Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 499–504

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

RECOGNITION OF 2'-DEOXY*ISO*GUANOSINE TRIPHOSPHATE BY HIV-1 REVERSE TRANSCRIPTASE AND MAMMALIAN CELLULAR DNA POLYMERASES

Michael J. Lutz,*[a] Jennifer Horlacher[a] and Steven A. Benner[b]

[a]*Department of Chemistry, ETH Zürich, Universitätstr. 16, CH-8092 Zürich, Switzerland*
[b]*Department of Chemistry, University of Florida, Gainesville FL 32611 USA*

Received 18 November 1997; accepted 23 January 1998

Abstract: HIV-1 reverse transcriptase (RT) incorporates 2'-deoxy*iso*guanosine triphosphate (d-*iso*GTP) opposite thymidine (T) in a DNA template and opposite uracil (U) in an RNA template about 10 times more efficiently than the eukaryotic DNA polymerase α, both in the absence and presence of dATP.
© 1998 Elsevier Science Ltd. All rights reserved.

Transposing the exocyclic amino and carbonyl functions of the two standard bases G and C yields a third Watson-Crick base pair between the non-standard bases *iso*guanine (*iso*G) and *iso*cytosine (*iso*C) (Fig. 1).[1] This non-standard base pair contributes to the overall stability of a duplex in complementary oligonucleotide strands.[2] Further, individual RNA and DNA polymerases have been found that catalyze template-directed incorporation of both non-standard bases into duplex DNA.[3] For example, the Klenow fragment from DNA polymerase I and AMV reverse transcriptase (RT) both incorporate d-*iso*GTP opposite d-*iso*C in a DNA template. T7 RNA polymerase incorporates *iso*G opposite a templating d-*iso*C.

However, the Klenow fragment of DNA polymerase I and T7 RNA polymerase also misincorporate *iso*GTP opposite T in a template, and both UTP and TTP opposite d-*iso*G in a template.[3] Further, T7 RNA polymerase evidently prefers to misincorporate UTP opposite d-*iso*G in a template even in the presence of excess d-*iso*CTP. The isoG-T mismatch was explained by the fact that d-*iso*G could adopt a minor tautomeric form (the O^2H tautomer) that constitutes approximately 10% of the total in aqueous solution[4] and that is complementary (in a Watson-Crick sense) to T in a DNA template and U in an RNA template (Fig. 1).

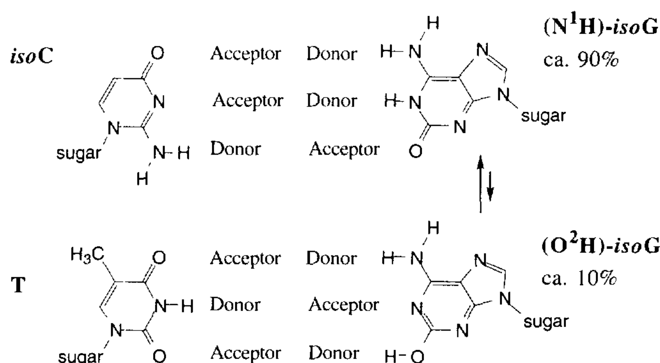

Fig. 1 The N^1H- and O^2H-tautomeric forms of *iso*G and their Watson-Crick base pairs with *iso*C and T.

* Novartis Pharma Inc., CH-4002 Basel, Switzerland, Fax (+41) 61 324 3036, michael.lutz@pharma.novartis.com

0960-894X/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(98)00057-2

More recent studies have shown that HIV-1 RT is generally more tolerant of non-standard bases than eukaryotic DNA polymerases.[5] This suggested that HIV-1 reverse transcriptase might incorporate *iso*G in its minor tautomeric form opposite U or T into the nucleic acid encoded by the retrovirus to a greater extent than eukaryotic polymerases would incorporate *iso*G opposite T into cellular DNA. In this way, *iso*G might be used to specifically target nucleic acids encoded by HIV-1 reverse transcriptase,[3] acting as "a tautomeric Trojan horse" causing the death of HIV-infected cells in the S-phase due to an inability of cellular polymerases to read a DNA strand containing d-*iso*G placed there by HIV-1 RT.[3] To test this strategy *in vitro*, HIV-1 RT and mammalian cellular DNA polymerases α and ε were examined for their ability to incorporate d-*iso*GTP opposite U in an RNA template and opposite T in a DNA template.

