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Incorporation into DNA of the base analog 2-aminopurine by the Epstein–Barr virus-induced DNA polymerase *in vivo* and *in vitro*

(lymphocyte nuclei/mutagenic nucleotide analog)

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ABSTRACT **The Epstein–Barr virus (EBV)-induced intracellular DNA polymerase was assayed *in vitro* for the ability to utilize the mutagenic nucleotide analog 2-aminopurine deoxyribose triphosphate (d2apTP), incorporating it as the corresponding monophosphate into DNA or poly[d(A-T)] template. Bacteriophage T4, lymphocyte α, and the EBV particle-associated DNA polymerases were assayed simultaneously for direct comparison. Unlike these three polymerases, which were capable of distinguishing between d2apTP and dATP with a strong preference for the latter, the EBV-induced DNA polymerase only weakly distinguished between dATP and d2apTP and incorporated substantial amounts of d2apTP into template. Detergent-treated lymphocyte nuclei undergoing a high level of EBV DNA synthesis were shown to incorporate the 2-aminopurine analog of dATP into viral DNA. The relative inability of the EBV-induced DNA polymerase to distinguish between the two purine nucleotides reported here is consistent with previous reports on the ready incorporation of other nucleotide analogs into DNA polymerases induced by other herpesviruses. Because most antiherpes agents currently in use or under study are nucleotide analogs, the viral mutagenic properties of these drugs should be examined.**

The Epstein–Barr virus (EBV) is a human herpesvirus that has been associated with two different human malignancies (1, 2). Some viral strains have been shown also to transform human B lymphocytes in culture (3, 4). The details of the replication of the EBV DNA are of considerable interest because viral DNA replication may play an important early role in the cell transformation process (5, 6). Like other herpesviruses (7–11), EBV induces a DNA polymerase that is associated with viral DNA replication (12–15). This enzyme is not found in uninfected cells or in infected, virus-containing, nonproducing cell lines (12). The EBV-induced DNA polymerase shares many structural and catalytic features with DNA polymerases induced by other herpesviruses (12–16). For example, both the EBV-induced and the herpes simplex virus (HSV)-induced DNA polymerases are sensitive to phosphonoacetic acid (12, 17–20) and have been shown to copurify through many purification stages with exonucleolytic activity (7, 18–20). It has been suggested that the HSV polymerase-associated exonuclease may fulfill an editing or error-correcting function, in analogy to the exonucleases associated with prokaryotic DNA polymerases (18, 19, 21).

We examined the utilization by the EBV-induced DNA polymerase of the adenine (6-aminopurine) base analog 2-aminopurine. This compound has been extensively studied with the DNA polymerase induced by the bacteriophage T4 (22, 23), which uses for DNA synthesis 2-aminopurine deoxyribose triphosphate (d2apTP) as a small percentage of utilized dATP. The amount of d2apTP incorporated (as the corresponding monophosphate d2apMP) by the T4 DNA polymerase has been shown to be inversely proportional to the error-correcting or proofreading ability of the T4 polymerase (22).

We report here that the EBV-induced DNA polymerase incorporated d2apTP and dATP at similar frequencies into activated calf thymus DNA or poly[d(A-T)] template, showing little ability to distinguish between the natural and analog nucleotides. This was in sharp contrast to the behavior of phage T4, lymphocyte α, and the EBV particle-associated DNA polymerases, which were studied in the same experiments for comparison and were clearly capable of distinguishing between the two nucleotides. The relative inability of the EBV-induced DNA polymerase to distinguish these two nucleotides was independent of a nucleotide turnover function that has been associated with the copurifying exonuclease (20). Experiments in an *in vivo* system of nuclei derived from detergent-treated lymphocytes showed d2apTP to be incorporated at a frequency lower than observed with the EBV-induced polymerase *in vitro*, but significantly higher than that observed with the three other DNA polymerases *in vitro*.

