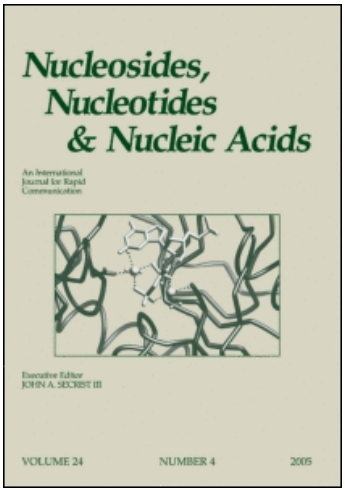
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

EFFECT OF SUBSTITUTING ARABINONUCLEOSIDES FOR DEOXYNUCLEOTIDES IN THE DNA PRIMING STRAND ON THE POLYMERASE ACTION OF HIV-1 RT

Anne M. Noronha[a]; Dominique Arion[b]; Michael A. Parniak[a]; Masad J. Damha[c]
[a] Department of Biochemistry and Molecular Biology, The Johns Hopkins University, Baltimore, MD, U.S.A. [b] Departments of Medicine, McGill University, Montreal, Canada [c] Departments of Chemistry, McGill University, Montreal, Canada

Online publication date: 31 March 2001

To cite this Article Noronha, Anne M. , Arion, Dominique , Parniak, Michael A. and Damha, Masad J.(2001) 'EFFECT OF SUBSTITUTING ARABINONUCLEOSIDES FOR DEOXYNUCLEOTIDES IN THE DNA PRIMING STRAND ON THE POLYMERASE ACTION OF HIV-1 RT', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1205 — 1209
To link to this Article: DOI: 10.1081/NCN-100002520
URL: http://dx.doi.org/10.1081/NCN-100002520

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1205–1209 (2001)

EFFECT OF SUBSTITUTING ARABINONUCLEOSIDES FOR DEOXYNUCLEOTIDES IN THE DNA PRIMING STRAND ON THE POLYMERASE ACTION OF HIV-1 RT

Anne M. Noronha,[1,*] Dominique Arion,[2] Michael A. Parniak,[2,*] and Masad J. Damha[1,†]

Departments of [1]Chemistry and
[2]Medicine, McGill University, Montreal, QC, Canada

Downloaded At: 12:24 26 January 2011

ABSTRACT

The ability of 5′-DNA-araN-3′ chimeras to serve as primers during HIV-1 RT-catalyzed DNA synthesis was assessed. It is shown that while the structural changes imparted by the arabinose units are minimal, the biological outcome is significant. For example, a DNA strand with arabinocytidine (araC) at the 3′-terminus was found to serve as a primer of DNA synthesis but significant pausing of HIV-RT was observed after the addition of 4 dNTP's. This phenomenon was not observed for the analogous DNA primer containing a riboC unit or an all-DNA strand.

Arabinonucleosides are stereoisomers of the naturally occurring ribonucleosides, differing only in the configuration at the 2′-position of the sugar ring (Fig. 1A). This seemingly "minor" alteration of the sugar ring leads to nucleoside analogues with remarkable biological activities, including anticancer and antiviral activities (1,2). Recent reports on arabinonucleic acids (ANA) are rapidly drawing attention with the main emphasis being the impact of 2′-stereochemistry (RNA *versus* ANA)

*Current address: Department of Biochemistry and Molecular Biology, The Johns Hopkins University, Baltimore, MD, USA.
†Corresponding author.

