JOURNAL OF VIROLOGY, Nov. 1989, p. 4913–4918
0022-538X/89/114913-06$02.00/0

Vol. 63, No. 11

In Vitro Mutagenesis of the Herpes Simplex Virus Type 1 DNA Polymerase Gene Results in Altered Drug Sensitivity of the Enzyme

JAMES T. MATTHEWS, ROBERT D. CARROLL, JOHN T. STEVENS, AND MARY L. HAFFEY*

Department of Virology, The Squibb Institute for Medical Research, Princeton, New Jersey 08543-4000

Received 20 April 1989/Accepted 20 July 1989

A mutation (asparagine 815 to serine 815) was introduced into the herpes simplex virus type 1 (HSV-1) DNA polymerase (pol). The HSV-1 pol enzyme in lysates of *Saccharomyces cerevisiae* cells expressing the mutant protein showed increased resistance to acyclovir triphosphate and increased sensitivity to phosphonoacetate but was not substantially altered with respect to sensitivity to phosphonoformate or aphidicolin. These results directly demonstrate that both resistance to acyclovir triphosphate and sensitivity to phosphonoacetate can be conferred by this mutation in the absence of other viral factors and that the yeast expression system can be used for structure-function studies on HSV-1 pol.

The herpes simplex virus type 1 (HSV-1) DNA polymerase (pol) is essential for viral replication and is an effective target for antiviral drug therapy (3, 4, 7, 27). Recent work from a variety of laboratories is aimed at understanding the relationship between the structure of the viral pol and its enzyme activity. Comparative protein sequence analysis of an increasing number of DNA pol enzymes has predicted regions of the enzyme involved in substrate interactions and pol activity (12, 13, 26, 32, 33). DNA sequence analysis of temperature-sensitive and drug-resistant HSV-1 mutants has identified specific point mutations in the pol gene, and marker rescue experiments have shown that mutations in the pol gene confer altered drug resistance or temperature-sensitive phenotypes (4, 8, 16, 17, 18, 31).

Recently, the construction of a *Saccharomyces cerevisiae* expression system which produces an active form of the HSV-1 (strain 17) DNA pol was reported (14). In the present study, a specific point mutation was introduced into the yeast–HSV-1 pol expression vector, resulting in a predicted change in the reported protein sequence (13, 28) at residue 815, converting asparagine to serine. This mutation was chosen because an identical mutation in the DNA pol gene had been reported by Larder et al. (22) in three different HSV-1 (strain SC16) mutants selected for resistance to acyclovir (ACV). The three mutants (TP4.4, TP4.1, and TP3.2) which they isolated exhibited various degrees of resistance to ACV (59- to 233-fold) and aphidicolin (APH) (2.5- to 4.3-fold) as well as various degrees of increased sensitivity to phosphonoacetate (PAA) (2- to 5-fold) and phosphonoformate (PFA) (1.4- to 5.2-fold) in plaque reduction assays (20–22). Those authors had demonstrated by marker rescue that ACV resistance could be transferred by a DNA fragment containing the mutated HSV-1 pol gene. In an earlier report (20), the HSV-1-specific DNA pol in lysates of cells infected by each of the three mutants was shown to exhibit increased resistance to ACV triphosphate (ACV-TP) and hypersensitivity to PAA. However, a direct relationship between PAA hypersensitivity and the mutation at residue 815 of the HSV-1 pol gene was not demonstrated. To explain the variations in drug resistance and sensitivity seen with the three mutants, Larder et al. (22) proposed that mutations in genes other than pol could be responsible for modulating the sensitivity of the viruses to ACV and/or PAA.

In the present study, the effects of the mutation on HSV-1 pol enzyme activity were evaluated in the absence of other viral gene products, and the sensitivity of the mutant DNA pol enzyme to APH (11) and PFA (10) was examined.