MATERIALS AND METHODS

Materials. [α-^{32}P]dATP (2,000–3,000 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) was from Amersham, and all unlabeled dNTPs were from Sigma. [^{3}H]d2apTP was synthesized by S. Watanabe as described (22, 23). The HR-1 cell line was from M. Nonoyama, and the Raji line was from Pfizer (New York).

EB Virus Replication in Superinfected Nuclei. The virus in 1 liter of supernatant of HR-1 lymphocyte culture was concentrated by centrifugation for 1 hr at 13,700 × *g*. This was suspended in 2 ml of RPMI 1640 medium (GIBCO) and was mixed with a pellet containing 4 × 10^{7} logarithmic-phase Raji cells. After equal distribution into four small flasks, adsorption was for 1 hr at 35°C with agitation after 30 min. After adsorption, 12 ml of RPMI 1640 medium and 0.32 ml of dialyzed, heat-inactivated fetal calf serum (GIBCO) were added to each flask, and the incubation was continued at 35°C. For the experiment shown in Fig. 1, cells were harvested 3, 4.5, 6, and 20 hr after superinfection by 5 min of centrifugation at 700 × *g*. Nuclei were prepared by detergent treatment as described (24) with the ATP regenerating system omitted (25) to maximize viral DNA synthesis under superinfection conditions. The incubation included 10^{7} nuclei in 0.5 ml containing 25 mM Hepes buffer (pH 8.0); 40 mM NaCl; 5 mM $MgCl_2$; 2.5 mM $CaCl_2$; 5 mM EGTA; 150 μM spermine; 500 μM spermidine; 125 mM sucrose; 1% dextran; 1 mM dithiothreitol; 0.2 mM each of dCTP, dGTP, and dTTP; and 4 μCi of [^{32}P]dATP per ml. After incubation at 37°C for 30 min, 2 ml of cold 150 mM NaCl/50

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Abbreviations: EBV, Epstein–Barr virus; d2apTP, 2-aminopurine deoxyribose triphosphate; d2apMP, 2-aminopurine deoxyribose monophosphate; HSV, herpes simplex virus.

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mM Tris·HCl, pH 7.4, was added, and the nuclei were pelleted, drained well, and frozen at −80°C.

The samples in Fig. 2 were prepared in the same way, except that all nuclei were made from cells harvested 20 hr after superinfection. These 0.5-ml incubation mixtures all received 0.3 μCi of [^{32}P]dATP and 30 nmol of unlabeled dATP in addition to the other ingredients. Tubes for the experiments in Fig. 2 *Right* also received 30 nmol of [^{3}H]d2apTP (20 μCi). All samples were lysed in the same way (25). Nuclear pellets were suspended in 2 ml of phosphate-buffered saline to which was added 1 ml of 3% sarkosyl/50 mM Tris·HCl, pH 8.5/15 mM EDTA, pH 8.0. The lysed, viscous suspensions were mixed with 0.6 ml of Pronase (stock solution of 10 mg of Calbiochem B-grade Pronase per ml preincubated 40 min at 37°C in 50 mM Tris·HCl, pH 8.5) and incubated at 37°C overnight. The digests were extracted once with buffer-saturated phenol and precipitated with 0.7 ml of 1 M sodium acetate (pH 5.0) and 7 ml of 100% EtOH at −80°C for 1 hr. The pellets from a 12,000 × *g* 20-min centrifugation were dissolved in 2.2 ml of 1 mM Tris·HCl, pH 8.0/1 mM EDTA, pH 8.0 and adjusted to a refractive index of 1.4000 and a final volume of 4.8 ml with CsCl. These tubes were centrifuged at 33,000 rpm for 20 hr at 18°C in the TV865 rotor in the OTD-50 ultracentrifuge (Sorvall) with controlled deceleration. Fractions (40) were collected from each tube. The profiles in Fig. 1 were obtained by spotting 10-μl samples from each fraction onto Whatman 3MM paper squares, followed by trichloroacetic acid and ethanol washing (12) and assay of radioactivity. The profiles in Fig. 2 were obtained by adding 10 μg of calf thymus DNA to each fraction, followed by precipitation of the full-fraction volume with 1 ml of 15% (wt/vol) trichloroacetic acid. The precipitates were collected onto GF/A filters (Whatman), washed with 0.1 M HCl, dried, and assayed for radioactivity.

