NOTES

Kinetics and Inhibition of Reverse Transcriptase from Human and Simian Immunodeficiency Viruses

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Reverse transcriptase from the simian immunodeficiency virus (SIV) was found to have kinetic behavior similar to that of enzyme from the human immunodeficiency virus (HIV). Michaelis constants for the substrates TTP and dGTP and inhibition constants for the inhibitors 3'-azido-3'-deoxythymidine 5'-triphosphate, 2',3'-dideoxythymidine 5'-triphosphate, and 2',3'-dideoxyguanosine 5'-triphosphate were obtained for SIV reverse transcriptase and were found to be similar to the corresponding values for HIV reverse transcriptase. Thus, the interaction of SIV reverse transcriptase with nucleotide analogs appears to be indistinguishable from that of the HIV enzyme, suggesting that SIV/simian acquired immunodeficiency syndrome (SAIDS) is a potentially good model of AIDS.

The development of new compounds to inhibit the replication of human immunodeficiency virus (HIV) relies on valid tissue culture and animal models of acquired immunodeficiency syndrome (AIDS). HIV has been successfully cultured in cells of human origin and transmitted to chimpanzees (1, 10–12, 15, 18, 20). Although chimpanzees can be infected by HIV, the status of the animals as an endangered species and the fact that HIV does not appear to cause the fatal immunodeficiency syndrome in infected animals severely limit their usefulness (1, 11, 12). Alternative animal models for AIDS are necessary to complement available tissue culture systems.

One animal model that resembles AIDS closely is the simian immunodeficiency disease (SAIDS) associated with a related retrovirus (SIV/simian T-lymphotropic virus type III) isolated from rhesus macaques (9). SIV has an approximately 40% nucleotide sequence homology with HIV type 1 (5), and it causes in infected monkeys a fatal immune deficiency disease that is clinically similar to AIDS (9). Thus, the SIV/macaque system represents an attractive animal model for the study of AIDS.

The viability of SIV/SAIDS as an animal model of HIV/AIDS depends on the similarity of the biochemical functions of SIV to those of HIV. The hallmark of the retroviral life cycle is expression of reverse transcriptase activity in infected cells. Inhibition of the retroviral enzyme RNA-directed DNA polymerase (reverse transcriptase) is one approach toward treatment of AIDS (2–4, 8, 14, 19, 22). An example of this approach is the drug 3'-azido-3'-deoxythymidine (AZT), which has clinical efficacy because in the nucleoside triphosphate form it preferentially inhibits viral reverse transcriptase (13, 19, 23). To compare the relative effects of nucleotides and their analogs on reverse transcriptase from SIV and HIV, we examined the kinetics and inhibition of enzymes from the two viruses.

Radioactive [methyl-3H]TTP (25 Ci/mmol) was purchased from New England Nuclear Corp., and [1',2',3',5'-H]dGTP (40 Ci/mmol) was purchased from Amersham Corp. Template primers poly(A)-oligo(dT)12–18 (1:1, A:dT ratio) and poly(C)-oligo(dG)12–18 (1:1, C:dG ratio), 2',3'-dideoxythymidine 5'-triphosphate (ddTTP), and 2',3'-dideoxyguanosine 5'-triphosphate (ddGTP) were from Pharmacia-PL Biochemicals, Inc. The inhibitor AZT 5'-triphosphate (AZTTP) was synthesized by established methods (16, 17). Bovine serum albumin, Nonidet P-40, and dithiothreitol were purchased from Sigma Chemical Co. Single-stranded-DNA-agarose was from Bethesda Research Laboratories, Inc. HIV type 1 (human T-lymphotropic virus type III lymphadenopathy-associated virus) and A3.01 cells were from Thomas Folks of the National Institutes of Health (10). SIVmac-infected HUT-78 cells were obtained from Ron Desrosiers of the New England Regional Primate Research Center (9).

For the preparation of HIV, A3.01 cells in log phase (~2 × 10³/ml in RPMI 1640 with 10% fetal calf serum and 30 μg of gentamicin per ml) were pelleted by low-speed centrifugation (3,000 × g, 15 min). The cells were suspended in fresh medium to a density of 2 × 10³/ml, and supernatant from an 8-day HIV type 1-infected culture was added (1:4, infected:uninfected [by volume]). The cells were incubated at 37°C with agitation for 3 h, diluted with an equal volume of fresh medium, and cultured at 37°C. The cell concentration was monitored daily and maintained at 10⁶/ml by the addition of fresh medium. Viral replication was monitored by daily assays of reverse transcriptase activity in the supernatant. When peak reverse transcriptase activity was achieved (ca. day 8 postinfection), the medium was cleared of cells by centrifugation (3,000 × g; 15 min), and the virus in the supernatant was pelleted by centrifugation at 110,000 × g.

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FIG. 1. Lineweaver-Burke plots for inhibition of SIV and HIV reverse transcriptases by ddGTP. Poly(C)-oligo(dG)12-18 and [3H]dGTP were the substrates, and reactions were performed as described in the text. (Top panel) Kinetics and inhibition of SIV reverse transcriptase; (bottom panel) kinetics and inhibition of HIV reverse transcriptase. V (initial rate) is in disintegrations per minute per minute of reaction time. Symbols: ■, no ddGTP; Δ, 0.05 μM ddGTP; □, 0.10 μM ddGTP. Inserts, Replots of slopes versus ddGTP concentrations to determine the $K_i$ of ddGTP for each enzyme.

