Herpes Simplex Virus-specified DNA Polymerase Is the Target for the Antiviral Action of 9-(2-Phosphonomethoxyethyl)adenine*

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9-(2-Phosphonomethoxyethyl)adenine (PMEA) is a new antiviral compound with activity against herpes simplex virus (HSV) and retroviruses including human immunodeficiency virus. Although it has been suggested that the anti-HSV action of PMEA is through inhibition of the viral DNA polymerase via the diphosphorylated metabolite of PMEA (PMEApp), no conclusive evidence for this has been presented. We report that in cross-resistance studies, a PMEA-resistant HSV variant (PMEA-1) was resistant to phosphonoformic acid, a compound which directly inhibits the HSV DNA polymerase. In addition, phosphonoformic acid-resistant HSV variants with defined drug resistance mutations within the HSV DNA polymerase gene were resistant to PMEA. Furthermore, the HSV DNA polymerase purified from PMEA-1 was resistant to PMEApp in comparison with the enzyme from the parental virus. Moreover, PMEA inhibited HSV DNA synthesis in cell culture. These results provide strong evidence that HSV DNA polymerase is the major target for the antiviral action of PMEA. Further studies showed that HSV DNA polymerase incorporated PMEApp into DNA in vitro, while the HSV polymerase-associated 3'-5' exonuclease was able to remove the incorporated PMEA. Thus, the inhibition of HSV DNA polymerase by PMEApp appears to involve chain termination after its incorporation into DNA.

Acquired immunodeficiency syndrome (AIDS)† has focused attention on the need for new and efficacious compounds that not only inhibit HIV, the etiological agent of AIDS, but also that are effective against the many opportunistic infections associated with AIDS. One such opportunistic infection is herpes simplex virus. Although many individuals are infected with herpes simplex virus, the disease is manifested to a much greater extent in AIDS patients.

Several new compounds have been synthesized by Holy and Rosenberg (1–3) that show potent antiviral activity against a wide spectrum of viruses. Fig. 1A shows the structure of one of these new compounds, PMEA. PMEA is an adenine nucleotide analog that contains an acyclic phosphonomethoxyethyl moiety in place of the phosphorolylated sugar (3). The phosphonate group is designed to be resistant to phosphatases (1, 3) and allows the drug to be metabolized, at least in vitro, to mono- and diphosphate forms in the absence of viral thymidine kinase (4). The diphosphorylated form of PMEA, which is analogous to a nucleotide triphosphate, is an inhibitor of HSV-specified DNA polymerase in vitro (5). Since the antiviral activity of PMEA does not depend on activation by viral thymidine kinase, it is effective against viruses that lack thymidine kinase (6). This is in contrast to other compounds, such as acyclovir, which require phosphorylation by viral thymidine kinase to achieve antiviral activity (7).

PMEA was reported to be active against several herpes viruses including HSV-1 and HSV-2 in cell culture (6, 8) and in mice (8) and Epstein-Barr virus in the virus-producing P3HR-1 and latently infected Raji cell models (9). In addition, PMEA has been reported to be effective against several retroviruses including HIV in cell culture systems (6, 10), Moloney murine sarcoma virus in cell culture (10) and in mice (11), and the LP-BM5 retrovirus in a murine acquired immunodeficiency syndrome model (12). Therefore, PMEA may have potential for use as an antiviral against HSV, Epstein-Barr virus, or HIV.

Because the diphosphorylated metabolite of PMEA, PMEApp, is a potent inhibitor of HSV DNA polymerase in vitro, it was suggested by Berta et al. (5), that HSV DNA polymerase is a possible target for the antiviral activity of PMEA. However, no conclusive evidence for this has been presented.

In view of the unique features of PMEA including its spectrum of activity and structure, the mechanism of the antiviral action of PMEA is of considerable interest. The present study was aimed at elucidating the target for the anti-HSV-1 action of PMEA. To this end, a PMEA-resistant HSV-1 strain denoted PMEA-1 was examined. PMEA-1 was cross-resistant to PFA, a compound which directly inhibits HSV DNA polymerase. In addition, PFA-resistant mutants with defined drug resistance mutations within the HSV DNA polymerase gene were cross-resistant to PMEA. Moreover, studies of the purified DNA polymerase from PMEA-1 confirmed that the enzyme was resistant to PMEApp and PFA. Furthermore, PMEA inhibited HSV DNA synthesis in infected cells. These results provide strong evidence that the target for the antiviral action of PMEA is the HSV DNA polymerase. Additional experiments showed that PMEApp
was incorporated into and removed from DNA by HSV DNA polymerase in vitro.

