55°C for all; enzyme used: 1 μL DV, 1 μL 9N, 0.4 μL Ph in DV-1, 9N-1 and Ph-1, respectively; 2 μL DV, 2 μL 9N, 0.4 μL Ph enzyme in DV-2, 9N-2 and Ph-2, respectively; Lane H1 was formed through a chain termination assay with 0.1 mM dATP, dGTP, dCTP, dTTP+ddTTP (1:1 molar ratio), 0.4 μL HIV-RT in 20 μL reaction volume, on the same template PF33; lane H2 with 0.1 mM dATP, dGTP, dTTP, dCTP+ddCTP (1:1 molar ratio) and conditions as H1.

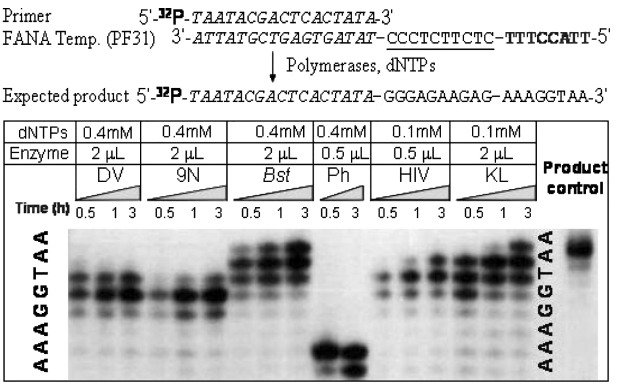


FIGURE 2 FANA as a template (PF31) for polymerase-directed DNA synthesis. Reaction volume 30 μL, dNTP and enzyme amounts/concentrations shown.

H1 and H2; Figure 1). This shows that 2′F-araTTP and 2′F-araCTP are substrates for most thermophilic DNA polymerases.

Next, a DNA-FANA chimeric template (PF31) was examined as a possible template for DNA synthesis by various DNA polymerases (Figure 2). The data shows that most enzymes can catalyze the extension of at least two dNTPs on the FANA template region. DV and 9N can incorporate up to 6 of the 8 dNTPs with a significant pause after 5 nucleotides. HIV-RT incorporated 7 nucleotides with a significant pause after incorporation of 5-6 nucleotides, whereas Ph. incorporated two dNTP maximally. Remarkably,

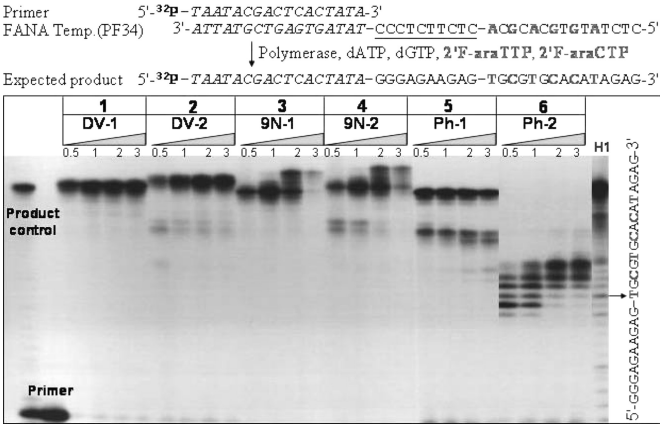


FIGURE 3 Incorporation of 3 × 2′F-araT and 3x 2′F-araC units catalyzed by DNA polymerases on a chimeric DNA-FANA template (40% FANA content after the running start sequence). Reaction conditions: 20 μL reaction volume at 55°C for all, 2 μL enzyme used; lane H1 were formed through a chain termination assay with 0.1 mM dATP, dGTP, dCTP, dTTP+ddTTP (1:1 molar ratio), 0.4 μL HIV-RT in 20 μL reaction volume on a DNA template with same sequence as PF34.

KL and *Bst* afforded significant full-length products incorporating all eight dNTPs on the all-FANA template (Figure 2).

Last, we designed another DNA-FANA chimera to test the possibility of using polymerases to generate a FANA-DNA chimeric product on a FANA-DNA template (PF34). Two sets of triphosphates were used in the primer extension reaction, namely, only dNTPs (odd groups; Figure 3) and dATP, dGTP, 2′F-araTTP and 2′F-araCTP (even groups in Figure 3). In the presence of only dNTPs, DV, 9N and Ph DNA polymerases all recognized PF34 as a template to afford full length all-DNA products. Efficient full-length product synthesis took place for DV and 9N only, with only modest posing observed after introduction of the last 2′F-araNTP. Ph could not generate full-length DNA-FANA product and significant pausing was observed after two FANA units were incorporated on this DNA-FANA template (group 6 in Figure 3).

In conclusion, 2′F-araNTPs appear to be excellent "dNTP mimics" and substrates of various DNA polymerases (e.g., thermophilic DNA polymerases), and that FANA-DNA chimeric templates are generally well recognized by these polymerase enzymes. Based on these findings and the well-known nuclease resistance and physicochemical properties of FANA,[8,9] we predict that 2′F-araNTP will be particularly useful in the generation of aptamers via SELEX and/or post-SELEX.

REFERENCES

1. Ellington, A.D.; Szostak, J.W. *Nature* **1990**, 346, 818–822.
2. Tuerk, C.; Gold, L. *Science* **1990**, 249, 505–510.
3. Brody, E.N.; Gold, L. *Rev. Mol. Biotechn.* **2000**, 74, 5–13.
4. Ichida, J.K.; Zou, K.; Horhota, A.; Yu, B.; McLaughlin, L.W.; Szostak, J.W. *J. Am. Chem. Soc.* **2005**, 127, 2802–2803.
5. Kuwahara, M.; Hanawa, K.; Ohsawa, K.; Kitagata, R.; Ozaki, H.; Sawai, H. *Bioorg. Med. Chem.* **2006**, 14, 2518–2526.
6. Kato, Y.; Minakawa, N.; Komatsu, Y.; Kamiya, H.; Ogawa, N.; Harashima, H.; Matsuda, A. *Nucleic Acids Res.* **2005**, 33, 2942–2951.
7. Elzagheid, M.I.; Viazovkina, E.; Damha, M.J. *Current Protocols in Nucleic Acid Chemistry* **2002**, 4(15), 1–22.
8. Noronha, A.M.; Wilds, C.J.; Lok, C.-N.; Viazovkina, K.; Arion, D.; Parniak, M.A.; Damha, M.J. *Biochemistry* **2000**, 39, 7050–7062.
9. Wilds, C.J.; Damha, M.J. *Nucleic Acids Res.* **2000**, 28, 3625–3635.