Partial Purification and Characterization of the Reverse Transcriptase of the Simian Immunodeficiency Virus TYO-7 Isolated from an African Green Monkey†

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ABSTRACT: The reverse transcriptase (RT) was partially purified by a newly developed procedure from the simian immunodeficiency virus TYO-7 isolated from an African green monkey (SIVagmTYO-7). The method comprised lysis of the virus with nonionic detergent followed by two centrifugations in isopycnic sucrose density gradients and one velocity sedimentation in a glycerol gradient. The enzyme exhibited a purity of 70–80% and showed an exceptional high specific activity of 135 nmol incorporation of dTMP per milligram of protein in 1 h with poly(rA)·oligo(dT) as template-primer (TP). The molecular weight of the native enzyme was estimated by velocity sedimentation analysis as 120K–130K. Investigation of the RT by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) showed that the active enzyme is a heterodimer composed of a 64- and a 50-kDa subunit. The two subunits were identified to be RT specific by Western blot analysis. In activity gels, both subunits exhibited enzymatic activity, whereby the 64-kDa subunit showed the predominant activity. The RT preferred the TP poly(rA)·oligo(dT) over poly(rC)·oligo(dG). With poly(rCm)·oligo(dG), only marginal activity was detected, and no activity was measured with poly(dA)·oligo(dT). The TP specificity was influenced by the reaction temperature. The highest activity was measured around the melting temperature of the TP used. Furthermore, the enzyme activity was more thermolabile when measured with poly(rA)·oligo(dT) than with poly(rC)·oligo(dG). To compare the specificity of RT inhibitors, their inhibition efficiency (IE) was defined as the ratio of the 50% inhibiting concentration (ID_{50}) obtained with the RT in viral lysates to the ID_{50} of purified RT.

Recently, several simian immunodeficiency viruses (SIVs), which belong to the lentivirus subgroup of the retroviridae, have been isolated from various Old World primate species (Daniel et al., 1985; Lowenstine et al., 1986; Fultz et al., 1986; Murphey-Corb et al., 1986; Otha et al., 1988). SIVs are related to the human immunodeficiency viruses HIV-1 and HIV-2 with respect to their morphology, cell tropism, and genomic sequence and organization. Sequence comparisons with SIV isolates obtained from African green monkeys (SIVagm) showed this virus group related about equidistantly to HIV-1 and to HIV-2 (Fukasawa et al., 1988; Sharp & Li, 1988). Moreover, some SIVagm isolates including SIVagmTYO-7 are able to infect rhesus monkeys and to induce pathological changes like persistent peripheral lymphadenopathy in the infected monkeys (Herchenröder et al., 1989). Hence, the infection provides an animal model for studies on vaccines and antiviral therapy relevant for AIDS.

To date, the structure and function of RTs of these SIV isolates have not been investigated. We have isolated and characterized the RT of SIVagmTYO-7. The results were compared to those reported for the RTs from HIV-1 and HIV-2 purified by conventional methods. Since the RT plays a central role in retrovirus replication, the enzyme is a target for antiviral therapy. Therefore, we examined the specificity of RT inhibition by polyanionic compounds, dextran sulfate, heparin, suramin, and some newly synthesized suramin analogues.

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Experimental Procedures

Reverse Transcriptase Assay. The reaction mixture (50 μL) contained 50 mM Tris-HCl, pH 7.8, 5 mM DTT, 0.6% Triton X-100, 1 μg of the respective TP poly(rA)·oligo(dT), poly(dA)·oligo(dT), poly(rC)·oligo(dG), or poly(rCm)·oligo(dG), a 15 μM sample of the corrsponding desoxynucleotide triphosphate (dNTP), 1 μCi of ^{32}P-labeled dNTP with a specific activity of 3000 Ci/mmol, and 1–10 μL of the RT sample. All template-primers were purchased from Pharmacia-LKB, Freiburg, the dNTPs from Boehringer-Mannheim, and the ^{32}P-labeled dNTPs from Amersham-Buchler, Braunschweig.

