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Identification and Some Properties of a Unique DNA Polymerase from Cells Infected with Human B-Lymphotropic Virus

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A new DNA polymerase and DNase activity were identified from cells infected with human B-lymphotropic herpesvirus (HBLV). DNA polymerase associated with HBLV infection was similar in its sensitivity to inhibition by pp_i analogs as other herpesvirus-specific DNA polymerases but was dissimilar in its inhibition by certain nucleoside triphosphates.

In recent years, several human viruses have been identified and related to a number of human diseases. Human B-lymphotropic virus (HBLV), a novel herpesvirus, was isolated from cultured human mononuclear cells from patients with lymphoproliferative diseases. Based on its ability to infect freshly isolated B lymphocytes, this virus was designated HBLV (15, 25). This virus can also infect several non-B types of human tissue culture cells (1). HBLV was morphologically classified as a herpesvirus on the basis of the icosahedral symmetry of the isolated virions and since it contained double-stranded DNA greater than 110 kilobases (3, 15, 25). The failure of HBLV DNA to hybridize to the genomic probes of other herpesvirus DNAs and vice versa clearly indicates that HBLV is a novel herpesvirus (25). Although no human diseases have so far been associated with HBLV, it is of interest to study the biochemistry and enzymology of this virus since very little of its biology is known. This information could aid in understanding more about this herpesvirus, identifying diseases which may be associated with HBLV, and identifying potential antiviral compounds should HBLV be linked to a specific disease.

All identified human herpesviruses such as herpes simplex virus type 1 or 2 (HSV-1 or -2) (10, 13, 17, 18, 23), Epstein-Barr virus (EBV) (22, 26), cytomegalovirus (CMV) (14), and varicella-zoster virus (8) are capable of inducing virally coded enzymes such as DNA polymerase and DNase in cells infected with these viruses. In this report, we present evidence that the infection of HSB-2 (a T-cell line) by HBLV also leads to the induction of DNA polymerase and DNase. The DNA polymerase induced by HBLV shares some of the properties of DNA polymerases induced by other human herpesviruses. However, it also has some unique features which distinguish it from other herpesvirus DNA polymerases.

HSB-2, a cell line susceptible to infection with HBLV (obtained from the American Type Culture Collection, Rockville, Md.) was used for this study. Infections were initiated by adding HBLV-infected HSB-2 cells to uninfected cells at a ratio of 1:20 (infected-uninfected). The cells were harvested 5 days postinfection, washed, and stored at −70°C. The infected cell pellet was freeze-thawed and then extracted with 500 μl of 100 mM Tris chloride (pH 7.5) containing 300 mM KCl (all buffers contained 2 mM EDTA, 2 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride, and 20% glycerol; all procedures were done at 4°C). The solution was allowed to stand on ice for 15 min and then centrifuged. The crude cell extract thus obtained was used for the enzyme assays. DNA polymerase assays were performed according to published procedures (9). DNA polymerase was assayed in the presence and absence of salt to distinguish between virus-induced and host DNA polymerase. One unit of DNA polymerase activity is defined as the amount of enzyme that catalyzed the incorporation of 1 pmol of dTTP per min into acid-insoluble product under the assay conditions. The concentrations of the inhibitors and the appropriate nucleotides were varied during the kinetic analyses, and 2 units of enzyme were used in each assay. The K_m and K_i values were determined by the linear regression method. DNase assays were performed by published methods (7). This assay is capable of detecting alkaline DNase as well as virus-induced DNA polymerase-associated exonuclease. For each point on the time course, 1×10^7 infected

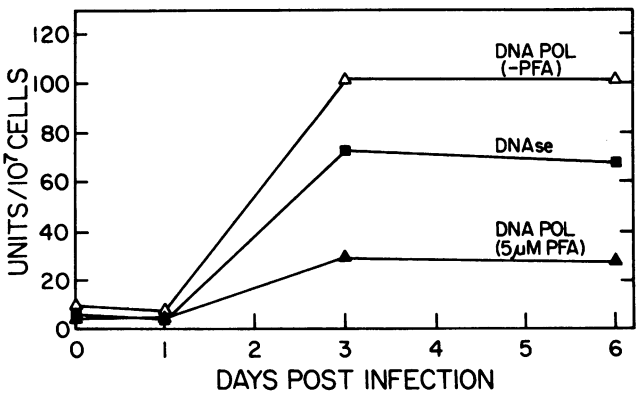

FIG. 1. Profile of the DNA polymerase and DNase activities following infection of HSB-2 cells with HBLV-infected HSB-2 cells (cocultivation, 20:1). POL, Polymerase; PFA, phosphonoformate.

