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Expression of Reverse Transcriptase Activity of Human T-Lymphotropic Virus Type III (HTLV-III/LAV) in *Escherichia coli*

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The *pol* gene from a biologically active clone of the human T-cell lymphotropic virus type III provirus was inserted into a bacterial expression vector. The resulting gene fusion induced the formation of active reverse transcriptase that could be readily detected in extracts of bacterial cells. The activity exhibited the template and divalent cation requirements of the authentic enzyme. These constructs will be useful for safe and rapid analysis of potential inhibitors of this important enzyme.

Acquired immune deficiency syndrome, a disease whose spread has now reached epidemic proportions, is characterized by a marked depletion of the cellular immune response. The causative agent of the disease is now firmly established as the human retrovirus known as human T-cell lymphotropic virus type III or lymphadenopathy virus (HTLV-III/LAV) (1, 6–8). Efforts to arrest the spread of this virus are being made on two broad fronts. These include the development of antiviral vaccines which might allow immunized individuals to resist infection and the development of antiviral drugs which would specifically retard or arrest viral replication. One potentially important target of such drugs is the virion-associated enzyme reverse transcriptase (3, 5, 12, 13). The HTLV-III enzyme has been purified in small quantities, and some of its properties are established. The enzyme has unusual template and divalent cation preferences (4, 10, 15), suggesting that specific inhibitors of the enzyme may be discovered. The isolation of such inhibitors would be promoted by the availability of larger quantities of the active enzyme.

To facilitate preparation of the HTLV-III enzyme, we prepared gene fusions between the *trpE* gene of *Escherichia coli* and the appropriate portion of the viral *pol* gene. A large DNA fragment that contains almost all of the *pol* gene was excised from a biologically active proviral clone (pHXBc2 [14]) and inserted into the pATH2 expression vector (17; T. J. Koerner, unpublished data) by fusion of the 5′ half of the *trpE* gene to the *pol* sequences in the correct reading frame (Fig. 1). Two duplicate clones of the resulting plasmids, pHRT22 and pHRT25, were selected for analysis. Parallel constructions were performed to insert the same *pol* sequences into the pATH3 vector in the wrong reading frame to form the control plasmids pHRT31 and pHRT32.

Bacterial cultures that contained various plasmids were grown in minimal medium, starved for tryptophan, and exposed to indoleacrylic acid to derepress the *trp* operon (11, 16–18). After 2 h, the cells were harvested, lysates were prepared, and crude extracts were assayed directly for reverse transcriptase activity (Fig. 2A) as described previously (16, 18). In these assays, portions of the extracts were incubated in reaction cocktails that contained 50 mM Tris hydrochloride (pH 8.3), 20 mM dithiothreitol, 60 mM NaCl, 0.05% Nonidet P-40, and 10 μM of the appropriate α-^{32}P-labeled deoxynucleotide (1 Ci/mmol) for 30 min at room temperature. The ability of the extracts to incorporate radioactive precursors into DNA was assessed by spotting 10 μl of the cocktail directly onto DEAE paper (DE81; Whatman, Inc.), washing away the unincorporated precursors, and exposing the paper to film (9). The extracts were tested on either of two substrates: poly(rA) (10 μg/ml) primed with oligo(dT) (5 μg/ml) or poly(rC) (10 μg/ml) primed with oligo(dG) (5 μg/ml; all homopolymers were from Collaborative Research, Inc.). Each substrate was tested with either of two divalent cations, Mg^{++} (10 mM) or Mn^{++} (1 mM). All cultures, no matter what plasmids they carried, showed considerable background activity on the poly(rA)-oligo(dT) substrate; only a slightly higher level of activity was apparent from cells that carried the pHRT22 and pHRT25 constructs (Fig. 2). In contrast, assays on the poly(rC)-oligo(dG) substrate gave dramatic results. Cells that carried the vector DNA showed essentially no background activity, whereas cells that contained pHRT22 and pHRT25 yielded high levels of activity (Fig. 2A, row Mg[GC]). The activity was highly specific for Mg^{++} as the divalent cation (compare Fig. 2A, row Mg[GC] with Fig. 2A, row Mn[GC]). This behavior was distinct from the activity of murine reverse transcriptase, which was most active on the poly(rA)-oligo(dT) template with Mn^{++} (column V). Control plasmids formed with fusions out of frame showed a trace of activity that was only slightly above background.