MATERIALS AND METHODS

Compounds—PMEA, PMEAp, PMEApp, HPMPA, HPMPApp, and ACGTP were generously provided by A. Holy and I. Rosenberg from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia. Acyclovir was a gift from Thomas Spector, Wellcome Research Laboratories, Research Triangle Park, NC. PFA was purchased from Sigma. Aphidicolin was obtained from the National Cancer Institute.

Cells and Viruses—HeLa S3 (ATCC CCL 2.2) and Vero (ATCC CCL 80) cells, originally from the American Type Culture Collection, Rockville, MD, were maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum and 100 μg/ml kanamycin in a 37 °C incubator with 5% CO2. The viruses used included HSV-1 strain KOS and HSV-2 strain 333. PMEA was a gift from V. Vonka, Czechoslovak Academy of Sciences, Prague, Czechoslovakia.

Preparation of Crude Extracts—Cells were harvested by scraping, rinsed with phosphate-buffered saline, and resuspended in extraction buffer which contained 0.3 M sodium phosphate, pH 7.5, 10% glycerol, 1 mM EDTA, 2 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. The samples were frozen and thawed three times followed by sonication and pelleting of the cell debris at 12,000 x g for 6 min. The resulting supernatant fraction was used for electrophoresis and DNA polymerase activity analysis.

Enzyme Assays—The HSV DNA polymerase standard reaction mixture contained 50 mM Tris-HCl, pH 8.0, 5 mM MgCl2, 0.5 mM dithiothreitol, 0.5 μg/ml heparin, and 100 μM dATP, dCTP, and dGTP, 5 μM [3H]dATP (ICN) at 2 Ci/mmol in a volume of 50 μl. For analysis of crude extracts, column fractions, and the determination of enzyme units, the KC1 was at 200 mM and the labeled substrate was [3H]dATP (ICN). Following the reaction at 37 °C for 15–30 min, 40–50 μl samples were spotted onto GF/A disks (Whatman) for the determination of thioracil-acetic acid insoluble radioactivity. One unit is defined as the incorporation of 1 pmol dATP per min into acid-insoluble material.

The assay for HSV DNA polymerase-associated 3′-5′ exonuclease was performed as described previously (22). The reaction mixture contained the same components as the HSV DNA polymerase assay in a volume of 0.2 ml except that the deoxynucleotides were omitted and the activated DNA was replaced with 10 μg/ml of 3′ terminally labeled activated calf thymus DNA (6 x 107 cpm/μg). The labeled DNA was prepared essentially as described (23) with [3H]dATP as the labeled nucleotide.

Protein concentrations were determined by using the Bio-Rad protein assay (Bio-Rad).

Polycyclamide Gel Electrophoresis and Western Blotting—SDS-polyacrylamide gel electrophoresis was performed essentially as described (16, 18). The assay for HSV DNA polymerase-associated 3′-5′ exonuclease was performed as described previously (22). The reaction mixture contained the same components as the HSV DNA polymerase assay in a volume of 0.2 ml except that the deoxynucleotides were omitted and the activated DNA was replaced with 10 μg/ml of 3′ terminally labeled activated calf thymus DNA (6 x 107 cpm/μg). The labeled DNA was prepared essentially as described (23) with [3H]dATP as the labeled nucleotide.

Protein concentrations were determined by using the Bio-Rad protein assay (Bio-Rad).

In Situ Lysis Agarose Gel Electrophoresis—In situ lysis agarose gel electrophoresis was a modification of a previous procedure (26). The body of the gel was 1% agarose while the gel surrounding the wells was 0.5% agarose and contained 2% SDS and 1 mg/ml protease K (Boehringer Mannheim). The gel and the electrophoresis buffer contained 0.3 M sodium phosphate, pH 7.5. The gel was placed into a CHEF-DR II pulsed field electrophoresis chamber (Bio-Rad) with the electrophoresis buffer. Approximately 1.5 x 106 cells, which were suspended in TBE with 15% Ficoll, 0.8 mg/ml RNase A, and 0.01% bromphenol blue were loaded into the wells. After 30 min, electrophoresis was started at 15 V. Two hours later, the voltage was increased to 150 V. The buffer was circulated through a water bath to maintain approximately 16 °C in the electrophoresis chamber. The total run time was 18 h with an A:B switching ramp of 60 to 90 s. Following electrophoresis, the DNA was stained with ethidium bromide and transferred to a Hybond-N nylon membrane (Amersham, Hungary) by using a vacuum blot apparatus (American Bioarrays, Hayward, CA) according to the manufacturer’s instructions. The blot was probed for HSV DNA with EcoR I fragment B (containing standard techniques (18)). After autoradiography with Kodak X-omat-RF film (Sigma), the bands were excised from the blot for scintillation counting.

