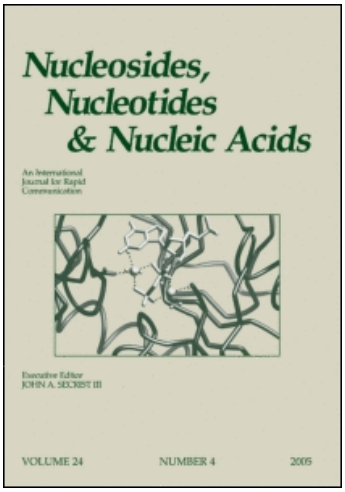


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# Does Interaction of dNTP Glycone with an Active Center of Reverse Transcriptases Takes Place? A Model for a Binding Site

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NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 735-738 (1995)

# DOES INTERACTION OF dNTP GLYCONE WITH AN ACTIVE CENTER OF REVERSE TRANSCRIPTASES TAKES PLACE? A MODEL FOR A BINDING SITE

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**Abstract:** A model for the active center of reverse transcriptases is discussed



A fine structure of active centers of different DNA polymerases is investigated by various methods including inhibitor analysis. The latter approach allowed to reveal some differences in their structures[1,2]. Summarizing the data obtained one can divide DNA polymerases into three groups: mammalian replicative DNA polymerases, which are shown to be the most specific, reverse transcriptases (RTs) of retro and hepadnaviruses, which seem to be the least specific, and mammalian repairing DNA polymerases which take an intermediate place.

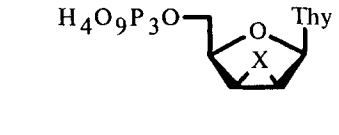
RTs can incorporate into 3'-termini of growing DNA chains modified dNTP with bulky substituents in 3'-position (for references see[1,2]), both in DNA-dependent and in RNA-dependent synthesis. As a rule, all these compounds are not incorporated by other DNA polymerases up to 100 fold higher concentrations.

The RTs also show decreased specificity towards glycone conformation of dNTP substrates. Among termination substrates of RTs one can mention 2',3'-dideoxynucleoside 5'-triphosphates with 2',3'-double bond[3], 2',3'-ribo- and lyxoepoxy[4], 2',3'-ribo- and 2',3'-lyxothiirane[5], and with 2',3'-double bond and other modifications in the glycone[6-9].

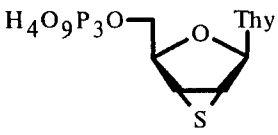
Based on the variability of the substrate's structure one could speculate what fragments of dNTP molecules could bind to [RT + template-primer] complexes in a special manner. Binding of a nucleic base of dNTP (with Watson-Crick complex formation) and a triphosphate part were shown to take

place. With the aim to investigate the glycone contribution to the binding process of dNTPs with [RT + template-primers] complexes we have synthesized model substrates of **1-6** types.

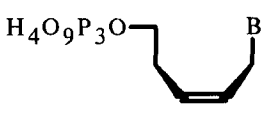
All these compounds were demonstrated to be termination substrates. For all of them we measured either $K_m$ of incorporation reaction in both [RT + DNA template-DNA primer] and [RT + RNA-template-DNA primer]



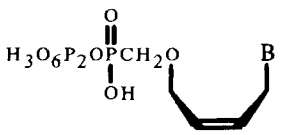

**1a** X=O, **1b** X=S



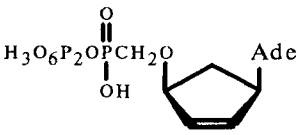

**2**



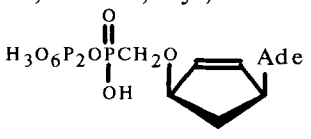

**3**, B=Ade, Cyt, Gua




**4** B=Ade, Gua, Cyt, Thy




**5**




**6**

complexes or their $IC_{50}$ in the DNA elongation reaction. All these data reflect the affinity of **1-6** to DNA synthesizing complexes. The $K_m$ and relative $V_{max}$ for **1-2** are shown in[5], and Table 1 demonstrates them for **5-6**[10]. $IC_{50}$ for **3**/dNTP (in moles) are 0.9-1.2[7] and for **4** - 1.3-1.4[11].

The analysis of these data together with some others from literature allows some conclusions.