DNA Polymerases and *in Vitro* Studies. The purification of the EBV-induced intracellular and the EBV particle-associated DNA polymerases have been described (refs. 12 and 20; unpublished data). The purifications involved DEAE-cellulose, phosphocellulose, and denatured DNA-cellulose chromatography. The lymphocyte α DNA polymerase was obtained from different fractions from DEAE-cellulose chromatography used for the EBV intracellular DNA polymerase. It was further purified from β and γ DNA polymerases by phosphocellulose chromatography (12) and was identified by substrate preference, sedimentation coefficient, and chromatographic behavior. The T4 DNA polymerase was a gift from S. Watanabe and M. Goodman and was purified by chromatography on DEAE-cellulose and phosphocellulose (22). The DNA polymerase assays containing [^{3}H]d2apTP were based on the assay conditions of Goodman *et al.* (12) and were carried out in 100 μl containing 80 mM Tris·HCl (pH 8.0); 14.3 μM each of [α-^{32}P]dATP (0.05 μCi per assay), dCTP, dGTP, dTTP, and [^{3}H]d2apTP (1.0 μCi per 1.43 nmol) (concentrations of nucleotide stock solutions were determined by *A* at 260 nm); 1 mM dithiothreitol; 0.05% Triton X-100; 180 μg of activated calf thymus DNA or 80 μg of poly[d(A-T)] (Miles) per ml; and 25 μl of enzyme sample (concentration adjusted with enzyme buffer). Each assay was incubated with an identical control in which Tris/EDTA buffer had been substituted for template to determine the level of template-independent nucleotide turnover. The incubations were for 12 or 24 min at 37°C, and the assays were then removed to ice.

Of the original 100-μl reaction volume, 80 μl was used to determine nucleotide incorporation as follows. Na pyrophosphate (200 mM) was added (0.2 ml), followed by 5 μl of bovine serum albumin (50 mg/ml) and 1 ml of 15% trichloroacetic acid. After incubation in ice-water for 20 min, the precipitates were collected by centrifugation at 1500 × *g* for 20 min, dissolved in 0.5 ml of 0.1 M NaOH, then reprecipitated in the cold, and redissolved. They were precipitated again and collected onto GF/A filters; the precipitates were washed several times with cold 0.5 M HCl and 70% ethanol, and the filters were dried and assayed for radioactivity.

Nucleotide Turnover. For nucleotide turnover determination, 20 μl of assay volume was removed to tubes containing 10 μl of a solution of 20 mM each of dATP and dAMP as markers and 5 μl of 250 mM EDTA. Of this mixture, 25 μl was spotted, 5 μl at a time, onto freshly washed polyethyleneimine-cellulose thin-layer chromatography plates. The plates were developed in 1 M LiCl, and the nucleotide marker spots were cut out (dATP and d2apTP comigrated closely, as did dAMP and d2apMP in this system; therefore, dATP and dAMP were the only markers used). Each spot was eluted by shaking in 1.0 ml of 0.5 M HCl at room temperature for 45 min. Scintillation fluid (5 ml) containing Triton X-100 was mixed in, and each sample was assayed for radioactivity.