Homogenization procedure—The homogenization procedure was performed essentially as described (20). The labeling kit was used recommended by the manufacturer.

Purification of HSV DNA Polymerase—HSV DNA polymerase was purified from HSV-infected HeLa S3 cells by sequential DEAE-, phospho-, and native DNA-cellulose chromatography as previously described (14).
PMEApp were added or omitted as indicated. The reaction volume was 25 μl. After a 30-min incubation at 37 °C, the reaction was terminated by the addition of 12.5 μl of 50 mM EDTA, 0.025% bromphenol blue in deionized formamide; the samples were placed in boiling water for 3 min and then placed on ice. Five microliters were loaded per lane on a 15% polyacrylamide/urea sequencing gel. After electrophoresis, autoradiography was performed with Kodak X-Omat-RP film.

PMEApp-terminated primer was generated by running a 50-μl reaction in which PMEApp was added at 50 μM in place of dATP. Ten units of Klenow enzyme (Boehringer Mannheim) was used as the DNA polymerase. This resulted in the extension of the original 17-base primer by 2 nucleotides with PMEA as the terminal nucleotide. The terminated reaction was run on a preparative sequencing gel, and the PMEA-terminated primer was excised and purified as described (28). The resulting primer was reannealed to single-stranded M13mp19 DNA and used in exonuclease assays. The exonuclease reaction mixture was the same as the primer extension reaction except that the deoxynucleotides were omitted and the M13 template-primer PMEApp were added or omitted as indicated. The reaction volume was 240 μl, and the PMEA-terminated primer was excised and purified as described (28). The value of KOS was 80 and 150 μM, respectively (Fig. 1B). These values are somewhat higher than those reported previously (8). This discrepancy may be due to different methodology or cell lines used.

RESULTS

**Sensitivity of HSV to PMEA**—Virus yield experiments in HeLa S3 cells showed that both HSV-1 and HSV-2 were inhibited by PMEA with IDₙ₀ values of 80 and 150 μM, respectively (Fig. 1B). These values are somewhat higher than those reported previously (8). This discrepancy may be due to different methodology or cell lines used.

**Cross-resistance Studies**—In virus yield assays, PMEA'⁻¹ was found to be highly resistant to PMEA, PFA, and acyclovir (Table I). Cross-resistance of PMEA' to acyclovir has been reported previously (13). Because PFA and acyclovir exert their antiviral action on the HSV DNA polymerase, these results suggest that PMEA resistance may be associated with alterations in the DNA polymerase. Consistent with this hypothesis, three independent PFA-resistant variants of HSV-1 (KOS), PFA'⁻¹, PFA'⁻², and PFA'⁻⁵ (14) were also resistant to PMEA (Table I). Drug resistance in each virus was associated with specific nucleotide substitutions within the HSV DNA polymerase gene (29). Thus, these results suggest that HSV DNA polymerase is the likely target for the antiviral action of PMEA.

In addition, Table I shows that PMEA'⁻¹ was more sensitive to aphidicolin. A previous report indicated that a common phenotype for HSV-1 mutants resistant to acyclovir with aphidicolin (30). Thus, it is possible that collateral sensitivity to aphidicolin may be a common phenotype for PMEA-resistant mutants.