The original vector, pMH202 (14), was modified to contain HSV-1 (strain KOS) pol sequences, yielding pRC203 (Fig. 1), so as to be consistent with ongoing biochemical studies with HSV-1 (KOS) in our laboratories. The chimeric pol gene encodes a protein which differs from the predicted HSV-1 (KOS) sequence at only one residue, serine 33 instead of glycine 33. To produce a vector with a unique *Pst*I restriction site for engineering mutations, we further modified pRC203 by deleting sequences downstream of the HSV-1 DNA in the multiple cloning site between *Xba*I and *Hin*dIII, creating pRC205 (not shown). A synthetic oligonucleotide, 5′-CCCGTACACCGAGCTACACACGACC-3′, was prepared by using an Applied Biosystems 380B DNA synthesizer and purified by chromatography on oligonucleotide purification cartridges (Applied Biosystems Product Bulletin, January 1988). The synthetic oligonucleotide was designed to create the exact mutation previously reported (22), changing residue 815 from asparagine to serine (Fig. 1). To facilitate mutagenesis, we subcloned a 1,200-base-pair *Sst*I-*Pst*I fragment of HSV-1 (KOS) DNA from the center of the pol gene into M13mp18 (34). Uracil-containing single-stranded bacteriophage DNA was prepared from this vector by using *Escherichia coli* CJ236 (Bio-Rad Laboratories); the synthetic oligonucleotide was used to prime second-strand DNA synthesis, and the double-stranded DNA product of this reaction was used to transform *E. coli* XL-1 Blue (Stratagene) as described by Kunkel (19). Phage were isolated from the resulting plaques, and phage DNA was screened for introduction of the correct mutation by DNA sequencing by the dideoxy chain termination method (29). A 1,200-base-pair *Sst*I-*Pst*I fragment of DNA from the M13mp18 vector containing the correct HSV-1 pol mutation and an 11-kilobase-pair *Sst*I-*Pst*I fragment from pRC205 were purified from a 1% agarose gel by standard methods (23). Both fragments were joined by using T4 DNA ligase to produce mutant pRC206.

Plasmid DNAs from pRC203 (wild type) and pRC206

* Corresponding author.

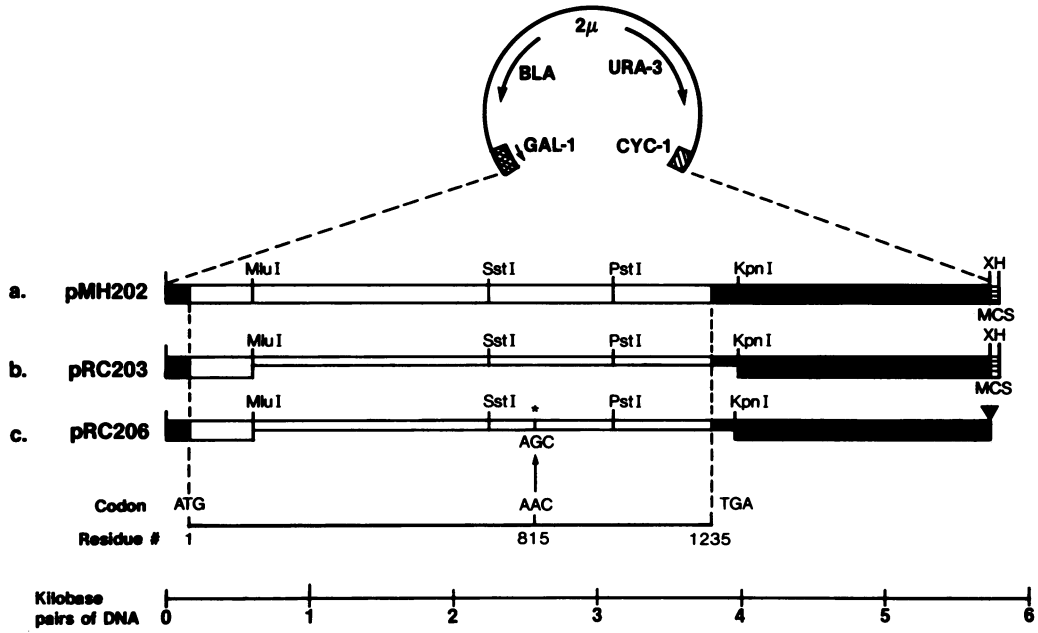

FIG. 1. Summary of the HSV-1 DNA pol-yeast expression vectors used in this study. GAL1, CYC1, URA3, BLA, and 2μm (2μ) are as previously described (14) and denote, respectively, the *S. cerevisiae* galactokinase-1 gene promoter, a synthetic oligonucleotide transcription termination sequence, a selectable marker for growth in *S. cerevisiae*, a selectable marker for growth in *E. coli*, and the 2μm *S. cerevisiae* replication sequence. Restriction endonuclease sites are *Mlu*I, *Sst*I, *Pst*I, *Kpn*I, *Xba*I (X), and *Hin*dIII (H). Open bars denote translated HSV-1 pol sequences; solid bars denote untranslated sequences. Thick bars are HSV-1 (strain 17) sequences; thin bars are HSV-1 (KOS) sequences. (a) The construction of pMH202 is described in detail elsewhere (14). (b) pRC203 is derived from pMH202: the pol region from *Mlu*I to *Kpn*I has been replaced by the analogous region of HSV-1 (KOS) DNA from pNN3, a plasmid constructed and generously supplied by M. D. Challberg (3). pRC205 (not shown) is derived from pRC203 by deletion (▼) of part of the multiple cloning site sequence (MCS) between *Xba*I and *Hin*dIII. (c) pRC206 is derived from pRC205 but contains the point mutation (*), as described in the text.