1. Absence of the 3'-hydroxyl in dNTPs does not markedly decrease affinity of the compounds to RT.

2. Introduction of a *cis* double bond between C2'-C3' does not decrease affinity of 2',3'-dideoxy-2',3'-didehydronucleoside 5'-triphosphates.

3. Nucleoside in such a modified dNTP can be either D and L enantiomer series.

4. Endocyclic oxygen in glycone can be replaced by $CH_2$-group or removed. The C5'-$OCH_2$ group also can be replaced by a C5'-$CH_2O$ group.

A general conclusion that glycone does not participate in binding of dNTP with [RT + template-primer] complex in a specific way is supported also by the data that nearly each atom of te glycone in dNTP can be either replaced or removed. Nevertheless, dNTP glycone in an enzyme active center serves as a framework for correct distance of the nucleic base in respect to triphosphate residue at a definite distance and at a definite torsion angle

Table 1. The $K_m$ values and ratios of $V_{max}$ for **5** and **6** to $V_{max}$ for dATP or ddATP in the reaction of DNA synthesis catalyzed by AMV or HIV RTs

| | [Enzyme +template-primer] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AMV RT + A* | | AMV RT+ B* | | AMV RT+ C* | | HIV RT + C* | |
| | $K_m$, μM | $V_{max}$/ $V_{max}$ (dATP) | $K_m$, μM | $V_{max}$/ $V_{max}$ (dATP) | $K_m$, μM | $V_{max}$/ $V_{max}$ (ddATP) | $K_m$, μM | $V_{max}$/ $V_{max}$ (ddATP) |
| **5** | 0.03 | 0.72 | 0.028 | 0.63 | 0.069 | 1.38 | 0.021 | 1.26 |
| **6** | 0.019 | 0.77 | 0.076 | 0.77 | 0.014 | 1.51 | 0.034 | 1.52 |
| dATP | 1.72 | 1.00 | 0.034 | 1.00 | - | - | - | - |
| ddATP | - | - | 0.21 | 0.83 | 0.19 | 1.00 | 0.19 | 1.00 |

*) Primer-template systems:

A. (d) GGGTCAGTGCTGCAACATTTTGCTGCCGGT...
(d) [5'-$^{32}$P] GACGTTGTAAAACG

B. (r) CGAGAGGGAAUACGCUGAGGACGUAA...
(d) [5'-$^{32}$P] ATGCGACTCCTGC

C. (r) CGAGAGGGAAUACGCUGAGGACGU
(d) [5'-$^{32}$P] GCTCTCCCTTATGCGTCTCC +dTTP + dGTP + dCTP

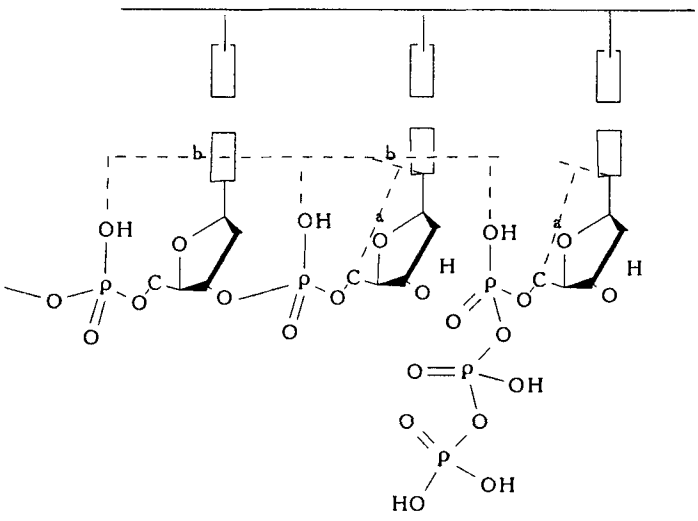


Figure 1

between a glycoside and C4'-C5' bonds (Fig.1, where: a - the distance between N1 (for pyrimidines) or N9 (for purines) and C5'; b - the distance between internucleoside phosphate groups). So, the introduction of C2'-C3' double bond into dNTP also decreases the nucleic base rotation barriers because it eliminates the interaction of a base with the C2'-hydrogen and facilitates the Watson-Crick base pair formation with the template[12,13]. Probably such decrease in base rotation barriers defines the higher affinity of 1-6 to DNA synthesizing complexes.

It should be mentioned that active centers of other DNA polymerases can have significant differences in comparison with those for RTs, and their analysis has to be made individually.

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