In an attempt to reduce the background of DNA polymerase activity on the poly(rA)-oligo(dT) template, the plasmids were transferred into a bacterial strain deficient in DNA polymerase I, *E. coli* C2110 (*his rha polA1*; gift of D. Figurski, Columbia University, New York, N.Y). These cells do not support replication of plasmid DNA, and transformants carry plasmid DNA integrated into the host chromosome only in one or a few copies. Extracts were prepared and assayed as before (Fig. 2B). PolA$^-$ cells that carried the vector plasmid pATH2 showed no detectable background activity on either template or with either divalent cation. These same cells carrying the pHRT22 construct showed high levels of activity in the presence of Mg^{++}; the activity

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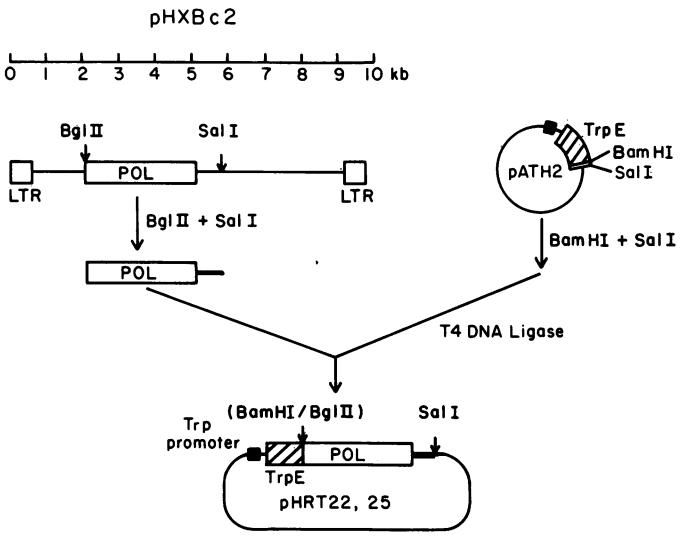

FIG. 1. Construction of active gene fusion. A 3.7-kilobase fragment of cloned proviral HTLV-III DNA was excised by cleavage with *Bgl*II plus *Sal*I and inserted into the expression plasmid at the *Bam*HI and *Sal*I sites. Cloning procedures were as described previously (16, 18). Parallel constructions were performed to insert the same *pol* sequences into the pATH3 vector in the wrong reading frame to form plasmids pHRT31 and pHRT32.

could be detected on either poly(rC)-oligo(dG) or on poly(rA)-oligo(dT), but there was slightly higher activity on the poly(rC)-oligo(dG) template. There was very little activity in the presence of Mn^{++} on either template. These properties of the activity duplicated precisely the behavior of the authentic HTLV-III reverse transcriptase enzyme as purified from virion particles (4, 10, 15).

To obtain a quantitative measure of the level of activity, assays were repeated on the poly(rC)-oligo(dG) template with Mg^{++}, and the amount of incorporated radioactivity was determined by scintillation counting. Assays lacking either primer or template showed only background activity (data not shown). Titration of the amount of extract in reactions allowed to proceed for 10 min showed that the assay was linear with up to 1 μl of extract (Table 1). Extracts of cells that carried the pHRT22 construct showed more than a 200-fold increase in specific activity over extracts from control cells. Analysis of the time course of the reaction, with 0.1 μl of extract, showed that the reaction was linear for more than 10 min (Table 2). These experiments also showed large increases in the specific activity of cells that carried the expression constructs relative to controls. C2110 $PolA^-$ cells with pHRT22 yielded about fivefold less activity than did $PolA^+$ cells that carried the same plasmid, presumably because there was a lower copy number of the plasmids in the $PolA^-$ cells.

In summary, we inserted a portion of the HTLV-III *pol* gene into a bacterial expression plasmid and demonstrated that the construct induces reverse transcriptase activity. The appearance of activity depends on the joining of the *trpE* and *pol* sequences in the correct reading frame and is independent of the bacterial *polA* gene. The resulting activity closely mimics the behavior of the authentic HTLV-III enzyme in the strong preference for Mg^{++} over Mn^{++} and thus differs sharply from the activity of the enzyme encoded by the murine leukemia viruses. Like the murine enzyme, the HTLV-III enzyme seems to be active in the form of a large fusion protein. Preliminary results with deletion variants of pHRT22 (data not shown) suggest that much of the *pol* gene can be removed without affecting activity but that the *trpE* portion of the construct is essential for good activity. We expect that these constructs and more active derivatives will be useful in the genetic analysis of the functions of the HTLV-III *pol* gene and in surveys for antiviral agents.