RESULTS

***In Vitro* Frequency of d2apTP Incorporation by the EBV-Induced DNA Polymerase.** The purification of the EBV-induced DNA polymerase through DEAE-cellulose, phosphocellulose (12), and denatured DNA-cellulose (20) has been described. Table 1 shows the frequencies of d2apTP incorporation (as d2apMP) into activated DNA template obtained with the intracellular EBV-induced DNA polymerase at various purification stages, EBV particle-associated DNA polymerase, cellular α DNA polymerase, and DNA polymerase from phage T4-infected *Escherichia coli*. The frequency of d2apTP incorporation obtained with T4 DNA polymerase was the same as previously observed (22). The lymphocyte α polymerase displayed a frequency of d2apTP incorporation somewhat lower than has been reported for calf thymus α DNA polymerase (23). Table 1 also shows the results of identical experiments performed with poly[d(A-T)] as template. The overall incorporation of both nucleotides was lower than in the presence of activated DNA template, but the ratios of incorporation of the two nucleotides were similar in all cases.

The frequency of d2apTP incorporation observed with the EBV intracellular DNA polymerase was considerably higher than that obtained with any other enzyme tested. The reactions in all cases were linear over the time period examined, and there was >80% of dNTPs remaining in the reaction mixture without significant change in the ratios of [^{3}H]d2apTP to [α-^{32}P]dATP.

DNA-Dependent Turnover of Inserted d2apTP and dATP by the Four DNA Polymerases. DNA-dependent turnover of nucleotides by prokaryotic DNA polymerases such as T4 DNA polymerase (22) is mediated by utilization of dNTP by the polymerase for incorporation into the template as dNMP. The associated exonuclease (26) can then remove the incorporated dNMP; thus, the triphosphate is turned over to monophosphate. The EBV-induced intracellular DNA polymerase (20) and the related HSV DNA polymerase (7, 18, 19) have been associated with exonuclease activity. Purified polymerase α has not been consistently associated with exonuclease. Such activities do not copurify with the EBV particle-associated DNA polymerase (unpublished data; present report). Because the EBV-induced DNA polymerase was capable of DNA-dependent turnover of normal deoxyribonucleotides, it was of interest to determine its ability to turn over d2apTP in a template-dependent fashion. Table 2 shows the percentage of nucleotide inserted that was turned over by each of the four DNA polymerases tested in the presence of activated DNA template. T4 polymerase, as previously reported (22), turned over both dATP and d2apTP, and a much higher percentage of d2apTP was turned over than was the case for the natural nucleotide, dATP.

Table 1. Incorporation of d2apMP and dAMP into two templates by the four DNA polymerases

Polymerase	d2apMP incorporated, pmol		Range		dAMP incorporated, pmol		Range		d2apMP/dAMP	
	A	B	A	B	A	B	A	B	A	B
EBV-induced,										
DEAE-cellulose-purified	39.8	2.5	3.7	0.4	105.3	7.0	11.0	0.5	0.38	0.36
Phosphocellulose-purified	3.6	0.2	0.4	0.1	9.5	0.6	1.5	0.2	0.38	0.33
Denatured DNA cellulose-purified	3.8	0.1	0.3	<0.1	11.8	0.2	0.9	<0.1	0.32	0.50
Lymphocyte α	1.0	0.2	0.2	<0.1	30.8	4.5	2.8	0.7	0.03	0.04
Phage T4	2.7	0.2	0.3	<0.1	61.3	21.7	3.7	7.3	0.04	0.01
EBV particle-associated	0.1	—	<0.1	—	4.3	—	0.7	—	0.02	—

Picomoles of nucleotide incorporated represent total incorporation in each 100-μl assay volume over 24 min at 37°C. Incorporation was linear for over 30 min. Specific activities of labeled nucleotides were determined by assaying the radioactivity of measured volumes of the polymerase reaction mixtures which had been dried on GF/A filters under the same counting conditions as the incorporation determinations. Specific activities were typically 100–120 cpm per pmol of [^{3}H]d2apTP and 350–400 cpm per pmol of [^{32}P]dATP. Picomoles incorporated are averages, and the range represents the difference between the average and the value farthest from the average. A, activated DNA template; B, poly[d(A-T)] template.