(30,000 rpm; 90 min; Beckman SW41 rotor). The virus pellet was suspended in phosphate-buffered saline containing 2% Nonidet P-40 and 10% glycerol.

For the preparation of SIV, SIVmac-infected HUT-78 cells were grown in RPMI 1640–10% fetal calf serum plus penicillin and streptomycin supplemented with glutamine at 100 mM. Cell cultures were seeded with infected/uninfected HUT-78 cells at a ratio of 3/2. Virus was harvested from spent media at 72 h postinfection. Cells were pelleted by centrifugation at 300 × g for 10 min, and the supernatant was further clarified by centrifugation at 10,000 × g in a Beckman 45 Ti rotor. The SIV particles were pelleted at 95,000 × g for

<table>
<thead>
<tr>
<th>Template primer</th>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
<th>Inhibitor</th>
<th>$K_i$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(A)-oligo(dT)12-18</td>
<td>[3H]dTTP</td>
<td>8.0</td>
<td>AZTTP</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>ddTTP</td>
<td>0.015</td>
</tr>
<tr>
<td>Poly(C)-oligo(dG)12-18</td>
<td>[3H]dGTP</td>
<td>4.7</td>
<td>ddGTP</td>
<td>0.009</td>
</tr>
</tbody>
</table>

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1 h and suspended in phosphate-buffered saline containing 2% Nonidet P-40.

Reverse transcriptases from the lysed preparations of HIV and SIV were purified by DNA-agarose chromatography. Lysates were diluted in column buffer (10 mM Tris-acetate [pH 7.0], 1 mM dithiothreitol, 10% glycerol) at 1:4 dilution for HIV (2-ml final volume) or 1:7 dilution for SIV (1.4-ml final volume) and applied to a single stranded-DNA–agarose column (1-ml packed volume equilibrated in column buffer). The enzyme activity was eluted with a gradient (100 ml) of 0 to 1.0 M NaCl in column buffer. Fractions (1 ml) were collected into tubes containing 200 μg of bovine serum albumin (10 μl of a 20-mg/ml solution), which improved enzymatic stability. Activities, which eluted at 0.25 to 0.30 M NaCl, were collected and stored at −70°C.

The assay for reverse transcriptase measured the incorporation of tritiated deoxynucleoside triphosphate into an acid-precipitable form. The assay mixture (80 μl) contained 50 mM Tris hydrochloride (pH 8.0), 5 mM MgCl₂, 150 mM KCl, 5 mM dithiothreitol, and labeled deoxynucleoside triphosphate. The template-primer concentration for [³H] thymidine incorporation was 15 μM poly(A)-oligo(dT)₁₂₋₁₈ (concentration in nucleotide base pairs); for [³H]deoxyguanosine incorporation, the concentration of poly(C)-oligo(dG)₁₂₋₁₈ was 188 μM in nucleotide base pairs. The concentrations of the template primers were chosen to be saturating for the enzymes. The high concentration of poly(C)-oligo(dG)₁₂₋₁₈ needed to achieve Vₘₐₓ was observed for both SIV and HIV enzymes. Inhibitors were added as required. After incubation at 37°C, 15-μl samples were removed at various times and processed as described previously (7).

Enzymes for this study were prepared from viral lysates by DNA-agarose chromatography. The final preparations of reverse transcriptases were free of nuclease activities and yielded linear reaction kinetics for up to 80 min of incubation time. The preparations were free of other viral proteins based on immunoblotting (Western hybridization) with HIV antisera.

The nucleotides AZTTP, ddTTP, and ddGTP are potent inhibitors of HIV reverse transcriptase, with Kᵢ/Kₘ ratios of 100 to 400 with respect to the corresponding deoxyribonucleoside triphosphates (6, 13, 21). inhibition data for the SIV enzyme have not been reported. The kinetics of inhibition of reverse transcriptase from HIV and HIV by ddGTP are shown in Fig. 1. The data indicate the competitive nature of the inhibition by ddGTP with respect to dGTP and afford nearly equal Michaelis and inhibition constants for the two enzymes. The Kᵢₛ of ddGTP were 5.4 μM for the HIV enzyme and 4.7 μM for the SIV enzyme. The Kᵢₛ of ddGTP were 0.011 and 0.009 μM for the SIV and HIV enzymes, respectively. The Kᵢ/Kₘ ratio for SIV reverse transcriptase was 490; for HIV, the ratio was 522.

The complete data for two primer templates and three inhibitors are summarized in Table 1. For all substrates and inhibitors tested, the SIV reverse transcriptase had values of Kᵢ and Kₘ that were very similar to those of the HIV enzyme. The values determined for the enzyme from HIV were in good agreement with those reported previously (6, 13, 21).

In addition to being potent competitive inhibitors of the enzymes, the compounds tested in this study are also chain terminators for the DNA polymerase reaction. Comparisons of chain termination for the two enzymes require further investigation.

In summary, the results indicate that the kinetic properties of reverse transcriptase from SIV are virtually identical to those of the enzyme from the human virus. In conjunction with data from animal studies, our data suggest that SIV-infected cells and animals are relevant models of AIDS and may be particularly suited to the development of drugs directed against HIV reverse transcriptase.

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LITERATURE CITED