To optimize the reaction conditions for the different template-primer combinations, various concentrations of KCl and $MgCl_2$ were examined. The reaction mixtures were incubated for 30 min at 42 °C. The reaction was stopped by the addition of 20 μL of 10% SDS. The mixtures were spotted on DEAE filters (Whatman, U.K.). Filters were dried for 15 min at 80 °C and washed 3 times with 5% Na_2HPO_4, twice with water, and once with ethanol. Filters were dried again, and the radioactivity was determined by liquid scintillation counting in a Packard Minaxi Tri-Carp Series 4000.

Two different assays were used to examine the specificity of RT inhibition of chemical compounds. The inhibitory potencies of the compounds were measured first with the RT contained in viral lysates (Jentsch et al., 1987) and then with partially purified RT with poly(rC)·oligo(dG) as TP. The IE was defined for each compound as the ratio of the ID_{50} obtained with the viral lysate versus partially purified RT. In these assays, the KCl concentration was kept at 30 mM as many compounds showed different inhibition characteristics at higher salt concentrations.

Enzyme Purification. Uninfected and SIVagmTYO-7-infected MOLT-4 clone 8 cells were kindly provided by Prof.

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Dr. M. Hayami, Kyoto. For enzyme purification, virus was harvested from 1.0–1.5 L of cell culture supernatant (Schneider et al., 1986) and suspended in 1.5 mL of buffer A (10 mM Tris-HCl, pH 8.0, 100 mM KCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, and 1% Triton X-100) by sonication with a Labsonic 2000, Braun, Melsungen, using a microtip for 3 × 5 s at 30 W. The virus was solubilized for 30 min at 4 °C, and the solution was clarified by centrifugation for 15 min at 10000*g*. The supernatant was layered on a 20–70% sucrose gradient in phosphate-buffered saline, pH 7.2 (PBS), and centrifuged for 15 h at 200000*g* at 4 °C. Twenty equal gradient fractions were collected and assayed for RT activity. Fractions with the highest activities were pooled, diluted to less than 20% sucrose, and adjusted to 1% Triton X-100. This solution was again centrifuged in a 20–70% sucrose gradient as described above. Fractions with the highest RT activity were pooled and dialyzed against 10 mM Tris-HCl, pH 8.0, to remove sucrose. The concentration of Triton X-100 was adjusted before to 1%, and the dialysate was stored on liquid nitrogen in 1-mL fractions. For further purification, every stored fraction was applied on a 10–40% glycerol gradient in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 500 mM KCl and centrifuged at 200000*g* for 15 h at 4 °C. Aliquots of the gradient fractions were analyzed by SDS–PAGE and for enzyme activity. Fractions exhibiting the highest specific activity were pooled, the Triton X-100 concentration was adjusted to 1%, and an equal volume of 10 mM Tris-HCl, pH 8.0, containing 1% Triton X-100 was added. Finally, this RT preparation was stored in aliquots on liquid nitrogen. Frozen aliquots were used only once, because refreezing and thawing possibly decreased the specific activity.

The molecular weight of the RT was estimated by comparison with apoferritin (450K), phosphorylase B (180K), and BSA (70K) run in parallel glycerol gradients. Prior to determination of protein concentration by the Bio-Rad microassay and analysis by SDS–PAGE, aliquots of the material obtained after different purification steps were dialyzed against 10 mM Tris-HCl, pH 8.0, and precipitated with ethanol.

Activity Gel Analysis. The catalytic activity of the RT was determined in gels after SDS–PAGE (Bertazzoni et al., 1986). A 6-mL 10% separating gel (4.5 × 3.5 × 0.15 cm) was prepared containing 8 μg/mL poly(rC)·oligo(dG). After polymerization overnight at 4 °C, the separating gel was overlayed with 1.5 mL of a 5% stacking gel. The virus was solubilized in 10 mM Tris-HCl, pH 8.0, and 1% Triton X-100 for 30 min at 4 °C. Unsolubilized material was separated by centrifugation at 10000*g* for 15 min. Thirty microliters of this solution was adjusted to 10% glycerol, 100 mM mercaptoethanol, and 0.1% SDS. For analysis of the partially purified RT, 30 μL of the glycerol gradient pool was adjusted to 0.1 mg of BSA (self-digested by incubation overnight at 37 °C followed by 5 min at 95 °C), 10% glycerol, 100 mM mercaptoethanol, and 0.1% SDS. Before the samples were loaded, they were incubated at 37 °C for 3 min. Electrophoresis was performed at room temperature for 2 h at 20 mA. Thereafter, the gel was incubated for 30 min at 42 °C in renaturation buffer (50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, and 1 mM mercaptoethanol) followed by incubation in renaturation buffer for 2.5 h at 4 °C including buffer changes every 30 min. The in situ enzyme assay was performed by incubating the gel overnight at 42 °C in 18 mL of reaction mixture containing 50 mM Tris-HCl, pH 8.0, 80 mM KCl, 4 mM $MgCl_2$, 2.5 mM DTT, and 2.5 μCi/mL [^{32}P]dGTP of specific activity of 3000 Ci/mmol. The gel was washed with an aqueous 10% TCA–3% sodium pyrophosphate solution, dried, and autoradiographed.