No significant nucleotide turnover was detected for polymerase α and the EBV particle-associated polymerase. However, the EBV-induced intracellular DNA polymerase turned over both dATP and d2apTP. Unlike T4 polymerase, the EBV polymerase turned over the analog and natural nucleotides at similar frequencies. Although total nucleotide incorporation was lower in the presence of poly[d(A-T)] template (Table 1), the percentage nucleotide turned over was substantially higher for both dATP and d2apTP (data not shown).

The nucleotide turnover function associated with highly purified EBV-induced DNA polymerase has been shown to be labile. Storage of the enzyme at −80°C for 7–10 days resulted in polymerase free of nucleotide turnover and nuclease activity (20). EBV-induced DNA polymerase that was free of nucleotide turnover activity showed a ratio of d2apMP/dAMP incorporation in the presence of activated DNA template that was identical to the ratios shown in Table 1.

Incorporation of d2apTP into Viral DNA in an *in Vivo* System. Raji B lymphocytes that have been superinfected with EBV undergo extensive EBV DNA synthesis (27), which has been shown to be mediated by the EBV-induced DNA polymerase (25, 27); in fact, by several hours after superinfection, little cellular polymerase remains, and the EBV polymerase is the predominate and sometimes the only species of DNA polymerase in these superinfected cells (ref. 15; unpublished data). Nuclei derived by detergent treatment of these superinfected cells continue to synthesize viral DNA if they are incubated with dNTPs (24, 25, 27). Nuclei were isolated from EBV-superinfected Raji cells at 3, 4.5, 6, and 20 hr after superinfection and were incubated for 30 min at 37°C in the described mixture of nucleotides and [α-^{32}P]dATP. The DNA was extracted and centrifuged in CsCl density gradients, a procedure that readily separates viral from cellular DNA (27, 28). The amount of labeled nucleotide incorporated into viral DNA in the nuclei increased steadily with increasing time (after superinfection) at which the nuclei were isolated (Fig. 1). At 20 hr after superinfection, a highly labeled peak of viral DNA was present in CsCl gradients. The viral origin of this DNA was shown by its characteristic density and by restriction endonuclease analysis of the DNA, which showed a characteristic viral DNA banding pattern in agarose gels (data not shown). The synthesis was mediated by the EBV-induced DNA polymerase, as shown by its complete inhibition by the addition to the nuclei of low concentrations of phosphonoacetic acid (10–20 μg/ml), an inhibitor of herpesvirus-induced DNA polymerases (18–20). Because of the active viral DNA synthesis, 20 hr after superinfection was chosen as a suitable time to isolate nuclei and to incubate them in the presence of [^{3}H]d2apTP and [α-^{32}P]dATP. As can be seen from the CsCl gradients (Fig. 2 *Right*), significant amounts of [^{3}H]d2apTP were incorporated into viral DNA in the nuclear system, and this incorporation increased linearly over the 15- and 30-min incubations.

Table 2. DNA-dependent nucleotide turnover in the presence of the four DNA polymerases

Polymerase	d2apTP turned over, pmol	Range	Turnover, %	dATP turned over, pmol	Range	Turnover, %
EBV-induced,						
A	5.5	1.9	12	13.6	2.2	11
B	0.7	0.1	16	3.1	0.2	25
C	1.9	0.5	33	4.4	1.7	27
Lymphocyte α	ND	ND	ND	0.3	0.1	1
Phage T4	9.5	1.2	78	54.9	1.9	47
EBV particle-associated	ND	ND	ND	ND	ND	ND

Assay conditions and nucleotide turnover determinations were as described in the legend to Table 1. Specific activities of labeled nucleotides were determined by spotting measured volumes of the polymerase reaction mixtures in the presence of nucleotide markers onto the same polyethyleneimine plates used for the turnover determinations. After chromatography of the plates in 1 M LiCl, the triphosphate and monophosphate spots from the reaction mixtures were cut out and counted using the same conditions as for the spots from the nucleotide turnover determinations. Specific activities were typically 320–350 cpm per pmol of [^{3}H]d2apTP and 100–150 cpm per pmol of [^{32}P]dATP. Percentage of nucleotide turned over was calculated by dividing nucleotide turned over by nucleotide turned over plus nucleotide incorporated (Table 1). Range was calculated as for Table 1. ND, not detectable; A, DEAE-cellulose purified; B, phosphocellulose purified; C, denatured DNA-cellulose purified.