Copyright © 2001 by Marcel Dekker, Inc. www.dekker.com

ORDER REPRINTS

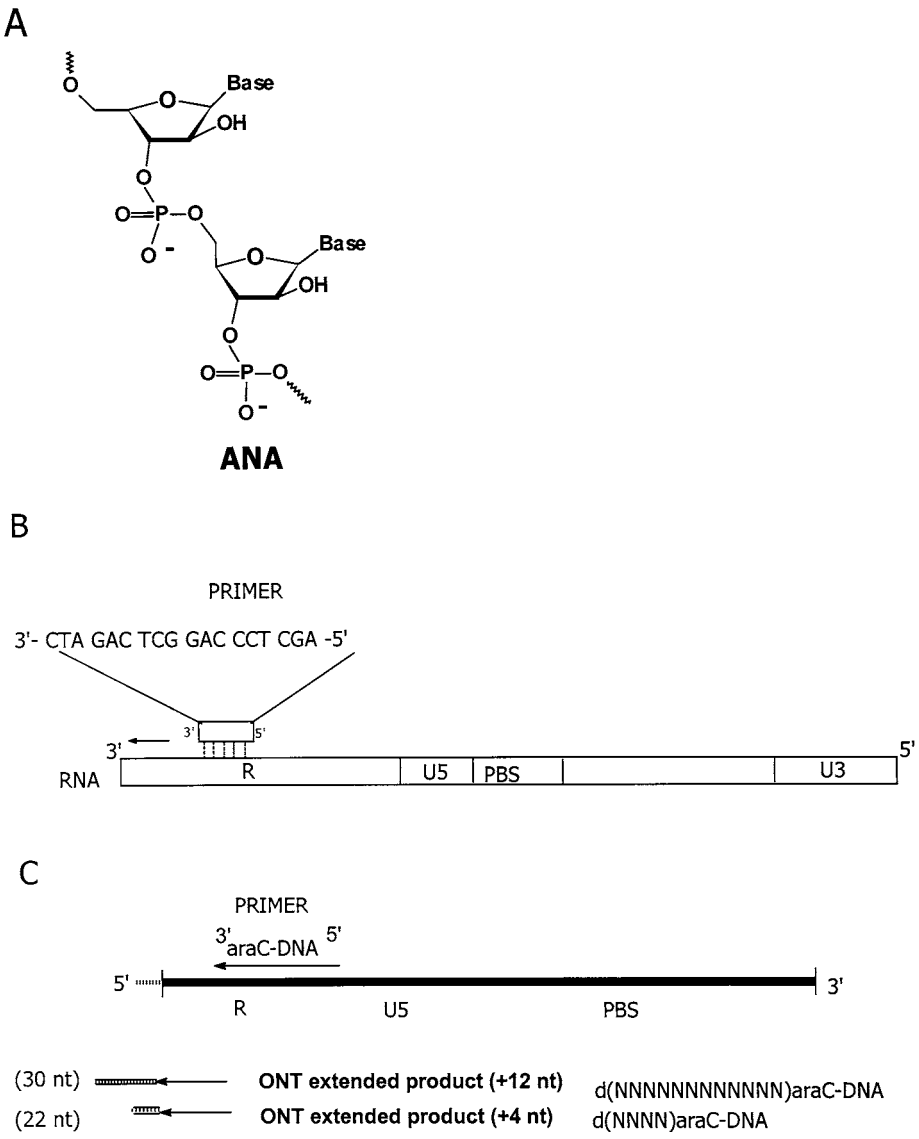


Figure 1. (A) Arabinonucleic acids. (B) The R region sequence of the HIV-1 genomic RNA. The sequence of the antisense primer molecule is 5′-AGC TCC CAG GCT CAG ATC-3′. (C) Schematic illustration of DNA polymerization products in the *in vitro* DNA polymerization reactions.

on physicochemical and biological properties (3). For example, both *E.coli* RNase H and HIV-1 RT associated RNase H cleave RNA strands of ANA:RNA duplexes, but not those of RNA:RNA duplexes (3a,c).

Chimeric oligonucleotides serve as excellent probes for studying the mechanism by which polymerases read-through the backbone of nucleic acid double

Downloaded At: 12:24 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

helices. Recent studies with chimeric RNA-DNA oligonucleotides have increased our understanding of the mechanism of substrate discrimination (rNTP *versus* dNTP) and extension of RNA transcripts by T7 RNA polymerase (4). Similar studies on HIV-1 reverse transcriptase (RT) have been reported (5). It is known that when HIV-1 RT encounters an altered nucleotide on the template strand, the enzyme may read through, pause or terminate synthesis (6). Primer extension may be accomplished with fidelity or with the insertion of a random or particular incorrect base while pausing (6). Termination may also occur before, at or after encountering the modified site on the template strand (6). It is not known whether DNA strands incorporating arabinonucleotides (araN) can serve as primers of RNA-dependent DNA polymerization, nor if they have been used to probe the mechanism of HIV-1 RT translocation.