A previous report indicated that PMEA' was more sensitive to HPMPA than was KOS (13). This is somewhat surprising since HPMPA differs from PMEA only by having a methoxy group on the alkyl side chain (3). However, our results with PMEA'⁻¹ confirmed that PMEA resistance was associated with HPMPA hypersensitivity (Table I).

| Virus | IDₙ₀ values as determined by virus yield assay |
|-------|---------------------------------------------|
|       | PMEA | PFA | Acyclovir | HPMPA | Aphidicolin |
|-------|------|-----|-----------|-------|-------------|
| KOS   | 80   | 160 | 14        | 50    | 4           |
| PMEA'⁻¹ | >2000 | 3000 | 380       | 14    | 0.1         |
| PFA'⁻¹ | 520  | 900 | 100°      | ND    | hs°         |
| PFA'⁻² | >1000 | >1000 | 100°     | ND    | hs°         |
| PFA'⁻⁵ | 370  | 830 | 78°       | ND    | hs°         |

ₙ From Ref. 16.
° ND, not determined.
° hs, hypersensitive. From Ref. 30, plaque reduction assay.

**Effect of PMEA on HSV DNA and Protein Synthesis**—Since the cross-resistance study suggested that HSV DNA polymerase was the target for the antiviral action of PMEA, HSV DNA synthesis in HSV-infected HeLa S3 cells treated for 8 h with different concentrations of PMEA was examined to see whether HSV DNA synthesis was blocked. For the HSV DNA analysis, whole HSV-1 (KOS)-infected cells were harvested and subjected to in situ lysis agarose gel electrophoresis as described under "Materials and Methods." After electrophoresis, the DNA was transferred to a nylon membrane and probed with HSV-1 EcoRI F fragment from plasmid pSG18. There was a dose-dependent decrease in the content of HSV DNA, indicating that PMEA interfered with HSV DNA synthesis (Fig. 2A). The decrease in the HSV DNA content correlated with the decrease in virus yield (Fig. 2B).

HSV protein synthesis as determined by Western blot analysis with anti-HSV-1 antibody, was affected very little under the same conditions used for the DNA synthesis experiment (Fig. 3A). While most of the proteins were not affected by the PMEA treatment, some of the protein bands appear to be reduced by the PMEA treatment. These bands most likely represent late proteins whose expression is decreased by blocking DNA synthesis (31). In addition, the activity of HSV DNA polymerase recovered in crude extracts from virus-infected cells was not changed appreciably by the PMEA treatment (Fig. 3B). Thus, while PMEA inhibited HSV DNA synthesis, the induction of HSV proteins as a whole was not significantly impaired. These results are consistent with the hypothesis that PMEA exerts its antiviral action by inhibiting the HSV DNA polymerase.

**Analysis of Purified HSV DNA Polymerase**—Since the previous results indicated that the HSV DNA polymerase was the likely target for the antiviral action of PMEA, the purified DNA polymerases induced by KOS and PMEA'⁻¹ were tested for sensitivity to the various inhibitors.

Fig. 4A shows that DNA polymerase from KOS was sensitive to inhibition by PMEApp, whereas the PMEA'⁻¹ enzyme...
HSV DNA Polymerase Is the Antiviral Target of PMEA

Fig. 3. Induction of HSV proteins in HeLa S3 cells treated with PMEA. Cells were infected with 3 plaque-forming units/cell of HSV-1 (KOS) and treated with PMEA for 8 h. Crude extracts then were prepared and used for SDS-PAGE with 50 μg of protein per lane followed by transfer to a nitrocellulose filter and immunostaining (A) or HSV DNA polymerase assays (B). HSV DNA polymerase activity was normalized for the protein content of the extracts. The bars indicate standard deviation for triplicate assays. Lanes 1–5, cells were treated with 0, 10, 25, 75, or 250 μM PMEA, respectively; M, mock-infected cells not treated with PMEA.

was resistant. Similarly, the PMEA'-1 DNA polymerase was resistant to PFA and ACGTP by at least 10-fold in comparison to KOS (Fig. 4, B and C). Although the PMEA'-1 virus was more sensitive to HPMPA, the purified PMEA'-1 DNA polymerase was somewhat resistant to HPMPApp (Table II). PMEA itself and PMEAp had little or no effect on the HSV DNA polymerase with at least 85–90% of activity at 10 μM concentration of either compound (data not shown).

In view of the resistance of PMEA'-1 DNA polymerase to PMEA, a more detailed kinetic study was performed. Kinetic measurements indicated that PMEA inhibition was competitive with dATP. This is consistent with another report (5). The K_0 of the PMEA'-1 enzyme for dATP was increased approximately 5.6-fold along with a 97-fold increase in the K_0 for PMEA in comparison to KOS (Table II). The net result was that the K_0/K_0 ratio for the KOS DNA polymerase was approximately 17-fold higher than for PMEA'-1 DNA polymerase. Thus, the enzyme data correlate well with the cell culture data and further support the idea that the HSV DNA polymerase is the target for PMEA via the diphosphorylated metabolite of PMEA.