When HIV-1 RT was incubated in the absence of dATP and in the presence of d-*iso*GTP with a [γ-^{32}P]-labeled primer/template-complex containing a single T in the single-stranded template overhang,[6] full length product was produced with very little pausing at the position where the template contained T (Fig. 2, lane 7). If d-*iso*GTP was omitted from the incubation mixture, elongation of the primer stopped at the templating T, and only trace amounts of full-length product were observed (Fig. 2, lane 6). The quantitative analysis[7] yielded that at least 91% of products elongated further than T in the template derived from specific incorporation of d-*iso*GTP opposite the templating T.

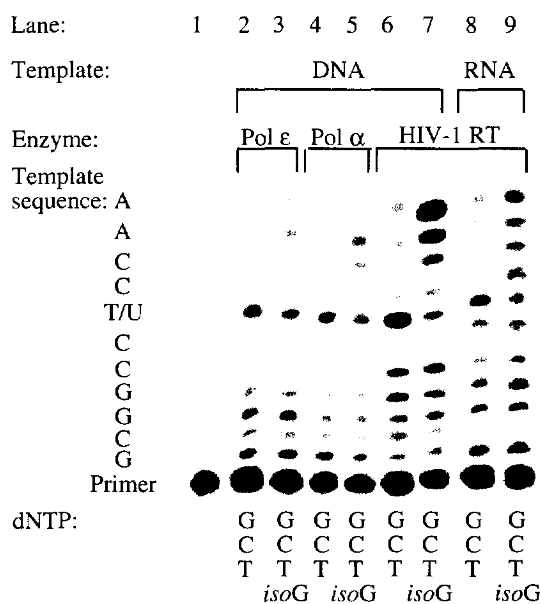

Fig. 2 Analysis of primer elongation of templates containing a single T (for DNA) or U (for RNA) in the presence of d-*iso*GTP by HIV-1 RT and cellular DNA polymerases α and ε by PAGE. Deoxyribonucleoside triphosphates present are indicated below (5 μM each). Incubations were performed at 37°C for 15 min with 0.15 pmol primer/template-complex containing a single T by using 0.02 units HIV-1 RT, 0.06 units DNA polymerase α and 0.05 units DNA polymerase ε in a final volume of 25 μl.

This experiment was repeated with an RNA template containing U (Fig. 2, lanes 8 and 9). Again in the absence of dATP, HIV-1 RT incorporated d-*iso*GTP efficiently opposite U in the RNA template, with little misincorporation of the standard nucleobases opposite U. The amount of products elongated further than U due to specific d-*iso*G-U base pairing was only about 60% under these conditions. This, however, might be explained by the reduced enzymatic activity of HIV-1 RT on the DNA/RNA primer/template-complex compared to its activity on the DNA/DNA primer/template-complex as indicated by the different amounts of unextended primer.

Mammalian cellular DNA polymerases were then examined to learn whether they would also incorporate d-*iso*GTP opposite T in a template. DNA polymerase α is believed to serve both as the initiating DNA polymerase and in the synthesis of the lagging strand during DNA replication.[8] When DNA polymerase α from calf thymus is challenged by a template containing a single T in the absense of both dATP and d-*iso*GTP (Fig. 2, lanes 4 and 5), smaller amounts of full length product (presumably arising from misincorporation of a standard nucleobase opposite the template T) were observed than seen in the parallel experiment with HIV-1 RT. PhosphorImager analysis suggested that when d-*iso*GTP was added, the amount of full length products increase by about 9%. A substantial fraction of this was due to prematurely terminated products. These suggest that the amount of incorporation of d-*iso*GTP opposite template T by mammalian cellular DNA polymerase α under these conditions is an order of magnitude lower than that seen with HIV-1 RT.

Similiar results were observed with calf DNA polymerase ε, an enzyme involved in gap-filling reactions, including completion of the lagging strand, nucleotide excision repair, and recombinatorial repair synthesis.[9] The data (Fig. 1, lanes 2 and 3) suggested that this mammalian DNA polymerase is an even poorer catalyst for incorporating d-*iso*GTP than DNA polymerase α. Only traces of products arising from elongation further than T were observed when d-*iso*GTP was present (Fig. 2, lanes 2 and 3). Approximately the same amount of products were found when d-*iso*GTP was omitted from the reaction mixture due to misincorporation of standard nucleobases.