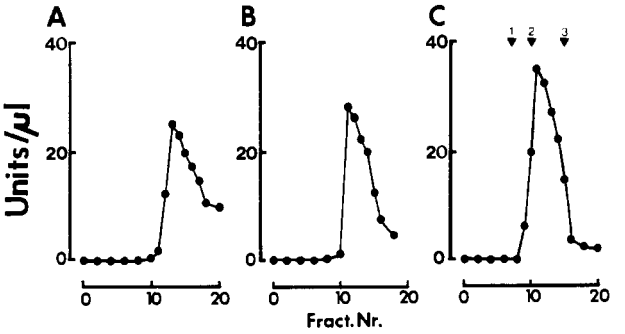

FIGURE 1: Partial purification of the SIVagmTYO-7 RT by three subsequent density gradient centrifugations. A detergent lysate of SIVagmTYO-7 was separated by centrifugation on two isopycnic 20–70% sucrose density gradient centrifugations (A and B). Fractions containing the peak RT activity were furhter purified by velocity sedimentation in a 10–40% glycerol gradient (C). Arrows indicate the position of (1) apoferritin (450K), (2) phosphorylase B (180K), and (3) bovine serum albumin (70K).

Immunoblot Analysis. Immunoblot analysis of the purified RT was performed according to the protocol of Bio-Rad (Bio-Rad Laboratories, München). The rabbit anti HIV-1 RT-specific antiserum was kindly provided by Dr. S. Le Grice, Hoffmann-La Roche AG, Basel.

Compounds for RT Inhibition. Dextran sulfate (M_r 500 000) was purchased from Serva, Heidelberg, heparin was from Sigma, München, and suramin was from Bayer, Leverkusen. NF 345 and NF 346 have been synthesized by methods described for the synthesis of suramin analogues (Nickel et al., 1986). The synthesis and the chemical properties of these compounds will be published elsewhere.

Results

Enzyme Purification. The objective of our study was the development of a simple and rapid purification procedure of the SIVagmTYO-7 RT which delivers partially purified, highly active enzyme in significant amounts. Routinely, the enzyme was prepared from virus harvested from 1.0–1.5 L of cell culture supernatant. The preparation procedure comprised lysis of virus in nonionic detergent followed by two isopycnic sucrose gradient centrifugations and one velocity sedimentation in a glycerol gradient. The yield of purified enzyme was about 0.5–1 μg/L of cell culture supernatant. After purification on the second sucrose gradient, the enzyme was stored in 1-mL aliquots on liquid nitrogen. For further partial purification, one batch was thawed and centrifuged in a glycerol gradient without loss of enzyme activity.

In Figure 1A–C, a typical activity profile throughout the preparation procedure is shown. The molecular weight of the native enzyme was estimated to be 120K–130K after glycerol gradient centrifugation (Figure 1C). Analysis of each fraction of the glycerol gradient by SDS–PAGE demonstrated two major bands with apparent molecular weights of about 64K and 50K in fractions 13–15. These fractions contained peak enzyme activity (Figure 2A). In fractions 14 and 15, in addition to the 64- and 50-kDa polypeptides, traces of p24, the major structural core protein of SIVagmTYO-7, and two polypeptides of 70 and 45 kDa could be detected. Further analysis was done with the pooled fractions 13–15. On immunoblots, an HIV-1 RT-specific antiserum reacted with the 64- and 50-kDa polypeptides in the pooled fractions (Figure 2B, fractions 13–15).

