Interaction of Epstein-Barr Virus DNA Polymerase and 5'-Triphosphates of Several Antiviral Nucleoside Analogs

JWO-FARN CHIOU AND YUNG-CHI CHENG*

Department of Pharmacology and Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

Received 29 August 1984/Accepted 6 December 1984

Epstein-Barr virus (EBV), a member of the herpesvirus family, is recognized as an important human pathogen and is closely associated with certain human malignancies (10, 11, 15). Thus, it would be useful to develop selective anti-EBV compounds. This approach taken in this laboratory is to design compounds based on the unique properties of virus-specified enzymes (2, 5). EBV is known to induce its own specific DNA polymerase (8, 14, 18). The properties of this EBV-specified enzyme are in many ways similar to the properties of herpes simplex virus (HSV) DNA polymerase, which are different from those of human DNA polymerases (1, 8, 9, 14, 19). It is logical, therefore, to consider EBV DNA polymerase as a target for developing anti-EBV agents.

Acyclovir (ACV) has been shown to have potent anti-EBV activity, although the action was readily reversible upon removal of ACV from the culture medium (6, 7). The action of ACV against EBV replication was suggested to be due to inhibition of EBV DNA polymerase by ACV-triphosphate (ACVTP), which is a potent inhibitor that competes with dGTP for binding to EBV DNA polymerase (7). In addition to the ability to be preferentially phosphorylated by HSV-specified thymidine kinase, several triphosphates of anti-HSV nucleoside analogs have higher binding affinities to HSV type 1 (HSV-1) and HSV-2 DNA polymerase than to human enzymes (3, 9, 12, 13), which could contribute to their selectivity of action against HSV. Based on the similarity of herpesvirus DNA polymerases, it is conceivable that some of these anti-HSV nucleoside analogs also have a selective anti-EBV activity, possibly caused by preferential interaction of EBV DNA polymerase with their triphosphate metabolites. Thus, we examined the effect of several anti-HSV nucleoside analogs against EBV replication in culture. 1-(2'-deoxy-2'-fluoro-5'-triphosphates of some compounds were examined. The enzyme used was highly purified according to procedures described previously by us to a specific activity of more than 4.0 × 10^5 U/mg of protein (20). The preparations of FMAUTP, FIACTP, FMACTP, DHPGTP, and ACVTP were previously published (13, 19). The results are shown in Table 1. It was found that FMAUTP, FIACTP, FMACTP, DHPGTP, and ACVTP could inhibit the incorporation of dTTP, dCTP, dGTP, and dGTP, respectively, into DNA templates catalyzed by EBV DNA polymerase. The Ki values for EBV DNA polymerase were lower than those for human a and b DNA polymerases (9, 13, 19). Our results with ACVTP were similar to those reported previously (Ki value of 15 nM) (7), whereas the results with FIACTP were significantly different from the Ki value of 32.2 μM reported by Allaudeen et al. (1). It is unclear why there was such a difference. The observation that Ki values for these compounds were lower against EBV DNA polymerase than against human DNA polymerase is consistent with our hypothesis and could be part of the reason for their selective anti-EBV action. The efficiency of triphosphate metabolite formation from these analogs in EBV-carrying cells is under investigation. To further investigate the action of these analogs on the EBV DNA polymerase-catalyzed reaction, we performed studies to examine whether each of these analogs could substitute for its competing deoxynucleoside triphosphate (dNTP), which is required for support of DNA chain elongation. These results are shown in Fig. 1. The drug or deoxynucleotide used was present at a concentration fivefold higher than the Km or Ki value described in Table 1. FMAUTP, FIACTP, and FMACTP could partially substitute for their competing deoxynucleotides in DNA chain elongation. This suggested that these analogs could be incorporated; however, the rate of further chain elongation was slow relative to the control rate. The incorporation of FIAC into DNA by HSV-1 or HSV-2 DNA polymerase was reported previously (1, 19). DHPGTP or ACVTP, instead of stimulating DNA synthesis in the absence of dGTP, inhibited the DNA chain elongation catalyzed by EBV DNA polymerase. These results are similar to those obtained when the interaction of DHPGTP or ACVTP with HBV DNA polymerase was examined (9, 13). These results suggest that the mode of action of both compounds with EBV DNA polymerase is the same as with HSV DNA polymerase. Both ACVTP and DHPGTP can be incorporated into the DNA template; however, the DHPG-
TABLE 1. Inhibition constants of analog dNTPs for EBV DNA polymerase