(mutant) were used to transform yeast strain Y-294 (2) by the method of Beggs (1) and selected for uracil prototrophy. Several of the resultant colonies were grown overnight in medium containing 2% raffinose (a noninducing sugar) and diluted into either 2% raffinose (controls) or 2% galactose medium as previously described (14). Expression of the HSV-1 pol gene in these vectors is tightly regulated by the GAL1 promoter and induced when yeast cells are grown in the presence of galactose (15). On the following day, cells were pelleted, and lysates were prepared and analyzed for the production of HSV-1 pol by immunoblotting and by an HSV-1 pol enzyme assay. Yeast and HSV-1 (strain Schooler)-infected and mock-infected HeLa cell lysates used in this study were prepared as previously described (14). Two new recombinant yeast strains, Y-RC203 and Y-RC206, derived from plasmids pRC203 and pRC206, respectively, were chosen for further study.

Figure 2 shows the results of an immunoblot assay of the cell lysates comparing the production of HSV-1 pol in the recombinant yeast strains with that in both the original yeast expression vector strain (Y-MH202) and infected HeLa cells. Control lysates derived from uninfected HeLa cells or recombinant yeast strains grown in raffinose were also included. The immunoblot assay was performed as previously described (14, 30). The primary antiserum was prepared against a synthetic peptide, designated P3, corresponding to residues 548 to 557 of the reported HSV-1 pol sequence (13, 28) and will be described in detail elsewhere (J. T. Matthews, J. T. Stevens, B. J. Terry, C. W. Cianci, and M. L. Haffey, submitted for publication). The immunoblot assay confirmed that both HSV-1-infected cells and yeast cells grown under inducing conditions (in galactose) contained HSV pol-immunoreactive polypeptide(s) of approximately 140 kilodaltons. Control lysates did not contain this protein, as expected. In addition, while the amount of alpha-pol enzyme activity in each of the lysates was similar, the HSV-1-specific pol activity was only found in lysates containing the HSV-1 pol enzyme and not in control lysates (Table 1). All of the pol enzyme assays reported in this study were performed as previously described (14). The HSV-1 pol assay mixture contained 5 μl of cell lysate (~25 μg of protein); 50 mM Tris hydrochloride (pH 8.0); 5 mM $MgCl_2$; 100 mM $(NH_4)_2SO_4$; 1 mM dithiothreitol; 5 μM each dATP, dCTP, and dGTP; 5 μM [^{3}H]TTP (200 cpm/pmol); 30 μg of nicked calf thymus DNA per ml; and 100 μg of bovine serum albumin per ml in a final volume of 50 μl. As shown in Table 1 and as reported previously, under these assay conditions the endogenous HeLa pol(s) (25) and the yeast pol(s) were not active (14). The alpha-pol assay mixture contained 5 μl of enzyme extract (~25 μg of protein); 10 mM Tris hydrochloride (pH 7.5); 10 mM $MgCl_2$; 10 mM $(NH_4)_2SO_4$; 0.1 mM dithiothreitol; 100 μM each dATP, dCTP, and dGTP; 100 μM [^{3}H]TTP (10 cpm/pmol); 40 μg of nicked calf thymus DNA per ml; and 2 mM spermidine in a final volume of 50 μl.

The HSV-1 pol enzyme in each of the lysates was examined for activity in the presence of increasing amounts of four known inhibitors of the HSV-1 DNA pol: ACV-TP, PAA, PFA, and APH. The mutant HSV-1 pol exhibited increased resistance to ACV-TP and increased sensitivity to PAA, relative to the wild-type enzyme (Fig. 3). The wild-type HSV-1 DNA pol 50% inhibitory concentrations determined in the present study for ACV-TP and PAA were approximately 0.8 and 180 μM, respectively. These values were higher than those previously reported with HSV-1

