Expression of Reverse Transcriptase from Feline Immunodeficiency Virus in Escherichia coli

THOMAS W. NORTH,1* GALE L. HANSEN,2 YAQI ZHU,1 JOHANNA A. GRIFFIN,2 AND CHENG-KON SHIH2

Division of Biological Sciences, University of Montana, Missoula, Montana 59812,1 and Department of Molecular Biology, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut 068772

Received 7 September 1993/Returned for modification 2 November 1993/Accepted 1 December 1993

Reverse transcriptase from feline immunodeficiency virus (FIV) has been cloned and expressed in Escherichia coli. We have purified this recombinant enzyme and shown that it is a 66-kDa protein that is indistinguishable from virion-derived FIV reverse transcriptase in sensitivity to the 5′-triphosphates of 3′-azido-3′-deoxythymidine and the four 2′,3′-dideoxynucleosides. The availability of large quantities of the FIV reverse transcriptase will allow more detailed physical and pharmacological studies.

Feline immunodeficiency virus (FIV) is a lentivirus that causes an immune deficiency in domestic cats that is very similar to AIDS in humans (13, 17). FIV-infected cats develop an AIDS-related complex-like disease which progresses to the final AIDS-like stage (6). Like AIDS in humans, the immune dysfunction in cats involves depletion of the CD4+ T lymphocyte subset, diminished in vitro lymphocyte proliferative response to phytohemagglutinin and interleukin-2, decreased humoral immune responsiveness to T-dependent immunogens, hypergammaglobulinemia, and diminished interleukin-2 production (1, 2, 12, 15, 16). These features make FIV a valuable animal model for studies of AIDS.

We have previously purified the FIV reverse transcriptase (RT) from detergent-lysed virions and shown that it is similar to the human immunodeficiency virus type 1 (HIV-1) RT in physical properties, catalytic activities (10), and sensitivity to the 5′-triphosphates of several nucleoside analogs that display anti-HIV activity, including zidovudine (3′-azido-3′-deoxthymidine) 5′-triphosphate, 2′,3′-dideoxynucleotides (ddATP, ddCTP, ddGTP, and ddTTP), and 2′,3′-dideox-3′-dideoxythymidine 5′-triphosphate (4, 9, 11). The FIV and HIV RTs are also similar in sensitivity to phosphonoformate but differ in that the FIV enzyme is not sensitive to other nucleoside inhibitors such as nevirapine (BIRG-587) and tetrahydroimidazo[4,5,1-j,k][1,4]benzodiazepin-2(1H)-one and -thione (TIBO) compounds, which are potent inhibitors of the HIV-1 RT. Although very similar to one another, the FIV and HIV RTs are quite different from the RT of avian myeloblastosis virus in susceptibility to antiviral nucleotide analogs (11).

Work with the FIV RT has been limited by availability of the purified enzyme, which has previously been purified from cell-free virus (10). Larger quantities are needed for physical studies, production of antibody reagents, and studies with heteropolymer templates. We report here the cloning and expression of the FIV RT in Escherichia coli and a comparison of the recombinant enzyme with the FIV RT purified from virus.

Construction of a clone expressing FIV RT. Plasmid DNA containing the entire FIV genome, pFIV-14 Petaluma, was obtained from the National Institutes of Health AIDS Re-
Purification of recombinant FIV RT. Recombinant FIV RT was purified from *E. coli* containing the clone pRTF14. Bacteria were grown in 1 liter of L broth (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter [pH 7.5]) at 37°C to an optical density at 550 nm of 0.55. IPTG was added to a final concentration of 1 mM, and incubation continued at 37°C for 3 h. Cells were harvested by centrifugation at 3,000 × g for 5 min, and all subsequent steps were carried out at 4°C. Cells were washed with 50 mM Tris-HCl, pH 7.5, containing 0.5 mM EDTA and 0.15 mM NaCl and then resuspended in 10 mM Tris-HCl, pH 7.5, containing 1 mM EDTA, 10% sucrose, 0.3 M NaCl, and 1 mM phenylmethylsulfonyl fluoride. NaCl was then added to a final concentration of 1 M, and cells were disrupted by sonic oscillation. Debris was removed by centrifugation at 30,000 × g for 30 min, and Triton X-100 was added to a final concentration of 0.2%. This mixture was dialyzed three times for a total of 12 to 16 h against 1.5 liter of 50 mM Tris-HCl, pH 7.9, containing 1 mM EDTA, 1 mM dithiothreitol, and 5% glycerol and then used for purification of RT by chromatography on DEAE-cellulose and then phosphocellulose, as previously described (10).