In addition to HSV DNA polymerase activity, PMEA, PFA, and ACGTP were tested for their ability to inhibit the polymerase-associated 3'-5' exonuclease activity. As with the DNA polymerase, 3'-5' exonuclease was inhibited by PMEA, PFA, and ACGTP with the PMEA'-1 enzyme being resistant in each case compared to the parental KOS enzyme (Fig. 5). Again, PMEA itself and PMEAp did not inhibit the exonuclease at concentrations up to 10 μM (data not shown). Also, it was noted that PMEA'-1 DNA polymerase had a 3- to 4-fold higher ratio of exonuclease to polymerase activity than the KOS enzyme (data not shown).

Incorporation of PMEA into DNA—Since the above results indicated that HSV DNA polymerase was the target for the antiviral action of PMEA, it was of interest to determine the mode of interaction between PMEA and HSV DNA polymerase. Specifically, primer extension analysis was used to determine whether HSV DNA polymerase was capable of incorporating PMEA into DNA. The template for these reactions was single-stranded M13mp19 DNA, and the primer was a 5'-32P-labeled 17-base oligonucleotide with the sequence 5'-GTAAAACGACGGCCAGT. Fig. 6A shows the result of this analysis. Lanes 1 and 2 are control reactions without PMEA, showing the addition of nucleotides to the primer. The enzyme appears to add the first 11 nucleotides in a distributive fashion followed by a more processive polymerization. In reactions with the same 17-nucleotide primer but with M13mp18 as the template, the pattern of DNA polymerization in general was more processive (not shown). Thus, the pattern of DNA polymerization seems to depend on the primer length, sequence of

| Virus   | dATP K_0 μM | PMEA K_0 μM | HPMPA K_0 μM |
|---------|-------------|-------------|--------------|
| KOS     | 0.23 ± 0.02 | 0.02 ± 0.13 | 10.5         |
| PMEA'   | 1.30 ± 0.05 | 2.13 ± 1.14 | 0.61         |

* Kinetics constants were determined from Lineweaver-Burk plots and replots of slope vs. V (Fig. II). Each value represents the mean of three determinations ± S.D.

TABLE II

Kinetic constants for purified HSV DNA polymerases

| Virus   | dATP K_0 μM | PMEA K_0 μM | HPMPA K_0 μM |
|---------|-------------|-------------|--------------|
| KOS     | 0.23 ± 0.02 | 0.02 ± 0.13 | 10.5         |
| PMEA'   | 1.30 ± 0.05 | 2.13 ± 1.14 | 0.61         |
template, and enzyme. Lane 3 is a misincorporation control without dATP or PMEApp. Lane 4 contains PMEApp in place of dATP and shows the incorporation of PMEApp at the end of the DNA. The faster migration of PMEA-terminated product compared to the dAMP-terminated product (lane 1) is expected based on the structure of PMEA, which does not have a complete sugar moiety.

Because HSV DNA polymerase was capable of incorporating PMEApp into DNA, it was of interest to determine whether the enzyme could remove the PMEA from the end of the DNA by the polymerase-associated 3'-5' exonuclease activity toward the 3'-5' exonuclease activity with the [32P]dTPP-labeled calf thymus DNA substrate. We are currently investigating these possibilities. This experiment also illustrates the increase in the exonuclease to polymerase ratio for the PMEA-1 enzyme, yet the exonuclease activity as reflected in the gel is very similar.

**DISCUSSION**

PMEA has recently been evaluated for its antiviral effects against several types of viruses. PMEA was active against herpes simplex virus, retroviruses including HIV, and Epstein-Barr virus. Thus, PMEA could be useful in AIDS treatment for both the underlying HIV infection and some of the common opportunistic infections (12). Whether PMEA will be effective and safe in the treatment of human disease remains to be determined.