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TABLE 1. K_m values of substrates for different DNA polymerases

Substrate	K_m (μM)						
	Human[a]		HSV-1 (KOS)[a]	HSV-2 (333)[a]	CMV (Towne)[a]	EBV (HR-1)[a]	HBLV
	α	β					
dATP	3.8		0.4	0.2	1.5	2.5	1.3 (± 0.15)
dGTP	6.0		0.2	0.2	0.5	0.7	0.1 (± 0.11)
dCTP	3.1	2.4	0.5	0.1	0.6	0.5	0.8 (± 0.23)
dTTP	7.0	8.6	0.5	0.2	0.6	0.5	0.8 (± 0.08)
Activated DNA (μg/ml)	15–17		7.2	7.5		8.2	6.7 (± 0.5)

[a] Reference 10.

cells were utilized, and to purify DNA polymerase, 1.6×10^8 infected cells were used.

The newly identified human herpesvirus HBLV, like other herpesviruses, was capable of inducing activities of DNA polymerase and DNase in infected cells (Fig. 1). In the mock-infected cells, there was no significant alteration of these enzyme activities through the time period studied (data not shown). The induction of the DNA polymerase and DNase activities correlated with the appearance of the HBLV-induced cytopathic effects.

To purify HBLV DNA polymerase, we mixed the crude extract with 3 ml of prewashed and preequilibrated (with 400 mM phosphate, pH 7.5) DEAE-cellulose. The slurry was mixed well and centrifuged. The supernatant was saved, and the resin was washed with 1 ml of 400 mM phosphate buffer and centrifuged, and this supernatant was saved. This was repeated two more times. The pooled supernatants were dialyzed against 25 mM phosphate (pH 7.5) and loaded onto a 15-ml DEAE-cellulose column (preequilibrated with the dialysis buffer). After the column was washed, it was eluted with a linear phosphate gradient (50 mM to 1 M). The fractions containing DNA polymerase activity (eluted at 0.45 M phosphate) were pooled, dialyzed, and loaded onto a 10-ml double-stranded DNA-cellulose column (preequilibrated with the dialysis buffer) and then eluted with a linear salt (KCl) gradient (0 to 1 M). The fractions containing DNA polymerase (eluted at 0.55 M KCl) were pooled and stabilized by dialysis against 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) (pH 7.5) buffer containing 30% sucrose and 20% ethylene glycol. Heat-inactivated bovine serum albumin (final concentration, 100 μg/ml) was added to the enzyme solution before dialysis. The DNase activity coeluted with DNA polymerase from DEAE-cellulose. However, we were unable to detect the activity in fractions obtained from the double-stranded DNA-cellulose column. Nearly a 200-fold purification of DNA polymerase was achieved by this scheme, as determined by its specific activity.

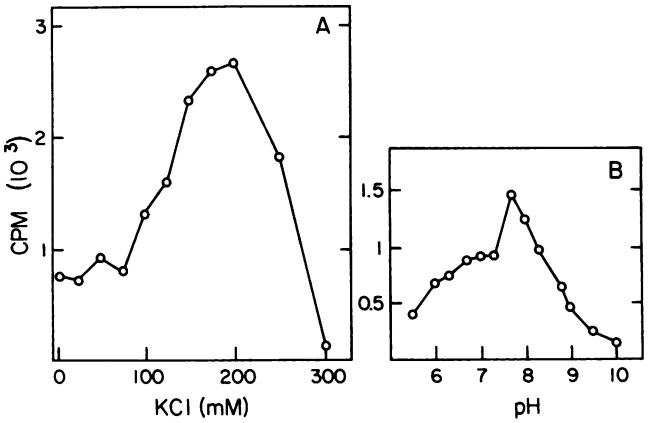

FIG. 2. Determination of the effect of salt (potassium chloride) (A) and pH optimum (B) of the HBLV-induced DNA polymerase activity. The salt was added to the reaction mix (pH 8) to give the desired concentration.

HBLV-induced DNA polymerase showed maximum stimulation of its activity at approximately 175 mM salt concentration, and its pH optimum appeared to be in the range of 7.7 to 8.0 (Fig. 2). On the other hand, host DNA polymerase (α) was inhibited by the salt, even at 100 mM. PP_i, analogs such as phosphonoformic acid and phosphonoacetic acid were capable of inhibiting this activity (50% inhibitory doses, 0.4 and 2.54 μM, respectively). All these properties were similar to those exhibited by other human herpesviruses-induced DNA polymerases (16, 19). The K_m values for its substrates were also determined, and although they appeared to be slightly higher than those for other herpesvirus-induced DNA polymerases, they were still much lower than substrate K_m values for human DNA polymerases (Table 1). Thus, like other herpesviruses, HBLV-induced DNA polymerase appears to be more efficient than host enzymes in competing for the nucleotides required for DNA synthesis.