During purification, fractions were assayed for RT activity with poly(rA)·oligo(dT) as a template-primer. Other homopolymer and heteropolymer templates were used in characterization of RT preparation. Poly(rA)·oligo(dT) 
16 poly (rC), oligo(dG) 
10 poly(dA-dT), and 2',3' -dideoxynucleoside 5'-triphosphates (ddATP, ddCTP, ddGTP, and ddTTP) were purchased from Pharmacia LKB, Piscataway, N.J. The zidovudine 5'-triphosphate (N 
3 dTTP) was provided by Wayne Miller of the Burroughs Wellcome Co., Research Triangle Park, N.C. The 2'-deoxyribonucleoside 5'-triphosphates (dATP, dCTP, dGTP, and dTTP) were purchased from Sigma Chemical Co., St. Louis, Mo. M13 DNA was provided by Brad Preston, Rutgers University, Piscataway, N.J. The isotopes used in this study, [methyl-3H]dATTP, [5-3H]dCTP, [8-3H]dGTP, and [8-3H]dTTP, were purchased from Dupont, NEN Research Products, Boston, Mass.

DEAE-cellulose chromatography yielded three peaks of activity during purification of RT. The first peak, which was eluted at approximately 180 mM NaCl, had a high level of activity with the DNA alternating copolymer template poly(dA-dT) and was presumed to be contaminated with *E. coli* DNA polymerase I. The second peak, which was eluted at approximately 300 mM NaCl, was active with poly(rA)·oligo(dT) but not with poly(dA-dT), as expected for the FIV RT. Fractions containing this peak of activity were pooled for phosphocellulose chromatography. We have not characterized the activity present in the third peak because it was eluted from the DEAE-cellulose column at a much higher salt concentration than the virion FIV RT. Further purification of the activity in peak 2 by chromatography on phosphocellulose yielded a single peak of activity at the position expected for the FIV RT (10).

We have analyzed the purified RT by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot (immunoblot) analysis using a monoclonal antibody (MAB) known to recognize the virion-derived FIV RT (Fig. 2). This antibody, MAB 42, which was obtained from mice immunized with the HIV-1 RT (5), cross-reacts with the FIV RT. MAB 42 recognizes two polypeptides of approximately 66 and 51 kDa in preparations of virion-derived FIV RT (lane 2). The recombinant FIV RT eluted from the phosphocellulose column contains only the 66-kDa protein (lane 1). This preparation of FIV RT was more than 98% pure as determined by SDS-PAGE and silver staining. The specific activity of the purified recombinant FIV RT was approximately 30,000 U/mg of protein. This is slightly lower than the value of 48,000 U/mg from which the activity of the virion RT was measured.

![TABLE 1. Template specificities of DNA polymerase activities](https://example.com/attachment.png)

<table>
<thead>
<tr>
<th>Template</th>
<th>FIV RT Activity</th>
<th><em>E. coli</em> DNAopolymerase I Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(rA)·oligo(dT)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Poly(rC)·oligo(dG)</td>
<td>60 ± 12</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>Poly(dA-dT)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

* The conditions for assay of RT using RNA homopolymers primed with complementary oligonucleotides were as previously described (9, 10). For assays with poly(dA-dT), the conditions were identical to those with poly(rA)·oligo(dT) except that poly(dA-dT) was used as a template-primer rather than poly(rA)·oligo(dT) and reaction mixtures contained both dATP and [3H]dCTP, each at 20 μM.