Here, we describe a study in which chimeric oligodeoxynucleotides containing arabinonucleotide residues (araN) were characterized for hybridization affinity and the ability to prime reverse transcription. Specifically, we wished to assess the efficiency with which HIV-1 RT extended a DNA-araC primer bound to an RNA template. The primers synthesized were 18-mer chimeric oligonucleotides complementary to the R-region of genomic HIV-1 RNA (Fig. 1B), the sequence of which are shown in Table 1. First, the ability of these oligomers to form duplexes with complementary RNA and DNA was determined from UV melting and circular dichroic spectroscopy. T_m values listed in Table 1 indicate that as deoxynucleotides near the middle of the strand were replaced by arabinonucleotides, T_m values with the target RNA were reduced slightly at *ca.* 0.5°C/substitution. The destabilizing effect was greater when the chimeras hybridized to a target DNA (ΔT_m *ca.* −2.3°C/araN). However, it should be noted that the apparent

Table 1. Thermal Stability of Duplexes Formed by DNA-ANA Chimeras with Target RNA and DNA Single Strands

Antisense Oligomer	Code	Antisense Sequence with ARA and Ribo Inserts	T_m (°C) RNA	T_m (°C) DNA
D18	I	5′-AGC TCC CAG GCT CAG ATC-3′	72.3	68.0
DNA-*araC1*	II	5′-AGC TCC CAG GCT CAG AT*aC*-3′	71.6	68.0
DNA-*araU2*	III	5′-AGC TCC CAG GCT CAG A*aU*C-3′	71.7	65.5
DNA-*araA3*	IV	5′-AGC TCC CAG GCT CAG *aA*TC-3′	71.7	65.5
DNA-*araG4*	V	5′-AGC TCC CAG GCT CA*aG* ATC-3′	69.9	64.0
DNA-*araA5*	VI	5′-AGC TCC CAG GCT C*aA*G ATC-3′	71.7	66.2
DNA-*araC16*	VII	5′-AG*aC* TCC CAG GCT CAG ATC-3′	71.5	65.0
DNA-riboC1	VIII	5′-AGC TCC CAG GCT CAG ATrC-3′	71.1	68.0
R18	IX	5′-AGC UCC CAG GCU CAG AUC-3′	84.6	66.2

All duplexes were 2.3 μM in concentration. Buffer: 140 mM KCl, 1 mM $MgCl_2$, 5 mM Na_2HPO_4 (pH = 7.2). Target RNA:GAU CUG AGC CUG GGA GCU, Target DNA:GAT CTG AGC CTG GGA GCT.

Downloaded At: 12:24 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

ORDER REPRINTS

destabilization caused by arabino units is significantly smaller than that created by mismatches at similar positions ($\Delta T_m = -5^\circ C$), suggesting that araN residues in these duplexes retain classical base-pairing interactions. This conclusion is also evident in the work of Beardsley and co-workers, who found that substituting dN for araN residues in the DNA strand of DNA:RNA and DNA:DNA duplexes resulted only in a moderate loss of stability (7).

The CD spectra of the DNA/araN:RNA hybrids closely resemble the spectra of unmodified DNA:RNA hybrids, all of which displayed characteristic "A-helix" patterns. The major difference seen among the spectra of the various DNA/araN:RNA hybrids was the magnitude of the negative Cotton effect at *ca*. 210 nm. The intensity of this peak gradually decreased as the araN insert was moved from the 3′-end towards the middle of the sequence, reaching a minimum when the araN residue was located at position −4. These observations suggest that while helix conformation of DNA/RNA hybrids is not significantly altered by an araN substitution, disruption of this particular hybrid sequence is greatest when the araN residue is located *ca*. 4 to 5 nt upstream from the DNA primer's 3′-end. Remarkably, these observations correlate well with ability of DNA/araC primers to partially block HIV-RT catalyzed DNA polymerization.