Because no d2apTP would preexist naturally within the cells, the *in vivo* frequency of d2apTP incorporation was determined by calculation from the specific activity of the [^{3}H]d2apTP added as substrate. In order to determine the effect of preexisting intracellular pools of dATP, unlabeled dATP was omitted from tubes run in parallel with those shown in Fig. 2. This produced an expected 20-fold increase in ^{32}P cpm incorporated, indicating that added nucleotide concentration was in ≈20-fold molar excess over the dATP that exists endogenously in the cells used. With the addition of unlabeled dATP to the tubes to make the total [^{32}P]dATP and [^{3}H]d2apTP of equimolar concentrations, the endogenous dATP would therefore not significantly change the ratio of [^{32}P]dATP and [^{3}H]d2apTP. It was important that these nucleotides remain approximately equimolar, be-

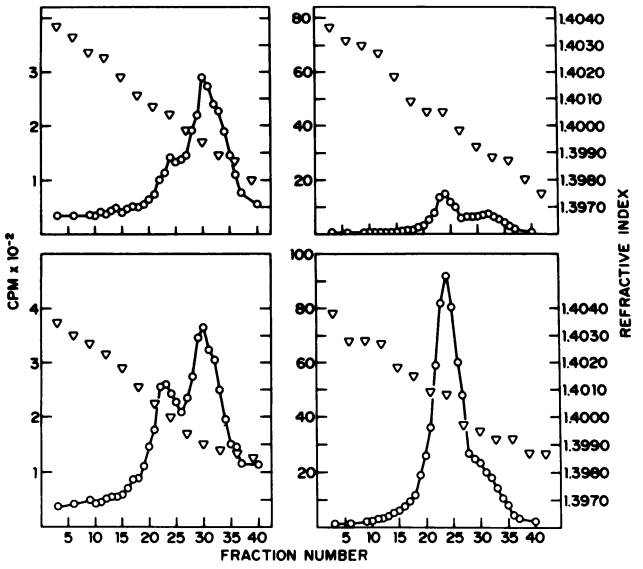

FIG. 1. CsCl density gradient centrifugation of DNA extracted from detergent-treated EBV-superinfected Raji cells. Raji B lymphocytes were superinfected with the HR-1 strain of EBV. The cells were harvested at 3 (*Upper Left*), 4.5 (*Lower Left*), 6 (*Upper Right*), and 20 (*Lower Right*) hr, converted into nuclei by detergent treatment, and incubated for 30 min in a reaction mixture with three unlabeled nucleotides and [α-^{32}P]dATP (2 μCi per 0.5-ml final volume) without addition of unlabeled dATP. The nuclei were then harvested and washed, and the DNA was extracted and sedimented in CsCl density gradients. ○, cpm × 10^{-2}; ▽, refractive index.