The mechanism of the antiviral action of PMEA has not been reported to date. A report by Vonka et al. (13) indicated
that PMEA′ was cross-resistant to acyclovir and araT. These results suggested the possibility that HSV DNA polymerase could be involved in PMEA resistance. However, since the activation of acyclovir and araT by viral thymidine kinase is required for antiviral activity, cross-resistance to these compounds is not necessarily due to HSV DNA polymerase alterations. Thus, PMEA′-1 was tested for cross-resistance to PFA. A body of evidence indicates that the antiviral target of PFA is the HSV DNA polymerase (for example Refs. 14 and 32). Furthermore, PFA does not require metabolic activation to achieve antiviral activity. Therefore, alterations that affect PFA sensitivity are very likely to involve HSV DNA polymerase itself. PMEA′-1 was found to be cross-resistant to PFA, which provides evidence that HSV DNA polymerase is involved in PMEA resistance.

In addition to testing PMEA′-1 for cross-resistance to PFA, several PFA-resistant mutants were tested for cross-resistance to PMEA. Each PFA-resistant mutant was cross-resistant to PMEA. These results provide additional evidence that HSV DNA polymerase is the likely target for PMEA since drug resistance for each PFA-resistant mutant has been mapped to specific nucleotide substitutions within the HSV DNA polymerase gene (29).

Since the cross-resistance data implicated HSV DNA polymerase in PMEA resistance, PMEApp was tested for its ability to inhibit HSV DNA replication in cell culture. The DNA synthesis experiment demonstrated that HSV DNA synthesis is largely unaltered, inhibition of DNA synthesis is likely to be a primary effect of PMEA. In addition, the decrease in HSV DNA correlated with the decrease in virus yield.

In order to determine whether the HSV DNA polymerase was indeed altered in PMEA′-1, the DNA polymerase was purified from HSV-1 (KOS) and PMEApp. A previous report indicated that PMEApp was a potent inhibitor of HSV DNA polymerase (5). When tested for sensitivity to PMEApp, the PMEApp′-1 enzyme was quite resistant compared to the KOS enzyme. The PMEApp′-1 enzyme was also resistant to PFA and ACGTP, in agreement with the cell culture data and confirming that the PMEApp′-1 DNA polymerase was indeed altered. These results provide an additional level of evidence that HSV DNA polymerase is the target for the antiviral activity of PMEA.

In addition to cross-resistance to PFA, PMEApp′-1 showed collateral sensitivity to aphidicolin. Collateral sensitivity of PFA-resistant mutants to aphidicolin has been previously reported, and it was suggested that combination chemotherapy with PFA and aphidicolin could be considered in HSV infections (30). In view of the common characteristics of PFA- and PMEApp-resistant HSV-1, the same reasoning may apply to the combination of PMEApp and aphidicolin. However, a study of several independently derived PMEApp-resistant mutants would be necessary to substantiate this point further.

It is important to note that our results do not exclude the possibility that other actions of PMEA may contribute to its antiviral activity. For example, PMEApp′-1 was reported to be an inhibitor of HSV ribonucleotide reductase (33). However, we conclude that HSV DNA polymerase is the primary target for PMEA.

Since these studies focused on HSV-1, the conclusions must be restricted to HSV-1. Thus, although the target of PMEA against HSV-2 is likely to be the HSV-2 polymerase, this remains to be confirmed.

In terms of the mechanism of PMEApp inhibition of HSV DNA polymerase, the primer extension analysis demonstrated that PMEApp is a substrate for HSV DNA polymerase in vitro and is incorporated at the end of the DNA strand. Since PMEApp does not have a 3′ hydroxyl group from which the DNA chain could be elongated, the addition of PMEApp to the DNA marks the termination of DNA strand elongation. Therefore, the exonuclease results indicate that the HSV DNA polymerase-associated 3′-5′ exonuclease activity is able to remove incorporated PMEApp. Thus, if the same processes occur inside the cell, PMEA would be continually misincorporated and removed by the HSV DNA polymerase resulting in inhibition of HSV DNA synthesis. Whether incorporation and removal occurs in the DNA of uninfected cells is not known, although cellular polymerases β and γ to a lesser extent α appear to be less susceptible to inhibition by PMEApp (5).

Finally, it is noteworthy that PMEA′-1 was more sensitive to HPMPA compared to KOS. This result is in agreement with Vonka et al. (13). Also, the difference in HPMPA sensitivity between KOS and PMEA′-1 was not reflected in the sensitivity of the purified polymerases to HPMPA. Indeed, the PMEA′-1 DNA polymerase was somewhat resistant to HPMPA. These results raise the possibility that HPMPA may have a different mechanism of action than PMEA. An independent study by Vonka et al. (13) led to a similar suggestion. We are currently investigating this possibility.

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