During the course of purification, we observed not only an increase in the specific RT activity but surprisingly also an increase of the total enzyme activity (Table I). The enzyme partially purified by glycerol gradient centrifugation showed

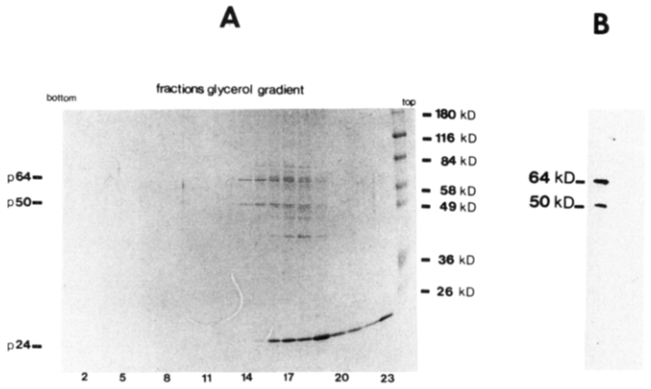

FIGURE 2: Analysis of the glycerol gradient centrifugation of the SIVagmTYO-7 RT. One milliliter of the peak fractions pooled after the second sucrose gradient centrifugation (Figure 1B) was separated on a 10–40% gycerol gradient. An aliquot of each fraction was ethanol precipitated and analyzed by SDS–PAGE (A) as well as by Western blot analysis of the glycerol gradient pool (fractions 13–15) with the rabbit antiserum to HIV-1 RT (B).

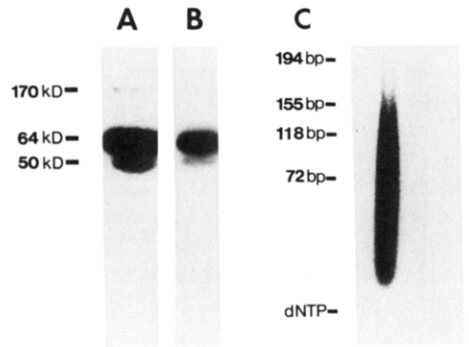

FIGURE 3: Detection of the SIVagmTYO-7 RT in activity gels. The gel contained poly(rC)·oligo(dG) as template-primer. Viral lysates (A) and partially purified RT (B) were examined. The reaction products eluted from the activity gels were analyzed by agarose gel electrophoresis.

Table I: Purification of the SIVagmTYO-7 Reverse Transcriptase

fraction	protein (mg)	total act. (units[a]/ fraction)	sp act. (units[a]/ μg)
viral lysate	3	42	0.014
(1) sucrose gradient	0.23	56	0.243
(2) sucrose gradient	0.14	99	0.707
glycerol gradient	0.0012	163	135.000

[a] One unit is defined as 1 nmol of [^{32}P]dTMP incorporation in 60 min at 42 °C with poly(rA)·oligo(dT) as template-primer.

an exceptionally high specific activity of about 135 nmol of dTMP incorporation per microgram of protein in 1 h. Contaminations of the purified enzyme with nucleases were not detected. When supercoiled pBR322 or pUC 18/19 DNA was offered as substrate, conversion into circular or linear plasmid forms was not observed within 24 h (data not shown).

By activity gel analysis, we could demonstrate that the 64- and 50-kDa polypeptides showed RNA-dependent DNA polymerase activity either in viral lysates (Figure 3A) or, for the first time, in partially purified RT (Figure 3B). In the viral lysate, an additional active band of M_r 170K was observed (Figure 3A). When the partially purified RT was investigated directly by activity gel analysis, no activity was detected accompanied with the 50-kDa polypeptide. Addition of self-digested BSA to the enzyme sample before electrophoresis results in the restoration of the 50-kDa enzyme activity. The use of protease inhibitors like phenymethanesulfonyl fluoride (PMSF) had no influence on the results obtained. When viral