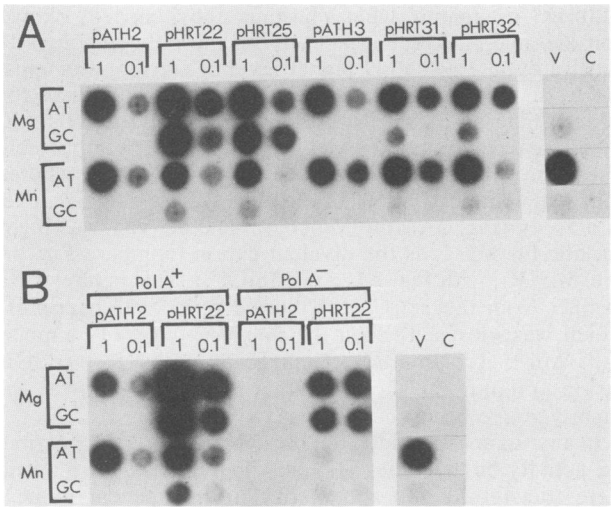

FIG. 2. Reverse transcriptase assays of crude bacterial extracts. (A) HB101 cells that carried the indicated plasmids were used. Either 1 or 0.1 μl of each extract as indicated was incubated in reaction cocktails that contained either Mg^{++} (10 mM) or Mn^{++} (1 mM) as indicated at the left. Rows labeled AT included substrates poly(rA) and oligo(dT), whereas rows labeled GC included substrates poly(rC) and oligo(dG). Columns labeled V and C show results of assays of authentic reverse transcriptase from Moloney murine leukemia virus and of medium from control cells. (B) Strains HB101 ($PolA^+$) and C2110 ($PolA1^-$) cells that carried the indicated plasmids were used. Assays and substrates were as in panel A.

TABLE 1. Titration of extracts[a]

Cell line	Vol of extract (μl)	dGTP incorporated (pmol/10 min)	Sp act (pmol/10 min per μg of protein)
HB101(pATH2)	0	0	
	0.02	<0.035	
	0.06	<0.035	
	0.1	<0.035	<0.02
	0.3	<0.035	
	1.0	<0.035	
HB101(pHRT22)	0	0	
	0.02	0.6	
	0.06	0.8	
	0.1	1.9	4.3 ± 0.07
	0.3	4.2	
	1.0	14.1	

[a] The indicated volumes of extracts were incubated in reaction mixes (total volume, 50 μl) that contained Mg^{++}, poly(rC), oligo(dG), and [α-^{32}P]dGTP. Reactions were carried out for 10 min. Background counts of 220 cpm present in reactions without extract were subtracted before calculation of the picomoles of nucleotide incorporated. Protein determinations, performed by the method of Bradford (2), gave a concentration of 4.4 mg/ml for HB101(pATH2) and 3.3 mg/ml for HB101(pHRT22). Specific activity of extracts was calculated from the slope of a least-squares fit of the titration data.

TABLE 2. Time course of incorporation[a]

Cell line	Time (min)	dGTP incorporated (pmol/μg of protein)	Sp act (pmol/μg of protein per 10 min)
HB101(pATH2)	0	0	
	1	<0.2	
	5	<0.2	
	10	<0.2	<0.1
	20	<0.2	
	40	<0.2	
HB101(pHRT22)	0	0	
	1	0.4	
	2	0.4	
	5	1.1	1.7 ± 0.1
	10	2.1	
	20	3.5	
	40	6.5	
C2110(pATH2)	0	0	
	1	<0.07	
	2	<0.07	
	5	<0.07	<0.03
	10	<0.07	
	20	<0.07	
	40	<0.07	
C2110(pHRT22)	0	0	
	1	0.004	
	2	0.06	
	5	0.2	0.30 ± 0.03
	10	0.2	
	20	0.6	
	40	1.2	

[a] 0.1 μl of crude extract from the indicated cultures was incubated in reaction mixes for the indicated times, and the incorporated counts were determined as in Table 1. Background radioactivity of 150 to 200 cpm was subtracted before calculating activities. Protein concentrations were as follows: HB101(pATH2), 4.4 mg/ml; HB101(pHRT22), 3.3 mg/ml; C2110(pATH2), 3.9 mg/ml; C2110(pHRT22), 5.2 mg/ml. Specific activities were determined as described in Table 1, footnote *a*.

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