Biochemical heterogeneity of reverse transcriptase purified from the AIDS virus, HTLV-III

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Received 11 December 1985

The reverse transcriptase from AIDS virus, HTLV-III, was purified and characterized. The purified enzyme has a very high affinity for template primers $(rC)_n \cdot (dG)_{12}$ and $(rCm)_n \cdot (dG)_{12}$ compared to that for $(rA)_n \cdot (dT)_{12}$. In addition, the HTLV-III reverse transcriptase was able to transcribe $(rAm)_n \cdot (dT)_{12}$ very efficiently. The ionic requirements are unique in the sense that HTLV-III reverse transcriptase prefers Mg^{2+} as divalent ions to transcribe $(rC)_n \cdot (dG)_{12}$ and $(rA)_n \cdot (dT)_{12}$. The M_r of the enzyme is 95000–98000. Unlike the HTLV-I reverse transcriptase, the HTLV-III enzyme is highly stable and has a much higher activity in the presence of $(rC)_n \cdot (dG)_{12}$; the V_{max} for HTLV-III reverse transcriptase is several-fold higher than that for HTLV-I enzyme. The enzyme activity of the purified reverse transcriptase from HTLV-III was resolved into two peaks on a preparative isoelectric column, one at pH 5.75 and the other at pH 6.25. This leads us to conclude that the reverse transcriptase of HTLV-III is biochemically heterogeneous.

HTLV-III *AIDS* *Reverse transcriptase* *Heterogeneity*

1. INTRODUCTION

Human T-cell leukemia/lymphoma virus (HTLV) was the first human retrovirus identified as an infectious agent etiologically associated with adult T-cell leukemia [1]. A related but distinct retrovirus was isolated from a T-cell variant of hairy cell leukemia [2]. Both the viruses show a tropism for T cells, particularly $OKT4^+$ cells, and have the capacity of immortalizing and transforming normal human T cells in culture [3,4]. These viruses have been subclassified as HTLV-I and HTLV-II, respectively. Because of their T-cell tropism, particularly for $OKT4^+$ cells, and other biological properties [5,6], the search for a similar retrovirus associated with the acquired immune deficiency syndrome (AIDS) was implicated. Gallo and his associates [7] succeeded in isolating and characterizing a retrovirus associated with AIDS, which was shown to be biologically and morphologically distinct from HTLV-I and HTLV-II. However, due to some homologic gene suequences between the AIDS-associated virus and HTLV-I and HTLV-II [8], this virus has been termed HTLV-III. Other investigators have also reported virus isolates with properties similar to those of HTLV-III, from patients with lymphadenopathy syndrome and AIDS [9].

Compared to the other T-lymphotropic retroviruses, HTLV-I and HTLV-II, the AIDS-associated viruses are non-transforming cytopathic viruses without immortalizing activity. Thus, virus replication is an important event in the progress of this disease, and the interruption of viral replication offers an important strategy for the control of AIDS. The development of reverse transcriptase inhibitors is, therefore, a useful approach in designing potential therapeutic agents against AIDS. For this reason, we have purified reverse transcriptase from the AIDS-associated virus

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

HTLV-III and characterized it biochemically. The purified reverse transcriptase from HTLV-III shows some novel features which we have not observed thus far in reverse transcriptases purified from human tumor cells and tissues [10]. Here we describe some of the distinguishing features of HTLV-III reverse transcriptase which may be useful for analyzing the virus-associated enzyme activity in biological specimens from AIDS patients, and in designing its specific inhibitors.

2. EXPERIMENTAL

The enzyme from HTLV-III-infected H9 cells, or HTLV-III suspensions (obtained from Dr R.C. Gallo and Dr P.S. Sarin, National Cancer institute, Bethesda, MD) was purified by successive chromatography on DEAE-cellulose (DE23 and DE52) and phosphocellulose columns. A single peak of high reverse transcriptase activity was eluted from the phosphocellulose column at a salt concentration of 0.175 M KCl. This peak of activity was unable to transcribe the template primers $(dC)_n \cdot (dG)_{12}$ and $(dA)_n \cdot (dT)_{12}$, indicating that no DNA-dependent DNA polymerase activities were present in this fraction. A quantitative analysis of the enzyme at various purification steps showed a 120–140-fold purification, starting from the crude fraction obtained after centrifugation at 170 000 × *g* [11].