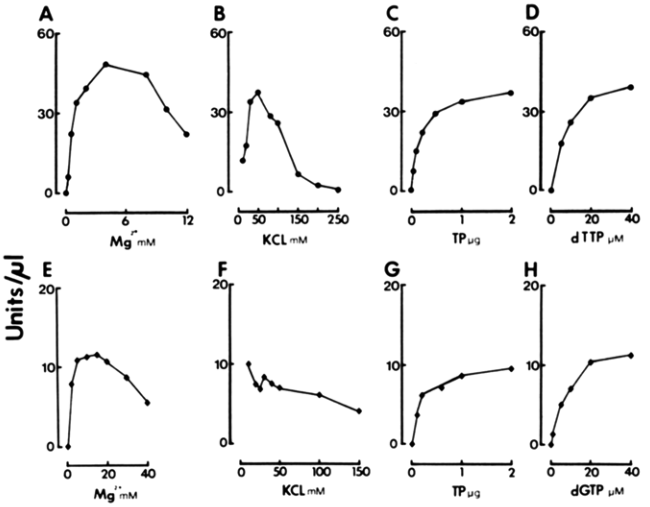

FIGURE 4: Optimization of the reaction conditions of the SIVagmTYO-7 RT. Concentrations employed of di- or monovalent cations as well as the template-primers and dNTPs are indicated in the figure. Poly(rA)·oligo(dT) (●) and poly(rC)·oligo(dG) (♦) were used as template-primers (TPs).

Table II: Template-Primer Specificity of the Partially Purified SIVagmTYO-7 RT

template-primer	[^{32}P]dNTP	enzyme act.[a] Mg^{2+} (mM)	enzyme act.[a] Mn^{2+} (mM)
poly(rA)·oligo(dT)	dTTP	37 (5)	4.7 (0.2)
poly(dA)·oligo(dT)	dTTP	0 (5)	0 (0.2)
poly(rC)·oligo(dG)	dGTP	9.3 (15)	0.2 (0.2)
poly(rCm)·oligo(dG)	dGTP	0.6 (4)	2.8 (0.1)

[a] Enzyme activity is defined as picomoles of [^{32}P]dNMP incorporated per microliter in 60 min at 42 °C with the appropriate template-primer.

lysate was examined under these conditions, the activity of the 50-kDa polypeptide represented 70% of the activity of the 64-kDa polypeptide and 8% in case of the partially purified RT. Reaction products eluted from the activity gels revealed a length of 50–100 nucleotides (Figure 3C).

Enzyme Characterization. Conditions for the reaction of the partially purified enzyme were optimized. Preliminary results showed that almost no activity was observed in the presence of Mn^{2+} as a divalent cation. Therefore, the concentration optimum of Mg^{2+} was determined. The highest activity was obtained at 5 mM Mg^{2+} with poly(rA)·oligo(dT) and at about 15 mM Mg^{2+} with poly(rC)·oligo(dG) (Figure 4A,E). The concentration optimum for KCl was about 50 mM for poly(rA)·oligo(dT) and quite broad between 10 and 100 mM for poly(rC)·oligo(dG) (Figure 4B,F).

The K_m values for the two TPs and their corresponding dNTPs were around 5 μg/mL and 9–14 μM, respectively (Figure 4C,G and Figure 4D,H). With poly(rA)oligo(dT), the optimum pH value was around 8.0. Interestingly, with poly(rC)·oligo(dG), a broad optimum between pH 8.0 and 9.5 was measured (data not shown).

Under optimized conditions, the freshly prepared enzyme showed the highest activity with poly(rA)·oligo(dT) as template-primer of about 37 pmol/μL followed by poly(rC)·oligo(dG) (Table II). There was only little enzyme activity with poly(rCm)·oligo(dG) and no activity with poly(dA)·oligo(dT), a TP preferred by cellular DNA polymerases (Table II).