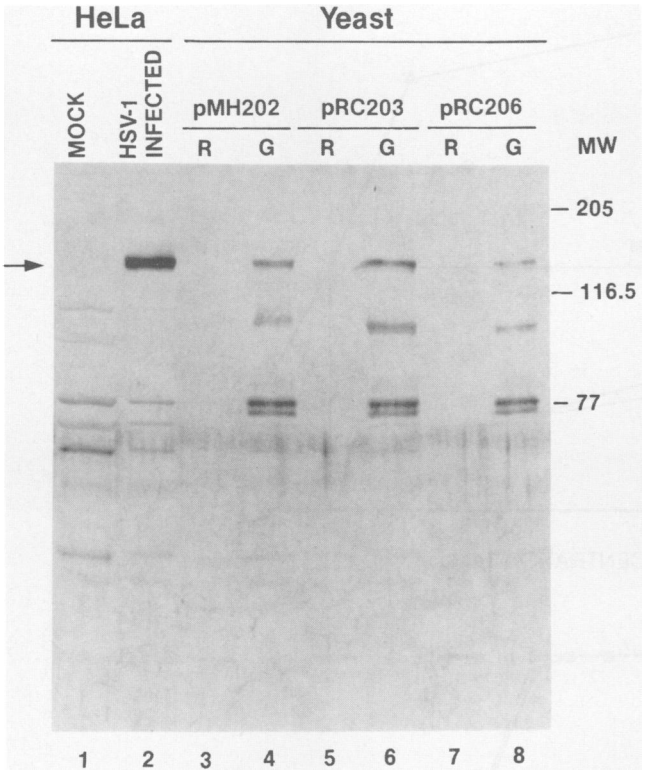

FIG. 2. Immunoblot of HSV-1 DNA pol in cell lysates. MW, Molecular weight in thousands. Lanes 1 to 8 contain about 50 μg (total protein) of lysate from the following: mock (1)- or HSV-1 (2)-infected HeLa cells; Y-MH202 yeast cells grown in raffinose (R) (3) or galactose (G) (4); Y-RC203 yeast cells grown in raffinose (5) or galactose (6); and Y-RC206 yeast cells grown in raffinose (7) or galactose (8). The primary antibody was a polyclonal rabbit antiserum (1:500 dilution) prepared against a synthetic pol peptide (residues 548 to 557), as described in the text. The secondary antibody was alkaline phosphatase-conjugated goat anti-rabbit immunoglobulins (1:500; DAKO). The arrow indicates the mobility of the ≈140-kilodalton HSV-1 pol polypeptide.

(SC16)-infected cell lysates (20). Differences between the two studies in either the infected cell extracts or in vitro enzyme assay conditions (or both), including the higher deoxynucleoside triphosphate concentrations used in our study, may account for this discrepancy. Despite this fact, the approximately 63-fold increase in ACV-TP resistance and the approximately 6-fold increase in PAA sensitivity shown for the mutant HSV-1 pol enzyme in the present study were comparable to the ranges previously reported for the three viruses (TP4.4, TP4.1, and TP3.2) containing this mutation at residue 815 in plaque reduction assays, 59- to 233-fold and 2- to 5-fold, respectively (20–22), and were within the same order of magnitude seen with the mutants in HSV-1 pol enzyme assays, 80- to 100-fold and 1.4- to 2.9-fold, respectively (20).

It was also of interest to determine whether this mutation affecting sensitivity to ACV-TP and PAA also directly affected resistance to two other inhibitors, PFA, a congener of PAA, and APH. Collateral resistance to PAA and PFA is frequently reported (8, 12, 17, 20, 21), and PAA hypersensitivity has been associated with APH resistance (21, 31). Interestingly, the mutation did not grossly affect the sensitivity of the enzyme to PFA and had only a marginal effect

TABLE 1. Results of pol enzyme assays

Lysate[a]	DNA pol activity[b] as determined by:	
	Alpha-pol assay	HSV-1 pol assay
HeLa		
Uninfected	837.1	1.0
Infected	818.2	27.5
pRC203		
Uninduced	858.6	0.6
Induced	748.0	38.45
pRC206		
Uninduced	850.2	1.0
Induced	814.4	36.0

[a] Each assay mixture contained 25 μg of protein.
[b] Expressed as picomoles of [^{3}H]TMP incorporated per reaction. Values are the averages of triplicate samples. Assays were performed as described in the text and elsewhere (14).

on the resistance to APH (less than twofold) (Fig. 4), underscoring the discrete nature of the change in the mutated pol protein. These results were in contrast to the increased sensitivity of the three mutant viruses (TP4.4, TP4.1, and TP3.2) to PFA (1.5- to 5.2-fold) and the increased resistance to APH (2.5- to 4.3-fold) in plaque reduction assays reported by Larder and Darby (21). It is possible that these differences could be accounted for by additional mutations in these viruses, as previously suggested (22). For example, mutations in the HSV-1 thymidine kinase or the major DNA-binding protein have been reported to influence sensitivity to antiviral drugs (5, 9). In contrast to the previous study (22), the only viral protein present in the enzyme extracts analyzed in the present study was HSV-1 pol. It is also possible that the six predicted amino acid sequence differences (17) between HSV-1 (SC16) pol (22) and HSV-1 (KOS) pol (13) could affect the sensitivity of the enzymes to antiviral drugs.