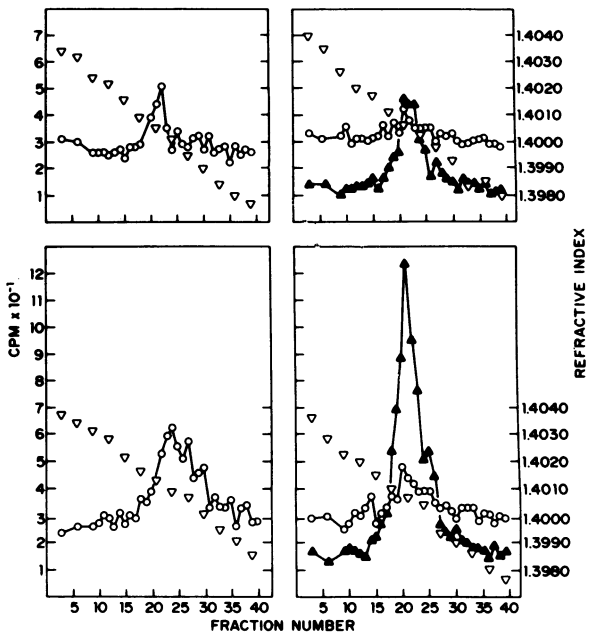

FIG. 2. CsCl density gradient centrifugation of DNA extracted from detergent-treated EBV-superinfected Raji cells incubated with d2apTP (*Left*). Raji cells were superinfected as for Fig. 1; cells were harvested at 20 hr after superinfection, subjected to detergent treatment, and incubated for 15 min (*Upper*) or 30 min (*Lower*) with three unlabeled nucleotides, 0.3 μCi of [α^{32}P]dATP, and 30 nmol of unlabeled dATP per 0.5-ml reaction mixture. (*Right*) Experiments receiving in addition 30 nmol of [^{3}H]d2apTP (20 μCi) per 0.5-ml reaction mixture. After incubation for 15 min (*Upper*) or 30 min (*Lower*), nuclei were harvested and washed, and the DNA was extracted and sedimented in CsCl density gradients. ○, [^{32}P]dAMP; ▲, [^{3}H]d2apMP; ▽, refractive index.

cause they compete for the same sites in the DNA (principally opposite thymine), and a large change in their ratios could affect their relative rates of incorporation. Polyethyleneimine-cellulose thin-layer chromatography of samples from each incubation showed at least 80% of each dNTP to be present after 15- or 30-min incubations, with no significant change in their ratio (data not shown). A change in this ratio would be unexpected in view of the approximately linear increase in both ^{32}P and ^{3}H incorporation, showing that the system is in a steady state.

The ratio of d2apMP/dAMP incorporated *in vivo*, which was calculated from data such as that shown in Fig. 2, was 0.1 for the 15-min sample and 0.15 for the 30-min sample. This was lower than the d2apMP/dAMP ratio observed with the purified EBV intracellular DNA polymerase but significantly higher than the *in vitro* frequency obtained with any of the other DNA polymerases tested. There also was significant inhibition of total *in vivo* EBV DNA synthesis after addition of the d2apTP to the nuclei.

DISCUSSION

We report here that the highly purified EBV-induced DNA polymerase, alone of four DNA polymerases tested, could distinguish only weakly between the natural base adenine and its analog 2-aminopurine. Only a weak discrimination between these purines was shown in nuclei undergoing EBV DNA replication. This polymerase incorporated the analog nucleotide regardless of the presence of an active but labile nucleotide turnover function, which has been attributed to the copurifying exonucleolytic activity (20).

The *in vitro* studies showed a ratio of d2apMP/dAMP incorporation into activated DNA template of ≈0.32–0.38 for the EBV-induced intracellular DNA polymerase at all levels of enzyme purification. The above value differed considerably from the ratio of ≈0.03–0.04, which was obtained for the cellular α DNA polymerase and for phage T4 DNA polymerase. The incorporation of d2apTP has been related to the error frequency of mutant phage T4 DNA polymerases, which produce increased or decreased mutation frequencies *in vivo*. For example, increased d2apTP incorporation and increased mutation rates are associated with the mutator T4 genotype (22). The level of incorporation of this nucleotide is considered an indicator of the error frequency of the T4 DNA polymerase because 2-aminopurine differs from adenine in positions on the molecule involved in base pairing (29). Herpesvirus DNA polymerases are known to incorporate purine nucleotide analogs such as adenine arabinoside (30) and acycloguanosine (acyclovir) (31) that are modified in the sugar moiety. Furthermore, the pyrimidine analogs 5-iodo-5′-amino-2′,5′-dideoxyuridine and 5-iodo-2′-deoxyuridine are incorporated into viral DNA by the HSV-induced polymerase (32).