DNA polymerase activity was measured by adding 20 μl of the enzyme fraction to a volume of 30 μl, which gave a final concentration of 50 mM Tris-HCl buffer (pH 8.0), 50 mM KCl, divalent ions (as indicated in table 1), 1 mM dithiothreitol, 20 μM each of complementary dNTP, or ^{3}H-labeled substrate and 1.25 μg template primer (as listed in table 1). The reaction mixtures were incubated for 30 min at 37°C. The reaction was stopped by the addition of 0.36 mg bovine serum albumin and 3 ml trichloroacetic acid (10%, w/v) containing 20 mM sodium pyrophosphate. Acid-precipitable material was collected on Whatmn GF/C glass fibre discs, washed and counted in a scintillation spectrometer. Each assay was carried out in triplicate.

The M_r of the purified reverse transcriptase was detefmined by gel filtration on an Ultrogel AcA 44 (LKB, Munich) column. The gel, as supplied by LKB, was poured into a column (1 × 40 cm) and equilibrated with buffer (pH 7.5) containing 50 mM Tris-HCl. Protein markers (collagenase, M_r 10900), bovine serum albumin (M_r 67000) and chicken albumin (M_r 45000) were purified before use. The enzyme activity eluted from the column was assayed in the presence of $(rC)_n \cdot (dG)_{12}$ using Mg^{2+} (15 mM) in the standard reaction mixture described above.

Preparative electrofocusing in a pH gradient was carried out at 4°C on 110 ml columns (LKB 8100-1). The sample (400–500 μg protein) was distributed throughout the column by mixing carrier ampholytes (Ampholines, LKB) of pH ranges 3–10 (1 part) and 5–8 (2 parts) in a 10–60% (w/v) glycerol gradient. Electrofocusing was performed at 1600 V. Fractions were collected and assayed in the presence of $(rC)_n \cdot (dG)_{12}$ using Mg^{2+} (15 mM) in the standard reaction mixture, as indicated in table 1.

3. RESULTS AND DISCUSSION

The purified and concentrated enzyme was tested with various template primers in the presence of different concentrations of divalent cations. These assays are useful in deriving information as to whether cellular DNA polymerases are present, and whether the enzyme has a type specificity; for example, mammalian viruses of the C-type have different ionic requirements from viruses belonging to the B-type. As follows from table 1, the purified enzyme transcribes $(rA)_n \cdot (dT)_{12}$, $(rAm)_n \cdot (dT)_{12}$, $(rC)_n \cdot (dG)_{12}$ and $(rCm)_n \cdot (dG)_{12}$; primer alone with either cation gives no activity. The pattern of template primer utilization by the enzyme distinguishes it from terminal deoxynucleotidyltransferase and host DNA polymerases. Another property unique to reverse transcriptase is its capacity to catalyze transcription of the viral RNA (70 S RNA). This was confirmed for the HTLV-III enzyme using a purified 70 S RNA from SSAV.

Except for the transcription of 2′-*O*-methylated templates, $(rAm)_n$ and $(rCm)_n$, all other template primers require Mg^{2+} for optimal activity. The fact that $(rC)_n \cdot (dG)_{12}$-dependent activity is several-fold higher than that catalyzed by $(rA)_n \cdot (dT)_{12}$ and is strictly magnesium-dependent constitutes a novel feature of the HTLV-III enzyme, compared to known properties of other C-type virus reverse

Table 1

Template primer requirements of the purified reverse transcriptase from HTLV-III

Template primer	^{3}H-labeled substrate	Enzyme activity per h (pmol/μg protein h)	
		Mg^{2+} (mM)	Mn^{2+} (mM)
$(rA)_n \cdot (dT)_{12}$	dTTP	15.45(0.5)	0.23(0.05)
$(rAm)_n \cdot (dT)_{12}$	dTTP	0.18(3.0)	94.77(0.05)
$(rC)_n \cdot (dG)_{18}$	dGTP	273.90(15.0)	0.74(0.075)
$(rCm)_n \cdot (dG)_{18}$	dGTP	38.17(3.0)	89.03(0.2)
70 S RNA (SSAV)	dGTP	1.79(10.0)	0
70 S RNA + $(dT)_{12}$	dGTP	4.78(10.0)	0
$(dT)_{12}$	dGTP	0.01(0.5)	0

Assays were performed with the same batch of enzyme using the same concentration. Viral RNA (70 S) was tested in the presence of 3 other cold dNTPs with the indicated radioactive dNTP. DNA polymerase activity was measured by adding 20 μl of the enzyme fraction to a volume of 30 μl, which gave a final concentration of 50 mM Tris-HCl buffer (pH 8.0), 50 mM KCl, divalent cations (as indicated), 1 mM dithiothreitol, 20 μM each of the complementary dNTP, or ^{3}H-labeled substrate and 1.25 μg template primer (as indicated). Other conditions are described in section 2