To determine whether d-*iso*GTP could compete with dATP for incorporation opposite template T with both HIV-1 RT and DNA polymerase α (Fig. 3), unlabeled DNA primer/template-complex (0.15 pmol) was incubated with RT or DNA polymerase α at different concentrations of [α-^{35}S] dATP (0.1, 0.5, 2, and 5 μM) and unlabeled d-*iso*GTP (0, 0.5, 1, and 5 μM). With both HIV-1 RT and DNA polymerase α, d-*iso*GTP suppressed the incorporation of radiolabeled dATP into oligonucleotide product.[10] However, the inhibitory effect was different both quantitatively and qualitatively for HIV-1 RT (Fig. 3a) and DNA polymerase α (Fig. 3b).

Quantitatively, d-*iso*GTP inhibited incorporation of dATP by HIV-1 RT much more effectively than with DNA polymerase α. At 5 μM concentrations of dATP, similar to those found *in vivo*,[11] 1 μM d-*iso*GTP was sufficient to decrease incorporation of radiolabeled dATP by 50% when HIV-1 RT was the catalyst. With polymerase α, an extrapolated 11 μM d-*iso*GTP is required to obtain the same level of inhibition. Qualitatively, double reciprocal plots show that d-*iso*GTP inhibits incorporation of dATP into DNA competitively with DNA polymerase α and largely uncompetitively with HIV-1 RT (data not shown). Kinetics of polymerases are, of course, complicated and more detailed studies are necessary. Nevertheless, these differences should allow the selection of a concentration of d-*iso*GTP that creates a wide range of selectivities with these polymerases in the future.

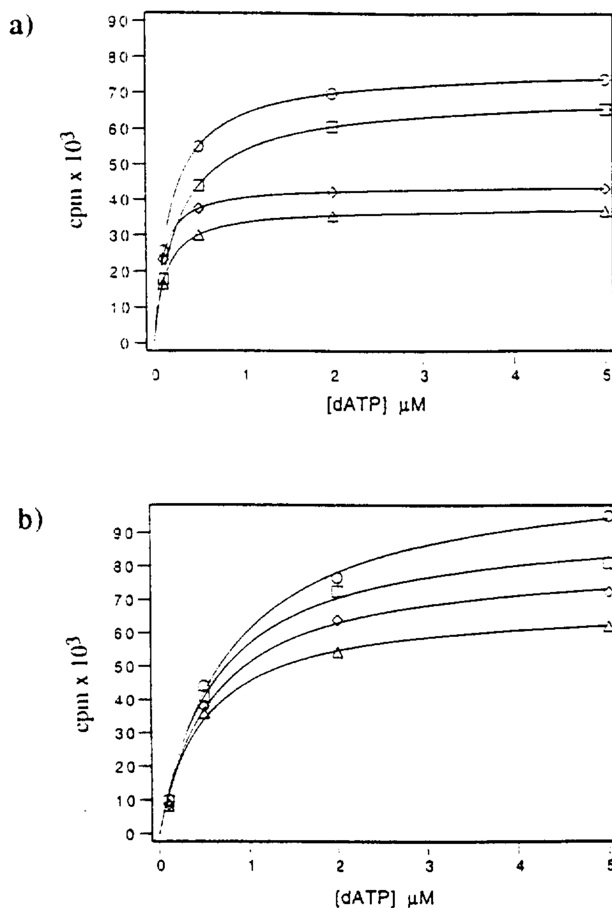

Fig. 3 Incorporation of [α-^{35}S] dATP opposite T in a template by (a) 0.02 units HIV-1 RT and (b) 0.06 units DNA polymerase α with varying concentrations of d-*iso*GTP (O: 0 μM, ❐: 0.5 μM, ◊: 1 μM, and Δ: 5 μM). Incubations were performed at 37°C for 15 min in a final volume of 25 μl with 0.15 pmol unlabeled DNA primer/template-complex containing a single T.

A final comment must be made concerning the stability of the *iso*G-T base pair. Recent thermodynamic and theoretical studies suggested that the *iso*G-T base pair is joined by only two hydrogen bonds and exists most presumably in a wobble geometry.[2] However, this study reveals that HIV-1 RT actually prefers d-*iso*GTP (approximately five fold) over dATP as a partner for T. When further correcting for the tautomeric equilibrium of d-*iso*G (about 10% of the total material consists of the O^{2}H tautomer in aqueous solution),[4] the *iso*G-T pair seems to be prefered some 50 fold more than the A-T pair. This, however, appears to be possible only if the O^{2}H-tautomeric form of d-*iso*G participates in the polymerization and forms a Watson-Crick base pair that is joined by three hydrogen bonds. Apparently, the stability of the *iso*G-T base pair could be higher in the active site of a polymerase than its thermodynamic stability would indicate.