Furthermore, we have examined the stability of the enzyme at 4 °C for 7 days. We obtained a more rapid decrease of the poly(rA)·oligo(dT)-dependent activity. After 2 days, the enzyme lost 20% of this activity whereas no activity loss was

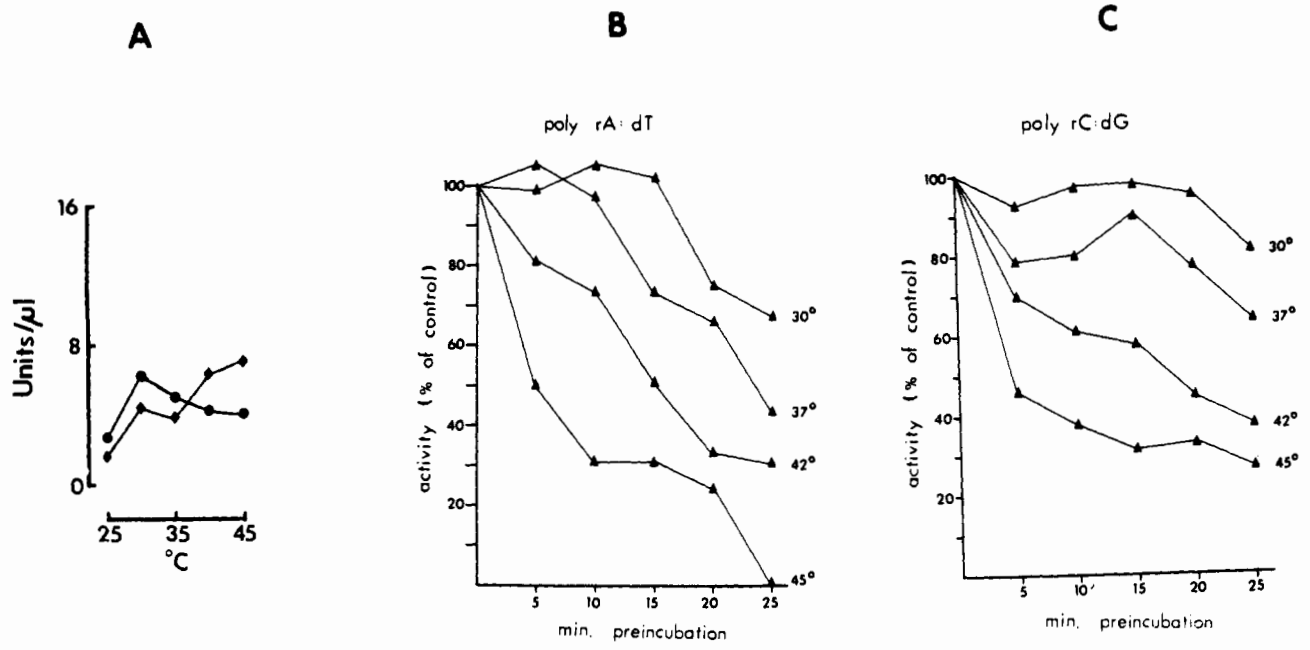

FIGURE 5: Thermolability of the partially purified SIVagmTYO-7 RT. The activity of the template-primer/enzyme complex was measured at the indicated temperatures with poly(rA)·oligo(dT) (●) and poly(rC)·oligo(dG) (♦) as template-primers (A). The thermolability of the RT reactivity with poly(rA)·oligo(dT) (B) and poly(rC)·oligo(dG) (C) was determined as described under Experimental Procedures. Prior to the reaction, the RT was kept at the indicated temperatures for various times.

observed with poly(rC)·oligo(dG) (data not shown). In a second type of experiment, we investigated the stability of the enzyme/template-primer complex at various temperatures ranging from 25 °C up to 45 °C with enzyme preincubated for 3 days at 4 °C. As shown in Figure 5A, the activity with poly(rC)·oligo(dG) was increased up to 45 °C, whereas the poly(rA)·oligo(dT)-dependent activity had an optimum at 30 °C. In another set of experiments, the thermolability of the purified RT was investigated by preincubation at different temperatures. The RT assay was then performed under standard conditions (Figure 5B,C). The enzyme was stable during preincubation for 15 min at 30 °C and for 10 min at 37 °C with poly(rA)·oligo(dT) and up to 20 min at 30 °C with poly(rC)·oligo(dG). At preincubation at 42 and 45 °C, the enzyme activity decreased continuously with increased preincubation time. Preincubation for 25 min at 45 °C destroyed the poly(rA)·oligo(dT)-dependent activity completely (Figure 5B). When poly(rC)·oligo(dG) was used, the RT activity was more stable during the preincubation at 42 and 45 °C. After a preincubation for 25 min at 45 °C, 20% of the poly(rC)·oligo(dG)-dependent activity was preserved (Figure 5C).