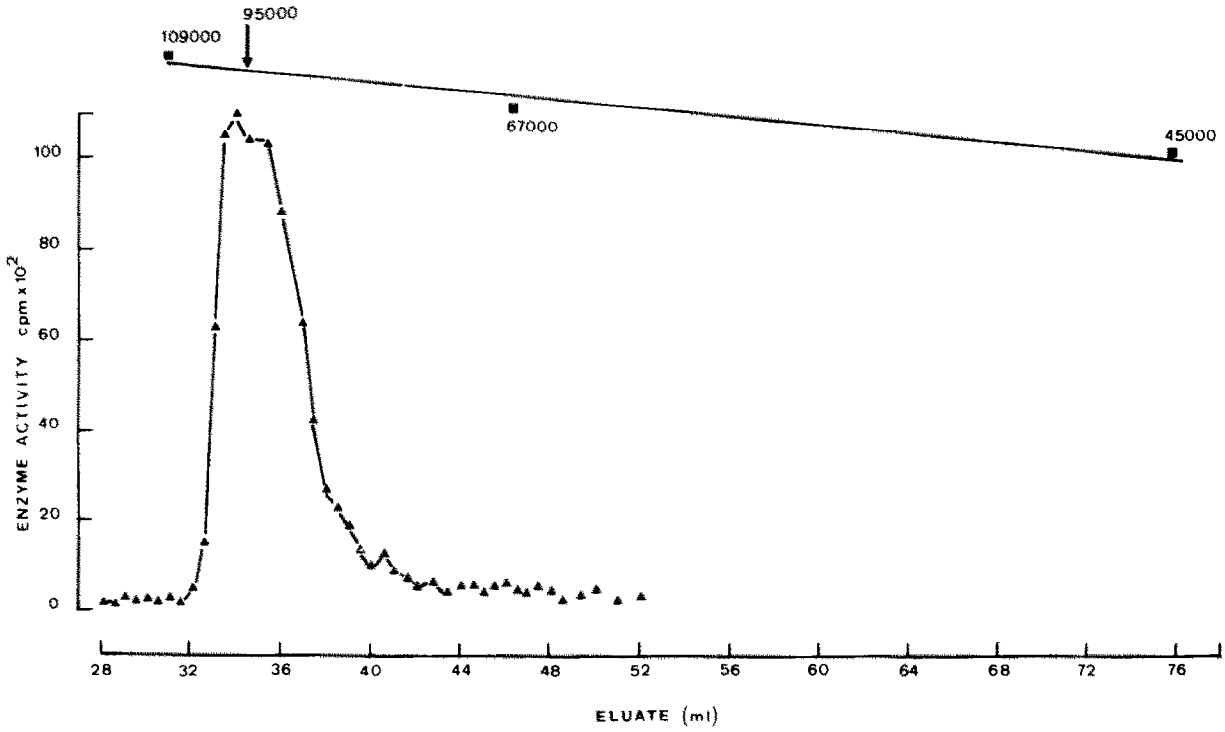

Fig. 1. Determination of the M_r of the HTLV-III enzyme by gel filtration on Ultrogel AcA 44. The enzyme activity eluted from the column was assayed in the presence of $(rC)_n \cdot (dG)_{12}$ using Mg^{2+} (15 mM) in the standard reaction mixture described in section 2.

transcriptases. In this respect, the enzyme resembles more closely reverse transcriptases from B- and D-type viruses.

The M_r of HTLV-II enzyme was determined by gel filtration (Ultrogel AcA 44) using purified protein markers (M_r 45 000–109 000). The HTLV-II enzyme activity was recovered as a single peak in the range M_r 95 000–98 000 (fig.1). With few exceptions [11,12], most of the C-type viral reverse transcriptases have an M_r of about 70 000. In this respect, the HTLV-III enzyme is another exception.

Our futher attempts to purify the HTLV-III enzyme obtained after phosphocellulose column chromatography led to unexpected results. The enzyme purified on phosphocellulose was subjected to isoelectric focusing on a preparative column. Quite unexpectedly, the HTLV-III reverse transcriptase activity resolved into two peaks, one at pH 5.75 and the other at pH 6.25 (fig.2). This was also confirmed by the analytical procedure, where the isoelectric focusing was carried out on gels. However, we believe that the isoelectric data from the preparative procedure, involving a direct measurement of the enzyme activity, are more reliable and leave no doubt that HTLV-III enzyme is heterogeneous with two isoelectric points, 5.75 and 6.25. In a parallel experiment, we investigated the isoelectric behavior of a purified reverse transcriptase from HTLV-I and failed to observe this type of heterogeneity. At present we cannot provide an explanation for the heterogeneity of HTLV-III reverse transcriptase. However, two sets of experimental observations are relevant to our