The results of the present study demonstrating increased sensitivity to PAA but not to PFA are somewhat surprising, as the two compounds are closely related structurally. While collateral resistance is often observed, one HSV-1 pol mutant, AraA^{r}9, was reported (12) to differ slightly from this trend in that it was sensitive to PFA but marginally resistant to PAA. Perhaps the mutation in the present study is another example of a variant from the general trend or perhaps generalizations on collateral PP_i analog resistance cannot be applied to the hypersensitivity phenotype. Our results suggest that there are differential characteristics of these two PP_i analogs which the HSV-1 pol can recognize and that the mutation at residue 815 affects the interaction of PAA with the HSV-1 pol but does not necessarily affect the PFA (or APH) sensitivity of the enzyme. Although it is possible that the yeast lysates contain components which interfere with the detection of APH resistance and PFA hypersensitivity, we do not believe that to be the case for the following reason. We have independently introduced (data not shown) another mutation (tyrosine 696 to histidine 696 [31]) into the yeast expression vector which is capable of conferring both APH resistance (eightfold) and PFA hypersensitivity (twofold) to the expressed enzyme, as measured in our assay.

The manner by which the mutation at residue 815 exerts its effect on enzyme activity or antiviral sensitivity is not clear. Other mutations conferring drug resistance or sensitivity sequenced in this region of HSV-1 pol (residues 797, 813, 821, and 841 [12]) serve to demonstrate that this region

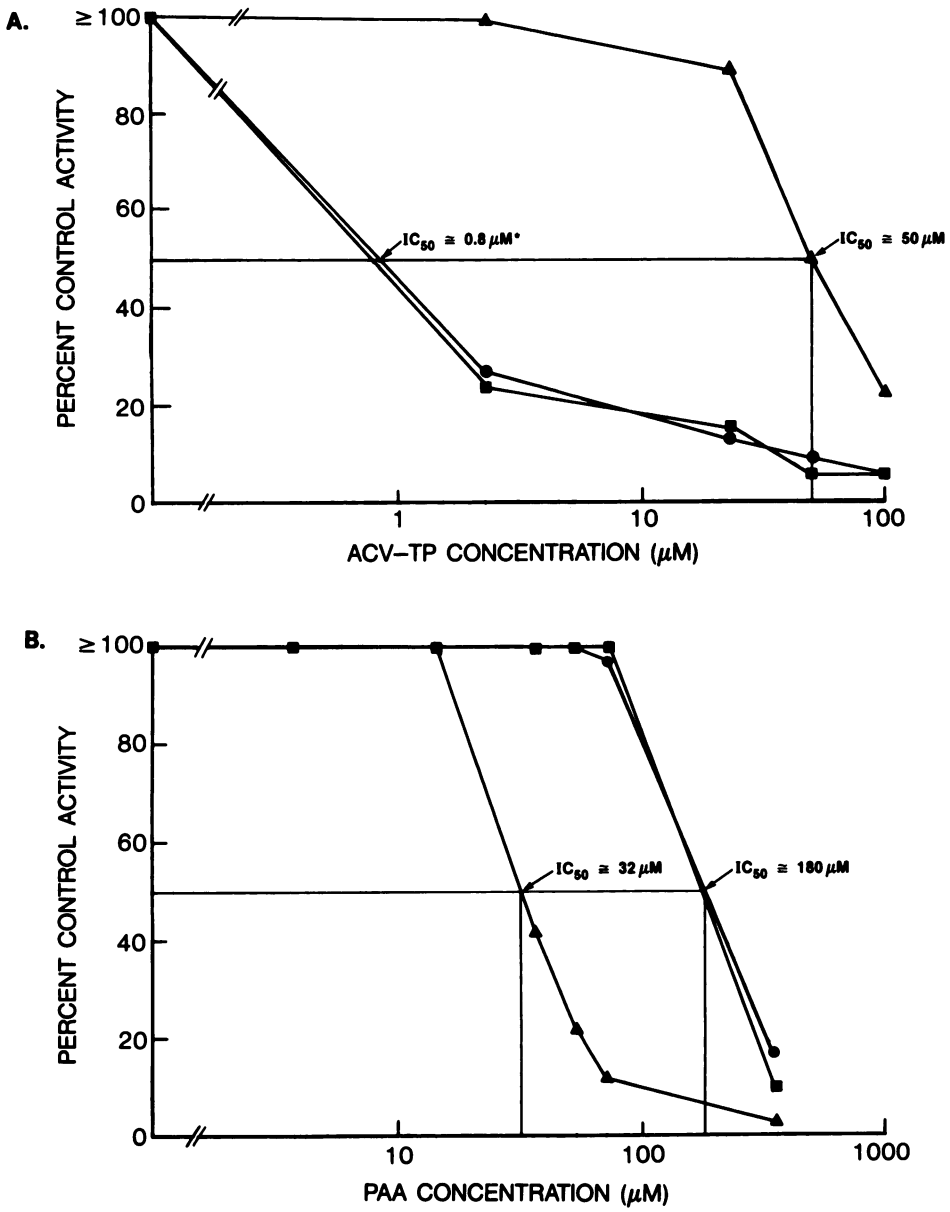

FIG. 3. (A) Inhibition of HSV-1 DNA pol activity by increasing amounts of ACV-TP. (B) Inhibition of HSV-1 DNA pol activity by increasing amounts of PAA. Symbols: ■, lysates from HSV-1-infected HeLa cells; ●, lysates from Y-RC203 cells grown in galactose; ▲, lysates from Y-RC206 cells grown in galactose. HSV-1 DNA pol enzyme assays were performed as described in the text and elsewhere (14). *, Value derived from a separate graph (data not shown). Control activity for each lysate is the result of an assay run without drug present. IC_{50}, 50% inhibitory concentration.