The ability of HBLV-induced DNA polymerase to utilize different types of DNA templates was investigated. This enzyme failed to use synthetic templates (final concentration; 0.5 U/ml) such as poly(rA) · $oligo(dT)_{12-18}$, poly(dG) · $oligo(dC)_{12-18}$, and poly(dC) · $oligo(dG)_{10}$. However, HBLV-induced DNA polymerase utilized poly(dA) · $oligo(dT)_{12-18}$ as a template (90% activity compared with activated calf thymus DNA). Activated, but not native or denatured, calf thymus DNA (final concentration, 40 μg/ml) could serve as a template (100% activity represents 28 pmol of incorporated [^{3}H]TTP). DNA polymerases induced by CMV (14) and

TABLE 2. K_i values of deoxynucleoside triphosphate analogs toward HBLV and other DNA polymerases

Analog	Competing substrate	K_i (μM)						
		Human[a]		HSV-1 (KOS)[a]	HSV-2 (333)[a]	CMV (Towne)[a]	EBV (HR-1)[a]	HBLV
		α	β					
ACV-TP[b]	dGTP	0.2		0.003	0.003	0.008	0.01	2.5 (± 0.35)
DHPG-TP[b]	dGTP	0.12		0.030	0.046	0.022	0.075	0.13 (± 0.05)
BVdU-TP[b]	dTTP	3.6	6.5	0.068	0.068			0.25 (± 0.04)
Aphidicolin	dCTP	1.3		0.071				0.5 (± 0.15)

[a] Reference 10.
[b] 5'-Triphosphate form of ACV, DHPG, and BVdU.

TABLE 3. Inhibition of HBLV DNA polymerase by triphosphates of various nucleoside analogs

Inhibitor (μM)	Competing nucleotide (0.625 μM)	% Inhibition[a]
3′-N_3TTP[b] (2)	dTTP	27
BVdU-TP (6)	dTTP	90
Acyclo ATP (6)	dATP	78
ddATP (6)	dATP	7
5-Fluoro-ara-CTP (6)	dCTP	55
ddCTP (6)	dCTP	15
ddGTP (6)	dGTP	13
DHPG-TP (5)	dGTP	76
ACV-TP (5)	dGTP	45

[a] The values represent the percent inhibition of the control, which contained no inhibitor and incorporated 28 pmol of [^{3}H]TTP in 30 min.
[b] 3′-N_3TTP, 3′-Azido-2′-dTTP.

EBV (16) share some of the properties with HBLV-induced enzymes.

The interaction of the triphosphates of several known antiviral nucleoside analogs (2, 5, 6, 8, 12, 24) with HBLV-induced DNA polymerase was examined (Table 2). The triphosphates of 9-(2-hydroxyethoxymethyl)guanine (ACV), 9-(3,1-dihydroxy-2-propoxymethyl)guanine (DHPG), and (*E*)-5-(2-bromovinyl)-2-deoxyuridine (BVdU) were observed to be competitive inhibitors of this enzyme with respect to dGTP, dGTP, and dTTP, respectively. The K_i values were quite different from those of other herpesvirus-induced DNA polymerases. Conversely, dideoxynucleotides such as ddATP, ddCTP, and ddTTP, which are potent inhibitors of reverse transcriptases (4, 11, 21), were found to be poor inhibitors of HBLV-induced DNA polymerase (Table 3). HBLV-induced DNA polymerase, like HSV-1 and HSV-2 (9)- and EBV (20)-induced DNA polymerase was also inhibited by aphidicolin, and the inhibition appeared to be competitive with dCTP. Based on the K_i value for ACV-TP (ACV 5′-triphosphate) and its ratio to the K_m value for dGTP, ACV may not be as selective against HBLV as it is against other herpesviruses such as EBV, whereas DHPG and BVdU may be more selective inhibitors of HBLV replication. At present, this is under investigation.

The properties described above indicate that the DNA polymerase induced by HBLV, a newly identified human herpesvirus, shares some properties with DNA polymerases induced by other herpesviruses. This suggests that the structures of DNA polymerases induced by known human herpesviruses are conserved. However, there exist several differences which distinguish between DNA polymerase induced by HBLV and those induced by other herpesviruses, and these differences could be utilized to differentiate different types of herpesviruses. Furthermore, the differences in the properties of this enzyme from the host enzymes could also be explored for developing selective anti-HBLV compounds, should a disease related to HBLV infection be identified. Although the enzyme obtained was not completely purified, the results suggest that HBLV-induced DNA polymerase is different from other herpesvirus-induced DNA polymerases. Therefore, further characterization is warranted.

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