Human Immunodeficiency Virus Reverse Transcriptase

**GENERAL PROPERTIES AND ITS INTERACTIONS WITH NUCLEOSIDE TRIPHOSPHATE ANALOGS***

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Using affinity purified human immunodeficiency virus (HIV) reverse transcriptase the reaction assay conditions were determined. The optimum incorporation of dTMP into the (rA),(dT)₁₀ template with HIV reverse transcriptase required 6 mM MgCl₂ and 80 mM KCl. The template specificity of HIV reverse transcriptase is quite different from those of the human γ-polymerase-associated reverse transcriptase or avian virus reverse transcriptase. The preferential inhibition of HIV reverse transcriptase as compared to human γ-reverse transcriptase was observed with several nucleoside analog triphosphates. The *Kᵢ* values for triphosphates with HIV reverse transcriptase ranged from 5 to 13 nm with decreasing effectiveness for 3'-fluoro > 3'-amino > 2',3'-dideoxy > 3'-azido groups. This study provides information on the structure activity relationships of the triphosphate and nucleoside analogs inhibitory effects on HIV reverse transcriptase versus human γ-polymerase-associated reverse transcriptase, and the possible mechanisms of action of 3'-azido thymidine and the 2',3'-dideoxynucleosides, and also identifies other nucleoside analogs for possible development as inhibitors of HIV.

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HIV-associated reverse transcriptase was recently purified using immunoadsorption chromatography. It is composed of two polypeptides with molecular weights of 66,000 and 51,000 (5). Preliminary studies of the properties of HIV reverse transcriptase, using a partially purified preparation, show that this enzyme appears to be quite different from other virus-associated reverse transcriptases. Since HIV reverse transcriptase appears to be critical for HIV replication, it presents a logical target for the development of selective antiviral compounds. A detailed study of its properties and its interaction with nucleoside triphosphate analogs will not only provide information on how to design new compounds, but will also help to explain the mechanism of some anti-HIV nucleoside analogs already developed. This report will describe the results of some of our studies.

**EXPERIMENTAL PROCEDURES**

**RESULTS AND DISCUSSION**

Several analogs of dTTP and dGTP were examined for their inhibitory activity against HIV reverse transcriptase and intrinsic reverse transcriptase of γ-DNA polymerase. The IC₅₀ values are presented in Table I. Interestingly, the replacement of the 3'-OH group by hydrogen, fluorine, amino, or azido groups yielded compounds with very potent inhibitory activities against HIV reverse transcriptase. Acyclic nucleotides also have good inhibitory activity. The replacement of hydrogen with either a hydroxyl or a fluorine group in the 2'-ara configuration improved the binding affinity of these dTTP analogs to the enzyme since the IC₅₀ values of ara-TTP and 2'-F-ara-TTP are less than [³H]dTTP concentrations used in the assay. Ara-CTP inhibited HIV reverse transcriptase only 5% at 2 µM employing (rA),(dT)₁₀ and [³H]dTTP as substrate, whereas others have shown this to be the IC₅₀ concentration for murine leukemia virus reverse transcriptase activity (13). Unfortunately, (rU),(da)₁₂₋₁₈ and (rG),(dc)₁₂₋₁₈ did not serve as efficient templates, which prevented us from estimating the inhibitory potency of ddATP and ddCTP against HIV reverse transcriptase.

It is conceivable that ddCTP or ddATP will also be able to exert the same degree of inhibitory potency against reverse...
The time course of the reaction catalyzed by viral reverse transcriptase with proper templates, which require either the (rA),(dT)lo or (rC),(dG)12 18, respectively, as a template. It should be noted that the spectrum of inhibitory activities of these analogs againt HIV reverse transcriptase were similar to our results in a qualitative but not quantitative sense. The observation of the selective antiviral activities of 2',3'-dideoxynucleosides and 3'-N3-dThd reported by other investigators (1,2) could be partially explained by the strong interaction of their nucleoside analog triphosphates with viral reverse transcriptase. We are currently investigating the potency of 3'-NH2-dThd and 3'-F-dThd against HIV in culture.

**REFERENCES**

**Supplemental Material: 1**

Human Immunodeficiency Virus Reverse Transcriptase: General Properties and Its Interaction with Nuclear Matrix Protein-1

Tung-chih Chang, Daniel O. Donner, Kenneth D. Naruse, M. J. Sarnataro, and Robert T. Ting

**EXPERIMENTAL PROCEDURES**

**Materials:**
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**Glass Microfiber Filters:** The 2.1 cm discs used for the reverse transcriptase and polymerase assay were purchased from Millipore Inc., Bedford, MA.

**Chemicals:** The 

**Isolation:**
- The 20% FCS and 20% FCS were gifts from Dr. William Powell, Yale University, New Haven, CT. The 20% FCS and 20% FCS were gifts from Dr. Peter Laposky, National Institutes of Health, Bethesda, MD. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO. The antiserum to HIV-1 was kindly provided by Dr. Robert Gallo, National Institutes of Health, Bethesda, MD. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

**Preparation of Melting Curve Analysis of Transcription:**
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**Preparation of Transcription Reactions:**
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**Preparation of Blank:**
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**Nuclear Matrix Protein:**
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**RESULTS:**
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**DISCUSSION:**
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**TABLE:**

<table>
<thead>
<tr>
<th>Template Specificity of Reverse Transcriptase</th>
<th>KVI RT</th>
<th>Y-58M polymerase</th>
<th>B63 RT</th>
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<tr>
<td><strong>Template</strong></td>
<td><strong>KVI RT</strong></td>
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**Figure 1:**

**Figure 2:**