is clearly involved in an interaction with antiviral drugs. Residue 815 falls within the highly conserved region III (12, 13, 32, 33) of eucaryotic pols. The wild-type HSV-1 Asn residue is highly conserved and is also found in pols which are resistant to ACV-TP, including cellular alpha-pol (33) and yeast pol I (26). Secondary structure analysis of the mutant pol as compared with the wild-type HSV-1 pol by several computer programs suggests only a modest change in predicted structure (6, 17, 24). The program of Chou and Fasman (6) predicts a slightly increased propensity for a turn at the mutated residue (Ser) relative to the wild-type HSV-1 residue (Asn) (Fig. 5). Coincidentally, a similar propensity for a turn is seen at the corresponding cellular alpha-pol residue (Asn), suggesting that secondary structure and not primary sequence interactions are involved in determining drug sensitivity at this site. Additional work will be required to elucidate the mechanism of increased resistance and sensitivity induced by this mutation in HSV-1 pol.

In conclusion, our results demonstrate that the yeast expression system can be used for structure-function studies on the HSV-1 pol and that independent of other viral factors, the mutation converting Asn-815 to Ser-815 can account, at least in part, for both the ACV-resistant and PAA-hypersensitive phenotypes of the three mutant viruses previously

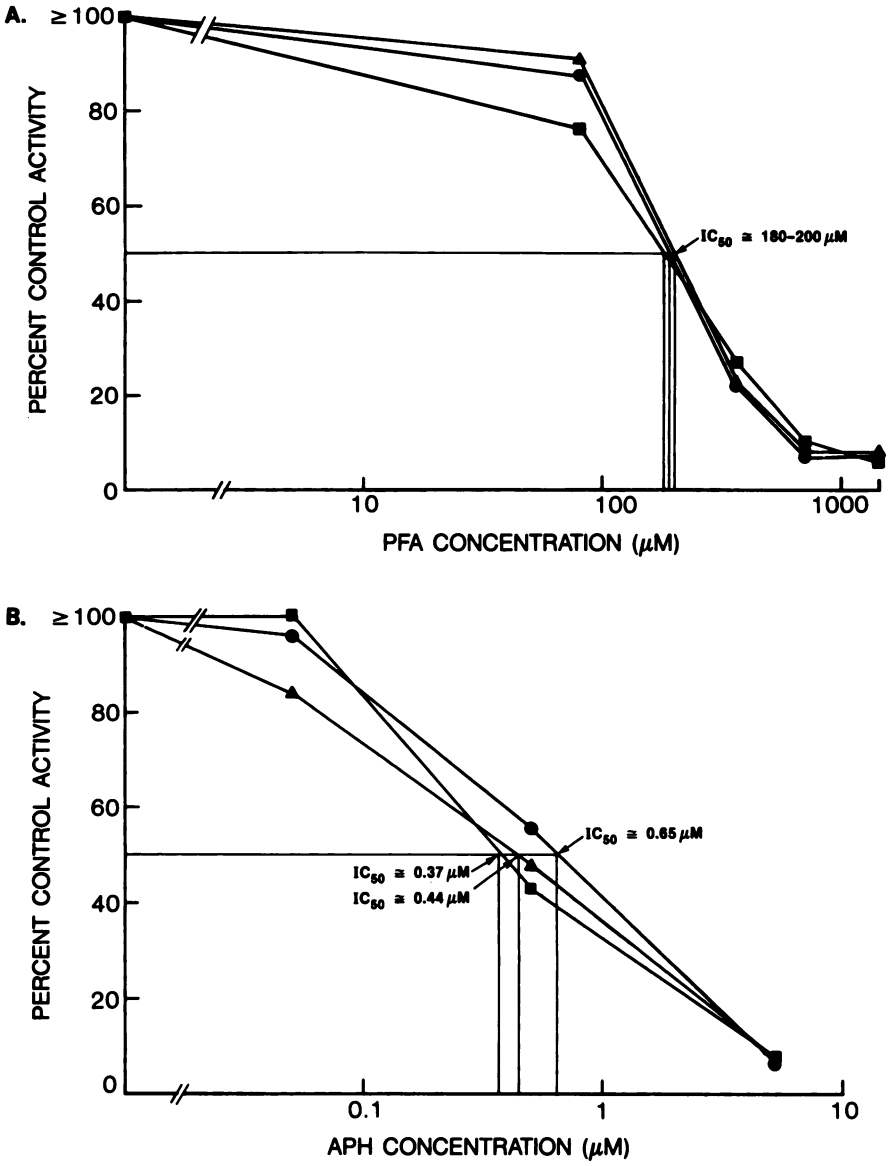

FIG. 4. (A) Inhibition of HSV-1 DNA pol activity by increasing amounts of PFA. (B) Inhibition of HSV-1 DNA pol activity by increasing amounts of APH. Symbols, enzyme assays, and abbreviations are as described in the legend to Fig. 3.

	HSV-1 DNA pol (wild type)	HSV-1 DNA pol (mutant)	Alpha-pol (human)
Residue	815	815	954
	↓	↓	↓
Amino acid	QAAIKVVCNSVYGFTGG	QAAIKVVCSSVYGFTGG	QKALKLTANSMYGCLFG
	αααααα	αααααα	αααααααα
Structure	βββββββββ βββββ	βββββββββ βββββ	βββββ
		τττ	τττττττ

FIG. 5. Chou-Fasman (6) structural analysis. Amino acids are given in the single-letter code. HSV-1 DNA pol and human DNA alpha-pol sequences are from Gibbs et al. (13) and Wong et al. (33), respectively. Conserved residues are underlined. α, Alpha helix; β, beta sheet; τ, turn. Analysis was done with Intelligenetics Suite, Version 5.2 (Intelligenetics, Inc.).

described (20–22). In addition, we show that this mutation does not necessarily or dramatically affect the sensitivity of HSV-1 pol to PFA or APH.

We gratefully acknowledge A. K. Field for support, encouragement, and critical reading of the manuscript. We thank M. D. Challberg for supplying pNN3 (3), the plasmid containing HSV-1 (KOS) DNA pol, B. J. Terry for preparation of ACV-TP, and K. Mazina for preparation of the synthetic oligonucleotide. We also thank J. Alcantara for preparation of the illustrations and both J. Baumann and M. Heilman for preparation of the manuscript.