Two other observations are (*i*) unlike T4 polymerase, the EBV-induced polymerase turned over d2apTP and dATP at comparable rates, although the pmol of d2apTP turned over were significantly less than those incorporated; and (*ii*) the presence or absence of the labile nucleotide turnover activity did not affect the high ratio of d2apMP/dAMP incorporation *in vitro*.

We concluded that the high d2apTP incorporation frequency was due to a high insertion frequency of the analog nucleotide by the EBV polymerase activity, whether or not the polymerase has unusually high error frequencies due to currently unspecified error correction mechanisms. It is also possible that the nucleotide binding site allows d2apTP incorporation because it is "sloppy" with nucleotide analogs in a way not related to its behavior with natural nucleotides. The polymerization by avian myeloblastosis virus DNA polymerase (reverse transcriptase) is error-prone (33), whereas the polymerization by deoxynu-

cleotidyl terminal transferase is random (34). Therefore, error-prone herpesvirus-induced DNA polymerases would not be unprecedented.

An approach to the study of polymerase fidelity with natural nucleotides is to determine the misincorporation of natural nucleotides by the polymerase into synthetic templates. Because deoxycytidine is highly susceptible to deamination (35), this experiment is best done by determining the frequency of the G·T mispair. However, certain herpesvirus DNA polymerases, including the EBV-induced polymerase, utilize poly[d(A-T)] poorly, at least under commonly used assay conditions (7, 12, 20; Table 1), so that even a relatively high dGTP misincorporation frequency could not be detected (unpublished data).

It is probable that other proteins in addition to the EBV DNA polymerase were involved in the replication of EBV DNA. The well-characterized prokaryotic systems of *E. coli* and bacteriophages T4 and T7 require at least four to six different proteins to replicate DNA in an *in vivo* fashion (36–38). No eukaryotic replicative system has been so extensively characterized, but an equal or greater degree of complexity is anticipated (39). Our *in vivo* studies on incorporation of d2apTP into EBV DNA in nuclei from detergent-treated lymphocytes showed that even in the presence of a full complement of viral and cellular proteins, the viral polymerase incorporated more d2apTP than any of the other polymerases incorporated *in vitro*.

It is noteworthy that the other heavily-studied herpesvirus-induced enzyme, thymidine kinase, also displays a lack of specificity in substrate compared with the cellular pyrimidine deoxynucleoside kinases. This class of herpesvirus-induced enzyme readily phosphorylates many 5-substituted pyrimidine deoxynucleosides and even purine deoxynucleosides that the cellular thymidine and deoxycytidine kinases ignore (40). The thymidine kinase induced by EBV (41) is no exception in that it phosphorylates 5-bromodeoxycytidine (unpublished data).

The significance of the studies reported here is that they provide further evidence for the hypothesis that herpesvirus-induced DNA processing enzymes are less discriminating than their host-cell counterparts. Such a lack of discrimination was suggested for the HSV-induced DNA polymerase on the basis of its ability to incorporate acyclovir into DNA (31). Most of the antiherpes drugs now in use or under investigation are nucleoside or nucleotide analogs that are incorporated into viral DNA or that resemble analogs that are known to be so incorporated. The best studied of these are acyclovir, adenine arabinoside, cytosine arabinoside, and iododeoxyuridine. d2apTP is mutagenic when incorporated into DNA because it forms a weak base pair with cytosine as well as thymine (29). In spite of the fact that most of these analogs are inhibitory to viral replication, we must consider the possibility that small but significant numbers of mutagenized virus arise when such drugs are used in infected cell systems or live animals. Such mutagenized herpesviruses could be detected in the form of drug-resistant viral strains or be present as other less readily detectable mutant forms.

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