Thus, whereas HIV-1 RT readily extends the antisense unmodified DNA primer I to form the expected 30 nt product (Fig. 1C), primer extension is significantly reduced for the DNA primer containing the araC at the 3′ end. In this case, significant pausing of HIV-RT was noted after the addition of the 4th DNA residue producing primarily 5′-DNA-araC-dNNNN-3′("+4 product") and only ∼10% of the expected full-length (30 nt) product. Pausing is also observed for the chimeric DNA primers containing araN at internal positions (e.g., −1, −2, −3 and −4). In all cases, the polymerization reactions produced the 'premature' 5′-dN. . .dN-araN-dNNNN-3′ (or "+4") product as well as the expected full-length 30 nt product (Fig. 1C). There seems to be no change in priming relative to the DNA control when the araN residue is placed at, and past, the fifth position (primers VI–VII, Table 1). Interestingly, a primer containing riboC at the 3′-end (DNA-riboC) was fully extended to the 30 nt product without accumulation of the corresponding "+4 product".

In conclusion these results indicate that when araX is located at or near the 3′ terminus of a DNA primer, the arabinose 2′-hydroxyl group, while not significantly affecting the stability of the primer terminus, interferes directly with the mechanism by which HIV-1 catalyzes phosphodiester bond formation. In addition, the results suggest that while the arabino 2′-OH does not causes *immediate* chain termination, the efficiency of subsequent steps, particularly the addition of the 5th dN nucleotide, is very much dependent on the sugar stereochemistry at position 2′ (ribo *versus* arabino). This may be due to either "physical blockage" of HIV-RT translocation when the enzyme encounters the arabinonucleotide residues, and/or subtle changes in helix conformation as demonstrated by our CD experiments.

Downloaded At: 12:24 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

REFERENCES

1. Kuchta, R.D.; Thompson, H.C. *Biochemistry*, **1995**, *34*, 11198–11203.
2. Bodey, G.P.; Freiereich, E.J.; Monto, R.W.; Hewlett, J.S. *Cancer Chemother. Rep*., **1969**, *53*, 59.
3. (a) Damha, M.J.; Wilds, C.J.; Noronha, A.; Brukner, I.; Borkow, G.; Arion, D.; Parniak, M.A. *J. Am. Chem. Soc*., **1998**, *120*, 12976–12977. (b) Noronha, A.; Damha, M.J. *Nucleic Acids Res*., **1998**, *26*, 2665–2671. (c) Noronha, A.; Wilds, C.J.; Viazovkina, K.; Lok, C.-N.; Parniak, M.A.; Damha. M.J. *Biochemistry*, **2000**, *39*, 7050–7062. (d) Wilds, C.J.; Damha, M.J. *Bioconjugate Chem*., **1999**, *10*, 299–305.
4. Huang, Y.; Beaudry, A.; McSwiggen, J.; Soussa, R. *Biochemistry*, **1997**, *36*, 13718–13728.
5. (a) Joyce, C.M *Proc. Natl. Acad. Sci. USA*., **1997**, *94*, 1619–1622. (b) Astatke, M.; Ng, K.; Grindley, N.D.F. Joyce, C.M. *Proc. Natl. Acad. Sci. USA*., **1998**, *95*, 3402–3407. (c) Boyer, P.L.; Sarafianos, S.G.; Arnold, E.; Hughes, S.H. *Proc. Natl. Acad. Sci. USA*., **2000**, *97*, 3056–3061.
6. Lorsch, J.R.; Bartel, D.P.; Szoztak, J.W. *Nucleic Acids Res*., **1995**, *23*, 2811–2814.
7. Mikita, T.; Beardsley, G.P. *Biochemistry*, **1988**, *27*, 4698–4705.

Downloaded At: 12:24 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Downloaded At: 12:24 26 January 2011

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081NCN100002520