LITERATURE CITED

1. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–109.
2. **Brugge, J. S., G. Jarosik, J. Andersen, A. Queral-Lustig, M. Fedor-Chaiken, and J. R. Broach.** 1987. Expression of Rous sarcoma virus transforming protein pp60$^{v\text{-}src}$ in *Saccharomyces cerevisiae* cells. Mol. Cell. Biol. **7**:2180–2187.
3. **Challberg, M. D.** 1986. A method for identifying the viral genes required for herpesvirus DNA replication. Proc. Natl. Acad. Sci. USA **83**:9094–9098.
4. **Chartrand, P., C. S. Crumpacker, P. A. Schaffer, and N. M. Wilkie.** 1980. Physical and genetic analysis of the herpes simplex DNA polymerase locus. Virology **103**:311–326.
5. **Chiou, H. C., S. K. Weller, and D. M. Coen.** 1985. Mutations in the herpes simplex virus major DNA-binding protein gene leading to altered sensitivity to DNA polymerase inhibitors. Virology **145**:213–226.
6. **Chou, P. Y., and G. D. Fasman.** 1978. Prediction of the secondary structure of proteins from the amino acid sequence. Adv. Enzymol. **47**:45–147.
7. **Coen, D. M., D. P. Aschman, P. T. Gelep, M. J. Retondo, S. K. Weller, and P. A. Schaffer.** 1984. Fine mapping and molecular cloning of mutations on the herpes simplex virus DNA polymerase locus. J. Virol. **49**:236–247.
8. **Coen, D. M., H. E. Fleming, L. K. Leslie, and M. J. Retondo.** 1985. Sensitivity of arabinosyladenine-resistant mutants of herpes simplex virus to other antiviral drugs and mapping of drug hypersensitivity mutations to the DNA polymerase locus. J. Virol. **53**:477–488.
9. **Coen, D. M., and P. A. Schaffer.** 1980. Two distinct loci confer resistance to acycloguanosine in herpes simplex type 1. Proc. Natl. Acad. Sci. USA **77**:2265–2269.
10. **Derse, D., K. F. Bastow, and Y.-C. Cheng.** 1982. Characterization of the DNA polymerases induced by a group of herpes simplex type-1 variants selected for growth in the presence of phosphonoformic acid. J. Biol. Chem. **257**:10251–10260.
11. **Dicicco, R. A., K. Chadha, and B. I. S. Srivastava.** 1980. Inhibition of herpes simplex virus-induced DNA polymerase, cellular DNA polymerase α, and virus production by aphidicolin. Biochim. Biophys. Acta **609**:224–231.
12. **Gibbs, J. S., H. C. Chiou, K. F. Bastow, Y.-C. Cheng, and D. M. Coen.** 1988. Identification of amino acids in herpes simplex virus DNA polymerase involved in substrate and drug recognition. Proc. Natl. Acad. Sci. USA **85**:6672–6676.
13. **Gibbs, J. S., H. C. Chiou, J. D. Hall, D. W. Mount, M. J. Retondo, S. K. Weller, and D. M. Coen.** 1985. Sequence and mapping analysis of the herpes simplex virus DNA polymerase gene predict a C-terminal substrate binding domain. Proc. Natl. Acad. Sci. USA **82**:7969–7973.
14. **Haffey, M. L., J. T. Stevens, B. J. Terry, D. I. Dorsky, C. S. Crumpacker, S. M. Wietstock, W. T. Ruyechan, and A. K. Field.** 1988. Expression of herpes simplex virus type 1 DNA polymerase in *Saccharomyces cerevisiae* and detection of virus-specific enzyme activity in cell-free lysates. J. Virol. **62**:4493–4498.
15. **Johnson, M., and R. W. Davis.** 1984. Sequences that regulate the divergent GAL1-GAL10 promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1440–1448.