RT Inhibition Studies. We have determined the IE of the polyanionic compounds dextran sulfate and heparin as well as suramin and its analogues NF 345 and NF 346 (Table III). Although the ID_{50}s of dextran sulfate and heparin were low, both have a high IE and thus inhibit the RT unspecifically. Likewise, suramin exhibits a high ID_{50} and a high IE and is also an unspecific RT inhibitor. However, the two suramin analogues gave better results. Compared to suramin, NF 346 exhibited a lower ID_{50} in the viral lysate, and, therefore, its IE was significantly improved. For NF 345, the ID_{50} measured in viral lysates was in the same range as that obtained with purified RT. Thus, the IE was nearly 1 as expected for a specific RT inhibitor.

Table III: Inhibition by Various Compounds of the SIVagmTYO-7 RT in Viral Lysates and of the Purified Enzyme

compound	M_r[a]	ID_{50} (µg/µL) lysate RT	ID_{50} (µg/µL) purified RT	IE[b]
dextran sulfate	500000	0.22	0.0044	50
heparin	23000	1.30	0.0740	17
suramin	1429	26	4	6.5
NF 346	1266	16	5	3.2
NF 345	1266	9	7	1.3

Discussion

In the present paper, we describe a novel purification procedure for the RT of immunodeficiency viruses which avoids column chromatography. The enzyme was partially purified from a detergent lysate of SIVagmTYO-7 by three subsequent density gradient centrifugation steps. The preparation procedure yields RT purified 70–80% with a specific activity about 5–500 times higher than reported by others (Weber & Grosse, 1989; Chandra et al., 1985). The most surprising result obtained by this purification method was the increase of the total activity throughout the procedure. So far, this phenomenon was not described for RTs. For example, Weber and Grosse (1989) have lost 89% of the total RT activity in the course of purification, and we have obtained similar results when we tried to purify the RT by column chromatography. Therefore, the increase of the total activity could be explained by avoiding column chromatography which damaged the RT. This was reported for murine leukemia virus (Moelling, 1974) and mouse mammary tumor virus (Dion et al., 1974). The molecular weights of 120K–130K for the native enzyme and about 64K and 50K for its two subunits were close to those reported earlier for HIV-1 and HIV-2 (Veronese et al., 1986; De Vico et al., 1989). In addition, we have demonstrated by Western blot analysis that the 64- and 50-kDa subunits react with a polyclonal antiserum to HIV-1 RT, and, therefore, we conclude that both polypeptides represent the RT subunits and that the active enzyme is a heterodimer. Different enzymatic activities of the two subunits have been described in the literature. Some groups claimed that only the larger subunit is active (Hansen et al., 1988; Starnes et al., 1988), whereas other reported activity of both subunits (Mous et al., 1987; Lori et al., 1988). We have found both subunits of the SIVagm RT to be enzymatically active, both in viral lysates as well as in the partially purified RT. As described for HIV-1 (Lori et al.,

1988), the larger subunit of the SIV RT exhibits the predominant activity. Taking into account the possibility that the 50-kDa polypeptide could be generated by proteolytic cleavage of the 64-kDa polypeptide by a contaminating protease, activity gel analysis was done in the presence of protease inhibitors, and no differences were obtained. Therefore, we believe that the activity is associated with the 50-kDa RT subunit demonstrated by SDS–PAGE. The significant lower activity of the 50-kDa polypeptide could be due to its lesser renaturation capacity or a lower intrinsic enzymatic activity. The former assumption was also supported by the fact that the 50-kDa RT activity in partially purified RT could only be demonstrated in the presence of comigrating, stabilizing protein (in this case BSA), which perhaps promotes renaturation as described for the DNA polymerase α (Karawya & Wilson, 1982; Karawya et al., 1983). Investigation of the eluted reaction products showed that polymerization occurred in the activity gels. The major reaction products range from 50 to 100 bp. The 170-kDa band found in the viral lysate could be the gag-pol precursor protein if one assumes for the gag precursor 55 kDa, 64 kDa for the RT, 34 kDa for the endonuclease, and 14 kDa for the protease (Ratner et al., 1985).