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_a$ (µM) of competing substrate</th>
<th>$K_i$ (µM)</th>
<th>$K_a/K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMAUTP</td>
<td>0.64 ± 0.13 (dTTP)</td>
<td>0.08 ± 0.005</td>
<td>8.0</td>
</tr>
<tr>
<td>FIACTP</td>
<td>0.52 ± 0.04 (dCTP)</td>
<td>0.95 ± 0.07</td>
<td>1.5</td>
</tr>
<tr>
<td>FMACTP</td>
<td>0.51 ± 0.04 (dCTP)</td>
<td>0.9 ± 0.14</td>
<td>0.6</td>
</tr>
<tr>
<td>DHPGTP</td>
<td>0.58 ± 0.08 (dGTP)</td>
<td>0.08 ± 0.02</td>
<td>7.3</td>
</tr>
<tr>
<td>ACVTP</td>
<td>0.58 ± 0.08 (dGTP)</td>
<td>0.007 ± 0.002</td>
<td>82.9</td>
</tr>
</tbody>
</table>

* Analog dNTPs were prepared as described previously (13, 19). $K_a$ values were determined from Lineweaver-Burk plots with five different concentrations of variable dNTP. $K_i$ values were determined by replotted Lineweaver-Burk plots which employed three drug concentrations. All compounds were assayed concurrently against radioactive competing substrates in amounts 0.3 to 2.0 times the $K_a$. Noncompeting dNTPs were present at 170 µM each. The assay conditions were as described previously (9), except that 150 mM KCl was added instead of 200 mM. Purified enzyme (0.16 U) was used for each assay. The assays had a reaction time of 20 min at 37°C. One unit of enzyme activity catalyzed the incorporation of 1 pmol of dATP per min in the absence of inhibitor.

terminated DNA is poorly elongated, whereas the ACV-terminated DNA is not elongated at all. In addition, ACV- or DHPG-terminated DNA could interfere with the DNA polymerase action by competing with unsubstituted DNA templates. Obtaining final proof of this hypothesis will require an analog with high radiospecificity.

In summary, these analogs are preferentially incorporated into DNA by EBV DNA polymerase, which could be part of the mechanism responsible for their selective anti-EBV action. Since ACV was incorporated only at the termini of growing DNA chains, whereas FMAU, FIACT, FMAC, and DHPG were incorporated in both terminal and internal positions, this could be part of the reason why the anti-EBV actions of FMAU, FIACT, and DHPG are less reversible than that of ACV (4, 17).

![Figure 1](http://aac.asm.org/)

**FIG. 1.** Effect of analog dNTPs on limited DNA synthesis catalyzed by EBV DNA polymerase. Synthesis of DNA by EBV DNA polymerase was assayed by using conditions identical to those described in Table 1, except that one dNTP (dCTP, dGTP, or dTTP) was omitted. Reaction mixtures contained 10 µM [3H]dATP (2 µCi/nmol), two other dNTPs at 100 µM each, and 0.16 U of EBV DNA polymerase. The effect of adding back the missing dNTP at a concentration fivefold above the $K_a$ value or adding a dNTP analog at a concentration fivefold above the $K_a$ value was examined. Symbols: (A) ○, no dTTP or analog added; ●, dTTP added; △, FMAUTP added; (B) ○, no dCTP or analog added; ●, dCTP added; △, FIACTP added; △, FMACTP added; (C) ○, no dGTP or analog added; ●, dGTP added; △, DHPGTP added; △, ACVTP added.

This work was supported by grant CA-19014 from the National Cancer Institute and by a gift from Syntex Laboratories, Inc., Palo Alto, Calif.

LITERATURE CITED