16. **Knopf, C. W.** 1987. The herpes simplex virus type-1 DNA polymerase gene: site of phosphonoacetic acid resistance mutation in strain Angelotti is highly conserved. J. Gen. Virol. **68**:1429–1433.
17. **Knopf, C. W., and K. Weisshart.** 1988. The herpes simplex virus DNA polymerase: analysis of functional domains. Biochim. Biophys. Acta **951**:298–314.
18. **Kouzarides, T., A. T. Bankier, S. C. Satchwell, K. Weston, P. Tomlinson, and B. G. Barrell.** 1987. Sequence and transcription analysis of the human cytomegalovirus DNA polymerase gene. J. Virol. **61**:125–133.
19. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotype selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
20. **Larder, B. A., and G. Darby.** 1985. Selection and characterization of acyclovir-resistant herpes simplex virus type-1 mutants inducing altered DNA polymerase activities. Virology **146**: 262–271.
21. **Larder, B. A., and G. Darby.** 1986. Susceptibility to other antiherpes drugs of pathogenic variants of herpes simplex virus selected for resistance to acyclovir. Antimicrob. Agents Chemother. **29**:894–898.
22. **Larder, B. A., S. D. Kemp, and G. Darby.** 1987. Related functional domains in virus DNA polymerases. EMBO J. **6**: 169–175.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Novotny, J., and C. Auffray.** 1984. A program for prediction of protein secondary structure from nucleotide sequence data: application to histocompatibility antigens. Nucleic Acids Res. **12**:243–255.
25. **Ostrander, M., and Y.-C. Cheng.** 1980. Properties of herpes simplex virus type 1 and type 2 DNA polymerase. Biochim. Biophys. Acta **609**:232–245.
26. **Pizzagalli, A., P. Valsasnini, P. Plevani, and G. Lucchini.** 1988. DNA polymerase I gene of *Saccharomyces cerevisiae*: nucleotide sequence, mapping of a temperature sensitive mutation, and protein homology with other DNA polymerases. Proc. Natl. Acad. Sci. USA **85**:3772–3776.
27. **Powell, K. L., and D. J. M. Purifoy.** 1977. Nonstructural proteins of herpes simplex virus. I. Purification of the induced DNA polymerase. J. Virol. **24**:618–626.
28. **Quinn, J. P., and D. McGeoch.** 1985. DNA sequence of the region in the genome of herpes simplex virus type-1 containing genes for DNA polymerase and the major DNA binding protein. Nucleic Acids Res. **13**:8143–8163.
29. **Sanger, F., S. Miklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
30. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
31. **Tsurumi, T., K. Maeno, and Y. Nishiyama.** 1987. A single-base change within the DNA polymerase locus of herpes simplex virus type 2 can confer resistance to aphidicolin. J. Virol. **61**:388–394.
32. **Wang, T. S.-F., S. W. Wong, and D. Korn.** 1989. Human DNA polymerase α: predicted functional domains and relationships with viral DNA polymerases. FASEB J. **3**:14–21.
33. **Wong, S. W., A. F. Wahl, P.-M. Yuan, N. Arai, B. E. Pearson, K.-I. Arai, D. Korn, M. W. Hunkapiller, and T. S.-F. Wang.** 1988. Human DNA polymerase α gene expression is cell proliferation dependent and its primary structure is similar to both procaryotic and eucaryotic replicative polymerases. EMBO J. **7**:37–47.
34. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.