The optimum concentrations for mono- and divalent cations as well as the Michaelis–Menten constants of the dNTPs and the TP are not significantly different from those of HIV-1 RT (Chandra et al., 1986; Wondrak et al., 1986; Cheng et al., 1987; Huber et al., 1989). In contrast to data reported with HIV-1 (Chandra et al., 1985, 1986; Cheng et al., 1987), the activity of freshly prepared enzyme was always higher with poly(rA)·oligo(dT) than with poly(rC)·oligo(dG). For the SIVagm RT, we were also unable to confirm the high activity with poly(rCm)·oligo(dG) in the presence of Mn^{2+} (Chandra et al., 1985, 1986). These discrepancies could be explained by the different purification procedures of the different enzymes or alternatively may be characteristic for the enzyme examined. For example, the RT from feline leukemia virus purified by phosphocellulose column chromatography changed its TP preference from poly(rA)·oligo(dT) to poly(rC)·oligo(dG) (Lüke, unpublished observation). In addition, the TP preference of the partially purified enzyme stored for 3 days at 4 °C is highly dependent on the reaction temperature. The enzyme activity with poly(rC)·oligo(dG) increased up to 45 °C whereas the activity with poly(rA)·oligo(dT) increased only up to 30 °C and decreased at higher temperature. This result could be due to the different T_m values of both TPs. Poly(rA)·oligo(dT) has a T_m value of 28 °C, and the highest enzyme activities were determined around this temperature (Chang et al., 1972; Modak et al., 1977; Rossignol et al., 1981). Poly(rC)oligo(dG) has a T_m value around 40 °C and therefore is more efficient at higher temperatures (Waters et al., 1974; Modak et al., 1977). However, the T_m value of the template-primer could not be the only reason for this temperature-dependent activity because freshly prepared enzyme at 42 °C exhibits a 3–4 times higher incorporation rate with poly(rA)·oligo(dT) than with poly(rC)oligo(dG). Therefore, we have performed thermal inactivation studies with the partially purified RT. The poly(rC)·oligo(dG)-dependent activity was more heat stable than the poly(rA)·oligo(dT)-dependent activity. After preincubation for 25 min at 45 °C, the poly(rA)·oligo(dT)-dependent activity was completely lost whereas 20% of the poly(rC)·oligo(dG)-dependent activity was still preserved. To our knowledge, this phenomenon has not been described yet, and more detailed studies are necessarry to explain this observation.

Since poly(rC)·oligo(dG)-dependent activity was more heat stable, we have used this TP to examine the specificity of RT inhibitors. The specificity of RT inhibition was expressed as the IE. This is the ratio of the ID_{50} obtained with the RT in viral lysates to the ID_{50} determined with purified RT. Assuming a simple inhibition model described by a bimolecular binding mechanism, one would find an IE of close to 1 for specific inhibitor binding. Therefore, the measurement of the enzyme activity required an excess of inhibitor and TP compared to the enzyme. IE values higher than 1 would then indicate an unspecific binding within the limits of this model. First we have determined the IE for dextran sulfate and heparin, two compounds reported to be very effective inhibitors of the HIV-1 RT (Nakashima et al., 1987). Both compounds had a relatively low ID_{50}s, but the calculated IE was high. Therefore, RT inhibition by these polyanions is unspecific and does not appear to be the relevant mode of action by which these compounds inhibit viral replication in culture (Jurkiewicz et al., 1989). Recently, Baba et al. (1988) reported that both compounds were most effective during the early event of the virus replicative cycle and perhaps interfere with the viral adsorption to the cell.

We have also determined the IE of suramin and two of its analogues. The RT inhibition of suramin was known before human retroviruses had been discovered (De Clerq, 1979). More recent studies have shown that RT inhibition by suramin is unspecific (Basu & Modak, 1985). The IE values which we have determined confirmed this latter observation and demonstrated the applicability of the IE. However, the complex chemical structure of suramin has been varied systematically. Many new analogues have been synthesized. Using the IE to express specificity, we have found among a collection of suramin analogues more specific RT inhibitors like NF 346 with an IE of around 3 as well as the very specific RT inhibitor NF 345 with an IE of around 1. Therefore, the IE is useful to rank RT inhibitors according to their specificity and may guide chemists to synthesize more active antiviral compounds.

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