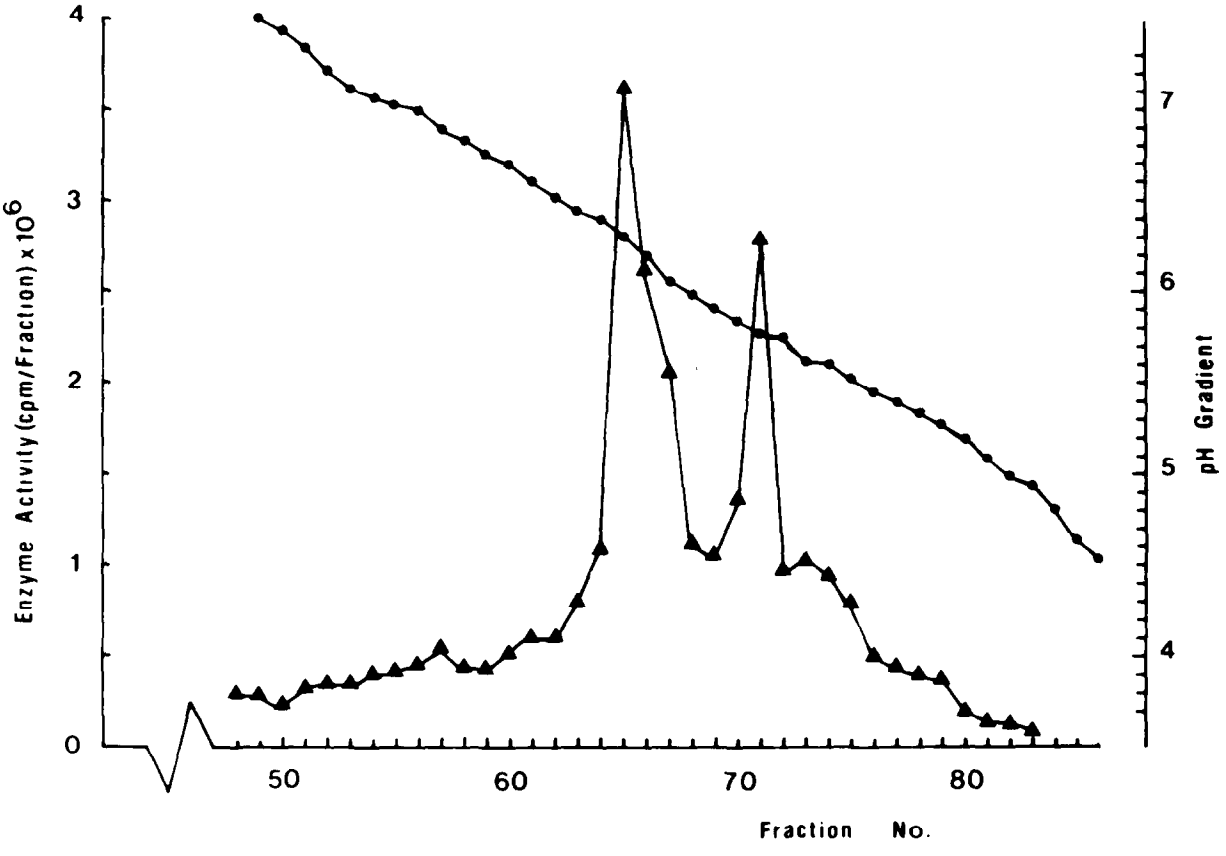

Fig.2. Profile of DNA polymerase activities after electrofocusing of the HTLV-III enzyme eluted from a phosphocellulose column. Experimental details are given in the text. The enzyme activity of the eluted fractions was measured in the presence of $(rC)_n \cdot (dG)_{12}$ using Mg^{2+} (15 mM).

results: firstly, a similar type of heterogeneity in the antigenic proteins of HTLV-III, especially P24, has been observed (Sarangadharan, personal communication): secondly, HTLV-III differs from HTLV-I in the amino-terminal region of the second open reading frame as the corresponding region of HTLV-I cannot encode a functional protease [13]. The second open reading frame (nucleotides 1629–4673) encodes the *pol* gene, the reverse transcriptase and a protease at its 5'-terminus, plus an endonuclease at its 3'-terminus [13].

ACKNOWLEDGEMENTS

We thank Dr Robert C. Gallo and Dr P.S. Sarin for helpful discussions, and for providing us with HTLV-III and H9 cells. We also thank Maria Dzwonkowski for her skilled technical assistance. This work was supported by a grant from Deutsche Krebshilfe eV.

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