* Values are means ± standard errors for three or more determinations and are normalized to activity with the template that gave the highest activity—poly(rA)·oligo(dT) for RT preparations and poly(dA-dT) for DNA polymerase 1. These values correspond to 3.9 ± 1.2, 2.6 ± 0.8, and 2.1 ± 0.2 mmol of deoxyribonucleoside monophosphate incorporated per h for the recombinant FIV RT, virion FIV RT, and *E. coli* DNA polymerase 1, respectively.

* FIV RT was purified as previously described (10).
TABLE 2. Kinetic constants for virion and recombinant RTs in reactions with poly(rA)·oligo(dT) as a template-primera

<table>
<thead>
<tr>
<th>FIV RT</th>
<th>$K_m$ for dTTP ($\mu$M)</th>
<th>$K_i$ (nM) for ddTTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virion</td>
<td>4.6 ± 0.6</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Recombinant</td>
<td>5.2 ± 0.8</td>
<td>4.2 ± 0.8</td>
</tr>
</tbody>
</table>

a Kinetic parameters ($K_m$ and $K_i$) were determined as previously reported (4, 9) by using intercept values calculated from double-reciprocal plots. The values are means ± standard errors for three or more determinations.

we estimated for the purified virion RT (10). The recombinant and virion RTs are different in that the former is a homodimer of p66 and the latter is a heterodimer of p66 and p51, and the apparent difference in specific activity may be attributable to this.

Comparison of recombinant and virion-derived FIV RTs. Comparisons of template specificities of recombinant and virion-derived FIV RTs are shown in Table 1. Both preparations effectively use poly(rA)·oligo(dT) and poly(rC)·oligo(dG) as template-primers but are inactive with poly(dA-dT)·poly(dA-dT). In contrast, E. coli DNA polymerase I uses poly(dA-dT)·poly(dA-dT) preferentially and has poor activity with the RNA homopolymer templates. These data demonstrate similar template specificities of recombinant and virion-derived FIV RTs and confirm that the recombinant FIV RT preparation is not significantly contaminated with E. coli DNA polymerase I.

Of particular importance to studies of antiviral agents is the degree of similarity of these two RT preparations in their sensitivities to antiviral inhibitors. Accordingly, we have compared the sensitivities of the two enzymes to a dideoxynucleotide (ddTTP) and to the active form of zidovudine (N3dTTP) in experiments with poly(rA)·oligo(dT) as a template-primer. As shown in Table 2, there is no significant difference between these two preparations of FIV RT with respect to $K_m$ for dTTP or $K_i$ for either inhibitor.

We previously showed that virion-derived FIV RT will effectively utilize φX174 DNA as a template and characterized inhibition of this reaction with analogs of dATP and dTTP (4). In order to extend this work, we have characterized reactions of recombinant and virion-derived FIV RT with M13 DNA, which is more readily available than φX174 DNA. The velocity of FIV RT with M13 DNA is 15 to 20% of that obtained with poly(rA)·oligo(dT), which is slightly higher than what we reported with φX174 DNA (4). We have determined $K_m$ values for all four deoxynucleoside triphosphate (dNTP) substrates and $K_i$ values for competitive inhibition by the four ddNTPs and N3dTTP. These data are summarized in Table 3. The recombinant and virion RTs were indistinguishable from one another in the ability to use this heteropolymeric DNA template.

We have not had sufficient quantities of virion-derived FIV RT to precisely determine the N and C termini. Also, it is not possible to infer these termini from the nucleotide sequence, because the virion RT is derived by proteolysis of a larger protein. Therefore, our selection of the region of the FIV pol gene to clone and express was based upon sequence homology with HIV-1 and likely sites for proteolysis. The enzyme we have cloned and expressed is a 66-kDa protein that is indistinguishable from the virion-derived FIV RT in catalytic activity and sensitivity to antiviral nucleotides. Our ability to purify large quantities of the recombinant FIV RT will make the enzyme more readily available and enable more detailed physical studies.

This work was supported by Public Health Service grant AI 28189 from the National Institute of Allergy and Infectious Diseases to T.W.N.

We thank John Miglietta and Anthony Shrutkowski for assistance with DNA sequencing.

REFERENCES


Downloaded from http://aac.asm.org on October 28, 2017 by guest