Acknowledgement

We thank U. Hübscher (University of Zürich) for kindly providing DNA polymerases α and ε in purified form and G. Maga (University of Zürich) for helpful discussions. We are indebted to G. Ott (University of Bayreuth) for the synthesis of the RNA template. M.J.L was supported by a scholarship from the DAAD in the program HSP II/AUFE.

References and Notes

1. Rich, A. On the Problems of Evolution and Biochemical Transfer. In Horizons in Biochemistry; Kasha, M., Pullman, B., Eds.; Academic Press: New York, 1962, pp. 103-126.

2. Horn, T.; Chang, C-A.; Collins, M.L. *Tetrahedron Lett.* **1995**, *36*, 2033; Roberts, C.; Bandaru, R.; Switzer, C.Y. *Tetrahedron Lett.* **1995**, *36*, 3601; Roberts, C.; Bandaru, R.; Switzer, C.Y. *J. Am. Chem. Soc.* **1997**, *119*, 4640.

3. Switzer, C.Y.; Moroney, S.E.; Benner, S.A. *J. Am. Chem. Soc.* **1989**, *111*, 8322; Switzer, C.Y.; Moroney, S.E.; Benner, S.A. *Biochemistry* **1993**, *32*, 10489.

4. Sepiol, J.; Kazimierczuk, Z.; Shugar, D. *Z. Naturforsch.* **1976**, *31c*, 361.

5. Horlacher, J.; Hottiger, M.; Podust, V.N.; Hübscher, U., Benner, S.A. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 6329; Lutz, M.J.; Held, H.A.; Hottiger, M.; Hübscher, U.; Benner, S.A. *Nucl. Acids Res.* **1996**, *24*, 1308.

6. 2'-Deoxy*iso*guanosine triphosphate (d-*iso*GTP) was synthesized as recently described (in ref 3). The DNA template (5' AACCTCCGGCGCTATAGTGAGTCGTATTA 3') and the primer (5' TAATACGACTCACT ATAG 3') were both synthesized by Microsynth (Windisch, Switzerland). An RNA template (5' AACCUCCGGCGCUAUUAGUGAGUCGUAUUA 3') was provided by G. Ott (University of Bayreuth, Germany). All oligonucleotides were purified by PAGE. The primer was labeled at the 5' end with Redivue™ [γ-^{32}P] ATP (Amersham) using T4 polynucleotide kinase (Life Technologies). Standard dNTPs were from Pharmacia and [α-^{35}S] dATP (400 Ci/mmol) was from Amersham. Purification of HIV-1 RT, annealing of the DNA primer with either the DNA or RNA template, and enzymatic assays were performed as recently described (in ref 5).

7. Radioactively labeled reaction products were quantified by using a PhosphorImager (Molecular Dynamics), with 5 h exposures and the ImageQuant software (Molecular Dynamics). To determine the lower limit of specific formation of the *iso*G-T(U) base pair, the amount of products past T(U) was quantified, divided by the total amount of radioactivity in the lane and expressed as percentage. To correct for non-specific misincorporation of standard nucleobases opposite T(U) the amount of misincorporation of standard dNTPs, determined in a control experiment, was subtracted. This corrected percentage was then reported to the percentage of read-through past T(U) when d-*iso*GTP was present in solution to yield the lower limit of specific formation. If no misincorporation of standard nucleotides opposite T or U occur, the percentage for specific *iso*G-T(U) base pair formation would be 100%.

8. Hübscher, U.; Spadari, S. *Physiol. Rev.* **1994**, *74*, 259.

9. Podust, V.N.; Hübscher, U. *Nucl. Acids Res.* **1993**, *21*, 841; Jessberger, R., Podust, V.N.; Hübscher, U.; Berg, P.J. *J. Biol. Chem.* **1993**, *268*, 15070; Aboussekhra, A.; Biggerstaff, M.; Shivij, M.K.; Vilpo, J.A.; Moncollin, V.; Podust, V.N.; Protic, M.; Hübscher, U., Egly, J.M.; Wood, R.D. *Cell* **1995**, *80*, 859; Shivij, M.K.; Podust, V.N.; Hübscher, U.; Wood, R.D. *Biochemistry* **1995**, *34*, 5011.

10. Radioactively labeled reaction products deriving from the incorporation of [α-^{35}S] dATP were quantified by Phosphor Imager analysis. The resulting counts per minute (cpm) were plotted against [dATP].

11. Reichard, P. *Annu. Rev. Biochem.* **1988**, *57*, 349.